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A) Adult Inborn Errors of Metabolism (001 to 037)

001 - Clinical Presentation of Late-Onset Multiple Acyl-CoA Dehydrogenase Deficiency

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Multiple acyl-CoA dehydrogenase deficiency (MADD) is an inborn error of metabolism affecting the catabolism of branched chain amino acids, lysine and tryptophan, as well as β oxidation of fatty acids. It results in impaired ATP synthesis, excessive lipid accumulation, and impaired gluconeogenesis. The condition arises from defects in either the electron transfer flavoprotein (*ETF A/B* gene) or ETF-ubiquinone oxidoreductase (*ETFDH* gene). Three phenotypes are recognized: neonatal onset with congenital anomalies, neonatal onset without congenital anomalies, and late onset. Late onset form age of presentation is variable, and symptoms range from muscular/cardiac to episodic vomiting. Diagnosis is based on urinary organic acid and plasma acylcarnitine profile. We describe a male presenting at 26 years of age with a vasculitic rash which worsened with fluctuating consciousness progressing to encephalitis. He had a partially compensated metabolic acidosis (pH 6.92, HCO_3^- 6.6, pCO_2 32.4), hypoglycemia (3.2 mmol/L), and decreased lactate (<0.3 mmol/L). Plasma acylcarnitine profile collected on day 3 showed low total 10 µmol/L (RR 21-70) and free 2 µmol/L (RR 13-56) carnitine with significant elevations in C_5 0.94 µmol/L (RR <0.28 µmol/L) and C_5 DC 1.55 µmol/L (RR <0.34 µmol/L). The pattern was suggestive of MADD; however, urine metabolic screen showed decreased glycine, but no markers of a fatty acid oxidation defect. Following treatment with IV carnitine, β-hydroxybutyrate, CoQ, riboflavin and glycine, total (52 µmol/L) and free (25 µmol/L) carnitine normalized, but medium-chain species showed significant elevations C_5 2.36 µmol/L, C_5 DC 4.10 µmol/L, C_6 0.58 µmol/L (RR <0.13 µmol/L), C_8 2.80 µmol/L (RR <0.24 µmol/L), C_{10} 4.78 µmol/L (RR <0.40 µmol/L). During this period, the patient also suffered subcortical hemorrhage requiring drainage, fluctuating GCS, renal failure requiring dialysis, and collapsed lung. His condition improved significantly both biochemically and clinically, and he was able to be discharged on day 24 and remains on the medications above and a low-fat diet. Mutation testing results for *ETF A/B* and *DH* are pending. The precipitating factors are unclear, but the underlying infection producing the vasculitic rash is suspected to be the trigger.

002 - Characterization of SLC6A8 Mutations in Creatine Transporter Deficiency (CTD)

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SLC6A8 gene mutations impair the transport of creatine into cells, and the effects of creatine deficiency are most debilitating in organs and tissues that require rapid high energy production, especially the brain. Creatine transporter deficiency (CTD) is an X-linked disease, thus males often have more severe phenotype than females. Patients with CTD have decreased or absent brain creatine, as measured by MRS, and increased creatine–creatinine ratio in urine. They present with mild to severe intellectual disability with prominent delays in speech and language development. CTD patients can also experience seizures with a delay in the development of motor skills. The prevalence of CTD is unknown; however, more than 250 affected individuals have been identified. Based predominantly on mutation data in the Leiden Open Variation Database (LOVD), we conducted a systematic search of studies in the human gene mutation database (HGMD) and PubMed, in which the pathogenicity of SLC6A8 mutation was known. Objective was to compile a current catalogue of pathogenic mutations in the SLC6A8 gene and correlate genetic information with symptomatic profile, thus advancing our investigation into potential biomarkers of CTD. From our search, we created an SLC6A8 gene pathogenic mutation database consisting of the location of the mutation, cDNA nucleotide substitution, corresponding protein change, type of mutation, and an assigned clinical phenotype. The most frequent types of mutations were nonsense (43), missense (41), and deletion (32), with 8 distinct de novo mutations. It is estimated that approximately 30% of

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SLC6A8 mutations are de novo mutations. The most reported clinical phenotypes were intellectual disability and mild intellectual disability. Mutations clustered around exons 6 through 12, with exon 9 having the most (16), next to exon 6 (14), exon 12 (12), and exons 7 and 8 with an equal number of mutations (10 each). SLC6 transporter family has the substrate binding site located at the core of the transporter, with the inner ring that forms the substrate binding site coded by exons 1, 3, 6, and 8. We strongly encourage researchers and clinicians to submit SLC6A8 gene variants to LOVD, as this database is the most expansive published database of pathogenic and nonpathogenic mutations of this gene and is a valuable resource for genetic counselors.

003 - In Vivo Models for Qatari Specific Classical Homocystinuria as Basis for Development of Novel Therapies

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Background: Homocystinuria is a rare inborn error of methionine metabolism caused by cystathionine β-synthase (CBS) deficiency. However, in Qatar the prevalence is 1:1800 due to a founder Qatari missense mutationc.1006C>T, in which arginine (R) is replaced with cysteine (C) at CBS p.336. Untreated homozygous patients are clinically severely affected with intellectual disability & multisystem complications. **Objectives:** To repair the R336C-CBS deficient enzymatic activity by different approaches using 2 in vivo models: (i) Yeast (S. cerevisiae) model: established by generating cbs−/− yeast strains express in trans, the wild-type (wt) human CBS (WY79p.hCBS strain) and the cbs-R336C (WY79p.R336C strain) and (ii) HEK293 T and HepG2 knock-in cbs-R336C cell lines: generated using CRISPR/Cas9 technique. **Results and conclusions:** In yeast, only WY79p.hCBS, but not WY79p.R336C strain, was able to grow in media lacking cysteine. Similarly, HEK293 T knock-in cbs-R336C cells failed to proliferate in media deprived from cysteine, demonstrating the blockage of cysteine formation due to the cbs-R366C mutation in both models. Native PAGE-gel analysis demonstrated similar expression levels of the cbs-R336C protein, but absence of the active tetrameric conformation present in both wt HEK293 T or WY79p. hCBS cells. We recently started screening of potential drug candidates that might restore R336C-cbs activity in these models. Our previous studies showed that the enzymatic activity of R336C-cbs protein (bacterial purified or crude cell homogenate) can be repaired using cysteamine, a drug used for treatment of cystinosis (*Hum Mol Genet*. 2015; 24: 7339). Next to cysteamine, therapeutic alternatives, including chaperones and gene therapy approaches, are currently under investigation.

004 - Long-Term Follow-Up in Women With X-Linked Adrenoleukodystrophy: A Prospective Cohort Study

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Background: The majority of women with X-linked adrenoleukodystrophy (ALD) develop myelopathy in adulthood. The onset of spinal cord disease is usually in the fifth decade. The proportion of symptomatic women increases markedly with age. Current treatment options are supportive only. As new treatments are under development, quantitative information on disease progression rates is vital for clinical trial design. However, data on the progression rate of myelopathy in women with ALD is limited. In the only published prospective follow-up study (n = 29; mean follow-up 9 ± 3 months) 2 myelopathy scales, the Japanese Orthopaedic Association (range 2-17 points) and Severity Score System for Progressive Myelopathy (range 0-100 points), revealed a decrease of 0.42 and 1.87 points per year, respectively. Longer follow-up and additional outcome measures are needed. The aim of this study was to prospectively assess long-term progression of myelopathy in women with ALD. Baseline results from this cohort were reported previously (Engelen et al *Brain* 2014). **Methods**: In this prospective follow-up study, we re-evaluated the cohort after 5 years. Primary outcome measures were symptomatic status, Expanded Disability Status Scale scores, AMC Linear Disability Scale scores and SF-36 (Quality of Life Assessment) scores. Patients were considered symptomatic when symptoms and signs suggestive of myelopathy were found during clinical assessment. Linear mixed models were used to evaluate progression of myelopathy. **Results**: All 46 patients were invited for follow-up between May 2015 and May 2017. Thirty-four (73.9%) were enrolled. Twelve patients were lost to follow-up: death (1/12), contact information unknown (1/12), comorbidity (3/12), and declined to participate (7/12). Median follow-up time was 7.83 years (range 6.42 -8.67 years). Nineteen of the 34 (55.9%) patients had myelopathy at baseline and 26/34 (76.5%) at follow-up. An exact McNemar's test determined a statistically significant difference in the proportion of patients with myelopathy at baseline and follow-up, $P = .016$. Further statistical analysis of the follow-up data is in progress. Results will be available before September 2017. **Conclusion**: This study will report new quantitative data on progression of myelopathy in women with ALD and will be the first to report after a long-term follow-up period of 7 years. These data will be vital in the development of new clinical trials.

005 - Hemodynamic and Catecholaminergic Response to Stress in Adult Patients With Phenylketonuria

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Introduction: Defective function of phenylalanine-hydroxylase in phenylketonuria (PKU) results in the accumulation of phenylalanine (Phe) and reduction of tyrosine (Tyr) in the blood. Tyr is the precursor for catecholamines, secreted in response to stress by the adrenal medulla and paraganglia. We hypothesized that PKU patients have reduced catecholaminergic, therefore physiologic response to sympathetic stress stimulation, and this reduction is in relation with adherence to the low phenylalanine diet and medical foods. **Method:** Twelve males with PKU (age 18-38 years) where included in this monocentric cross-sectional study. They were divided into 2 groups based on their annual Phe values, with the cut-off point being the upper limit of 600 μmol/L: on diet (n = 6) and loose diet (n = 6). Their response to sympathetic stimulation was compared to 10 age-matched healthy controls. After a period of 30-minute resting, basal plasma norepinephrine (NE), epinephrine (E), Phe, Tyr, blood pressure, and heart rate where recorded. Subsequently the subjects were exposed to two sympathetic stress stimulations: cold pressor (CPT) and isometric-handgrip test (HGT). Plasma NE, E, Phe, Tyr, blood pressure, and heart rate were measured right after the test and compared to the basal values. **Results:** No significant difference was observed in basal values and sympathetic stress-induced hemodynamic changes between PKU patients and controls. The surge of NE level was higher in the control group (CPT: 95.7 ± 94.8 pg/mL, HGT: 113.6 ± 109.6 pg/mL), compared to on diet group (CPT: 88.6 ± 41.2 pg/mL, HGT: 55.2 ± 41.2 pg/mL), and loose diet group (CPT: 34.4 ± 33 pg/mL, HGT: 60.7 ± 37.8 pg/mL). E level changes showed a similar pattern with during the CPT in control group (32.4 ± 37.5 pg/mL), compared to on diet group (36.4 ± 32.3 pg/mL), and it was lower in the loose diet group (13.3 ± 6.5 pg/mL). Following the HGT, the control group showed higher E level increase (34.9 ± 39.2 pg/mL), compared to the on-diet group (20 ± 12.8 pg/mL) and loose diet group (6.8 ± 9.4 pg/mL). **Conclusion:** The hemodynamic response to stress was comparable in both PKU groups and controls. Lower surges of NE and E levels in response to stress suggest altered catecholamine metabolism in PKU patients, that could be influenced by metabolic control.

006 - Assessment of the Effect of Once Daily Nitisinone therapy on 24-h Urinary Metadrenalines and 5-Hydroxyindole Acetic Acid Excretion

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Introduction: One of the major metabolic consequences of treatment with nitisinone in Alkaptonuria (AKU) and Hereditary Tyrosinemia type-1 (HT1) is that circulating tyrosine concentrations significantly increase. It has been postulated that hypertyrosinemia may contribute to neurodevelopmental delay in HT1. As tyrosine is the metabolic substrate required for the biosynthesis of catecholamine neurotransmitters, it possible that these metabolites may also be altered in AKU due to hypertyrosinemia. Herein we report the concentrations urinary normetadrenaline (NMA), metadrenaline (MA), 3-methoxytyramine (3-MT) (catecholamine metabolites), and 5-hydroxyindole acetic acid (5-HIAA, metabolite of serotonin) as surrogate markers of the catecholamine and serotonin metabolic pathways. **Materials and Methods:** Urine samples analyzed were from subjects included in the SONIA-1 clinical Trial. The concentrations of urinary NMA, MA and 3-MT, and 5-HIAA were determined by liquid chromatography tandem mass spectrometry. Interassay coefficient of variation was <10% for all analytes measured. **Results:** Urine samples from 36 patients were available for inclusion in this study; 7 patients received no treatment (4 male, mean age (±SD) 46.3 (16.4) years) and 29 patients received a daily dose of nitisinone [1 mg (n = 7, 6 male, mean age 45.9 (10.9) years), 2 mg (n = 8, 5 male, mean age 43.9 (13.7) years), 4 mg (n = 8, 5 male, mean age 47.3 (10.7) years) and 8 mg (n = 6, 4 male, mean age 53.8 (8.3) years)]. 3-MT concentrations increase significantly ($P < .01$, at all doses) following nitisinone therapy, but not in a dose-dependent manner. NMA concentrations decreased ($P < .05$, at all doses) following nitisinone therapy at all doses. A large proportion of patients had NMA, MA, and 3-MT concentrations outside of the normal reference range prenitisinone therapy. Following nitisinone therapy, NMA concentrations were within the normal reference range, however 3-MT concentrations were outside of the reference range in all patients. 5-HIAA concentrations decreased following nitisinone therapy, and were significantly lower at a daily dose of 8 mg ($P < .05$). **Conclusions:** This study shows that there is an alteration in the catecholamine and serotonin metabolic pathways in AKU patients post-nitisinone therapy.

007 - The Severe Morbidity of Iconic Disease Alkaptonuria (AKU): Data From the United Kingdom National Alkaptonuria Center

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Alkaptonuria (AKU) is a rare genetic deficiency of homogentisate dioxygenase (HGD), characterized by high circulating homogentisic acid (HGA), some of which is deposited in connective tissue as a pigment, a process termed ochronosis. AKU being a genetic disease is present from birth but in the pediatric age group is mostly asymptomatic and considered a chemical curiosity. Data on 50 patients (20 female: 53.1+14.9 years; 30 male: 47+13.9 years) with AKU visiting the United Kingdom National Alkaptonuria Centre in Liverpool for assessment is presented. Some of the morbidity is due to HGA itself in the form of lithiasis with prevalence of renal and prostate stones of 26% and 60%, respectively. Osteopenia is present in 64%. There were 37 fractures in the cohort. There were 28 ruptures mainly of muscle and tendons. 58% had aortic valve disease. 18 of the 50 had joint replacements (63 in all). We highlight the considerable morbidity to emphasize that AKU is not a benign disease.

008 - Acute Manifestation of Classical Homocystinuria After Major Trauma in an Adult Patient Requiring Parenteral Nutrition

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Background: Homocystinuria due to cystathionine beta synthase (CBS) deficiency presents with a wide clinical spectrum. Treatment aims at reducing homocysteine levels by using vitamin B_6, possibly methionine-restricted diet, betaine, and/or folate and vitamin B_{12} supplementation. Currently no nutritional guidelines exist for parenteral nutrition (PN) of acute metabolic decompensation of CBS in critically ill patients. **Objective and design:** Review of literature and case report of a 22-year-old female with a history of untreated hyperhomocysteinemia and pulmonary embolism, who was admitted in the intensive care unit, following a motor vehicle accident (MVA) complicated by intestinal perforation, requiring total PN. **Results:** Total homocysteine (tHcy) level prior to MVA was 350.2 μmol/L (normal 5-15). Amino acid profile showed a methionine level of 952 μmol/L (normal 20-40) and low cystine and folate. Intravenous high dose vitamins B6, B12, and, folinic acid were started. Enteral nutrition being contraindicated on 5th day, PN was compounded, limiting methionine intakes, and using high dose n-3 polyunsaturated fatty acids (PUFAs). The patient remained comatose. Under tailored PN, tHcy and methionine levels decreased slowly, associated with neurological recovery. Enteral nutrition was introduced on day 28. At discharge the patient had no neurologic sequelae, was on normal diet, vitamin B6 therapy, stable normalized tHcy, and methionine levels. **Conclusions:** By reporting successful nutritional management of a decompensated CBS deficiency using tailored PN with limited methionine intake and n-3 PUFA addition, we would like to underscore the fact that standard PN solutions are not adapted for CBS deficient critical ill patients. High methionine levels (> 800 μmol/L) being potentially neurotoxic, there is an urgent need to improve our knowledge of acute nutritional management.

009 - A Case Report on a MPS II Patients With Parkinson-Like Symptoms

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Mucopolysaccharidosis type II, MPS II or Hunteŕs syndrome, is a chronic and progressive disease related to a dysfunction on lysosomal activity. The molecular manifestation of this disease is a deficiency on iduronate-2 sulfatase enzyme. This deficiency interferes on recycling and metabolic pathways of mucopolysaccharides or glycosaminoglycans into lysosomes. These events lead to a multisystemic organ dysfunction. More than 300 mutations have been reported on the *IDS* gene as a cause for this disorder. MPSs are sometimes related to other complex diseases like neurological disorders. Tubulinophaties are a heterogeneous group of conditions related to a large spectrum of malformations of cortical development. Recent studies have reported critical effects of tubulins and microtubule-associated proteins involvement in malformations of cortical development. A male patient showing globose abdomen, splenomegaly, dysmorphic facial features, tremors and signs related to Parkinson's disease, no perinatal or significant findings in the family history, normal karyotype, and radiographic findings of long bones and signs of disostosis. Exome sequencing was performed on the iIlumina platform and single-nucleotide variants were found on *IDS* and *TUBB3* genes. The variant for *IDS* was located on position X:148579779 (c566-567 T>TT) leading a truncated protein product. *TUBB3* variant was located on position 16:89986117 (C>T), and identified as rs1805007 with a pathogenic effect. Literature reports support a low prevalence of tubulinopathies related to *TUBB3*. In conclusion, it is important to identify the genotype by exome sequencing in patients with MPS manifestations in order to avoid misinterpretations and wrong diagnosis.

010 - Diagnosis of Gaucher Type 1 Disease Among Adult Patients With Splenomegaly, Avascular Osteonecrosis and/or Hip Replacement of Unknown Cause: Preliminary Results

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Objectives: Gaucher disease (GD) is one of the most frequent lysosomal storage disorders, and hepato-splenomegaly, bleeding, and bone disease are the most common presenting features. Early diagnosis and specific treatment of these patients can improve their quality of life and modify the natural history of this disease. However, an increased number of patients remain undiagnosed until adulthood. The objective of this study is to diagnose GD in adult patients with splenomegaly, avascular osteonecrosis, and/or hip replacement of unknown cause. **Methods:** Eleven Spanish hospitals were enrolled in this observational and retrospective study. Clinical histories of patients with splenomegaly (SM), avascular osteonecrosis (AVN), and/or hip replacement (HR) (age <50 years) of unknown cause, were reviewed. Enzymatic activities of glucocerebrosidase (GC) and chitotriosidase (CT) were evaluated on dry blood spot samples. **Results:** A total of 3653 clinical histories were reviewed but only 521(14.3%) patients met inclusion criteria [143(27.5%) with SM, 238(45.7%) with HR and 140(26.9%) with AVN] and 248(47.6%) of those [66(26.6%) with SM, 99(39.9%) with HR and 83(33.5%) with AVN] agreed to participate in the study. Seven (2.8%) patients [3(42.9%) with SM, 3(42.9%) with HR and 1(14.3%) with AVN] showed low GC activity, but the genetic analysis revealed that they were not affected with GD. On the other hand, 6 (2.4%) patients showed high activity of chitotriosidase and normal GC activity [2(33.3%) with SM, 3(50%) with HR and 1(16.7%) with AVN]. Only 1 patient with SM showed a low GC activity and a significant increase of CT at the same time. **Conclusions:** Even though 7 patients showed a low GC activity on dry blood spot samples, only 1 patient showed a low GC activity and high CT activity at the same time and was diagnosed of GD.

011 - Three-Year Experience of Pediatric Metabolic Physicians With Adult Inpatient Consultations

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Physicians specialized in inborn errors of metabolism (IEMs) are a small and growing group. In many regions, as in Turkey, physicians caring for adults have not specialized in metabolic diseases, and pediatric metabolic physicians continue to care for patients of all ages. With this study, we aimed to evaluate the spectrum of adult inpatient consultations to the pediatric metabolic department at a major metabolic center in Turkey retrospectively. Inpatient consultations to the pediatric metabolic department regarding patients over 18 years of age during the 2014-2016 study period were included. A total of 48 patients were consulted. 14 of them already had an established diagnosis of an IEM, 2 of which were consulted before child labor and 4 before surgery. The remaining 34 (age 19-63) were consulted for suspicion of an IEM (21 consults in 2016). Majority of consultations were from the departments of neurology or internal medicine and none from psychiatry. Among these 34 patients, 21 had neurological findings, followed by 12 patients with musculoskeletal, 8 with nephrological, and fewer with other findings. A definite diagnosis of an IEM was reached in 11 of 34 patients (32.3%): 2 were diagnosed with L-2-hydroxyglutaric aciduria, and 1 each with metachromatic leukodystrophy, Fabry disease, Hunter syndrome, mitochondrial neurogastrointestinal encephalopathy, X-linked adrenoleukodystrophy, Leber's hereditary optic neuropathy, classical homocystinuria, carnitine palmitoyl transferase-II deficiency, and cerebrotendinous xanthomatosis. Specific metabolic treatment was started in 10 patients. It is well-established that IEMs in adults mainly present with neuropsychiatric problems. Scarcity of psychiatric findings and consultations from the psychiatry clinic may suggest that IEMs with solely psychiatric presentations may have been missed. The fact that a third of adults consulted for suspicion of an IEM received a definite diagnosis reveals that consultations to pediatric metabolic physicians regarding adults are extremely relevant. The diagnostic yield may have been even higher if metabolomic or genomic approaches could have been used. Workshops by the pediatric metabolic department may have raised awareness of IEMs, increasing the number of consultations over the years. IEMs in adults is a challenging issue, which pediatric metabolic physicians must try to tend to until physicians caring for adults in their region specialize in this ever-growing field.

012 - A Case Report of Recurrent Urolithiasis Owing to Cystinuria Diagnosed in Adulthood

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Introduction: Cystinuria is a primary aminoacidopathy and genetic condition characterized by tubular defect in the reabsorption of cystine (Cys) and dibasic amino acids (DBA), with an incidence of 1 in 2500 to 1 in 16,000, according to the population origin; this disease caused by mutations in the genes: SCL3A1 and SLC7A9 present a complex inheritance pattern, however it can be described clinically as: phenotype I (recessive pattern) with normal aminoaciduria presented in heterozygotes patients, phenotype II (partially dominant) with a moderate increase in the urinary excretion of Cys and DBA, finally phenotype III which show high excretion of Cys and DBA caused by a dominant mutation in context of autosomal dominant pattern with incomplete penetrance. Without diagnosis and treatment, patients present recurrent stone formation due to the low solubility and subsequent accumulation of Cys, resulting in obstructive uropathy, pyelonephritis, and, rarely, renal failure. **Materials and methods:** We present, a middle-age woman with clinical suspicion of cystinuria using the medical history and paraclinical tests to confirm the diagnosis. **Results:** A 46-year-old woman, descendant of non-consanguineous parents, attends to the genetic consult for the first time remitted by the nephrologist, with no family history of lithiasis. She has a clinical history of bilateral cataracts and mild hypoacusia and debuted at the age of 20 years with an episode of bilateral urolithiasis, until now she required several invasive interventions owing to recurrent urolithiasis; the metabolic study for the calculi was made reporting the presence of Cys, then 24-hour urine test for Cys was performed, presenting 10 times higher than the normal range (618 mg/24 h) and a chromatography for amino acids, positive for bands of Cys, Lysine, Arginine, Histidine and Ornithine, with those result the molecular test was performed for the genes SLC3A1 - SLC7A9. **Discussion:** In this case, we suspect a phenotype II due to moderate excretion of Cys in the 24-hour urine test, which usually oscillates between 600 and1400 mg/day in this range patients may form calculi. Prevent new renal calculations, avoid the growth and dissolve the present calculi are the cornerstone of treatment for this we are using in this patient: abundant oral fluids to avoid urinary supersaturation of Cys in urine (<250 mg/L at pH of 4.5-7.5), dietary restriction (methionine, sodium intake) and potassium citrate as alkalizing agent.

013 - Sustained Radiographic and Functional Improvements With Asfotase Alfa Treatment for up to 7 Years in Children With Hypophosphatasia

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Children with hypophosphatasia (HPP) treated with asfotase alfa in a Phase 2 study (NCT00952484) and its open-label extension (NCT01203826) experienced significant improvements in skeletal mineralization and physical function that were sustained through 5 y of treatment [1]. Results through 7 years of treatment are reported here. Children with HPP aged 6 to 12 y at baseline were initially randomized to receive asfotase alfa 6 or 9 mg/kg/wk SC for 6 months and continued in an open-label extension where they received asfotase alfa 3 mg/kg/wk SC, later increased to 6 mg/kg/wk. Radiographs of hands/wrists and knees were assessed using the Radiographic Global Impression of Change (RGI-C) scale and Rickets Severity Scale (RSS). Additional outcome measures included growth; functional ability/disability (6-Minute Walk Test [6MWT]; Bruininks-Oseretsky Test of Motor Proficiency, 2nd Edition [BOT-2] Strength and Agility Composite Standard Score; Child Health Assessment Questionnaire Disability Index [CHAQ-DI]); and safety. Data are reported as median (minimum, maximum) change from baseline, except for RGI-C which is a measurement of change from baseline (range: -3 [severe worsening] to $+3$ [complete/near complete healing]). All 12 children who entered the extension phase received asfotase alfa for up to 7 years' total exposure. Improvements in HPP-related skeletal manifestations were documented and sustained through end of study (final median [min, max] RGI-C score: 2.8 [2.0, 3.0], $P = .0005$; RSS score: -2.8 [-5.0, -0.5], statistical testing not performed). Improved growth (height Z-score: 0.8 [-0.2, 1.9], $P = .0007$; weight Z-score: 1.1 [0.3, 3.4], $P = .0004$) and function (% predicted 6MWT: 26.7 [1.1, 56.1], $P = .0006$; BOT-2 Strength and Agility score: 23.0 [7.0, 30.0], $P < .0001$; and CHAQ-DI: -0.9 [-1.8, 0], $P = .0004$) were also sustained through end of study. All patients had mild to moderate injection site reactions (e.g., erythema, macule, lipohypertrophy); 1 event of injection site atrophy was assessed as severe. No serious adverse events, including deaths, were reported. Improvements in HPP-related skeletal manifestations, growth, and functional ability with asfotase alfa in children with HPP persisted at 7 years of treatment. Treatment was

generally well tolerated. [Data previously presented at the International Conference of Children's Bone Health, June 10–13, 2017.]

Reference

1. Whyte MP, Madson KL, Philips D, et al. Asfotase alfa therapy for children with hypophosphatasia. *JCI Insight*. 2016;1(9):e85971.

014 - Biochemical and Physical Function Outcomes At 5 Years of Treatment With Asfotase Alfa in Adolescents and Adults With Hypophosphatasia: Phase 2 Study Results

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This phase 2, open-label, randomized, dose-ranging study (NCT01163149) evaluated the safety and efficacy of 5 years of treatment with asfotase alfa in adolescents and adults with hypophosphatasia (HPP). Treatment with asfotase alfa 0.3 or 0.5 mg/kg/day SC was compared with no treatment (control) for 6 months in patients aged 13 to 66 years. All patients (treatment and control) then received asfotase alfa 0.5 mg/kg/day SC, which was increased after 6 to 12mo to 1 mg/kg 6x/wk (lowest effective dose). The primary safety outcome was tolerability. Coprimary efficacy outcomes were median change at 6 months in plasma inorganic pyrophosphate (PPi) and pyridoxal-5'-phosphate (PLP) levels. Other measures included 6 Minute Walk Test (6MWT) and Bruininks-Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2). Data from treatment groups were pooled and reported as median (min, max). Nineteen patients were randomized (6 aged 13-<18 y; 13 aged $\geq$18 years); 15/19 (79%) completed 5 years of treatment; 1 withdrew due to injection-site hypersensitivity and anaphylactoid reaction (1 episode each). No deaths occurred. The most common treatment-emergent adverse events were injection-site reactions. Decreases in PPi were numerically greater and decreases in PLP were statistically significant at 6 months of asfotase alfa treatment (n = 13) vs. controls (n = 6): PPi, −2.2 μM (−4.4, 0.3) vs. −0.2 (−6.8, 1.1; P = .0715); PLP, −255 ng/mL (−1467, −17) vs. 11 (−374, 346; P = .0285). Decreases were sustained at 5 y (n = 16): PPi, −3.0 μM (−5.2, 7.8); PLP, −284 ng/mL (−1580, −25). 6MWT distance walked improved from 355 m (10, 620; n = 19) before treatment to 450 m (280, 707; n = 13) at 5 y of asfotase alfa treatment, increasing from 76% predicted (42, 101; n = 15) to 88% predicted (62, 137; n = 11). BOT-2 Running Speed and Agility total point score was 6.5 (0, 39; n = 16) before treatment and improved by 4.0 (−5, 18; n = 11) at 5 years. BOT-2 Strength total point score was 13.5 (0, 33; n = 18) before treatment and improved by 3.5 (−9, 9; n = 12) at 5 years. Asfotase alfa was generally well tolerated. The coprimary outcome measure (changes in PPi and PLP at 6 months) was not met. Asfotase alfa significantly decreased circulating PPi and PLP levels and improved physical function in adolescents and adults with HPP through 5 years' of treatment. [Data previously presented at: 44th European Calcified Tissue Society (ECTS), May 13-16, 2017; International Conference of Children's Bone Health (ICCBH), June 10-13, 2017.]

015 - DOORS: A Dysmorphic and Syndromic Sensorineural Deafness Caused by 2-Ketoglutarate Dehydrogenase Deficiency

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DOORS is an acronym for deafness, onychodystrophy, osteodystrophy, mental retardation, and seizures. The syndrome has been classified into 2 types: the first one characterized by organic acid abnormalities, early seizures, progressive blindness, deafness, and early death in some cases; type 2 is not accompanied with organic acid abnormalities and is associated with mild neurological involvement. Nevertheless, the literature review suggests that the division is not clear. DOORS (MIM 220500) is caused by homozygous or compound heterozygous mutation in the *TBC1D24* gene on chromosome region 16p13, leading to a deficiency of 2-ketoglutarate dehydrogenase, which is part of the of the 2-oxoglutarate dehydrogenase complex. This is located within the mitochondrial matrix and converts 2-oxoglutarate to succinylCoA and CO2. Case report: A 10-year-old boy was referred to the Genetics Unit due to sensorineural hearing loss, delayed psychomotor development, short stature, and mild dysmorphisms. The patient is the only child of a consanguineous couple. After an uneventful pregnancy, the patient was born at 36 weeks, presenting neonatal hypoglycemia. Newborn hearing screening was abnormal bilaterally. At the age of 3 months, sensorineural bilateral deafness

was diagnosed, and the patient underwent cochlear implant surgery. The patient never had seizures. At physical examination at 8 years of age, the patient had proportionate short stature, mild facial dysmorphism, onychodystrophy, and short fingernails. The ophthalmologic examination revealed pale optic nerve and astigmatism (>7 diopters). The neurological examination revealed a mild degree of ataxia and hyporeflexia of inferior limbs. Abdominal ultrasound and echocardiography were normal. The X-rays of hands and feet revealed global hypoplasia of the terminal phalanges. Chromosomal analysis revealed a normal male karyotype. Considering the clinical and imaging findings, the hypothesis of DOORS was considered. The urinary organic acids analysis showed an increase of 2-ketoglutarate acid. This case exemplifies an inborn error of metabolism presenting as syndromic sensorineural deafness through the disturbance of 2-ketoglutarate dehydrogenase complex activity. DOORS is also one of the dysmorphic syndromes with a demonstrable biochemical abnormality. The analysis of organic acids may be a diagnostic clue and supportive of a clinical diagnosis if molecular analysis is not available.

016 - Blood Spot Testing for (Late Onset) Pompe Disease

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Introduction: Pompe disease is classified by age of onset and presentation. Late-onset Pompe disease (LOPD) generally presents with progressive limb-girdle and respiratory muscle weakness. Diagnosis is confirmed following blood spot enzyme assay by a second method, either mutation or glucose tetrasaccharide analysis. **Methods:** Measurement of acid α -glucosidase activity in dried blood spots was performed using two different methods: from 2000 to 2016, acid α-glucosidase activity was measured using a 4-methylumbelliferyl (4MU) labeled substrate, in a fluoro-immunometric assay based on the immuno-capture assay of Umapathysivam et al. (2001). From 2016 testing has used the multiplex tandem mass spectrometry method (6-PLEX LC-MSMS, Perkin Elmer) of Zhang et al. (2010) that measures activities for six different lysosomal storage disease enzymes, including α -glucosidase. Measurement of glucose tetra saccharides was performed by derivatization and measurement by combination hydrophobic/ion exchange chromatography prior to analysis by mass spectrometry. Mutation analysis of the *GAA* gene was performed using either Sanger or Next Generation Sequencing, the latter confirmed by sequencing. **Results:** Between 2000 and 2016, approximately 1500 high-risk samples were screened for Pompe disease with 77 positives, a diagnosis rate of 5.1%, approximately 5 patients per year. Of these, 33 were infantile Pompe disease, with 44 LOPD. Since May 2016, a further 480 patients have been screened for Pompe disease using the MSMS method. In the past year, 15 patients have to date screened as positive, with 9 already confirmed as having Pompe disease by either molecular testing or urine tetrasaccharide measurement, a diagnosis rate of 1.9%. Of these 9 patients, 8 are LOPD, with only a single infant being diagnosed in this time. Six adults are still to be confirmed by a second test. **Discussion:** The number of patients presenting for testing follows the recent Australian government funding for enzyme replacement therapy (Myozyme (alglucosidase alfa)) for adults with LOPD. The ease of submitting a dried blood spot sample for testing has contributed to an increase in test requests, however this may also reflect an increased recognition of this condition. These patients may have already been diagnosed clinically, but in the absence of a treatment, there was little benefit in a formal diagnosis.

017 - Pyruvate Dehydrogenase E1-Alpha Deficiency, A Case Report

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Background: Pyruvate dehydrogenase E1-alpha deficiency or congenital lactic acidosis is an energy defect inborn error of metabolism. It is an entity with genetic heterogeneity with at least 10 genes involved in the phenotype. Most cases are caused by mutations in the E1-alpha subunit gene on the X chromosome. Prevalence is unknown, with approximately 200 patients described in the literature. **Objective:** To report a case of pyruvate dehydrogenase E1-alpha deficiency. **Results:** A 20-year-old female patient with pyruvate dehydrogenase E1-alpha with no familial history for genetic or metabolic diseases, congenital malformations, or intellectual disability recorded, including her 3 siblings. Positive familial consanguinity, parents are second cousins. Developmental milestones were normal through childhood and infancy. At 12 years old, the patient started with peripheral weakness that worsened over time. Before diagnosis she had expressionless facies, generalized incapacitating weakness, and dysphagia. CPK: 711 U/L, muscular biopsy with a dysferlin deficiency, urine organic acids with high 2-hydroxibutiric acid, 2-hydroxivaleric, 3-hydroxibutiric, 3-hydroxyisovaleric, and acids from Krebs cycle: lactic, succinic, fumaric, pyruvic, isocytric, ketoglutaric. She is under ketogenic-like diet and takes carnitine, Q-coenzyme, and timine. After treatment, all symptoms improved mildly. **Conclusion:** We described the physical and biochemical phenotype of a patient with pyruvate dehydrogenase E1-alpha deficiency. Our patient improved her symptoms after diagnosis and treatment.

018 - The First Experience of Successful Treatment of Adult Patients With Late Diagnosis of BH4-Dependent Hyperphenylalaninemia

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Two female siblings, 31 yo and 27 yo, were presented with mild hyperphenylalaninemia diagnosed at their first year of life but noncurable by phenylalanine-free diet. Both patients had progressing symptoms such as seizures, dystonia, hyper salivation, depression, and insomnia. One of them displayed a light pigmentation. At the puberty, a severe deterioration of their condition occurred as abnormal movement, impaired tone and posture, hypertonia of extremities, and drug-resistant seizures. Based on these clinical signs and symptoms, BH4 deficiency was suspected and treatment with "Nakom" was administrated at the patients ages of 26 yo and 22 yo followed by a slight positive effect. Molecular genetic testing of PTS gene showed two mutations (pThr106Met and p. Asn72Lys). Despite initial low blood phenylalanine levels (290.6 and 286.8 mkg/mL, correspondingly), the patients (aged 24 yo and 28 yo) were referred to 3-day BH4 loading test with a commercially available formulation of BH4 (sapropterin dihydrochloride, Kuvan) at the dose of 20 mg/kg. BH4 loading test detected BH4 dependence: decrease in phenylalanine level by around 90.0% in both patients after eight hours of implementation. Aiming determination an effective dose of Kuvan, we tested parameters of neurotransmitters (including blood levels of 5-OH-tryptophan, serotonin and tryptophan, daily urine levels of vanillylmandelic acid (VMA), homovanillic acid (HVA, and 5-hydroxyindoleacetic acid (5-HIAA)) after administration of 5 mg/kg and of 10 mg/kg, each course lasted 5 days Normalization of all metabolites occurred at the dose of 10mg/kg which had been using for the further treatment. Upon 1 year of treatment, MRT of the brain showed positive changes. Currently, the elder sister is married, she was taking Kuvan throughout her uneventful pregnancy and gave birth to a healthy baby. **Conclusions:** (i) Disorders of BH4 synthesis, even those with low levels of blood phenylalanine, might develop eventually severe neurological symptoms due to disturbance of neurotransmitters metabolism. These disorders may be prevented by timely administration of replacement therapy with tetrahydrobiopterin. (ii) BH4 loading test should be indicated even for mild hyperphenylalaninemia to exclude neurological complications. An alternative approach would be molecular genetic testing of genes for enzymes involved in the synthesis and re-synthesis of BH4

019 - Evaluation of Respiratory and Motor Functions in Patients With Late-Onset Pompe Disease in Slovakia

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Objective: Pompe disease (PD) is an autosomal-recessive, lysosomal storage disorder. The deficiency of α-glucosidase results in an accumulation of glycogen in lysosomes mainly in skeletal muscle and in myocardial cells. Clinical presentation of juvenile and adult form (late- onset Pompe disease-LOPD) is connected with limb girdle weakness, dyspnea on exertion, and respiratory failure. Symptoms of respiratory muscle weakness can precede the limb girdle in one third of LOPD patients. Respiratory and locomotive functions are weakly correlated in LOPD. Affected respiratory muscles comprise the diaphragm in particular. The diaphragmatic dysfunction is attributed to myopathic changes and accumulation of glycogen in neurons. **Methods:** We have regularly evaluated respiratory and motor functions in 6 Slovak adult patients with PD (5 women and 1 man, average age in time of diagnosis 42.8 years) and in 1 patient with juvenile form (7 years old in time of diagnosis). All 6 patients have been treated with ERT (enzyme replacement therapy). 1 patient ceased the treatment on his own request. We have evaluated vital capacity (VC) in upright and in supine position by spirometry. Motor functions have been evaluated by The Quick Motor Function Test (QMFT). **Results:** In a long-term observation of the motor functions in our patients we have confirmed improvement in 4 patients on ERT. In 1 patient, the motor functions are stabile without significant change. In 1 patient, we are witnessing constant deterioration. The motor functions of the patient who have ceased the ERT have significantly worsened. By comparing results of VC in upright and in supine position we are estimating significant diaphragmatic weakness. The patient with the juvenile type of disease has both VC parameters at reference range. In the patient who has ceased the ERT we have observed deterioration in VC but not as significant as in locomotive functions. **Conclusion:** The weak correlation between respiratory and motor functions, which we have confirmed also in our group of patients, indicates a need for routine, serial evaluation of both functions in LOPD patients. The evaluation of VC in upright and supine position by spirometry is one of the recommended, readily available methods. It is suitable for evaluating of diaphragmatic dysfunction, and therefore, to estimate the progression of the disease. The QMFT is easy-to-use method for clinical assessment and for long-term follow-up of motor functions of LOPD patients.

020 - Early and Prodromal Neurodegenerative Markers in Adult PKU Patients

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Background: PKU patients identified by newborn screening are now entering in the "new era" of middle age. Brain aging may increase the risk for neurological decline in PKU patients, given the co-presence of risk of phenylalanine aggregation, metabolic abnormalities, and oxidative damage. The purpose of our study was to evaluate neurodegenerative markers in adult PKU patients. **Methods and patients:** Inclusion criteria: classical PKU (Phe levels >1200 μmol/L) patients older than 30 years and age-matched controls (Ctrl, n = 25). **Investigations:** Extensive neurological evaluation, neuropsychological testing, 3 T structural and functional MRI (analyzed on single-subject and by voxel-based morphometry-VBM), sensory and motor evoked potentials, blood, and urine analyses. Cerebrospinal fluid (CSF) concentrations of neurodegenerative markers were also evaluated in a subset of patients who underwent lumbar puncture. **Results:** A total of 21 early treated PKU patients (mean age 38.5 + 5.7 years, range 30-46 years) with different dietary treatment regimens entered the study. All subjects presented with heterogeneous cognitive impairment, ranging from very mild single-domain to severe multidomain deficits associated with parkinsonism/tremor in some cases. VBM analyses showed gray matter atrophy in subcortical structures and bilateral frontal and temporal structures in adult PKU compared to Ctrl. Sensory and motor evoked potential were abnormal in patients without any diet. CSF analyses in 12 patients showed no Alzheimer-specific alteration (Tau/Abeta42 ratio). Several patients present with at least one prodromal Parkinson disease deficit, such as hyposmia, depression, urinary dysfunction, or constipation. **Conclusion:** Our results in a small group of 21 patients show that neurological impairment in early treated adult PKU patients is highly heterogeneous and that a subgroup of patients has positive early neurodegenerative markers. Larger studies are pivotal in order to understand the role of diet, activity, and brain training in progression of neurological impairment in adult PKU patients.

021 - Oligosymptomatic Cerebrotendinous Xantomatosis (CTX): A Case Report

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Cerebrotendinous Xanthomatosis (Mc Kusick 213700) is an autosomal recessive disorder caused by the deficiency of sterol 27-hydroxilase that results in reduced production of bile acids, predominantly chenodeoxycholic acid (CDCA), and in the increased formation of intermediates such as cholestanol and 27-carbon bile alcohols. The classical clinical presentations of CTX are Achilles tendon xanthomas, cataracts, chronic diarrhea, and neurologic dysfunction. CTX is treatable with CDCA, which inhibits abnormal bile-acid synthesis and slows the progression of the disease. We report a CTX oligosymptomatic patient whose diagnosis was suspected by an orthopedic traumatology specialist. MIV, 23-year-old female was referred to IEM Unit by her orthopedic traumatologist because of CTX suspicion. At the age of 14, she had right ankle pain when walking and was diagnosed as having sprain. After 15 days of rest, contralateral ankle pain began and a new sprain was diagnosed. Bilateral plaster was indicated with no improvement. After a new evaluation, Achilles tendon biopsy was performed. She received no treatment. Through the following years, she developed bilateral Achilles tumors that impaired her for wearing shoes, feet movements, and gait. Ankle MRI performed at 23 years old revealed thickening of the Achilles tendon compatible with xanthomas. Brain MRI, ophthalmologic, neurologic, and cardiology evaluations were normal. Surgery treatment was indicated. Orthopedic traumatologist suspected CTX. Histopathology of the tumor revealed fibroxanthoma. Urinary bile acid profile was diagnostic for CTX. Genetic test revealed homozygous for c.1183C>T (p.Arg395Cys). Chenodesoxycholic acid has been indicated. We emphasize the importance of the suspicion by the specialist that allowed the diagnosis and the indication of the specific treatment aiming at avoiding the progressive deterioration characteristic of this disease.

022 - Quality of Life of Patients With Lysosomal Storage Disorders in Slovakia

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Objective: Lysosomal storage disorders (LSD) are genetically determined disorders of degradation of macromolecules mostly on the basis of insufficient activity of lysosomal enzymes. They are generally associated with pediatric patients and the harsh course of diseases. Over time, due to new knowledge and the improvement of diagnostic methods, we are capable of discovering milder forms of diseases, especially in adults. Thanks to continuously improving healthcare and in some cases of diseases, the possible therapeutic effects, the number of patients with LSD is rising. **Methods:** To evaluate quality of life (QoL) of adult patients with LSD. Our group of patients consisted of 31 patients, 15 of which were women and 16 were men. The age ranged from 20 to 75 years while the average age was 40.5 years. Our group consisted of patients: Gaucher disease (GD): 9, Fabry disease (FD): 8, Niemann- Pick type C (NPC): 7, Pompe disease (PD): 4, and Mucopolysaccharidosis (MPS): 3. For assessment of QoL, we have used The World Health Organization Quality of Life (WHOQOL) - BREF questionnaire in Slovak language. **Results:** The average time to complete the questionnaire was 11 minutes. 21 patients (68%) were able to fill in the questionnaire by themselves. 10 patients (32%) needed assistance. Regarding the education, 24 patients (77%) graduated from high school and 2 (7%) from university. Only 1 patient was without education. 12 patients (39%) had some form of occupation, and 2 (7%) were students in time of testing. Among our group, 6 subjects (19%) were disabled and the same number needed assistant care. By evaluating QoL, we have observed that the majority (58%, 18 patients) rated their QoL neither poor nor good. Higher QoL was reported by FD patients, in contrast, the least satisfied were NPC patients. 39% (12 subjects) were dissatisfied with their health, however majority of FD and GD patients were satisfied with their health. **Conclusion:** During the last few years, the emphasis is given on the evaluation of the factors that influence the patients' perception of QoL. The tendency to evaluate the QoL is due to the interest for the provision of quality of health care. By using the questionnaire method, we were able to evaluate how adult patients with rare chronic progressive disease manage their tasks and how they perceive their QoL.

023 - Hyperoxaluria Type 1 And the Transplant as an Option

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Primary hyperoxaluria type 1 (PH1) is an autosomal recessive disorder of glyoxylate metabolism, characterized by an excess of oxalate, secondary to a deficiency of glyoxylate aminotransferase (AGT). Individuals have a variable clinical presentation from recurrent nephrolithiasis, nephrocalcinosis, to renal terminal disease with systemic oxalosis. Liver or liver/renal transplantation is an acceptable option for disease therapy or perhaps cure. We present a case report of primary hyperoxaluria type 1 and the importance of an adequate study of related donor in patients with the end-stage renal disease. **Case Presentation:** We present a female patient, 38 years old, who has a history of several episodes of nephrolithiasis since the age of 6 years. At 32 years old, she was diagnosed with stage 4 renal failure that progress to nephrocalcinosis and end-stage renal disease. At 38 years old, she had ulcers in the skin and biopsy reports of deposits of oxalate crystals in small vessels. According to this finding, we requested *AGXT* gene sequencing, which identified a homozygous mutation c.33dupC, (p.Lys12GlnfsTer156). The patient has a family history of one affected sister who died with a diagnosis of end-stage renal disease and background of nephrolithiasis. **Discussion:** PH1 leads to accumulation of oxalate crystals in different tissues; the kidney is the first and the most compromised organ, developing end-stage renal failure. Usually in autosomal recessive diseases, heterozygous are asymptomatic, but some can present mild symptoms. In this specific situation, in which the sister is the possible donor of the kidney, it is necessary to carry out the study of gene sequencing in order to determine her carrier condition. Published reports with heterozygote patients reveal that these patients may present mild elevation of oxalate levels and patients present failure in renal transplantation secondary to compound heterozygous mutations. **Conclusion:** In this autosomal recessive disorder, the cure is based on the renal and hepatic double transplant, however in the case of a related donor, it is important to carry out complete sequencing of the gene to discard carrier donors in order to preserve to the maximum the transplanted organs.

024 - CSF Biogenic Amines Depletion and Brain Atrophy in Adult PKU Patients

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Introduction: Biogenic amines synthesis in PKU patients with high blood phenylalanine (Phe) concentration is impaired by inhibition of tyrosine- and tryptophan hydroxylase activity and by competition with other large neutral amino acids LNAA at the blood–brain barrier. The biogenic amines CSF levels have never been tested in adult patients and might explain the neurological alterations and cognitive impairment in the aging PKU population. **Method**: Neurotransmitter analysis (5-HIAA, 5-HTP, DOPA and HVA), amino acid analysis in CSF, and plasma was performed in 12 early treated PKU patients >30 years of age and 14 healthy controls. Voxel-based morphometry (VBM) was used to test the correlation between gray matter (GM) atrophy and CSF amine levels corrected for age, gender, and educational levels (uncorrected $P < .005$) . The study was approved by ERB of the Universities of Tübingen and Heidelberg, Germany. **Results**: Phe was increased in CSF and strongly related to plasma Phe levels (0.84, $P < .001$) and current treatment. 5-HIAA and 5-HTP were significantly reduced in PKU patients compared to controls, while L-Dopa and HVA were reduced only in a subset of patients with high Phe concentration. Adult PKU patients presented frontotemporal gray matter atrophy, possibly related to low neurotransmitter concentrations especially of the serotoninergic pathway. Specifically, the reduction in 5-HIAA and 5-HTP correlated with frontal and occipital gray matter atrophy, respectively. **Conclusion**: We could show for the first time the relationship between high blood and CSF Phe levels, low biogenic amines in CSF, and gray matter atrophy regions in treated adult PKU patients. The results may contribute to further treatment recommendation in adult PKU patient. Further studies are needed in order to understand the clinical relevance of these findings.

025 - Successful Management of Pregnancies in Patients With Inherited Disorders of Ketone Body Metabolism

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Patients with succinyl-CoA 3-oxoacid CoA transferase (SCOT) deficiency and 3-hydroxy-3 methylglutaryl (HMG)-CoA lyase deficiency are at increased risk of developing metabolic acidosis and hypoglycemia during pregnancy, delivery, and postpartum period. This can be fatal if not treated appropriately. Pregnancy in such patients should be managed in a specialist center by a multidisciplinary team including metabolic physician, high-risk obstetrician, and metabolic dietician. We report two pregnancies in women with SCOT deficiency and HMG-CoA lyase deficiency, which were successfully managed at this tertiary care center. The patient with SCOT deficiency had recurrent ketoacidosis due to severe nausea and vomiting requiring several hospital admissions during pregnancy, while the patient with HMG CoA lyase deficiency remained metabolically stable. Both patients, nevertheless, had normal delivery of live born infants and had uneventful post-partum period.

026 - Partial OTC Deficiency in a Symptomatic Urea Cycle Disease Carrier

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Introduction: OTC deficiency (ornithine transcarbamylase) is a urea biosynthesis disorder with X-linked recessive inheritance, characterized by recurrent and often fatal hyperammonemic encephalopathy in affected males, but carrier females are usually asymptomatic. The present report is a symptomatic heterozygous female with OTC deficiency. **Case description:** The index case is a 36 years old female which was referred for genetic counseling with her nonconsanguineous husband, because their first male newborn was suspected as affected by urea cycle disease (death occurred at 8 days of life). She had a good school performance and reached university level. Her mom describes her as a person with "chronic sleep." She described herself as chronically protein intolerant with recurrent vomiting episodes during childhood and teens years. OTC gene analysis showed a missense heterozygous mutation *OTC*: c.1005G>A (p.Met335Ile) mutation (NCBI: rs281865553) and confirmed her status as a carrier of ornithine transcarbamylase deficiency. The couple choose for their next pregnancy in vitro fertilization followed by preimplantation diagnosis, and they have a healthy noncarrier female. She used a diet with fruits and restriction of animal proteins. Sometimes she has apathy after she ate her protein restricted food. Actually, she has recurrent episodes of headache, nausea, epistaxis, and blurred vision. High ammonium levels were detected 107 μg/dL (18.7-86.9) with normal hepatic function. Physical examination shows an asthenic habitus with overweight, short hair, and thin, brittle, and fluted nails. **Discussion:** OTC partial deficiency have been described in males and females from infancy to adulthood with diverse signs and symptoms as developmental delay, intellectual disability, attention-deficit hyperactivity disorder and executive function deficits, fine motor ability, cognitive flexibility, and inhibition ability, even in mild forms of the disease after a sudden hyperammonemic crisis, may change rapidly in a life-threatening situation. We report a young woman with *OTC* mutation which was identified after the death

of a previous affected son. She lived with a long history of nonidentified signs and symptoms of partial OTC deficiency as episodes of irritability, nausea, episodic vomiting and lethargy, chronic rejection to proteins, and headache. Greater than 90% of partial OTC deficiency survives beyond five decades with decrease of hyperammonemic episodes with long-term treatment.

027 - Brain MRI Characteristics and Scoring in Adult Onset Krabbe Disease

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Introduction: Krabbe disease is a recessively inherited lysosomal storage disease due to decreased galactocerebrosidase activity. Adult onset is rare, however probably underdiagnosed. Brain MRI showing a leukodystrophy is of great value to achieve diagnosis. We aimed at performing for the first time a systematic analysis of brain MRI features in adult onset Krabbe disease (>10 years old). **Methods:** We collected the first available brain MRI of patients seen in the reference center for lysosomal diseases. We also contacted authors of published articles describing adult onset Krabbe disease patients and ask them to share brain MRI data. A score was established to describe the brain MRIs, with quantification according to severity (from 0 to 4 for nonfascicular structures and from 0 to 2 for fascicular structures). Two neuroradiologists first scored separately the MRIs, then reached a consensus for final scoring. **Results:** Thirteen patients were included in the study. Pyramidal tract was the most frequent structure showing abnormal T2 hypersignal (100% of patients), however with some distinctions along the tractus: medial precentral gyrus (100% of patients, mean score 2.8/4), lateral precentral gyrus (77%, 1.77/4), and corona radiata (100%, 1.7/2) were highly abnormal whereas internal capsula (69%, 0.96/2), mesencephalon (46%, 0.69/2), pons (31%, 0.42/2), and spinal bulb (0%, 0/2) were quite spared. Other sus tentorial white matter (WM) localizations were also found abnormal: occipital WM (92%, 2.15/4), optic radiations (69%, mean score 1.03/2), frontal WM (69%, 1.5/4), temporal WM (61%, 1.15/4), and parietal WM (15%, 0.23/4). 9/13 patients (69%) had corpus callosum hypersignal especially in isthmus (69%, mean score 1.38/2), body (38%, 0.77/2), and splenium (31%, 0.61/2), whereas genu was always normal. Finally, medial lemniscus was the most frequent abnormal structure found in posterior fossa (9/13 patients, 69%, mean score 0.96/2). **Conclusion:** Upper pyramidal tract, occipital WM, optic radiations, corpus callosum isthmus, and median lemniscus were the structures most frequently found with abnormal T2 hypersignals. This study should improve awareness of Krabbe disease in adult patients with leukodystrophy.

028 - Clinical Phenotypes of Adult Onset GM2 Gangliosidosis

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Objective: GM2 gangliosidosis is a recessively inherited lysosomal storage disorder characterized by GM2 ganglioside accumulation due to impaired catabolism. Genetic defect may affect HEXA gene for Tay Sachs disease (TSD) or HEXB gene for Sandhoff disease (SD). Both TSD and SD can be screened by measurement of hexosaminidases activity in leukocytes, which is found very low. GM2 gangliosidosis may begin at any age, with different described clinical forms from early childhood to adult onset disease. The adult onset (>10 years old) form was mainly reported as case reports in literature. To clarify the neurological phenotype of the adult onset disease, we studied the cohort of French GM2 patients and reviewed all reported patients in literature. **Methods:** After request of French laboratories involved in GM2 biochemical and genetic testing, 13 French patients were included in our study (4 SD, 9 TSD). A systematic review of literature found 46 additional patients (13 SD and 33 TSD) adequately described to be included. Clinical, radiological, and genetic data were collected using a standardized questionnaire filled by French clinicians and from literature analysis. **Results:** Age at onset of neurological deterioration was 21.4 years ($\pm$10.4; 10-51). Many patients (90%, 29/32) had previous subtle developmental motor symptoms in childhood. Weakness due to a lower motor neuron disorder was the most frequent neurological manifestation: 95% of patients had a lower limb weakness (always first proximal) and 43% upper limb weakness. Other manifestations were cerebellar ataxia of gait (52%) and upper limb dysmetria (40%), psychiatric signs (35%, mostly psychosis), cognitive decline (30%), dysarthria (65%), and dysphagia (18%). Lower limb weakness, psychiatric signs and cerebellar ataxia were present in 55%, 25%, and 15% of patients, respectively, as part of the initial clinical picture. Ten patients needed use of a wheelchair, no death was reported. TSD and SD had a quite similar presentation, except that SD patients had much less psychiatric symptoms (8% vs 45%), less cognitive decline (13% vs 36%), and more

dysphagia (42% vs 8%). **Conclusion:** Adult onset GM2 gangliosidosis has heterogeneous clinical manifestations with a core frequent and early symptom being lower limb weakness due to a lower motor neuron disorder. Patients may also present with psychiatric symptoms and cerebellar ataxia. Clinical evolution usually leads to a severe gait disability.

029 - Quality of Life in Adult Patients With Leukodystrophies: The Impact of Perceived Fatigue

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Background: Leukodystrophies are a group of individually rare diseases that when combined are responsible for a substantial burden. Their prevalence and clinical impact in adult patients is being increasingly recognized. However, there are few studies concerning health-related quality of life (QoL) in these patients. **Objective:** To study the clinical factors related to QoL in adult patients with leukodystrophies. **Methods:** We included adult patients followed in our center with a diagnosis of leukodystrophy and without cognitive impairment (MMSE>15). Demographics and clinical data were collected from clinical files. We assessed QoL with the SF-36 health survey questionnaire and possible associated factors with the Modified Fatigue Impact Scale (MFIS), the Pittsburgh Sleep Quality Index (PSQI) and the Hospital Anxiety and Depression Scale (HADS). **Results:** We included 8 patients (2 with Krabbe disease, 2 with Cerebrotendinous xanthomatosis, 2 with X-linked adrenoleukodystrophy and 2 with Vanishing white matter disease), 4 female and 4 male, with a mean age of 42.63 ($\pm$ 10.65) years and a mean disease duration of 24 ($\pm$ 17.67) years. The mean EDSS score was 4.62 ($\pm$ 2.01). There was a significant negative correlation between the psychosocial subscale of MFIS and better SF-36 mental ($r = -0.84$, $P = .008$) and physical ($r = -0.71$, $P = .048$) scores. There was also a significant positive correlation between the physical subscale of MFIS and disease duration ($r = 0.72$, $P = .042$). There were no significant correlations between SF-36 mental/physical scores and disease duration, EDSS, PSQI and HADS scores as well as MFIS total, physical, and cognitive scores. **Discussion:** The psychosocial impact of fatigue appears to be a more significant factor in the QoL of adult patients with leukodystrophies than disability, physical, or cognitive burden. A larger disease duration seems to impact the physical but not the psychological fatigue burden. These findings could lead to more specific interventions in this group of patients in order to improve QoL, focusing on the social dynamics and integration. However, due to the small sample studied, further investigations are needed to confirm this hypothesis.

030 - Phenotypic Heterogeneity in Gaucher disease: Variable Parkinsonism in Patients With the Same Genotype

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Background: Gaucher disease (GD) is a lysosomal storage disorder caused by autosomal recessive mutations in the *GBA* gene, leading to a deficiency of glucocerebrosidase. It is clinically classified into 3 phenotypes based on the presence and progression of primary central nervous system alterations. However, even in patients with the same mutations in the *GBA* gene, there is a significant phenotypic heterogeneity with variable clinical presentation. **Objective:** We characterize 3 patients with the same *GBA* genotype but very different degrees of neurological and systemic involvement. **Results:** The first patient is a 76-year-old female who presented asymmetric parkinsonism when she was 60-year-old with steady progression since then but without any systemic involvement. Neurological examination also revealed cognitive impairment and distal dystonia of the upper limbs. DaTscan imaging showed a bilateral dopaminergic deficit. She is currently bedridden and fully dependent in activities of daily living. The second patient is a 54-year-old female with adult-onset crisis of bone pain. At 48-year-old she developed an asymmetric parkinsonism with severe progression and poor response to dopaminergic treatment. Neuro-ophthalmologic evaluation revealed ocular apraxia with increased latency of saccadic eye movements. DaTscan imaging showed a bilateral dopaminergic deficit. She started enzyme replacement therapy, without clinical improvement. The third patient is the 57-year-old brother of the second patient who presented adult-onset leukopenia, thrombocytopenia, and bone disease, with severe crisis of bone pain. He later developed diastolic heart failure with decreased ability to perform activities of daily living. Neurological examination only revealed increased latency of saccadic eye movements. In molecular genetic testing, all patients were homozygous for the p.N409 S mutation. **Discussion:** We present three patients with the same genotype but a clinical presentation ranging from only neurological to only systemic involvement. Although the genotype of our patients is classically found in patients with systemic clinical findings without neurological involvement, it has increasingly been associated with

parkinsonism. The underlying basis for this association remains to be established, but a role for ambient and epigenetic factors has been proposed. Our findings support current proposals that the clinical presentation of GD comprises a continuum of phenotypes.

031 - Quality of Life in Adult Portuguese Patients With Mitochondrial Diseases: Initial Validation of an Assessment Instrument

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Background: The role of health-related quality of life (QoL) in patient outcome measures is being increasingly recognized as an important part of clinical evaluation. Despite the availability of QoL scales, there is a need to develop disease-specific measures like the Newcastle Mitochondrial Quality of life measure (NMQ). **Objective:** To assess QoL in adult patients with mitochondrial diseases and the initial development and validation of a Portuguese version of the NMQ (NMQ-P). **Methods:** We included adult patients with a diagnosis of mitochondrial disease followed in our center. Patients with cognitive impairment were excluded. Demographics and clinical data were collected from clinical files. We translated and retroverted the original NMQ. The psychometric evaluation of the NMQ-P encompassed assessment of internal consistency, reliability, validity, sensibility, and a correlation with the Portuguese version of the SF-36v2. **Results:** 33 patients were included, with a mean age of 56.48 ($\pm$ 15.96) years, a female (72.7%), and adult-onset in (67.7%) predominance. The most common phenotype was chronic progressive external ophthalmoplegia (69.7%). NMQ-P had adequate divergent and group validity for most domains. Internal consistency of each domain exceeded the success rate threshold. Reliability was good/excellent in most domains, with the exception of Food and Digestion, Muscle Stiffness, and Diabetes. The NMQ-P is not sensible to variations due to age or age of onset. Domain scores correlate with similar SF-36v2 domains, showing convergent validity and good construct validity. Evaluating the factors associated with QoL, the mobility domain had worse scores in males ($P = .022$). The presence of Diabetes was associated with worse family, emotion, and social scores ($P = .028$, $P = .059$, and $P = .025$, respectively). There were no significant correlations between any parameter of QoL and current age, age of onset, phenotype, or other clinical findings. **Discussion:** We developed a culturally adapted version of NMQ to the Portuguese adult population. This instrument is a reliable and valid assessment tool but more studies are needed to evaluate its test-retest reliability and sensibility. The presence of diabetes seems to be an important factor associated with worse quality of life, mainly regarding emotion and interpersonal relationships. The knowledge of clinical findings associated with different parameters of QoL may lead to a more specific and effective clinical approach.

032 - Two Adult Brothers With Mental Retardation of Metabolic Origin, Suspected by the Magnetic Resonance Imaging (MRI)

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Objective: We would like to present the case of 2 adult brothers who presented mental disability in late childhood and the diagnosis of a metabolic disease was suspected by the typical findings in the MRI. **Case:** The older brother is 23 years old, he first came to our clinic when he was 14 years old, with no prenatal or neonatal history, his development was normal, besides a language delay, until the age of 8 years old, when he presented a strange behavior, isolation, seizures, and developmental regression (IQ = 36). The CT showed leukodystrophy, and the MRI suggested L-2-hydroxyglutaric aciduria. This diagnosis was confirmed by the urine organic acids. Later on, he developed ataxia, tremor and aggressiveness. He is in a protein restricted diet (0,8 gr/kg), L-carnitine (50 mg/kg/day), and riboflavin supplementation. The younger brother is 19 years old and had his first visit to our metabolic clinic at the age of 10 years, he had a history of in utero growth restriction, and he was born by a cesarean at 35 weeks of gestation. He had a developmental and language delay, IQ = 47, right hemiparesis and gait disturbances. His CT showed unspecific changes, but his urine organic acids confirmed an L-2-hydroxyglutaric aciduria. He is also in a protein restricted diet (0,8 gr/kg), L-carnitine and riboflavin supplementation. **Discussion:** We would like to emphasize that in the differential diagnosis of a mental retardation, specially in adolescence or late childhood, it is important to include the inborn errors of metabolism, and in the case of the L-2-hidroxyglutaric aciduria the neuroimages (MRI) have a key role for the suspicion and diagnosis strategy. It is also important to mention that besides the nutritional management, the patients continue with a progressive neurological deterioration, as it is described in the literature.

033 - Pregnancy Unmasking Sialidosis Type I

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Aims: To describe a patient with sialidosis type 1 during prenatal management. **Methods:** Clinical history assessment and charts review. **Results:** The patient was referred for specialized

management of epilepsy during pregnancy, at age of 17 years. At physical examination, she presented mioclonus of motor intention, which motivated an investigation of syndromes related to this condition. She is the first child of nonconsanguineous parents with unremarkable family history for genetic disorders, visual impairment or epilepsy. Her birth and perinatal period were without clinical changes. The cognitive and motor development were normal, and she had age-appropriate school performance. Ophthalmologic examination presented cherry red macular spots. Extensive laboratory investigations revealed unremarkable results, but chromatography of spontaneous urine samples showed an oligosaccharide pattern compatible with sialidosis. Analysis revealed mildly elevated excretion of bound sialic acid. Beta galactosidosis in fibroblasts had activity in the lower limit and chromatographic profile of amino acids was normal. Sequencing of the gene *NEU1* revealed 2 pathogenic missense mutations at exon 4: g.7336C>T (p.R214C) e g.7345G>A (p.V217 M). The pregnancy and delivery progressed without special remarks. Currently, she is 23 years old and she has ataxia, multifocal myoclonus, and controlled seizures; her son is 5 years old and has a normal psychomotor development. **Conclusion:** Sialidosis type 1 presents with variable phenotypes and autosomal recessive inheritance. This is the first case report of pregnancy in a patient with sialidosis. The proper clinical examination is essential for accurate diagnosis and clinical follow-up.

034 - Adult Onset Adrenomyeloneuropathy With Delayed Diagnosis

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Objective: To present the case of an adult onset adrenomyeloneuropathy who, despite a family history and a typical clinical presentation, had a delayed diagnosis. **Case:** 25 years old male who came to the adult metabolic clinic at the age of 24 years of age. His older brother had the diagnosis of a childhood cerebral form of X-linked adrenoleukodystrophy and had died in 2006. At that moment, a diagnosis of Addison syndrome had been made to our patient, so a determination of very long-chain fatty acids was requested. He did not show up for clinical evaluations until the age of 24, because he referred to be asymptomatic until then. However, after a car accident he started with gate abnormalities and instability, sphincter disturbances, and sexual dysfunction. A brain and spine magnetic resonance imaging did not show any special findings. The results of the long chain fatty acids and the clinical manifestations were consistent with adrenomyeloneuropathy. He has no treatment and presents a progressive deterioration. **Discussion:** This is a quite common inborn error of metabolism in adults, but it keeps being underdiagnosed. This patient could have been diagnosed due to the family history and the tests requested before clinical manifestations had appeared. Although, we are not sure if the early diagnosis would have changed the course of the disease.

035 - Glucose Transporter Type 1 Deficiency Syndrome: Report of a 30-Year-Old Patient With Atypical Clinical Presentation of Spastic Paraparesis and Ataxia

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The phenotypic spectrum of glucose transporter type 1 deficiency syndrome (Glut1) is now known to be a continuum that includes the classic phenotype as well as dystonia 9, dystonia 18, atypical childhood absence epilepsy, myoclonic astatic epilepsy, and paroxysmal nonepileptic findings, such as intermittent ataxia, choreoathetosis, dystonia, and alternating hemiplegia. We aim to present an interesting case regarding a patient that was referred to a Medical Genetics clinic at the age of 11 years due to gait imbalance and seizures started at 2 years of age and controlled since then. Until the age 29, he was submitted to an unsuccessful extensive metabolic and genetic evaluation. Due to megaloblastic anemia, proteinuria and low vitamin B12 levels, he has been treated as a patient with transcobalamin defect, with improvement in the laboratory findings, although the movement disorder characterized by ataxia, spastic paraplegia, and dystonia worsened with time. In order to define the molecular diagnosis, the patient underwent to a multigene NGS panel focused on treatable inborn errors of metabolism, which detected a heterozygous variant in *SLC2A1*. After the genetic confirmation of GLUT1 deficiency, the past medical charts were reviewed and a spinal tap, done when the patient was 2 years old, showed significant decrease in the glucose levels (23 mg/dL). Interestingly, this finding was not considered relevant in the past. Nowadays, at 30 years of age, the patient has dystonic movements, spasticity, mild cognitive decline, and is wheel chaired. Soon, he will start on a ketogenic diet. Our case report shows an uncommon nonepileptic manifestation of GLUT1 deficiency, presented in less than 10% of the affected individuals. The patient has a milder phenotype, characterized by paroxysmal dyskinesia including intermittent ataxia, choreoathetosis, dystonia, and alternating hemiplegia. We think that it raises the awareness of an interesting inborn error of metabolism that can present with atypical symptoms in adults and should be included in the differential diagnosis of movement disorders.

036 - Globotriaosylsphingosine (Lyso-Gb3) as a Biomarker for Cardiac Variant (p.N215 S) Fabry disease

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Introduction: Fabry disease (FD) is a multisystemic X-linked lysosomal storage disorder caused by the deficient activity of

α-galactosidase-A enzyme, leading to a progressive accumulation of glycosphingolipids in various body tissues. The *p.N215 S* mutation is considered an atypical variant which usually leads to a late-onset cardiac phenotype. Recent consensus guidelines acknowledged the use of globotriaosylsphingosine (Lyso-Gb_3) as a potential diagnostic marker for FD. The current study aims to characterize the clinical features and evaluate the sensitivity and specificity of plasma and urinary Lyso-Gb_3 levels in Fabry patients with the cardiac variant *p.N215 S*. **Methods:** Thirty-four Fabry patients with the late-onset cardiac variant *p.N215 S* were enrolled with 62 classical Fabry patients and 109 healthy controls. Lyso-Gb_3 and was extracted from plasma and urine samples using solid phase extraction and then analyzed using an HPLC-MS/MS-based method. A clinical workup included blood samples, echocardiography, 12-lead ECG, 24-hour Holter, and magnetic resonance imaging. **Results:** The spectrum of clinical features in *p.N215 S* mutation extends beyond cardiac involvement. Both genders of Fabry patients with late-onset *p.N215 S* mutation showed significantly higher levels of plasma Lyso-Gb_3 compared to controls ($P < .0001$), but the levels were significantly lower compared to classical Fabry patients ($P < .0001$). Urinary Lyso-Gb_3 levels in Fabry males with *p.N215 S* cardiac variant were significantly higher than in control males ($P < .0001$). **Conclusion:** Our study has shown that elevated plasma Lyso-Gb_3 is a diagnostic hallmark for FD patients with the cardiac variant *p.N215 S*, similar to classical Fabry patients. Furthermore, their spectrum of clinical involvement is not limited to the heart and includes cardinal features and other major organ involvement usually considered typical of classical FD.

037 - From Children to Parents (reverse genetics): CBS deficiency, a "Miss" and Heterogeneous Disease

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A newborn female (patient A) was sent to genetic evaluation due to polydactyly. She was the only child of a nonconsanguineous couple. The prenatal period was uneventful. Eventually, his father was also found to present polydactyly. The mother (patient B), 20 years old, was diagnosed as having Marfan syndrome at childhood (lens dislocation, high stature, and normal neuromotor development); she had 3 siblings: a twin sister who died at an early age due to anencephaly, a brother who died at 13 yo due to "heart problems," and an older sister presenting psychiatric problems and history of thrombosis (patient C). Metabolic investigation confirmed the diagnosis of Classical Homocystinuria (HCU) in patients B and C. Those cases illustrate the natural history of late-diagnosed HCU patients, and the heterogeneity and the lack of awareness by health professionals about this disease. Although the pregnancy of patient A was not associated to a bad outcome, HCU untreated patients have a higher risk of complications during this period. Also interesting is that this family was sent to genetic evaluation because of a common congenital malformation occurring in a newborn (a finding not related to HCU), which suggests metabolic adult patients may be at a smaller risk to be at suspicious of having a genetic disease either because of age or because of the absence of severe anatomic abnormalities.

B) Novel Diagnostic/Laboratory Methods (038 to 065)

038 - A Novel Mutation of Idursulfatase gene in a Libyan Child With a Mucopolysaccharidosis Type II

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Background and Aim: Hunter syndrome is an X-linked form of mucopolysaccharidosis—a lysosomal storage disorder—due to deficiency of iduronate-2-sulfatase (IDS) enzyme which is controlled by the *IDS* gene. As a result, accumulation of partially degraded heparan and dermatan sulfate occur which leads to progressive malfunction of various tissues and organs. Diagnosis should be based on physical criteria followed by enzyme level and finally confirmed by cytogenetic analysis. In this report, a patient with Hunter syndrome with a novel mutation was presented. His family pedigree was investigated. **Case report:** A 5-year-old male Libyan child was sent to our center when aged 3 years because of his coarse fascial features. Our patient presented coarse fascial features, large size (appearing more than his growth usual charts), attractive walking manner that clarify the joint contracture, and obvious speech delay. His history was significant by herniotomy and ongoing tonsillectomy. Abdominal and cardiac ultrasonography, skeletal X-ray, and brain and spine CT were evaluated. Enzyme levels were below the normal range. Molecular analysis of *IDS* revealed the hemizygous variant c.381C>G(p.Gly127Gly) in exon 3. Therapy initiation was based on his enzyme levels. Genetic results were uncertain, accordingly his enzyme therapy was held for 2 weeks while his mother was analyzed and identified as a carrier for the same mutation c.381C>G(p.Gly127Gly). **Conclusion:** Awareness of physician must be strengthened because of early detection and management will decrease the patient physical disabilities, although cytogenetic and physical examination will still be the leader.

039 - Accurate Enzymatic Assays for Distinguishing Affected From Pseudodeficiencies for Diagnosis and Prognosis of Lysosomal Storage Diseases

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Objective: We developed liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays to measure enzymatic

activities in leukocytes with unprecedented accuracy for post-newborn screening analysis of lysosomal storage diseases. **Methods:** High accuracy LC-MS/MS assays were developed to measure the lysosomal enzymes GALC, GAA, IDUA, and LAL in leukocytes (for analysis of Krabbe, Pompe, MPS-I, and Wolman diseases). **Results:** The analytical range of the new LC-MS/MS enzyme assays (assay response for the high activity samples, i.e., leukocytes from healthy donors, divided by the assay response for the blank) are >200. By comparison, conventional fluorometric assays display analytical ranges of <5. The high analytical range of the LC-MS/MS assays increases the accuracy of the measurement such that small differences in enzymatic activity close to zero activity can be measured. By using lysates from LCL lymphoblasts, we show that enzymatic activities in the 0% to 1% of normal range, in 0.2% increments, can be accurately measured by LC-MS/MS. With these LC-MS/MS assays, we show that disease-affected versus pseudodeficiency patients can be separated into different reference ranges. In the case of Pompe disease, we can separate infantile versus late onset disease for the first time based on activity of GAA in leukocytes. **Conclusions:** High accuracy LC-MS/MS assays of lysosomal enzymes in peripheral blood leukocytes is useful for post-newborn screening analysis of lysosomal storage diseases. For the first time, affected versus pseudodeficiencies and early versus late onset diseases can be resolved into separate reference ranges.

040 - The Diagnostic Yield and Novel Genetic Findings by Clinical Exome Sequencing in Highly Consanguineous Middle Eastern Families With Suspected Mendelian Disorders: Experience With 508 Samples

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Background: Mendelian disorders in consanguineous populations are not only common but may be diagnostically challenging due to the increased probability of novel alleles expressing themselves atypically as well as dual molecular diagnoses. Clinical exome sequencing (CES) lends itself readily to these challenges. **Methods**: We performed CES in 508 probands referred to the Clinical and Metabolic Genetics at Hamad Medical Corporation-Qatar from April 2014 to December 2016. Importantly, CES was a first-tier molecular test in the majority of cases. **Results**: Of the 508 patients enrolled, a clear genetic diagnosis (pathogenic or likely pathogenic mutation relevant to the phenotype) was made in 242 (47.6%), consanguinity and positive family history were associated with a higher diagnostic yield reaching up to 56% (Odds Ratio: 2.16 [95% CI, 1.2-3.6], $P = .02$). A dual or triple molecular diagnosis was identified in 35 (7%) of the cohort. Two homozygous mutations in the same gene, compound heterozygous variants/mutations and copy number variants, were identified in 3 (0.5%), 13 (2.57%), and 4 (0.7%), respectively. An apparently recessive mutation in genes hitherto only linked to dominant phenotypes was identified in 2 cases. We also highlight interesting variants in 23 novel candidate genes, which could explain the clinical presentation but require additional confirmation. Interestingly, the diagnostic rate was found to be significantly higher in the singleton -CES 84/131 (66%) cases vs trio-CES 36/71 (50%) (Odds Ratio: 1.7[95% CI, 0.96- 3.1], $P < 0.04$) in children aged from 6 to 18 years than others. Reanalysis of "negative" cases revealed 30/124 (24%). Most families opted not to receive ACMG secondary findings but among those who agreed 20, only one had such a finding. **Conclusion**: We attribute the high diagnostic rate we observed in this study compared to other studies in part to the high rate of consanguinity and our reanalysis of "negative" cases in light of newly published literature. Our data corroborate a growing body of evidence in support of considering CES as a first-tier molecular test in patients with suspected Mendelian phenotypes.

041 - A Novel Method for Screening of Mitochondrial Disorders

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Background: Diagnosis of mitochondrial disorder is often suspected on clinical grounds. However, confirmation of diagnosis is often challenging and time consuming. There is lack of good screening tool in order to determine which patients will likely benefit from more comprehensive and exhaustive studies. Blood lactate is the most commonly used parameter but it is neither sensitive nor specific. JC1 dye concentrate in mitochondria and forms aggregates. The aggregate formation changes fluorescent emission characteristic of the dye. In this pilot study, we have studied the shift in fluorescent emission characteristic as indicator of change in mitochondrial membrane potential which accurately predicted presence or absence of a mitochondrial disease in all patients tested. **Methods:** 5 patients and controls were tested. Lymphocytes were separated and incubated with JC1 dye. Emission characteristic of JC1 was studied in flow cytometer. A change suggestive of decrease in JC1 aggregate was considered indicative of a decreased mitochondrial membrane potential. Molecular genetic tests were performed for confirmation of diagnosis. **Results:** For those patients where flow cytometry indicated decreased mitochondrial function, molecular genetic test resulted in a confirm diagnosis. The emission characteristics

in control and patients with clinical suspicion of mitochondrial disorder but negative molecular diagnostic work up was similar. **Discussion:** Diagnosis of a mitochondrial disorder is a clinical conundrum. Oftentimes, it is challenging to select subgroup of patients among the larger group of "suspected mitochondrial disease patients" which are more likely to benefit from a detailed and exhaustive work up. A screening tool which is sensitive and specific will help select these patients and will result in efficient utilization of resources. This pilot study although performed in small group of patients, is promising and can potentially be a useful screening tool in clinical practice.

042 - LC-MS/MS Measurements of Urinary Guanidinoacetic Acid and Creatine: Method Optimization by Deleting Derivatization Step

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Creatine deficiency syndromes include three hereditary diseases affecting the metabolism of creatine (Cr): Guanidinoacetate methyltransferase deficiency, arginine glycine amidinotransferase deficiency, and the deficiency of creatine transporter. These pathologies cause a brain creatine deficiency responsible of non-specific neurological impairments with mental retardation. LC-MS/MS measurements of guanidinoacetic acid (GA) and Cr in urine and plasma are useful for the diagnosis and the identification of one of the deficits. The analysis of these polar and basic molecules is difficult. For the study of these substances, which are not retained on standard column, the reference method requires a derivatization step. To overcome this long and fastidious derivatization, an ion pairing method was chosen. The principle is to add an opposite charging ion to the molecules of interest in the two mobile phases to form an uncharged complex. The opposite charging ion will interact via its hydrophobic chain with the organic stationary phase causing a stronger retention of the complex. **Materials and methods:** A range of calibration was chosen to frame the biological and pathological values. Those solutions, the internal quality controls, and the urines of the patients were diluted to 1/20th in an aqueous solution of internal standard. After agitation, they have been analyzed by LC-MS/MS. To validate this method, the COFRAC (comité français d'accréditation) recommendations has been used as reference and adapted to the specific criteria of chromatographic dosages.

Results: The coefficient of variation (CV) realized on one day were representative of repeatability of the results while CV realized on several days were representative of reproducibility. Every of them were lower than 15% which proves the accuracy of our method. Precision, estimated by external quality samples, was good with biases lower than 15%. The comparison between our method and the reference method showed the same results. Furthermore, the time to prepare the samples before LC-MS/MS was shortened, moving from 2 hours with the former technique to 30 minutes with this new method. This is a technical timesaving of 75% combined to a significant technical simplification. **Conclusion:** In the dosage of GA and Cr by LC-MS/MS, the use of ion-pairing technique we have developed leads to a technical simplification of the method and to a saving of time. It appears to be an optimization of the current method.

043 - A Targeted Lipid Panel in Plasma for the Diagnosis of Niemann-Pick Types A, B and C Disease, Gaucher Disease, and Cerebrotendinous Xanthomatosis

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Objective: To develop an effective and efficient method that simultaneously measures in plasma seven biomarkers for rare diseases including Niemann-Pick A, B, and C diseases, Gaucher Disease, and Cerebrotendinous Xanthomatosis (CTX). **Methods:** In a microcentrifuge tube, 100 μL of plasma is mixed with internal standard containing d_7- cholestane-3β,5α,6β-triol (COT), d_7-7-ketocholesterol (7-KC), and d_5-glucosylsphingosine (GPSY). Acetonitrile is added to the tube in order to precipitate proteins. Following centrifugation, supernatant is transferred to a 96-well filter plate. The filter plate is eluted into a 96-well plate by centrifugation and the filtrate then evaporated under nitrogen. Following reconstitution, the sample is subjected to liquid chromatography-tandem mass spectrometry analysis to measure COT, 7-KC, GPSY, lyso-sphingomyelin (LSM), LSM-509, 7α-hydroxy-4-cholesten-3-one, and 7α, 12α-dihydroxycholest-4-en-3-one. The analysis is performed on a SCIEX API 3200 tandem mass spectrometer paired with an Eksigent Expert MicroLC 200 micro flow liquid chromatographic system (5.5 minutes per sample). **Results:** Preanalytical (specimen stability, anticoagulants, hemolysis, lipemia, icterus, and fasting) and analytical (recovery, precision, linearity, reference ranges, limits of detection, specificity, sample stability, and alternate HPLC column) factors were evaluated, demonstrating acceptable performance for clinical testing. The analysis of control ($n = 266$), Gaucher ($n = 25$), CTX ($n = 5$), NPAB ($n = 14$), and NPC ($n = 21$) yielded 100% clinical sensitivity for each condition. Additional conditions (Gaucher heterozygotes, Refsum, Sitosterolemia, SLO, and cholestatsis) were analyzed to assess clinical

specificity. Clinical specificity was found to be 100% for all conditions except NPC, where cholestasis may yield a false-positive result. **Conclusion:** We have developed an efficient and effective LC-MS/MS assay for the diagnosis of NPA, NPB, and NPC disease, Gaucher disease and CTX. Cholestasis may result in false-positive results for NPC, even with the inclusion of LSM 509 which is a sensitive but not specific marker for NPC.

044 - Simultaneous Quantification of Amino acids in Serum by HPLC for the Diagnosis of Aminoacidemias in Cuban Patients

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Aminoacidemias are metabolic diseases characterized by an excess of amino acids and organic acids in biological fluids. These diseases are caused by an enzymatic deficit in the metabolism of amino acids and present heterogeneous clinical manifestations and variable severity. The early diagnosis of the specific entity is crucial. A reverse phase analytical method was validated by HPLC with precolumn derivatization with sodium phenylisothiocyanate for the simultaneous quantification of 13 amino acids in serum; our aim was to use it in the diagnosis of aminoacidemias. This evaluated specificity, linearity, accuracy, and precision of the method. The validated method was used in the diagnosis of aminoacidemias in pediatric patients with clinical suspicion of the disease. It was also used to perform the biochemical follow-up of the diagnosed patients, in order to assess the efficacy of dietary treatment. The method is specific for amino acids, is linear in the evaluated concentration ranges for each amino acid ($r > 0.99$ and $r^2 > 0.98$), accurate (% recovered 90-106% and % recovered CV <15%), and precise (% CV <15%). The introduction of the method in the laboratory allowed the diagnosis of maple syrup urine disease (3 patients) and nonketotic hyperglycinemia (3 patients). The HPLC method for the simultaneous quantification of amino acids in serum met the validation criteria for bioanalytical methods and their introduction allowed the diagnosis of aminoacidemias.

045 - Performance Evaluation of Newborn Screening Tests in the SUMA® Automatic Analyzer SUMAutoLab

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SUMAutoLab is an automatic analyzer that can be used for neonatal screening purposes. A complete evaluation of this instrument is necessary in order to demonstrate their acceptability. Tests performance parameters should be assessed for evaluating the consistency in the results produced by the instrument. **Objective:** To validate SUMA Technology assays for newborn screening using the SUMAutoLab analyzer. **Materials and Methods:** The evaluation was made for Newborn Screening Tests: UMELISA TSH NEONATAL, UMELISA T4 NEONATAL, UMELISA 17OH Progesterone NEONATAL, UMTEST GAL. and UMTEST PKU. Intra- and interassay variation coefficients (CVs), recovery, limit of detection (LOD), sample carry over (CO), and the influence of the incubators position were determined. Finally, a comparison between SUMA semiautomatic technology and SUMAutoLab using 490 newborn samples was made by linear regression analysis. **Results:** The intra- and interassay CVs were in the range of 4% to 9% and 5% to 11%, respectively. The average percent recovery for each test was over 90%, with individual percentages fluctuating between 90 and 109%. CO didn't significantly affect the results. LOD were 1 mUI/L blood (TSH), 9.2 nmol/L serum (T4), 3.8 nmol/L (17 OHP), 0.09 mmol/L blood (Gal) and 75 µmol/L blood (Phe). A good agreement was obtained for all tests ($r^2 > 0.84$) when comparing the semi-automatic technology with SUMAutoLab. **Conclusions:** SUMA technology assays for newborn screening can be performed in the SUMAutoLab analyzer. Furthermore, the instrument offers advantages, such as, it uses low volumes of reagents, reduction in the processing time and the number of handling errors by operators, which increase the reliability in the results.

046 - Development of a Clinical Metabolomics Platform: Biomarker Characterization in Inborn Errors of Metabolism

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Introduction: The implementation of mass spectrometry has been a valuable technological advance in the diagnosis and follow-up of patients with inborn errors of metabolism (IEM). Over the last years, untargeted mass spectrometric analysis of metabolites, that is, metabolomics has become a powerful tool for biomarker identification and the study of disease mechanisms. We aim to develop a metabolomics platform for the simultaneous and unbiased analysis of different plasma metabolites in order to facilitate IEM diagnosis and biomarker discovery. **Methods:** Plasma samples from controls (n = 7), and subjects with medium-chain acyl-CoA dehydrogenase deficiency (MCADD, n = 7) and maple syrup urine disease (MSUD, n = 7) were analyzed using reverse phase chromatography coupled to an iFunnel Q-TOF mass spectrometer

operated in positive or negative ion mode. Batch recursive metabolite feature extraction was performed using Profinder software, followed by differential metabolite analysis and identification using Mass Profiler Professional software. Unpaired t-tests were performed for MCADD and MSUD against the controls with a Benjamini-Hochberg FDR correction applied (P corr < .05). **Results:** We identified 8 compounds significantly elevated in MCADD. In MSUD, we found one compound significantly elevated and 6 significantly decreased. We confirmed the elevation of compounds known to be associated with each disease state, including octanoylcarnitine, hexanoylcarnitine and octanoic acid (P corr < .001) in MCADD, and alloisoleucine (P corr < .05) in MSUD. Furthermore, we were also able to identify a potentially novel compound significantly elevated in MCADD (mass@RT: 302.2149@18.98). Further studies are underway to annotate and confirm the identity of this metabolite. **Conclusions:** We have demonstrated that our untargeted metabolomics platform correctly identifies the known disease markers of MCADD and MSUD. In addition, we also discovered a potential new biomarker for MCADD. Future work includes hydrophilic interaction liquid chromatography in both positive and negative ionization modes at low and high pH in order to have the most extensive assessment of compounds from the human metabolome. Furthermore, studies of more patients with different IEM and development of a robust data analysis model are needed to fully implement untargeted metabolomics into the clinical diagnosis of IEM.

047 - Validation of UMELISA TSH Neonatal® Using Technical Procedure at Room Temperature (20-25 °C)

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UMELISA TSH Neonatal is a quantitative immunoassay for neonatal screening of Congenital Hypothyroidism in dried blood spots. Perfectioning the assay procedure is an important strategy to enhance the performance of the test in laboratories. Functional characteristic assessment allows to demonstrate the reliability of a new analytical procedure. **Objective:** To evaluate the main functional characteristics of UMELISA TSH NEONATAL using an immunoreaction temperature of 20 °C to 25 °C. **Materials and Methods:** The evaluation was made using Quality Controls (CDC, Atlanta) samples from Cuban National Screening Program and in-house controls. Intra- and interassay variation coefficients (CVs), recovery, limit of detection (LOD) and limit of quantification (LOQ), were determined. In addition, a comparison between the current method (37 °C) and the method at a new immunoreaction temperature using 481 newborn samples was performed by linear regression analysis. **Results:** The intra- and interassay CVs were in the range of 2% to 4.1% and 2% to 4.7%, respectively. The average percent recovery for in-house controls was 98.5 ± 2.3%, with individual percentages ranging between 95% and 101%, for CDC was 96.3 ± 3.3%, with individual percentages ranging between 91% and 102%. LOD and LOQ were 1.5 and 4.5 mUI/L blood, respectively. A good agreement (r^2> 0.99) and concordance (0.99) were obtained. **Conclusions:** These results show that there is no difference in the estimation of the concentration between both immunoreaction temperatures, and the new procedure does not affect the performance parameters. Furthermore, the new UMELISA TSH NEONATAL offers a simpler method with good analytical characteristics.

048 - UMELISA TIR Neonatal: Immunoenzymatic Ultramicroassay for the Determination of Immunoreactive Trypsin (IRT) Levels in Dried Blood Spots on Filter Paper

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Introduction: Cystic fibrosis (CF) is a severe autosomal recessive disorder. It's caused by mutations in the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene. Early diagnosis of CF can be carried out by determining high IRT blood values in newborns. **Objectives:** A sandwich ultramicroELISA test for determining IRT levels in newborns from dried blood spots on filter paper samples is described. **Material and Methods:** Plates covered with monoclonal antitrypsin antibodies and a monoclonal antibody-alkaline phosphatase conjugated are used in the assay. The effect of elution, sample processing time, birth weight, and gestational age on TIR levels was evaluated. **Results:** The assay is carried out within 20 hours. The useful rank of the curve is 0 to 500 ng/mL and the lowest detectable concentration is 4.8 ng/mL. Intra and interassay variation coefficients were lower than 10%. The recovery mean value was 98.3 ± 4.3%. Cross reactivity with proteins structurally related to IRT (a2-macroglobulin, a1-antitrypsin, and human quimotrypsin) was lower than the detection limit of the assay. It was carried out a study with 3953 samples from the Cuban Newborn Screening Program. A mean concentration value of 12.6 ± 11.2 ng/mL was obtained. Higher IRT values were obtained when samples were eluted overnight. Preterm and underweight babies showed slightly higher IRT values than normal babies. Regression analysis showed a good correlation with the commercially available AutoDELFIA® Neonatal IRT kit (n = 4033, r = .83, P < .01). Ten IRT confirmed samples showed IRT levels higher than 70 ng/mL. **Conclusions:** UMELISA TIR Neonatal is a precise and accurate assay that can be used for the neonatal screening of CF.

049 - Validation of Quantitative Proton Nuclear Magnetic Resonance (q1H-NMR) Spectroscopy for the Clinical Measurement of Urinary Galactitol

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Background: The measurement of galactose-1-phosphate uridyltransferase (GALT) activity and galactose-1-phosphate (GAL-1-P) in erythrocytes are standard biochemical tests to diagnose and follow-up patients affected with classical galactosemia. Urinary galactitol has been shown to be a better indicator of the long-term outcome of the disease. However, the quantification of galactitol in urine by gas-chromatography/mass spectrometry (GC-MS) is expensive, time consuming, and not widely available. **Objectives:** The aim of this study was to clinically validate Proton Nuclear Magnetic Resonance (1H-NMR) Spectroscopy method for the quantification of urinary galactitol by assessing precision, accuracy, sensitivity, and specificity. **Methods:** Normal urines (n = 50), urines from patients hospitalized at neonatal and pediatric intensive care units (NICU and PICU, n = 10), and from individuals affected with galactosemia (n = 10) were treated with phosphate buffer and analyzed on an AVANCE III HD 600 MHz NMR Bruker spectrometer equipped with a cryo-probe CPQCI 1H-31P/13C/15 N, a Gilson 215 liquid handler and a SampleJet^{TM} autosampler. NMR spectra were processed with TOPSPIN 3.5pl2. Galactitol and creatinin quantifications were performed using ASSURE software and 5 mmol/L benzoic acid solution as external standard. **Results:** The galactitol signal was detected in all urines samples from galactosemia patients and was undetectable in normal urines and in urines from patients hospitalized in PICU and NICU. The linear range spanned from 1 to 50 mmol/L. The limit of detection is 0.7 mmol/L. The between run imprecision for galactitol measurement (Coefficient of Variation, CV) is 2.2% and 3.3% at concentrations of 10 mmol/L and 1 mmol/L, respectively (n = 20). The between run CV for creatinin measurement is 3.3% at concentrations of 5 mmol/L and 10 mmol/L (n = 20). There is a good correlation between ^{1}H-NMRS and GC-MS galactitol measurements and between creatinin concentrations measured by ^{1}H-NMRS and the routine automated clinical assay. The technologist time to process 10 samples is 15 min and the total turn-around time from sample reception to result reporting is 1 hour. **Conclusions:** Quantitative ^{1}H-NMR is a fast, cost-efficient, and reliable technology to detect and quantify urinary galactitol in clinical settings.

050 - Potential Role of Targeted Next-Generation Sequencing in the diagnosis of Niemann-Pick Disease Type A/B: Experience of a Brazilian Reference Center for Lysosomal Diseases

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Niemann-Pick disease types A and B (NPA/B), also known as acid sphingomyelinase deficiency (ASMD), are caused by mutations in *SMPD1* gene. Until now more than 220 mutations have been reported in NPA/B patients. The suspicion is usually raised by clinical features and diagnosis obtained by specific biochemical tests including measurement of ASM enzyme activity in dried blood spots, leukocytes, cultured skin fibroblasts, chorionic villus, or amniocytes. Recently, the genetic testing by Sanger sequencing and targeted next-generation sequencing (TNGS) were introduced in our laboratory routine for most lysosomal diseases. We report here the diagnostic work-up in a 15-year-old patient, initially referred for the investigation of Niemann-Pick C disease, who presented unexplained hepatosplenomegaly, with numerous histiocytes in the bone marrow biopsy. Biochemical tests were performed in the patient, including measurement of oxysterols and of the activity of chitotriosidase in plasma, and of lysosomal acid lipase activity in leucocytes, all these results within normal range. The Fillipin test in growing skin fibroblasts was inconclusive. According to our protocol, ASM activity in cultured skin fibroblast was measured, showing an activity of 1.25 nmol/h/mgprot (reference value: 49-72), a result compatible with ASMD diagnosis. This multistep process took approximately 12 months until the diagnosis was reached, in part due to the request of new samples. A previous validated TNGS panel was utilized as second-tier diagnostic approach, including the genes *NPC1*, *NPC2*, *LIPA*, *SMPD1*, *GBA1*, *PSAP* and *CHIT1*, related to some lysosomal disorders with visceromegaly as common clinical manifestation. We found 2 pathogenic variants in *SMPD1 gene*: p.Arg610del (c.1826_1828delGCC) and p.Asp420 fs (c.1259delA), the last one being a novel mutation. Both variants were confirmed by Sanger sequencing. Turnaround time for TNGS panel in our laboratory is 2 to 4 weeks. Here, we show that the TNGS panel is a sensitive tool, and may have a faster turnaround time compared to the biochemical approach, demonstrating the potential role for diagnosis of NPA/B in our service. Multigene panel next generation sequencing panels, with confirmation of the functional impact of the findings by

biochemical tests, can speed up the definition of the diagnosis, allowing the faster introduction of the appropriate management to the patient and family.

051 - Multiplex Glycosphingolipid Analysis Show Plasma Gb4 and Gb2 Discriminate Female Fabry Patients Better than Lyso-Gb3

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The glycosphingolipid (GSL) Gb_3 is elevated in Fabry disease due to markedly decreased or absent α-Gal A enzyme activity caused by mutations in the α-galactosidase A (*GLA*) gene. The down- and upstream effects of reduced alpha galactosidase activity on other glycosphingolipids (Gb_1, Gb_2 and Gb_4) in the GSL degradation pathway have not been investigated previously. We have developed a 10-minute multiplex LC-MS/MS GSL assay to quantitate 200+ GSL's and their isoforms. This can potentially diagnose Fabry, Sandhoff, and Gaucher disease. We have applied this assay to Fabry patient plasma and urine samples to correlate plasma Gb_3 and lyso-Gb_3 with urine Gb_3 and the other GSL's. Our aim is to investigate the relationship of these 4 glycosphingolipids in Fabry disease in plasma compared to urine and whether they can be informative for the detection of female Fabry patients or response to kidney damage. Plasma GSLs (Gb_1-Gb_4) were prepared from 50 µL of plasma. Plasma lyso-Gb_3 was prepared from 100 µl of plasma. Urine GSLs (Gb_1-Gb_4) were prepared from 50 µL of urine. Extraction solutions containing internal standards for Gb_3 and Gb_2 were added. After extraction, samples were reconstituted in methanol prior to analysis on a Xevo TQ-S mass spectrometer and analyzed using multiple-reaction monitoring (MRM). Data were acquired for lyso-Gb_3 analogues and Gb_1 (glucosylceramide), Gb_2, Gb_3 and Gb_4 (globotetraosylceramide) isoforms to create a profile of Fabry GSLs. Sixty-eight Fabry adult patient samples were analyzed and were grouped according to ERT status, sex, and eGFR stage. Multiple correlation analysis of all isoforms for Gb_3 and lyso-Gb_3 in plasma and urine showed good correlation with each other in both plasma and urine. However, expression of hydroxylated forms C:16 and C18 Gb_3 were independent of Gb_3 but demonstrated a correlation with lyso-Gb_3 in urine but not plasma. There was a poor relationship between urine and plasma Gb_3. Multivariate analysis revealed total plasma Gb_4 and C18 Gb_2 were better than lyso-Gb_3 in detecting female patients however lyso-Gb_3 still showed better response to ERT. There was no relationship with decreasing kidney function with any of the GSLs. Concentrations of urinary hydroxylated isoforms of Gb_3, Gb_2, and Gb_1 are independent of the levels of the unmodified GSL. The origin of these hydroxylated species of GSL is unknown but may be due to other biological processes. Plasma is better than urine for detecting females using Gb_4 and Gb_2.

052 - Congenital Adrenal Hyperplasia: Mutation Detection of CYP21A2 Gene in Cases From Southern Brazil

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Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders caused by a defect in one of the enzymes involved in cortisol biosynthesis. In 90% of cases, CAH is due to steroid 21-hydroxylase deficiency. The concentration of 17-hydroxyprogesterone (17OHP), one 21-hydroxylase substrate, is measured in newborn screening programs using biochemical tests. However, the levels of 17OHP can vary by influence of different factors, causing false-positive or false-negative results. Molecular biology assays have been used to clarify and assist such cases. **Aims:** This work aimed to perform molecular assays to detect mutations in the *CYP21A2* gene in cases of biochemically suspected CAH from Southern Brazil and to compare the findings with other population studies. **Methods:** Between 2014 and 2016, blood samples from 166 cases with high levels of *17-OHP* identified during the newborn screening were genotyped in order to elucidate diagnosis. Three different molecular methods were used to detect mutations on the *CYP21A2* gene. The SNaPshot assay was developed to simultaneously detect twelve-point mutations in the *CYP21A2* gene (p.Arg409Cys, p.Gln319Ter, p.Arg357Trp, p.Leu308PhefsTer6, p.Val237Glu, IVS2-13A/C>G, p.Ile173Asn, p.Pro31Leu, p.Pro454Ser, p.Val282Leu, p.Gly111ValfsTer21 and p.His63Leu). The direct sequencing assay was used to confirm point mutations indicated by the Snapshot developed method. Complementing the investigation, the multiplex ligation-dependent probe amplification (MLPA) assay was used to search for large deletions and gene conversions. **Results:** The Snapshot assay was performed for all cases of biochemically suspected CAH and the MLPA technique was additionally performed for 24 cases. We found 84 pathogenic alleles in 48 cases; p.Val282Leu (30.1%) and IVS2-13A/C>G (23.8%) were more frequent mutations. The frequencies for most mutations did not differ from those of mutated alleles described in other South American studies, with exception of p.Pro31Leu and p.Val282Leu that we found $P < .001$. A new variant T in IVS2-13A/C>G was identified in two patients using the Snapshot assay. *Conclusions* The resulting tested protocols allowed us to detect twelve mutations in the *CYP21A2* gene and help us to elucidate the complicated phenotypes of CAH. Furthermore, the assays proposed allowed us to obtain the first Southern Brazilian mutation frequencies concerning the *CYP21A2* gene.

053 - Automated Nuclear Magnetic Resonance Spectroscopy (NMR) Urinary Analysis: A New Approach for Selective Screening of Inborn Errors of Metabolism (IEM)

Claire Cannet[1], Lars Beedgen[2], Friedrich Karl Trefz[2], Juergen Guenther Okun[2], Claus-Dieter Langhans[2], Hartman Schäfer[1], Manfred Spraul[1], Dorothea Haas[2], Stefan Koelker[2], and Georg F. Hoffmann[2]

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Background: Selective screening of IEM is mainly performed by GC/MS, HPLC/MS, IEC, and other methods. Work up of samples and analyses are time consuming and not all methods are applied in a sample with unspecific symptoms like "autism" or mental disability. As technology and automation in NMR analysis have greatly improved, we applied NMR as first-line method for selective screening of IEM using ERNDIM proficiency testing for proof of principle. **Methods**: To 900ul urine 100 µL buffer was added and analyzed with a Bruker IVDr System at 600 MHz. Spot urine samples of 420 healthy children and adolescents were used as a reference. 16 urine samples of the ERNDIM proficiency testing program were analyzed using a panel of 150 metabolites tested in the reference group. **Results**: In 15 of 16 samples, the correct diagnosis could be made: 2 normal findings, 6 organic acidurias, 3 aminoacidopathies, 1 MCAD and 1 dihydropyriminidase deficiency, 1 sialidosis I, and 1 odontohypophosphatasia. A patient with glutaric aciduria type I (low excretor) could also correctly be diagnosed. A patient with aromatic acid decarboxylase deficiency could not be diagnosed (as it was the case in 17 out of 20 laboratories using other methods). As decided by ERNDIM this became an "educational sample". **Discussion**: The advantage of NMR is the broad spectrum of analytes which can be measured in a single run without time consuming work up of samples and without running additional methods. The reproducibility of results enables not only targeted but also untargeted analysis showing statistical deviations from the reference profiles.

054 - Automated Quality Assessment in Networks of Neonatal Screening Laboratories with SUMA Technology Using the SIGMA, CVI, SDI Estimators

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Quality Control in the Networks of Neonatal Research Laboratories guarantees the operation of the Health Programs reducing the occurrence of false-positive and false-negative results. The present work shows the results obtained with the development and implementation of Computer Systems developed for the Quality Assessment of laboratories with SUMA Technology based on the calculation of the Sigma, SDI (standard deviation index), and CVI (coefficient variation index). Using current computer tools, the automatic extraction of the results of laboratory controls, sending and automated calculation of evaluative results, reducing the execution time of corrective measures and guaranteeing the confidentiality of the results. **Objective:** Carry out the automated quality evaluation in laboratories networks using SUMA Technology using the SIGMA, SDI, and CVI estimators. **Methods:** Three computer systems were developed: 1. Diagnostic laboratories, 2. Supervision dedicated to control a network of diagnostic laboratories of SUMA Technology and 3. Server System for the reception, evaluation and sending of results automatically (without the intervention of personal). **Results:** The results obtained have demonstrated the efficacy of the Sigma, CVI, and SDI estimators in the evaluation of the quality of the laboratories, reducing the number of laboratories indicated deficient by other previously used estimators. The automation of extraction, calculation, and delivery of the results from a Server Software allows correcting errors of measurement in a short time, which positively influences the quality of the results obtained in the Programs of Neonatal Research with SUMA Technology that are carried out in different countries. **Conclusions:** The results obtained have demonstrated the efficacy of the Sigma, CVI, and SDI estimators in the evaluation of the quality of laboratories. The automation of extraction, calculation, obtaining and sending of the results from a Server Software allows correct the errors of measurement in a short time which has a positive influence on the quality of the results informed in the Programs of Neonatal Screening Laboratories with SUMA (Ultra Micro Analytic) Technology.

055 - Screening for Fabry Disease: More Than 50,000 Samples of DBS During Seven Years of Experience in São Paulo, Brazil

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Fabry disease (FD, OMIM#301500) is one of the X-linked lysosomal storage disease that could be treated by enzyme replacement therapy, which reinforces the need of accurate diagnostic methods for this disorder. A methodology to screen for FD in large populations, allowing a better outcome with earlier initiation of therapy, has been developed using a fluorometric assay for α-galactosidase A, the enzyme with functional activity deficient in FD, on dried blood spots (DBS) on filter paper. **Methodology:** DBS samples from patients undergoing dialysis for chronic renal failure in Brazil were analyzed from

October 2009 through May 2017 at the Laboratory of Inborn Errors of Metabolism, in São Paulo, Brazil. The analysis was performed using a synthetic fluorogenic substrate according to the method described by Müller et al. 2010. For quality control reasons, β-galactosidase activity is also performed in all samples with low α-galactosidase A activity. **Results:** We received 50 279 samples from different regions of the country. The mean age was 55.4 years (SD 15.7; range from 0 to 102 years old), and 95.2% of the patients were males. DBS results were considered normal when enzyme activity was higher than 2.5 μmol/L blood/hour until 2012 and 2.2 μmol/L blood/hour from 2013. Among all DBS samples we identified 2.3% of positive results. We had some samples rejected due to low quality (3.3%). Samples with α-galactosidase A activity below our reference range were sent to molecular analysis or a new blood sample was requested to analyze α-galactosidase A activity in leukocytes. **Conclusion:** Once end-stage renal disease is responsible for significant morbidity and mortality among patients with FD, screening throughout dialysis centers can be a first step into an unknown genetic diagnosis for these patients and families. Besides overcoming the hurdles in blood sample transportation from remote regions, DBS samples can be easily analyzed since the method is reliable and warrant the sample stability. However, other confirmatory assays are needed to establish FD diagnosis.

056 - Renal Function in Fabry Disease: The Importance of the Evaluation of Multiple Parameters

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Fabry disease (FD) is an X-linked metabolic storage disease due to the deficiency of alpha-galactosidase A (a-GAL) which causes accumulation of glycosphingolipids, causing impairment especially throughout renal, cardiac, and neurological systems. Enzyme replacement therapy (ERT) is recommended when signs, symptoms, or laboratory changes appear. ERT can improve the quality of life for FD patients, including stabilization or improvement in renal function. **Methodology:** Samples of blood and urine of 40 FD patients, diagnosed by biochemical and molecular analysis, were collected during clinical follow-up at the Institute of Genetics and Inborn Errors of Metabolism from March 2016 through February. For urine samples, proteinuria and microalbuminuria were quantified. Serum and urinary creatinine were measured and estimated glomerular filtration rate (eGFR) was calculated. Two control groups, one without renal loss (paired by age and gender) and other in dialyses treatment for renal disease, both without FD confirmed by enzymatic DBS analyses of a-GAL, were selected by convenience. **Results:** The mean age of 40 FD patients was 41 years (range from 18 to 76 years), 32.5% males and 72.5% on ERT. Clinical history of renal disease was prevalent in 52.5% of FD, including 12.5% that had kidney transplant and 7.5% on dialyses treatment. The results (mean $\pm$ SD) for serum creatinine (1.46 $\pm$ 2.35), urinary creatinine (45.82 $\pm$ 15.07), proteinuria (31.97 $\pm$ 56.25), and microalbuminuria (45.05 $\pm$ 48.78) show that 67.5% patients had at least one nonstandard parameter, including 8 patients without history of renal impairment. The eGFR by Cockcroft Gault (105.66 $\pm$ 50.10; mL/min) and MDRD simplified (97.82 $\pm$ 45.15; mL/min/1.73m^2) formulas support the identification of chronic kidney disease. Although all results of serum creatinine from control group without renal disease were in normal range, 41.5% had one urinary quantitative parameter altered. When two urinary parameters were considered, no individual in the control group had nonstandard results compared to 40% of FD patients, including 4 patients without ERT ($P < .05$, Kruskal-Wallis test). All results of serum creatinine from renal group were increased, as expected. **Conclusion:** Renal evaluation, one of the main markers for diagnosis and prognosis of FD patients on ERT, could be complex and controversial. We suggest that is very important to analyze more than one renal parameter, both in diagnosis and in follow-up of FD patients.

057 - Dynamic Next Generation Sequencing Improves Diagnosis for Fetal Hydrops

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Introduction: Non-immune hydrops fetalis (NIHF) is defined as an abnormal accumulation of fetal fluid in 2 or more extravascular compartments, when hematological and infectious causes have been excluded. Prognosis is generally poor, with 70% to 90% perinatal loss. The National Referral Laboratory has classically performed biochemical testing for a number of inborn errors of metabolism (18 disorders), predominantly lysosomal storage diseases (LSDs) for pregnancies in families with recurrent NIHF. This has now been replaced by a custom next generation sequencing (NGS) hydrops gene panel. **Method:** Our initial hydrops panel was designed to cover 48 genes associated with fetal hydrops on the Illumina Trusight 1 clinical exome. The number of genes interrogated has since been increased following literature reviews of new genes associated with hydrops. Our current hydrops panel (version 3) covers 112 genes on the Roche Medical Exome platform. Results: Biochemical testing of 114 samples from Australasia and overseas between 1991-2013 resulted in a diagnosis rate of 11%. The most frequent diagnosis was Mucopolysaccharidosis type VII (MPS-VII) with six cases. All of these conditions were in autosomal recessive disorders with 1 in 4 risk for subsequent pregnancies. To date NGS testing of 20 samples from May 2014-May 2017 has resulted in 7 diagnoses, a rate of 35%. A Noonan syndrome diagnosis was made in 5 cases, with 2 biochemical diagnoses: Gaucher disease and MPS-VII. Most

in-utero detected cases of Noonan syndrome represent *de novo* mutations, with a significantly reduced recurrence risk. **Discussion:** Prior to the introduction of molecular testing, biochemical testing was restricted to recurrent fetal hydrops because of the complexity and expense. A single NGS testing workflow for hydrops is both quicker, less expensive and can easily cover a greater proportion of diseases/genes that present clinically with NIHF. In addition, future in silico reanalysis of previously unsolved cases should result in additional diagnoses.

058 - IEMbase: Knowledgebase and Mini-Expert Platform for the Diagnosis of Inherited Metabolic Diseases: Validation, Implementation and Performance

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Purpose: Recognizing individuals with inherited diseases is challenging as signs and symptoms often overlap those of common medical conditions. Focusing on inborn errors of metabolism (IEMs), many of which amenable to treatment, we present a method that brings the knowledge of highly specialized experts to professionals involved in early diagnoses. We introduce IEMbase (www.iembase.org), an online expert-curated IEM knowledgebase combined with a prototype diagnosis support (mini-expert) system. **Methods:** Disease-characterizing profiles of biochemical markers and clinical symptoms were extracted from an expert-compiled IEM database. The extracted IEM profiles were transferred to a nascent knowledgebase to populate. To ensure interoperability with external databases, the profiles were linked to UniProt, NCBI Gene, Kyoto Encyclopedia of Genes and Genomes, Genetic Testing Registry, and GeneReviews. Using this knowledgebase, a mini-expert system algorithm was developed using cosine similarity and Human Phenotype Ontology-based semantic similarity. The system was evaluated using 190 retrospective cases with established diagnoses, collected from 15 metabolic centers. **Results:** IEMbase provides 563 well-defined IEM profiles and matches a user-provided phenotypic profile to a list of candidate diagnoses/genes. In addition, it provides differential diagnoses, along with biochemical test profiles and disease prevalence information to help users determine further steps in the diagnosis. The mini-expert system matched 62% of the retrospective cases to the exact diagnosis and 86% of the cases to a correct diagnosis within the top five candidates. The use of biochemical features in IEM annotations resulted in 41% more exact phenotype matches than clinical features alone. **Conclusion:** IEMbase offers a central IEM knowledge repository for genetic diagnostic centers and clinical communities seeking support in the diagnosis of IEMs and serve as model for other genetic conditions. Educational modules and a mobile App are in development.

059 - UPLC-HR-MS Assisted Non-Targeted and Targeted NMR Based Screening of Urine

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Background: Nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) are well proven spectroscopic techniques for the quantification of known and structural elucidation of unknown metabolites in body fluids, i.e., urine or blood serum samples. Both techniques, however, can be utilized also in a completely non-targeted approach. This enables the identification of intake of xenobiotic substances like drugs or detecting endogenous metabolites at unusual high concentration connected to, e.g., inborn errors of metabolism. Both techniques provide complementary data to achieve this goal. **Methods**: Urine samples were prepared for NMR by adding 10% of deuterated buffer solution to the sample and for the MS by diluting 200 µL of urine with the same volume of 2 mmol/L NaN_3 solution for submission to a UPLC-TOF-MS (ultra-performance liquid chromatography-time of flight mass spectrometry) system. The raw data obtained from the UPLC-MS system were subjected to statistical analysis software and the bucket table was then exported to an Excel spread sheet for further analysis. **Results**: Evaluation of the data yielded the tentative assignment of metabolites connected with the intake of drug substances or to the presence of highly concentrated endogenous metabolites. Combined statistical analysis with the NMR data revealed corresponding NMR resonances which could then be used for targeted analysis. For this, commercially available reference components were spiked to the suspicious urine samples to prove their presence. The NMR was then used to quantify the target molecules. **Discussion**: The method was applied to 500 urine samples sent to the metabolic laboratory for exclusion of an inborn error of metabolism. While the MS data were used to correlate meta data, e.g., intake of drug substances or diseases with the presence of xenobiotic or endogenous metabolites, the combined statistical analysis of NMR and MS data was used to identify resonance lines of target molecules in the NMR spectrum for subsequent targeted analysis. Examples for this analytical approach will be shown.

060 - A New Approach for Congenital N-Glycosylation Disorders (CDG) Diagnosis Through the Characterization of Intact Transferrin Isoforms by Two-Dimensional LC-MS

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Different approaches are nowadays devised for applying mass spectrometry (MS) in the characterization of the congenital N-glycosylation disorders (CDG). Most of them target the transferrin (TRF) by looking at its differently glycosylated forms. Some methods exploit the TRF digestion with the subsequent characterization of the generated glycol-peptides. Some others look at the intact TRF forms but after a convenient isolation like planar electrophoresis or immunoaffinity. All the above methods look slightly inadequate for a routine implementation either for labor cost in the sample manipulation or for the use of expensive antibodies. Hereby we present a different approach exploiting a 2-dimensional LC-MS for characterizing the intact TRF forms and by enabling the injection of the serum just diluted. For achieving the scope, a special fluidic system has been devised in order to accomplish the isolation of the TRF forms through an anionic chromatographic separation, followed by a capture on a reverse phase trap, the latter conveniently pre- and post-washed for either avoiding any contamination of the downstream ESI-source by the anionic-chromatographic eluent and for minimizing any carry-over effect. The procedure accomplished in 12 minutes (injection-to-injection) has demonstrated to be very robust in running just-diluted serum samples on a routine scale without any clogging effect. The resulting molecular weight-distribution spectrum clearly shows all the TRF-isoforms, both the normally glycosylated ones and the carbohydrate-deficient forms (CDT). Since this approach has to be correlated to the widely used electrophoretic methodology, hereby presented method is now extensively exploited for building a statistical survey for retrieving the pertinent thresholds. So far, more than 12 positive cases have been confirmed with a calculated % CDT ranging between 15 and 52% for CDG, while the normal values were found up to now between 1.3% and 5.3%. In terms of labor cost for sample preparation and in robustness of the instrumental setting, the protocol has demonstrated to be suitable for a large routine screening.

061 - Drug Repurposing: What If All Marketed Drugs Could be Tested on Every IEM?

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Apteeus is proposing "STOP Orphan," a custom and individualized drug discovery program. For the first time, an initiative will give patients the opportunity to start and participate in a research program dedicated to their disease and for the discovery of a treatment. This will reconcile time of research with their lifetime. Apteeus is making possible collaborative projects that imply patients through patients' association, clinicians, and researchers. The collaborative project consists in (1) developing an in vitro assay based on patient cells obtained from a skin biopsy and which reproduce the pathophysiological mechanisms responsible for the symptoms; (2) testing, on cells from several patients, a unique collection of marketed drugs from all around the world, in the hope that some could unsuspectedly correct the cellular deficiency responsible for the symptoms; and (3) compiling pharmacokinetics and safety properties to assess the opportunity for using an already marketed drug as a clinical candidate in a clinical trial or a compassionate use protocol. This process called "drug repurposing" is clearly less risky and patients' access to treatment is significantly faster than in case of a new drug. We are initiating the collaborative projects with medical doctors and established researchers in the field. In collaboration, we agree on a scientific plan that is systematically challenged by KOL for any monogenetic affection. In many inherited metabolic diseases, the functional defect caused by the genetic mutation is common to all cells of the body. It then makes possible the development and the use of assays on skin fibroblasts or myoblasts in culture. Moreover, these functional in vitro assays are often already used for diagnosis of IEM diseases. That makes the link between in vitro assays and pathophysiology very strong, and the results directly transposable to the clinic. Apteeus has already settled conditions for mitochondrial, peroxisomal, and lysosomal disorders. The main outcomes of STOP Orphan projects are drug candidates, in vitro assays for testing molecules and for better diagnosis, a better understanding of the biology, the identification of new drug targets, and the biobanking of biological samples that can be used in any new project on the disease. We will describe STOP Orphan process implemented for a 6-year-old boy suffering from a deficiency in ACOX1 peroxisomal enzyme and which is currently treated through a compassionate use protocol.

062 - Diagnostic Capability of Next Generation DNA Sequencing With A 450 Gene Panel for Inborn Errors of Metabolism

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Objective: Next-generation DNA sequencing has been widely used for the molecular research as well as diagnosis of genetic diseases and provides cheaper and higher throughput alternative compared to traditional Sanger sequencing. This update

study was performed to investigate the efficacy and reliability of a custom next-generation sequencing panel including nearly all the major genes (450 genes) listed in SSIEM 2012 classification. **Methods:** A total of 61 patients who had clinical and laboratory findings highly suggestive of an inborn error but who did not have a definite diagnosis even after a significant number of metabolic tests, individual enzyme, or genetic assays were included into the study. Semiconductor sequencing technology (Ion Torrent; Thermo Fischer Scientific) was used for the analysis. **Results:** A total of 18 patients were diagnosed with an inborn error of metabolism for having previously defined or novel mutations. Three cases were diagnosed with nucleus encoded mitochondrial disorder, one case with ketolysis defect, one case with tyrosinemia type I, one case with 3-methylglutaconic aciduria, one case with dihydrolipoamide dehydrogenase deficiency, one case with neurodegeneration with brain iron accumulation, one patient with cystinuria, one patient with optic atrophy, ophthalmoplegia, myopathy, ataxia and neuropathy syndrome, one patient with histidinemia, one patient with glycogen storage disease type IX, one patient with Krabbe, one patient with medium chain acyl CoA dehydrogenase deficiency, one patient with metachromatic leukodystrophy, one patient with Segawa Disease, one patient with Bartter Syndrome, and one patient with Fish-Eye Disease. **Summary:** The 450-gene metabolic disease panel is a promising diagnostic tool and could be considered before exome sequencing in the molecular diagnostic algorithm for rare inborn errors.

063 - Alternative Method for Lactic Acid Quantification in Biological Samples by Using Isotopic Dilution GCMS

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Lactic acid determination in biological samples by gas chromatography-mass spectrometry (GC-MS) has been previously described by Meyer (2011) and Rocchiccioli (2002). Both methods are fast and simple and after derivatization with bis-N, O-trimethylsilyl trifluoroacetamide were analyzed with EI MS in selective ion monitoring mode. The methods differ in the use of internal standard as reference molecule for quantification, while Meyer's protocol use 1,3 propyleneglycol Rocchiccioli use a synthetized internal standard 3[^{2} H]-(2 R)-Lactic Acid. We have assessed lactic acid quantification by SIM mode in GCMS using (3,3,3-D3)-L-Lactic Acid as internal standard without consistent results. We observed loss of natural L-Lactic acid in presence of deuterated lactic acid. Some of these inconsistences have been reported. In order to evaluate an alternative labeled Internal Standard for Lactic quantification, we used isotopic orotic acid (1,3-15N2), a reference molecule used in routine form orotic quantification. By using this alternative standard, we found that linear calibration was quantitatively possible and in a reproducible manner as much as for lactic acid present in plasma and urine sample. Also. we could measure lactic acid levels in the conditioned cell culture medium as example for its wide application in clinical and investigation.

064 - Kinetic Study of Plasmatic Alpha-Galactosidase A: Critical Variables for the Diagnosis of Fabry's Disease

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Introduction: Human alpha-galactosidase A (α-Gal A) is a lysosomal enzyme with a homodimeric structure of approximately 101 kDa, which is responsible for catalyzing the galactose of oligosaccharides, glycoproteins, and glycolipids during the catabolism of macromolecules, with globotriaosylceramide being the principal substrate cleaved. α-Gal A is a lysosomal enzyme which is deficient in Fabry disease. Fabry disease is a sphingolipidosis which chronic kidney failure (CKF) is the most important cause of morbidity and mortality. **Objectives:** The aim of this study was to understand the kinetic characteristics of α-Gal A and establish quality control parameters for the assay of α-Gal A activity in human plasma. **Material and methods:** The kinetic features were defined using fluorometric procedures. Reproducibility and fluorescence stability were also evaluated. **Results:** The enzyme was thermolabile, with a 71.09% reduction in activity from initial levels after 1 minute of preincubation at 60°C. After 15 minutes of preincubation, activity was only 2% of the original level. The activity of the α-Gal An enzyme increased progressively according to incubation time. The α-Gal A enzyme is extremely sensitive to variations in pH. Optimum pH was 4.8, and activity levels were significantly different at all other values. The K_m value for the α-Gal A enzyme was 1.007 mmol/L, and maximum reaction velocity was 30.9 nmol/h/mL. There was a significant decrease in the activity of the samples of the enzyme stored at 4°C after 5 or 6 months of storage. However, no change in activity levels was recorded in any month for samples stored at −20°C or −70°C. As measured by the fluorescence of the samples, mean enzyme activity varied between 21.47 ± 4.87 and 22.49 ± 5.08 nmol/h/mL over the 24-hour period following the end of the assay. The differences between values were negligible (non-significant), indicating that fluorescence remained stable for up to 24 hours following the assay. **Conclusions:** This is the first kinetic study of the α-Gal A enzyme from plasma using the same procedures as the diagnostic test, with original data on quality control. The understanding of the kinetic parameters of the enzyme, and its in vitro behavior will be important for the improvement of the diagnosis of Fabry's disease in the laboratory as well as providing a baseline for future analyses

of the kinetics of the α-Gal A of individuals affected by mutations of this enzyme.

065 - Diagnosis and Monitoring of Patients With Glycoprotein Storage Disorders by Novel UPLC-MS/MS Oligosaccharide Analysis

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Majority of clinical laboratories utilize thin layer chromatography (TLC) to measure urinary free oligosaccharides (FOS) to identify patients with a variety of inborn errors of metabolism including the glycoprotein storage disorders, Pompe disease, and more recently several congenital disorders of glycosylation. However, TLC is not an optimal assay as it is not quantitative and lacks the sensitivity and specificity of a clinical diagnostic test. We developed a novel, rapid UPLC-MS/MS method to measure urinary FOS using reducing end labeling. The relative concentration of nine disease-specific oligosaccharides is determined by comparison to the peak area of a single internal standard. As an initial validation, we analyzed 51 urine samples from a patient cohort encompassing 8 LSDs: aspartylglucosaminuria (n = 10), fucosidosis (n = 4), alpha-mannosidosis (n = 21), beta-mannosidosis (n = 1), beta-galactosidase deficiency (n = 8), Sandhoff disease (n = 2), sialidosis (n = 3), and galactosialidosis (n = 2), which were collected as part of the Glycoproteinoses Natural History Study or through routine diagnostic testing. Age-specific normal ranges were developed using 110 samples from unaffected controls. An increased abundance of the disease-specific oligosaccharide was identified in all 51 affected individuals. When compared to age-matched controls, the elevations ranged from 5- to 2100-fold, with fucosidosis (1285-fold), sialidosis (426-fold), galactosialidosis (265-fold), and aspartylglucosaminuria (154-fold) showing the widest dynamic range. Urine samples from patients with alpha-mannosidosis, fucosidosis, and beta-mannosidosis post-bone marrow transplantation had significantly lower oligosaccharide levels compared to untreated patients, indicating that this assay can be used to evaluate the efficacy of future treatments. We have also analyzed 80 urine samples from patients with Mucolipidosis types II, II/III, or III and identified at least one FOS abnormality in all ML patients and were also capable of differentiating between MLII and MLIII patients. Identification of significant elevations in urinary FOS specific for Pompe disease (Glc4) and 2 types of congenital disorders of glycosylation suggest the assay can be used as a broad screen for an increasing number of inborn errors of metabolism. Based on the data accumulated so far, our assay is a significant improvement over TLC and is capable of avoiding false positives due to dietary or medication related metabolites.

C) Newborn Screening (066 to 151)

066 - Development of a Novel 24-Plex Newborn Screening Assay

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Objective: We developed a multiplex tandem mass spectrometry (MS/MS) assay to measure enzymatic activities and biomarkers for newborn screening and diagnosis of 24 diseases (lysosomal storage diseases, galactosemia, biotinidase deficiency, and cerebrotendinous xanthomatosis). **Methods:** A series of enzyme substrates were prepared that allowed the assay of the enzymatic activity of lysosomal and other enzymes in dried blood spots (DBS). The assay uses liquid chromatography-tandem mass spectrometry (LC-MS/MS). In addition to enzymatic products, we also measured biomarkers in the same assay for a subset of the diseases. **Results:** LC-MS/MS enzymatic activity assays were developed for the following diseases: Pompe, Gaucher, Fabry, Niemann-Pick-A/B, Krabbe, Mucopolysaccharidoses (Types I, II, IIIA, IIIB, IVA, VI, and VII), Wolman disease, Neuronal Ceroid Lipofuscinosis (CLN1 and CLN2), Galactosemia (GALK, GALT, and GALE), and Biotinidase. In the same LC-MS/MS, we also measure the following biomarkers: C26-lysoPC (X-ALD), psychosine (Krabbe), glucosyl-sphingosine (Gaucher), lysosphingomyelin (Niemann-Pick), lyso-Gb3 (Fabry), bile derivatives (Cerenbrotendinous xanthomatotic and Niemann-Pick-C), and sulfatides (Metachromatic Leukodystrophy). All of these enzymatic products and biomarkers are analyzed in a single LC-MS/MS run lasting 2.3 minutes and is thus appropriate for high throughput newborn screening. A pilot study for a subset of the 24-plex is ongoing in the Washington state newborn screening laboratory. Data are available for ~50 000 DBS. The results show that the number of false positives is very low, thus showing that newborn screening for these conditions is feasible. **Conclusions:** Newborn screening for 24 inborn errors of metabolism is possible using LC-MS/MS. The assay performs well in a newborn screening laboratory including robustness and high throughput. The method is flexible in that all 23 conditions or any subset can be assayed depending on the needs of the newborn screening laboratory.

067 - Genetic Variants of Glucose-6-Phosphate Dehydrogenase (G6PD) in Brazilian Children With Positive Neonatal Screening for G6PD Deficiency, and Correlation With Neonatal Jaundice

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The objective of the current study was to identify the types of variants in the G6PD gene in a group of children screened through the Neonatal Screening Program (NSP) in the Federal District and correlate these data with the presence of neonatal jaundice. **Methods:** Oral mucosa samples were collected from eighty boys and four girls diagnosed with G6PD deficiency through the NSP in January and February of 2014, whose parents signed an informed consent form. The majority of the newborns presented with residual enzyme activity of around 50% (moderate deficiency). All representatives of the children filled out a questionnaire with relevant details regarding family history, history of neonatal jaundice, and therapy. Molecular analysis was carried out using real-time PCR (allelic discrimination). The G202A and C563 T mutations in the G6PD gene were analyzed using specific primers and probes. **Results:** Seventy of the 84 families were unable to provide information regarding ethnic origin of the child, 13 claimed indigenous descent, and 1 claimed Portuguese and Spanish descent. 60.7% of the children presented with neonatal jaundice, 76.5% presented at 48 hours post-natal, and 29% required phototherapy. Molecular analysis identified a high proportion (98.8%) of neonates positive for the G202A mutation (variant G6PD A-): 79 boys were hemizygous and 4 girls were homozygous for this mutation. Only one boy presented the Mediterranean C563 T mutation. Analysis of the correlation between genotype and presence of neonatal jaundice was compromised by the intense predominance of the G202A mutation in the sample group. **Conclusions:** This is the first study carried out in the population of individuals with G6PD deficiency in the Federal District of Brazil. Although the sample group studied was relatively small, the high prevalence of a single mutation suggests that G6PD deficiency in the population of the Federal District is principally due to the G202A mutation. Neonatal jaundice was prevalent among G6PD deficient children. The absence of cases of heterozygous females in the sample group may reflect the inability of neonatal enzyme screening to detect G6PD deficiency in these cases.

068 - Prediction of Congenital Hypothyroidism Based on Initial Neonatal Screening for Thyroid-Stimulating Hormone at Southwest Colombia (January 2006-March 2017)

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Congenital hypothyroidism (CH) is the most common endocrine disease in newborns. It is related to mental impairment and growth retardation. Most infants with CH are normal at birth, emphasizing the importance of screening programs. The optimal screening-TSH cutoff level is critical to ensuring that CH cases are not missed. Some programs choose a standard TSH screen cutoff, often in the 15-30 mIU / L range and consider all newborns below the cutoff as negative for CH. **Objective:** To evaluate the predictive value of TSH levels determined by the ultramicroanalytic system (SUMA) for diagnosis of CH in Southwest Colombia for the period 2006 to 2017. **Methods:** A longitudinal, retrospective, analytical, descriptive study on statistical information of newborns between January 2006 and March 2017, reported by a laboratory that perform neonatal screening for CH in the southwestern Colombian region and is part of the Program for the External Evaluation of TSH Performance Administered by the National Institute of Health. TSH was determined using the UMELISA-TSH diagnostic kit (Tecnosuma S.A, La Habana, Cuba); Heterogeneous immunoenzymatic assay, sandwich type. The indicators for neonatal screening were measured in two aspects: positive screening tests (TSH levels above 15 mIU / L) and true positives (confirmed by serum TSH and free T4 measurement). **Results:** In the initial screening and follow-up data from 180,650 infants born, there were 1632 positive screening tests (0.90% live births) of which 28 were true positives (1.71%). Twenty-six percent in the 15-19.9 mIU / L range were true positives. Seventy-four percent of neonates with an initial TSH screening of $\geq$ 20 mIU / L and 97% of those with $\geq$ 30 mIU / L were later confirmed to have CH. **Conclusion:** Newborn screening for CH is one of the major achievements in preventive medicine. Infants with TSH levels between 15 and 19.9 mIU / L have a significant risk (26%) of having CH. The true high frequency in newborns with initial TSH values $\geq$ 20mIU / L suggests that this group should be expedite further assessment and treatment. Screening positives with TSH value (15-19.9 mIU / L) need to be examined to determine if they have transient or permanent CH. The goal of early diagnosis and treatment is to minimize neonatal central nervous system exposure to hypothyroidism, as rapidly as possible.

069 - Informing Parents About a Suspected IMD Diagnosis Following Screening: Key Lessons

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The study objective was to determine the views and experiences of healthcare professionals and parents on communication and interaction during the period of confirmatory testing following a positive screening result. Research indicates that positive screening results can have a negative effect on family relationships, parental depression, and relationships with health-care professionals. The period of confirmatory testing following a positive screening result in particular can cause significant anxiety for the families as they wait for news. The communication and support provided during this time may

have a lasting impact on families. Semistructured interviews were undertaken with 10 parents of children who had received a positive ENBS result and 11 health-care professionals who had been involved with the diagnosis and support of parents. Key themes were identified through thematic analysis. The results highlighted the need for careful communication of results to parents, rapid turnaround of results, and a consistent approach. It was clear that parents expect and try to source reliable information online at this time. A need to explore interventions to support family relationships and review the workload and scheduling implications for health-care professionals was highlighted. The development of an app to provide family support and facilitate communication between the family and health-care professionals is now being explored. An initial codevelopment session was run with parents to produce some priorities for the app design. Media content is being developed to explain the confirmatory testing period to parents for 3 conditions (MSUD, HCU, and GA1). In the long term, as well as information, it is expected that a self-management resource may be useful to parents and allow services to provide an ongoing support system. As technology enables newborn screening for a larger number of conditions, there is an increasing need to consider and mediate the potential negative effects. The findings from this study point to a number of elements within the path through confirmatory testing that are difficult for parents and may be suitable for support online and through a smartphone app.

070 - To Screen or Not to Screen? Experiences With Candidate Newborn Screen Conditions Across 3 States

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Newborn screening (NBS) is a state-based public health program identifying babies with critical, treatable inherited conditions at birth across the United States. Rapid advances in screening, diagnostics, and therapeutics have dramatically expanded the number of candidate conditions for blood spot screening. While recommendations are made by a federally supported entity, the Advisory Committee on Heritable Disorders in Newborns and Children, ultimately every state is responsible for determining, financing and supplementing additions to their programs. This has led to great variability in process and timeline for new disorders, particularly the lysosomal storage diseases, which some states have added and others have decided to exclude. Our Newborn Screening Follow-Up Program serves as a referral center for three jurisdictions, each with different disease pipelines. This has presented some unique challenges and valuable learning opportunities. Herein we summarize our active involvement in this process in the District of Columbia, Maryland, and Virginia.

071 - Hemoglobin Kawachi: First Case Found in Uruguay

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Introduction: Hemoglobinopathies are a group of autosomic recessive congenital disorders, which affect synthesis and structure of hemoglobin (Hb). These diseases are seen frequently in Mediterranean area and Africa. Most Uruguayan population is European descendant, and according to the most recent national census, 8% are Afro descendant. Uruguay started in 2013 a nonselective neonatal hemoglobinopathies screening pilot program. Since then, 50 000 samples are analyzed and 2 cases of these disorders are found per year. At the moment, we process every sample by HPLC where we find many rare profiles. **Objective:** To show the results found of a rare hemoglobin variant through newborn screening. **Materials and Methods:** We analyzed whole blood samples obtained from heel prick on filter paper Whatman S&S 903 of babies with more than 40 hours of age. The newborn screening was carried out by HPLC-nbs variant (Biorad), a second sample was analyzed by IEF (Perkin Elmer), and the results were confirmed by molecular studies. **Results:** We found a sample analyzed by HPLC which shows 4 peaks: Hb F, double peak in window associated to Hb A, and a rare Hb at retention time like Hb D. This sample correspond to a girl who was born healthy, with 40 gestational weeks and 3080 g of birth weight. After on1e month, we received a second sample to confirm the previous results. HPLC showed the same profile as the first sample. IEF showed 4 bands: Hb A, Hb F, and two rare Hb. Molecular studies detected a heterozygous mutation in codon 44 (CCG > CGG). This is compatible with the replacement of Proline to Arginine. This genotype is compatible with Hb Kawachi. **Discussion and Conclusions:** A case of Kawachi hemoglobin was reported in Japan 1982 by Hara et col. The case report in this abstract is the first found in Uruguay by Newborn screening. The case detected has no family history of hemoglobinopathies or known decency to suggest a rare hemoglobin. The profile observed by the screening is very uncommon and force to perform confirmation studies not only to characterize the rare hemoglobin variant but also to discard the presence of other mutation that contribute to a pathologic case. Due to the racial mix in our population and the inconclusive results that could be found in neonatal screening, we need to establish confirmatory molecular studies in our laboratory, to complete

the diagnosis and to determine if the cases that present abnormal profiles are effectively pathogenic or not.

072 - Investigating Applications of Next Generation Sequencing for Newborn Screening

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In newborn screening (NBS) programs, dried blood spot (DBS) samples are used to identify babies with rare, often fatal, but treatable disorders. Biochemical analysis is performed at low cost with short turnaround times. However, it can be challenging to predict disease severity and appropriate treatment in asymptomatic individuals or for disorders lacking a suitable biochemical analyte or enzyme assay. This project is investigating the use of next generation sequencing (NGS) to: (1) improve the diagnostic and prognostic utility of existing UK NBS programs and (2) assess the technical feasibility of using NGS as a primary or adjunct screening test. **Methods:** For aim 1, a genotype–phenotype database is being developed to establish correlations between genetic mutations, biochemical changes, and phenotypes for NBS disorders. For aim 2, several methods have been tested to optimize DNA extraction from DBS samples for NGS. Library preparation, sequencing, analysis, and reporting steps are also being automated for high-throughput NBS. **Results:** For aim 1, database development, genotype, biochemical, and phenotypic information is being collected from 180 healthy controls, 130 screen identified patients, and 130 clinically identified patients with the NBS disorders MCADD, MSUD, HCU, PKU, GA1, and IVA. A data entry interface and database has been developed to permit analysis of complex genotype–phenotype correlations. For aim 2, an automated DBS extraction method has been optimized to produce DNA of sufficient quality and quantity for NGS. Other steps including sample receipt, sequencing, and reporting have been tested with robotic platforms and automated analysis pipelines. It is envisaged that the whole process can be offered as a low cost, high-throughput and accurate methodology capable of reporting 1000 to 2000 samples per week. Our aim is to test this as proof of concept by the summer of 2017 **Conclusions:** This project will potentially improve the effectiveness of existing NBS programs by providing prognostic information for patients and families. It will also offer a valuable foundation for further developments, particularly for disorders lacking a suitable biochemical screening test. Use of NGS technology as a primary or adjunct test will have a significant impact on health-care systems worldwide and on other areas of medicine. This project was awarded Health Innovation Challenge Funding from the Wellcome Trust and Department of Health.

073 - Newborn Screening for Arginase Deficiency in the U.S.: Where Do We Need to Go?

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Arginase 1 catalyzes the final step of the urea cycle, hydrolyzing arginine to ornithine and urea. When arginase 1 is mutated, arginine accumulates (hyperargininemia). Arginase 1 deficiency (ARG1-D) is rare with an estimated frequency of $0.5/10^6$ to $1.1/10^6$ live births. A newborn screening test with a low specificity would cause an unacceptably high false-positive rate. Failure of early detection and delays in clinical management frequently results in irreversible cognitive and neurological complications which may be treatable. Currently, ARG1-D is recommended as a secondary target for newborn screening in the U.S. Elevation in status to a "core" condition on the Recommended Uniform Screening Panel (RUSP) would result in more babies being screened, diagnosed, and treated. We surveyed all state newborn screening programs and the District of Columbia in order to ascertain current ARG1-D screening activities of the 51 U.S. programs. We confirmed that the screening laboratory methodologies and algorithms vary widely. Most screening programs rely on arginine concentrations to determine patient risk for hyperargininemia, but action levels vary. Some programs include ratios of arginine to other amino acids but a consistent approach to their use does not appear to exist. While many programs include hyperargininemia as a screening requirement, some do not, and in others the laboratory screening protocol may detect hyperargininemia even though it is not formally a part of the screening panel. Although hyperargininemia screening exists in 76% of U.S. jurisdictions, an overall lack of harmonization in screening methodologies suggests that national data sharing to evaluate arginine cutoffs, and the value of various ratios in establishing a model screening algorithm are needed. We report here as well data from the California Newborn Screening program which demonstrate that all 9 cases of Arginase 1 Deficiency in the past 6 years would have been detected using a low cutoff value of 50 μmol/L, and the ratios of arginine to ornithine and arginine to phenylalanine × isoleucine as suggested by the R4 S program developed at the Mayo Clinic. The sensitivity and specificity were each approximately 100%, meaning that there were no false positives in 5.4 million births. Arginase 1 deficiency can be screened with very high efficiency and is a candidate to be added to the RUSP.

074 - Late Diagnosed Phenylketonuria

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Introduction: Phenylketonuria is the most prevalent disorder caused by an inborn error in amino acid metabolism, and it is the first disease that has a successful treatment that prevents intellectual disabilities. It is the first disorder included in neonatal screening programs in the world and in Argentina. Furthermore, newborn screening is a highly favorable cost-effective test when the screening test is well done. Classical PKU is caused by phenylalanine hydroxylase that catalyzes the conversion of the essential amino acid L-phenylalanine to L-tyrosine. **Objective:** To identify patients with PKU who have not been diagnosed by newborn screening tests. Description of the clinical presentation of PKU. Analysis of the causes and potential implications for newborn screening programs. The historical background of PKU and of neonatal screening tests are briefly described. **Materials and methods**: We analyzed patients with PKU followed up in the Hospital Garrahan from 2000 to 2015 We found a case series of patients with PKU that have not been diagnosed by means of the newborn screening test and we compared them. We analyze the Public Health Care policies and the laws that regulate the screening tests in Argentina. **Results and Conclusion**: Three patients were diagnosed with classical PKU of late diagnosis and presented mental disability. The three cases were from Neuquén, Argentina. The neonatal screening tests had reported as "negative" and the three samples had been taken early. If the screening programs are to be effective it is necessary to have uniform health-care policies with national coverage with an efficient system of coordination, training, education, evaluation and statistics. It is essential to know the impact of not identifying these patients. We have noticed that the failure of the newborn screening tests resulted in three patients with intellectual disabilities, two of them totally dependent on their families and the health-care system.

075 - Enzyme Activities of Lysosomal Storage Disorders in a Neonatal Population of Japan

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Lysosomal storage disorders (LSDs) are caused by the defective enzyme activities in the lysosomes characterized by the accumulation of oligosaccharides, glycolipids, sphingolipids, and mucopolysaccharides. Growing evidences have suggested that the earlier detection of the affected individuals followed by an immediate initiation of appropriate therapy during the presymptomatic period usually results the better therapeutic outcomes. For this reason, simultaneous determination of multiple enzyme activities seems to be more favorable to neonatal screening for LSDs because of the low prevalence of an individual LSD. Thus, in this study, we examined the assay procedure of 6 LSD enzyme activities by LC-MS/MS-based method. Within our assay conditions, the accumulation of enzyme products was almost linear for 0 to 20 hours at 37°C and over 0% to 100% enzyme activity when CDC-provided dried blood spots were used for quality control. The values of coefficient of variance within a day and between days were less than 25%. Importantly, the enzyme activities of healthy individuals were higher than those of disease-confirmed individuals. These results suggest that the levels of enzyme activities of these LSDs of a neonatal population in Japan were comparable to the recent report [Elliott S et al *Mol Genet Metab* 118 (2016): 304-309], further extending an evidence that 6-plex LSD enzyme assay provides a promising analytical procedure for neonatal screening.

076 - Glycosaminoglycan Assay as First-Tier for MPS Newborn Screening

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Mucopolysaccharidoses (MPS) are lysosomal storage disorders characterized by progressive accumulation of glycosaminoglycans (GAGs) due to deficiency of specific lysosomal enzymes. All types of MPS are chronic and progressive with extensive range of clinical manifestations highlighting a need for early diagnosis, especially as many of these conditions are now treatable. This pilot study analyzed 17 467 dried blood spots (DBS) from general newborns and 14 DBS from newborn patients with MPS (7MPS I, 2 MPS II, and 5 MPS III). Disaccharides were produced from polymer GAGs by digestion with chondroitinase B, heparitinase, and keratanase II. Heparan sulfate (0 S, NS), dermatan sulfate (DS), and mono- and di-sulfated KS

were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). Median absolute deviation (MAD) was used to determine cutoffs to distinguish patients from controls. Cutoffs were defined as median + 7x MAD from general newborns. The cutoffs were as follows: HS-0S> 90 ng/mL; HS-NS> 23 ng/mL, DS> 88 ng/mL; monosulfated KS> 445 ng/mL; disulfated KS> 89 ng/mL and ratio di-KS in total KS> 32%. All MPS I and II samples were above the cutoffs for HS-0 S, HS-NS and DS, and all MPS III samples were above cutoffs for HS-0 S and HS-NS. The rate of false positives for MPS I and II was 0.07% based on a combination of HS-0 S, HS-NS, and DS and for MPS III was 1.3% based upon a combination of HS-0 S and HS-NS. Combination of levels of two or more different GAGs improves separation of MPS patients from unaffected controls, indicating that GAG measurement is a potentially valuable tool for first-tier newborn screening of MPSs, especially by the fact that a single test can screen for several distinct MPS. Positive samples for the first tier will be followed by enzyme assay (second tier). Early detection will also affect the choice of the most adequate treatment as well as reduce mortality, morbidity, and public health costs. This GAG-assay also enables treatment efficacy measurements.

077 - Prevalence of Hemoglobinopathies in a Heterogeneous Population of Bogotá, Colombia: A Pilot study

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Introduction: The term hemoglobinopathies refers to a group of genetic conditions that result in the abnormal production of hemoglobin or decrease in its rate of production. Hemoglobinopathies are the most common group of genetic disorders worldwide, with a higher prevalence in the black population. In Colombia, several studies have been carried out on the Afrodescendant population. However, the search for abnormal hemoglobin has not been performed in heterogeneous (Mestizo) populations. **Methods**: We analyzed, with isoelectric point electrophoresis, 594 samples of umbilical cord blood, collected on filter paper. The samples were initially used for the screening of congenital hypothyroidism by the Screening Program of the Secretary of Health at Bogotá, Colombia (4.7110° N and 74.0721° W). **Results**: From 586 samples of mestizo population from Bogotá (8675 feet, 2644 m above sea level), we found two individuals with HbS [β6(A3)Glu → Val; HBB: c.20A > T]. Implying, an allele frequency of A = 1170, and T = 2. A genotype frequency of A/A = 584, (99.7%) and a genotypic frequency of A/T = 2 (0.33%). **Discussion**: The proportion of abnormal hemoglobin found is low compared with the high prevalence areas of Colombia, like the Pacific coast, where the prevalence oscillates from 9% to 21%. The low incidence of abnormal hemoglobin in Bogotá is due to the predominance of mestizo population (Indigenous and Caucasian), and probably some other factors like selective pressure (altitude and temperature) and low migration of black people to the capital city. There is controversy about hemoglobinopathies screening in areas of low prevalence or low proportion of black people in the population. However, the detection of two mestizo carriers in this study and the potential increase in migration of black people to urban areas of the country indicates the necessity of studies that determinates the true prevalence of hemoglobinopathies in geographic areas traditionally considered no affected and the necessity of broadening screening programs to all the country.

078 - Decreased Citrulline on Newborn Screening in a Carrier for Carbamoylphosphate Synthetase I Deficiency

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Decreased citrulline is reported by some newborn screening (NS) laboratories as a marker for proximal urea cycle defects: N-acetylglutamate synthetase (NAGS), carbamoylphosphate synthetase (CPS), and ornithine transcarbamoylase (OTC) deficiencies. The plasma amino acid pattern of low citrulline, increased glutamine–citrulline ratio, and low arginine in association with increased urinary orotic acid is diagnostic for OTC deficiency, whereas the same pattern in the absence of orotic aciduria suggests CPS1or NAGS deficiency. Citrulline may also be low in some mitochondrial disorders and in patients with intestinal disorders such as short gut syndrome. We present an asymptomatic term female identified to have low citrulline (3 μmol/L; N>5) on her second NS performed at age 9 days, confirmed by follow-up testing in plasma at age 21 days (Citrulline: 7 μmol/L, Arginine: 54 μmol/L, Glutamine: 644 μmol/L, Alanine: 301 μmol/L). Plasma ammonia and urine orotic acid levels were normal. Subsequent amino acid analyses revealed persistently low citrulline, despite citrulline supplementation at 30 mg/kg/day and 90 mg/kg/day, initiated at 5 and 7 months of age, respectively. Citrulline remained low until the dose was increased to 143mg/kg/day at age 8 months. Sequencing and deletion/duplication analysis of the *OTC*, *NAGS*, and *CPS1* genes revealed a novel, likely pathogenic variant, c.1165-2A>G, in *CPS1*. While it is possible that the patient has a mild pathogenic mutation that escaped detection in trans with this allele, she is most likely a carrier for CPS1 deficiency. We refrained from mitochondrial testing as her brother succumbed to a mitochondrial disorder which was ruled out in this patient by prenatal testing. In conclusion, we

add carrier status for *CPS1* deficiency to the list of causes for low citrulline identified by NS. Currently, there is no consensus on the usefulness of low citrulline as a marker for proximal urea cycle defects because its positive predictive value is low. Whether to perform further evaluation for mitochondrial disorders in these individuals also remains controversial, as the age of onset of symptoms is variable and no preventive treatment exists. Thus, careful consideration should be given to the approach for follow-up evaluation of a low citrulline level identified by NS in asymptomatic infants, as the cost and unnecessary anxiety caused by additional testing may out-weigh the potential benefits of such testing.

079 - Results of the Neonatal Screening Program in Cuba: 31 Years of Experience

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Introduction: The Neonatal Screening Program in Cuba began in 1986 with the introduction of the UMELISA TSH and UMELISA T4 reagents for the study of congenital hypothyroidism. In 2000, UMTEST PKU was included in the early diagnosis of Phenylketonuria and in 2005, the UMELISA 17OH Neonatal Progesterone, UMTEST GAL, and UMTEST Biotinidase, in the detection of congenital adrenal hyperplasia, galactosemia, and diotinidase deficiency, respectively. These tests, developed and produced at the Immunoassay Center in Havana, Cuba, guarantee the screening of inborn errors of metabolism to more than 100 000 children each year. **Objective:** To evaluate the results of the Program and its impact on the National Health System. **Methods**: A retrospective descriptive study was conducted in the period 1986 to 2016 of the statistical reports of medical records (model 241-509-01 SUMA Technology) and analyzed the program's effectiveness indicators and evaluated the performance of SUMA services. According to the results of the External Quality Control. Statistical methods were applied for the analysis of the results. **Results:** In 31 years, 9 727 847 newborn exams have been performed, of which 1037 children with different metabolic disorders have been diagnosed and treated in time. The follow-up of the indicators of effectiveness showed at the end of 2016 that more than 95% of the samples were collected before the sixth day of birth, transferred to the laboratories before 72 hours, and tested immediately. The coverage of the program reaches 99% of the children born, and it extends to 169 Specialized Centers of Integral Active Research (CEPAI), that participate in the program of external evaluation of the quality and are located with technological support, in the National Network of the National Health System. **Conclusions:** Since the beginning of the program, life has been saved and guaranteed normal neurocognitive development for all children diagnosed. A high level of well-being has been guaranteed to the family and society as a whole. It has achieved the necessary technological sovereignty that allows strengthening and giving continuity to the national program with excellent results in the indicators of effectiveness. The external control program monitored the work of the laboratory network, giving credit to the results.

080 - Interlaboratory Comparison of Bloodspot Total Homocysteine Results for Expanded Newborn Screening Second-Tier Testing in England and Wales

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The objective of the study was to ensure comparability of results between the 3 laboratories providing bloodspot total homocysteine analysis for newborn screening second-tier testing in England and Wales. Expanded newborn bloodspot screening to include homocystinuria (HCU; cobalamin non-responsive cystathionine beta-synthase deficiency) commenced in January 2015 in England and Wales. Bloodspots found to have a methionine $\geq$50 μmol/L are referred for second-tier total homocysteine analysis. Patients are identified as "screen positive" if the bloodspot total homocysteine is $\geq$15 μmol/L. The bloodspot total homocysteine methods are calibrated using aqueous L-homocysteine calibrators. Second-tier testing is currently provided by 3 centers: Viapath at St Thomas' Hospital (London), Birmingham Children's Hospital (Birmingham), and University Hospital of Wales (Cardiff). When expanded newborn bloodspot screening was introduced, no external quality assessment (EQA) scheme was available for total homocysteine. Over a 2-year period, 48 bloodspots were distributed between the three testing laboratories. Bloodspots were made from whole blood hemolysate spiked with L-homocysteine at six concentrations (to give 0, 5, 10, 20, 40, and 80 μmol/L homocysteine). Bloodspots were stored at −20°C and were stable over this time. The six bloodspot levels were distributed in a semi-randomized pattern, with each level distributed eight times over the period. The results were collated and analyzed to show between-laboratory performance. The coefficient of variation between laboratories was lowest close to the screening cutoff (12% at 16.5 μmol/L) and highest at low concentrations (37% at 4.2 μmol/L, base material). The mean recovery of total homocysteine from the bloodspots was approximately 30% for spiked concentrations between 10 and 80 μmol/L but was lower at 21% for the 5 μmol/L spike. To conclude, while the between-laboratory variation close to the screening cutoff has been shown to be acceptable in England and Wales, the recovery of total homocysteine from spiked bloodspots is low. The current lack of independent EQA provision for second-tier newborn screening tests is not ideal; the

proposed pilot EQA scheme to be provided by ERNDIM is therefore welcomed.

081 - External Quality Assessment of Neonatal Screening in Cuba: A Three decades Overview

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For over three decades we have been applying an External Quality Assurance Program (EQAP) as an important analytical support to more than 150 laboratories that currently perform Neonatal Screening in Cuba, using SUMA® technology to more than 98% of newborns. The aim of this report is to present the results of the implementation of this EQAP scheme and its influence on quality performance of neonatal screening programs in our country. Control samples with different concentrations values of every analyte (TSH, T4, 17-OH Progesterone, GAL, and Phe) were delivered every 6 months to the participant. The results of variables as Average Index of Variance (AIV) and Average Index of Accuracy (AIA), with a monthly, semestral an annual periodicity were statically evaluated with specifically designed software. At their introduction, relatively high values were observed for both indexes with every test; later on, with gained experience and analytical technical assistant when needed, the indexes were progressively improved, reflecting a better precision within and between laboratories (coefficient of variations lower than 10% and 15%, respectively). Our results sustain the importance of the implementation of a quality control scheme as a component of a screening program supported on a laboratory network, providing a mean of supervision of the analytical performance and managing for quality improvement.

082 - Next Generation Sequencing and the Slovenian Newborn Screening Program

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Many countries in Europe have an expanded newborn screening (NBS) program using tandem mass spectrometry, while it is still not implemented in any south-eastern European country. Since the expansion of current screening programs is one the major goals of health-care programs in south-eastern Europe, we conducted a pilot study of expanded NBS in Slovenia with 10 048 included participants. 85 of them were chosen based on abnormal metabolites measured in newborn dried blood spots (DBS) and were analyzed at a metabolic follow-up consisting of the following analyses: acylcarnitines and amino acids in dried blood spots, amino acids in plasma and organic acids in urine. 75 participants, out of 85 analyzed at metabolic follow-up, were analyzed with next generation sequencing (NGS) for selected inborn errors of metabolism (IEM) using a specifically designed gene panel. Altogether, glutaric academia type 1 was confirmed in one patient who was a compound heterozygote for two known causative *GCDH* variants. A patient with very long-chain acyl-CoA dehydrogenase had two heterozygous *ACADVL* variants; one known disease causing variant and one indel, namely, c.205-7_-8delCTinsGC predicted to be causative. Nine participants were found with elevated metabolites characteristic for 3-methylcrotonyl-CoA carboxylase deficiency. 2 of them had known causative homozygous variant in *MCCC1*, while the other 7 participants were heterozygous and 2 of them had a novel genetic variant c.149_151dupCCA (p. Thr50dup). Two participants, one with classical PKU and one with hyperphenylalaninemia, were already confirmed within the existing screening program. Our study demonstrates that cumulative incidences of IEM in Slovenia are similar to the incidences in developed European countries, which have an expanded NBS program running for years. NGS is nowadays being implemented into NBS, either as a first-tier screening test or as a follow-up test of abnormal screening results. NGS as a confirmatory testing proved to be a valuable tool in our study as it explained the abnormal metabolites in DBS, enabled the differentiation between truly affected patients and mere heterozygotes and improved the turnaround time of genetic analyses.

083 - Average Value of 17-OH Progesterone in Different Weights in the INSTITUTO MEXICANO DEL SEGURO SOCIAL Guanajuatós (IMSS) Population

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Introduction: 17-OH progesterone is a test to evaluate congenital adrenal hyperplasia (CAH), which include a group of enzymatic disorders of the adrenal gland that produce an alteration in the synthesis of cortisol and aldosterone, with high levels of androgenic precursors. This is a statistic analysis to find the average value and standard deviation in the 17-OH progesterone test (17- OHP4) for different weights. The sample was the INSTITUTO MEXICANO DEL SEGURO SOCIAL Guanajuatós (IMSS) population. **Objective:** To determine the average value of 17-OH progesterone and its standard deviation (SD) in newly born, male and female, with weigh lower than 1500 g, between 1500 g to 2499 g and higher than 2500 g.

Methodology: Analysis of results since January 2012 to October 2016 with a total of 117 582 newly born, equivalent to 90.0% of the total of processed samples. The remaining 10.0% results belong to newly born with incomplete medical chart. Results: For female newly born: with a weight lower than 1500 g, the average value was 99.59 nmol/L and an SD 62.28; weight between 1500 g to 2499 g had a value of 43.33 nmol/L and SD of 31.39. For the weight over 2500 g, the value was 28.61 nmol/L with an SD of 14.97. For male newly born, with a weight lower than 1500 g, the average value was 112.75 nmol/L and SD of 71.96; weight between 1500 g to 2499 g had a value of 51.27 nmol/L and SD of 37.28. For the weight over 2500 g, the value was 30.6 nmol/L with an SD of 16.64. **Conclusions:** There is no difference between the average value for both genders. Nevertheless, the concentration of 17-OH progesterone is higher in a lower weight. This is related with the under developed of the hypothalamus, hypophysis and adrenal gland.

084 - Development and Evaluation of a Protocol for Diagnosis Confirmation of Cases With Abnormal Newborn Screening Results for Mucopolysaccharidosis I

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Introduction: Mucopolysaccharidosis I (MPS I), an inherited metabolic disorder from the group of Lysosomal Storage Diseases (LSDs), is caused mainly by defects in the *IDUA* gene, leading to alpha-L-iduronidase (IDUA) deficiency and consequently to intracellular accumulation of glycosaminoglycans (GAGs), dermatan and heparan sulfate. MPS I has been proposed for inclusion in early detection programs, such as newborn screening (NBS), due to the availability of treatment options and the advantage observed with its early introduction. While several studies have established techniques suitable for NBS of LSDs, including MPS I, and mass screening studies are underway in some NBS programs, few has been discussed on further procedures, such as the process for confirmatory diagnosis in newborns with an abnormal result at the initial testing of NBS for MPS I. **Objective**: To develop a protocol for the confirmatory diagnosis of cases that screened positive at the NBS programs for MPS I, considering its potential future use in Brazil and other countries. **Methods**: Protocol development and evaluation was based on the WHO Handbook for Guideline Development and The Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. Procedures including literature search were systematically performed at Cochrane Database, Database of Abstract of Reviews of Effects, Cochrane Central Register of Controlled Trials, PubMed, and WHOLIS. Where no reports were found, expert opinion was consulted and included. Relevant evidence was selected, synthesized and evaluated, to then formulate the recommendations. **Results:** Based on evidence interpretation, confirmatory diagnosis of cases with abnormal results in NBS programs for MPS I should be based on IDUA activity, urinary GAGs and *IDUA* gene analyses. GRADE evidence profiles were created based on these analyses that guided the formulation of initial eight recommendations, of which five were considered strong recommendations. Likewise, two diagnosis strategies combining the above analyses were identified and compared. Overall, evidence was evaluated as of moderate or low quality. Lack of randomized trials and systematic reviews were observed as limitations of evidence in the study. **Conclusion:** We provided an evidence-based protocol intended to facilitate decision-making during the confirmatory diagnosis process in newborns, likely to be asymptomatic, who will result screened positive in NBS programs for MPS-I.

085 - Improvement in the Sensitivity of Newborn Screening for Fabry Disease Among Females Through the Use of a High-Throughput and Cost-Effective Method, DNA Mass Spectrometry

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Background: Many female carriers of Fabry disease could develop severe morbidity and mortality. However, by our own estimation, around 80% of female newborns are missed by our current enzyme-based screening. **Objective**: Our team aim was to develop an improved cost-effective screening method that detects Fabry disease among female newborns. **Methods**: In Taiwan, based on a database of 916 000 newborns, approximately 98% of Fabry patients carry one of only 21 pathogenic mutations. An Agena iPLEX platform was designed to detect these 21 pathogenic mutations using only a single assay panel. **Results**: A total of 54 791 female infants were screened and 136 female newborns with the IVS4+919G>A mutation and one female newborn with the c.656T>C mutation were identified. Compared with the results obtained using the enzyme-based newborn screening approach, around 83% of female newborns have been missed by the current newborn screening.

Through a family study of the IVS4 female newborns, 30 IVS4+919G>A adult family members were found to have left ventricular hypertrophy. Ten patients have received an endomyocardial biopsy and all had significant globotriaosylceramide (Gb3) accumulation in their cardiomyocytes. Now, all of them have started to receive enzyme replacement therapy. **Conclusions**: We have demonstrated that the Agena iPLEX assay is a powerful tool to screen for females with Fabry disease. Through this screening, we also have identified many disease-onset adult family members who were undiagnosed for Fabry disease. This screening will help them to receive treatment in time before severe and irreversible cardiac damage has occurred.

086 - Incidence of Six Lysosomal Storage Diseases in a Mexican Newborn Screening Program

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Lysosomal storage diseases (LSD) are a group of more than 50 genetic disorders in which there is a decrease or an absence of activity, inadequate lysosomal enzyme, or carrier proteins biogenesis producing progressive accumulation of precursor metabolites within the lysosomes that result in cellular dysfunction and multiple organ system failure. Currently, there are no comprehensive studies that show the incidence or prevalence in Mexico for LSD. We retrospectively analyzed 44 784 newborn screening (NBS) reports storage in our database between January 26, 2012, and April 27, 2017. Each sample was placed on filter paper and processed by tandem-mass spectrometry at PerkinElmer Genetics (Bridgeville, PA). Six different LSD were studied, including Pompe disease, Fabry disease, Gaucher disease, mucopolysaccharidosis type I (MPS-I), Niemann-Pick type A/B disease, and Krabbe disease. The protocol followed to get an LSD diagnosis began with an NBS first sample showing a decreased enzymatic activity, a second sample with an abnormal result, performing as confirmatory testing serum enzymatic activity and/or gene sequencing. For the 44 784 NBS reports analyzed, 21 882 were female and 22 902 were male. On average, the NBS was performed at 5.27 days of age ± 5 days. After the second sample, we found 45 LSD abnormal results. The diagnosis was confirmed in 24 newborns (1 for Niemann-Pick, 15 for Pompe, 7 for Fabry, and 1 for MPS-I). Furthermore, 6 newborns were identified as heterozygous (2 for Niemann-Pick, 3 for Pompe, and 1 for Krabbe). The last 15 newborns were ruled out after a normal confirmatory test. The false-positive rate for the NBS was 0.047%. On the other hand, the positive predictive value was 53.33%. Noteworthy that the newborns confirmed with an LSD were diagnosed approximately at 54 days ± 27 days after the collection of the first sample. The incidence of the 6 LSD studied in a Mexican newborn population was 5.36 cases per 10 000 newborns, being Pompe disease the most frequent (3.35:10 000). Carrying out an expanded newborn screening that includes these LSD has allowed us to establish an early diagnosis, initiate an appropriate and timely treatment in order to improve their quality of life. Moreover, genetic counseling was given. In our experience, a prompt diagnosis is achieved thanks to a close follow up by the integration of all parties participating in the NBS program (laboratory, medical staff, parents, and social workers, among others).

087 - Efficient and Effective Newborn Screening for Early Infantile Krabbe Disease

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Newborn screening (NBS) for Krabbe disease (KD) has been conducted primarily by measuring galactocerebrosidase (GALC) activity and employing molecular genetic analysis of the GALC gene as a second-tier test. Based on NBS data from New York the incidence of KD is unexpectedly low (1:500 000 in NY), while many individuals (ca. 1:5600) with reduced GALC activity and genotypes of uncertain significance are detected and subjected to follow up testing. We and others have shown previously that Psychosine (PSY) is a marker of active KD and can be measured in dried blood spots (DBS). Therefore, we applied it as a second-tier test to NBS for KD along with post-analytical interpretive tools created using Collaborative Laboratory Integrated Reports (CLIR; https://clir.mayo.edu) and molecular genetic analysis of *GALC*. Among more than 60 000 newborns screened, we identified one case with reduced GALC activity (0.18 nmol/mL/h; first percentile of controls: 1.36), a high CLIR score for Krabbe disease (788; informative >30), significantly elevated PSY (61 nM; controls <10), and a genotype including a heterozygous pathogenic deletion, a heterozygous likely pathogenic frameshift mutation as well as a heterozygous and a homozygous pseudodeficiency allele. The NBS specimen was received in the laboratory on the fourth day of life (a Saturday), the report was released on the sixth day of life, the patient was admitted to the Pediatric Blood and Marrow Transplant Program at Duke University Medical Center on the seventh day of life, received a hematopoietic stem cell transplant (HSCT) on the 23rd day of life and was discharged to home on the 104th day of life. At now 5 months old the patient is developing appropriately for age with minor transplant related concerns. Based on our experience to date, we postulate that NBS for early infantile KD is possible without false positive results and without the need for molecular genetic testing in the NBS laboratory. The biochemical genetic screening approach

enables rapid identification of early infantile KD which is of utmost importance to ensure best possible outcomes of HSCT. As our case illustrates, 7-day operation of the NBS laboratory is also required to achieve best outcomes. Whether a biochemical-genetics-only approach is sufficient to detect all cases with later onset variants of KD remains to be determined. Meanwhile relevant stakeholders must continue an open and honest discussion about the achievable goals of NBS for KD.

088 - Newborn Screening for Inborn Errors of Metabolism by Tandem Mass Spectrometry in Cali, Colombia

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Inborn errors of metabolism (IEM) are genetic disorders causing serious, degenerative, chronic diseases affecting different organs and metabolic systems leading to mental retardation, physical disability and death. Laboratory diagnose of these diseases has evolved through the last two decades from identification of individual disorders using thin layer chromatography, enzymatic methods and high-performance liquid chromatography, to the recent use of tandem mass spectrometry (MS/MS) which allows simultaneous evaluation of multiple metabolites associated with IEM with high sensitivity, low false-positive rates, and high throughput. MS/MS was recently implemented in Cali (Colombia) to determine amino acids, acylcarnitines, succinylacetone, and galactose IP levels in dried blood spots (DBS) of newborns. In a pilot cross-sectional study conducted recently, blood samples of 891 healthy neonates from Cali (n = 523) and Quibdó (n = 368) were analyzed for 57 analytes which allow the diagnosis of >40 different IEM. Blood samples ($\sim$50 mL) from healthy neonates 2-18 days of age (52% male and 48% female) were processed by stable isotope dilution method using Isotopically labeled internal standards from Cambridge Isotope Laboratories, Inc., and analyzed in a 3200 QTRAP MS/MS equipment (AB Sciex). The method showed to be linear, precise and accurate; age-related differences on the concentration levels of amino acids and acylcarnitines were observed, whereas no significant differences by gender were found. Simultaneously, newborns with clinical risk of IEM were tested and two Propionic acidemias, one Methylmalonic acidemia and one Glutaric acidemia Type II, were identified, the last with a fatal outcome. A second DBS and a urine samples were taken from those patients for a second analysis in Cali as well as at the Santiago de Compostela's Clinic Hospital in Spain and results were confirmed. This is the first report about the implementation of a method by MS/MS to analyze characteristic metabolites for IEM diagnosis in Colombia. Detection of these 4 positive IEM cases suggest that the prevalence of these diseases might be most frequent that expected. As follow up we are currently initiating a study to determine the prevalence of metabolism-dependent diseases determined by MS/MS in a sample of 30 000 healthy corresponding to $\sim$58% of the total of newborns/year and 1500 risk cases in four municipalities of Valle department in alliance with the State Health Secretary.

089 - Galactosemia as Cause of Neonatal Sepse in Newborn Without Previous Risk Factors for Infections

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Galactosemia is a metabolic disorder diagnosed in the neonatal period through simple screening tests ("test of the foot"), incidence is 1:18000 (classical form) ranging up to 1: 50000 (depending on the source searched), with no predilection for sex. It is a genetic disease of the autosomal recessive type, which is part of the group of inborn errors of metabolism. It is characterized by the inability to convert galactose to glucose and consequently the accumulation of galactose-1-phosphate in nerve, liver, kidney, and crystalline tissue cells. Galactosemia can be classified into three subtypes. Newborns with galactosemia are more prone to septicemia, with significant frequency of neonatal deaths caused by septicemia by gram negative germs, mostly multiresistant and mainly *E. coli*. The aim of this work is to illustrate, through a case report, the possibility of galactosemia as a facilitator of late neonatal sepsis in a newborn vaginal birth, term, without complications, who was discharged within 48 hours and returned later with clinical and laboratory findings of severe neonatal sepsis. The newborn does not have any risk factors for neonatal sepsis and prenatal exams within normal limits. Cycles of wide-spectrum antibiotic therapy, mechanical ventilation, and vasoactive amines were required. The associated cholestasis, the patient's poor response to treatment, crop results, difficult to control hypoglycemia, and the severity of the case were factors that called attention to the possibility of the presence of the associated diagnosis of inborn metabolism error. Suspecting is the key. It was an observational work whose methodology was the retrospective analysis of the medical chart of this newborn and bibliographic review on the subject. In addition, the inclusion of galactosemia screening in the National Neonatal Screening Program ("foot test") could increase the chance of early diagnosis by improving quality of life both by introducing dietary measures and by increasing cases eligible for

outpatient follow-up. It would also increase the chance of prevention of possible complications, including those related to severe neonatal sepsis and the incidence of neonatal deaths. Neonatal sepsis is an important cause of death in this age group. It leads not only to emotional sequelae in families as possible socioeconomic sequelae. Any effort to improve the causal diagnosis and to be able to help in prevention and treatment is of extreme value for neonatology.

090 - G6PD Deficiency in Newborns at the Rafael Calvo Maternity Clinic in Cartagena de Indias: Pilot Study

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Objective: To determine the frequency of two of the most common genetic variants of G6PD deficiency (A and A -), by real-time polymerase chain reaction (qPCR) in a population of newborns at the Clínica Maternidad Rafael Calvo of the city of Cartagena de Indias, Colombia. **Methods:** Fifty-one dried blood spot samples from a population of newborns at the Rafael Calvo Maternity Clinic in Cartagena de Indias, Colombia, were analyzed and randomly selected to perform an initial survey of the study population and designed primers. Samples were obtained from umbilical cord blood and deposited on Whatman filter paper. The blood samples from Whatman paper were cut out and subjected to a DNA extraction process using InstaGene Matrix, for further amplification and qPCR analysis in order to determine the frequency of 2 mutations for G6DP A and A- in the respective Neonatal population**. Results:** Of the 51 analyzed samples, a total of 11 (21.7%) showed positive results for G6PD deficiency, the frequency of the two selected genetic variants was distributed as follows within the population, 8 samples (15.7%) with positive results for the genetic variant A and 3 samples (5.9%) positive for the genetic variant A-, with negative overall results for these mutations of 40 samples (78.4%). Conclusion: In this pilot study, a high frequency of two of the most common genetic variants of the enzyme deficiency of G6PD was demonstrated in a population of neonates born in the city of Cartagena, Colombia. This care center captures a high number of births not only coming from this city, but also from nearby cities and counties of the department of Bolivar, in which there are continuous reports of Malaria a disease closely related to this enzyme disease. These initial results are the first reports of G6PD deficiency in newborns in the Northern Colombian zone and motivate to continue studying this alteration with a view to obtain more complete results that demonstrate the general panorama of the genetic variants of G6PD present not only in the city of Cartagena, but also in the Colombian Caribbean. **Acknowledgments:** To Colciencias and the University of Cartagena for the financial support for the project 1107-569-33704.

091 - "Are We Doin' it Good, Boss?": Maintaining Quality Newborn Screening

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The NSW Newborn Screening Program aims to maintain and improve a high-quality screening program. There are many things to consider when assessing what is "quality newborn screening." A newborn screening program encompasses pretesting; sample testing and patient follow-up. Quality improvement involves looking at all aspects of our program. The NSW Newborn Screening Program is under constant review to improve the quality of what we do. We observe aspects that are both internal and external to the laboratory. As a program, we deal with what happens before the sample is collected, collection of the sample, testing of the sample, delivering results, and follow-up till diagnosis or exclusion of a disorder. Each aspect is reviewed separately using methods that are diverse and suitable for that aspect. This can be through database information, questionnaires, community awareness, observation. Each aspect of the process is reviewed by relevant members of our team to look for ways to improve the quality of our program. ISO15189 accreditation processes assess quality of the scientific portion of the program. The NSW Newborn Screening Program is accredited with National Association of Testing Authorities, Australia, and quality certified. Currently, we use Human Genetics Society of Australasia key performance indicators to assess some other aspects of screening quality. Percentage of specimens in transit for 4 days or less increased from 78% in 2007 to a peak of 87% in 2014 and in 2016 was 85%; where the target is 95%. Percentage of unsuitable samples has decreased from 0.88% in 2007 to 0.54% in 2016, where the target is <0.5%. The median day of specimen collection was day 3 in 2007 and day 2 in 2016, where the target is 95% collected between 48 and 72 hours of age. Quality is not static and needs to be evaluated frequently. It is important to schedule review and, if appropriate, update all aspects of newborn screening, not just scientific methods.

092 - Metagene (www.metagene.de): Knowledgebase for Diagnostic Support of Inborn Errors of Metabolism (IEM)

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Introduction: There is an increasing number of newly described genetic diseases with complex metabolic changes and a broad spectrum of clinical symptoms. 20 years ago, we first started developing a web-based knowledge base for diagnostic support

of IEM, which now has been updated in form and content. **Method**: Construction of a relational web-based database running on different platforms with Internet browsers and smartphones. 533 metabolic diseases and differential diagnoses, 1547 clinical symptoms, 657 metabolites and metabolic findings (like metabolic acidosis, hyperammonemia, etc.), and 4228 references are implemented. Typical pathologic concentrations of metabolites in various biologic fluids are available. Search functions for multiple combination of clinical symptoms and laboratory findings are possible. Clinical and detailed metabolic findings, diseases with links to OMIM, ORPHANET, Brenda (enzyme database), and gene locus (NCBI database) are possible as far as available. Terminology is standardized according to OMIM and other databases.

Results: 1. Search function for **clinical symptoms**. The more specific the symptoms, the better the yield. "Cataract" results in 42 metabolic diseases. In contrast, under the group "skin lesions", "eczematoid skin rush", only 2 metabolic disease but additional 2 differential diagnoses are displayed. 2. Search function for **metabolites**. Again, the more specific the metabolite, the more comprehensive are the results. With "Hypoglycemia" 92 diseases, with "3-hydroxyisolovalerylcarnitine" only 5 metabolic diseases are associated. 3. **Combined search function**: e.g.: "hepatomegaly" together with "lactate" provides 23 diseases. Addition of "creatine kinase" 6, and addition of "uric acid" only two IEM. **Conclusion**: The knowledgebase www.metagene.de is open to public and a nonprofit project. It supports training for young professionals, and is functioning as "watch dog" for more experienced metabolic clinicians. Further plans will include multi coauthor ship and support by sponsors to ensure continuous development and quality assessment.

093 - Congenital Hypothyroidism in Nicaragua: Etiologic Classification, Imaging, and Molecular Characterization of Diagnosed Children in 2005-2015

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Objective: To evaluate the effectiveness of the newborn screening program for congenital hypothyroidism in Nicaragua, etiological classification, and molecular characterization of children diagnosed in the period of 2005 to 2015. **Materials and methods:** Screening for congenital hypothyroidism (HC) was performed by quantifying TSH using a validated ELISA (enzyme-linked immunosorbent assay). The cutoff level was 20 µUI / mL. Suspicious children were located for retesting, and the positives were followed clinically and analytically. Imaging studies were performed using a Digital Ultrasonic Diagnostic. A total of 40 children with thyroid dysfunction (HC) were studied genetically for etiological and molecular classification. Mutations for the *TSHR*, *PAX8*, *TPO*, *Tg*, and *DUOX2* genes were analyzed by PCR techniques followed by sequencing. **Results:** Of the 271,969 children screened for HC in the period of 2005 to 2015, 80 cases of congenital hypothyroidism were identified, representing a prevalence of HC of 1: 3399 newborns. Overall coverage was 71% and a positive predictive value of 84% was achieved for the screening program. The mean time to start treatment was 57 days. Ultrasonography revealed that 31% of the patients had normal-sized glands, 55% had severe hypoplasia, and 13.8% had a goiter. Mutations were identified in 27.5% (11 patients). For the *TPO* gene, 3 mutations, 2 known mutations (c 1274A> G (p.N425 S), c. 2578G> A (p.G860 R) and an unknown mutation (c.962C> A (p.T321 N)) were identified. Five patients with mutations in homozygosis and 5 in compound heterozygosis. A patient carries an unknown mutation in the *TSHR* gene in homozygosis (c.1192T> G (p.C398G)).

094 - The Inclusion of ADA SCID in Expanded Newborn Screening by Tandem-Mass Spectrometry

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Adenosine deaminase (ADA) deficiency is an inherited disorder that causes severe combined immunodeficiency (SCID). The early-onset form is rapidly fatal in infancy because of severe infections. Patients with delayed or late-onset form suffer of multiple recurrent infections that may lead to permanent organ damage or death in childhood or adulthood. Irreversible brain damage may have already occurred before diagnosis is done. Gene therapy, bone marrow transplantation, or enzyme therapy might be effective if performed early. ADA-SCID meets the criteria for inclusion in newborn screening (NBS) program. The quantification of T cell receptor excision circles (TRECs) generated during maturation of T cells can identify newborns with early-onset ADA SCID on dried blood spots (DBS) collected at birth. In 2011, our group reported elevated level of adenosine (Ado) and 2-deoxyadenosine (dAdo) in DBS from newborns with ADA SCID early onset by tandem-MS analysis. However, it was not clear whether patients with delayed or late-onset form can be identified at birth before symptoms appear. Since 2011, we tested ADA metabolites in the our NBS panel, identifying an ADA affected newborn. Patient was confirmed by *genetic testing* as delay/ late onset ADA SCID. She had normal TREC levels. For this reason, the neonatal DBS from other 8 patients with delayed or late onset ADA-SCID were retrospectively analyzed by tandem-MS to

evaluate levels of Ado and dAdo, and TREC levels were quantified by real-time PCR. All patients showed adenosine values between 1.08- 25 µmol/L (n.v. <2) and 2-deoxyadenosine values between 0.11-2.7 µmol/L (n.v. <0.1) with ratio Ado/dAdo >2.2. Finally, all 8 patients had *undetectable* or low *TREC levels* at the diagnosis but normal TREC test on neonatal DBS. We also analyzed DBSs from 21 newborns with early-onset form showing Ado levels between 0.15 and 47.2 µmol/L (n.v. <2) and dAdo values between 0.46 and 55.7 µmol/L (n.v <0.1) with ratio Ado/dAdo <2.2. In this study, we report how *ADA SCID patients* can be *identified* at birth measuring biomarker levels by tandem-MS. In addition, Tandem-MS assay allows an easy inclusion of ADA SCID markers within the expanded newborn screening panel, worldwide performed. Most recently, we have validated a new tandem-MS test for the inclusion of Purine Nucleoside Phosphorylase deficiency, another SCID suitable for NBS by mass spectrometry.

095 - Additional Diagnoses Coming to Attention via Newborn Screening for Phenylalanine Hydroxylase and Biotinidase Deficiencies

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In Turkey, a country with a population of approximately 80 million, nearly 1.5 million babies are born every year. Currently, phenylalanine hydroxylase (PAH) deficiency and biotinidase deficiency are the 2 inborn errors of metabolism in the newborn screening (NBS) program, which has a coverage rate of 99.8%. In this retrospective study, we evaluated the infants referred to a major metabolic center in Turkey in 2016 due to positive NBS for one of these two disorders, who received additional or unrelated diagnoses as a result of follow-up after NBS. A total of 371 infants were referred in 2016 due to suspicion of PAH or biotinidase deficiency. Among 232 infants with elevated phenylalanine (Phe) in NBS, 98 were found to have normal Phe, 121 had mild hyperphenylalaninemia not requiring treatment, and 13 received treatment for phenylketonuria (PKU). Of the 139 infants referred for suspicion of biotinidase deficiency, 40 had normal biotinidase activity and 99 were started on biotin treatment. 8 infants (2.16%) received additional diagnoses as a result of physical and laboratory examinations with one case each of classical galactosemia, I-cell disease, hypertriglyceridemia, citrin deficiency, acute lymphoblastic leukemia, Down syndrome, severe acute malnutrition, and spinal muscular atrophy (SMA) type I. Six of these infants were given specific treatment for their conditions. Only 1 of the 8 infants was diagnosed with the condition that was suspected in NBS. (Patient with SMA also had PKU.) Only galactosemia and citrin deficiency had been previously associated with transient hyperphenylalaninemia. Infants with false-positive NBS results are commonly disregarded in clinics and discharged without even a physical examination. This study underlines the importance of history taking and physical examination in every newborn a physician encounters. Diagnosis in most, if not all, of these infants would have been delayed or missed had a thorough physical examination not been performed. Metabolic physicians should consider assessing all infants referred from NBS for possible unrelated or comorbid medical conditions regardless of the outcome of metabolic work-up.

096 - Tierra del Fuego, Antarctica, and South Atlantic Islands: Analysis of the Newborn screening program (1996-2016)

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Aim and scope: In Argentina, newborn Screening for diagnosis of phenylketonuria (PKU), congenital hypothyroidism (HC), cystic fibrosis (CFP), galactosemia (GAL), congenital adrenal hyperplasia (HSC), biotinidase deficiency (Def.B), Chagas disease, and syphilis is mandatory by national law in public and private maternities; in agreement with this law, the province of Tierra del Fuego, Antartida, and Islas Del Atlántico Sur (TDF-AIAS) and their public maternities participate in the *Programa Nacional de Fortalecimiento de la Detección Precoz de Enfermedades Congénitas* (PNFDPECM). The purpose of this study was to perform a quantitative assessment of the newborn screening (NBS) over the last 20 years (1996-2016) in the province TDF-AIAS in terms of full coverage, diagnostic, treatment, and development of the NBS. **Materials and methods:** A descriptive study of data provided by the Provincial Direction of Epidemiology, PNFDPECM, and medical histories of NBS detected as positive. We measured coverage rate, cumulative frequency, and treatment assessment. **Preliminary results:** In public establishments of TDF, AIAS happened 51.2% of the total provincial births and the PNFDPECM had an average coverage rate of 98.15% (29 869 newborns studied). 22 positive cases were detected for congenital diseases: 18 cases of HC (with female predominance 2:1), 2 cases of heterozygous deficit of biotinidase, 2 cases of FQP, and 2 cases of false negatives (FN). The incidence of these pathologies was as expected. There are no official data of private maternity hospitals. **Preliminary conclusions:** According to these data, we suggest reinforcement alertness in preterm NBS and monochorionic twins to prevent FN and designed an only province registration to value coverage, diagnostic, treatment of newborn screening in all provincial newborns.

097 - Flow Injection Analysis—A Novel Approach for the Second-Tier Estimation of Methylmalonic Acid on Dried Blood Spot to Improve Newborn Screening for Methylmalonic Aciduria

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Introduction: Newborn screening for the detection of Methylmalonic aciduria (MMA) and propionic acidemia (PA) relies on the detection of elevated propionyl carnitine (C3) in the dried blood spot by tandem mass spectrometry (TMS). As C3 being a nonspecific marker whose elevation could be transient, it cannot be included as a pathognomonic marker. So, we designed this study to increase the specificity for the diagnosis of methylmalonic aciduria and propionic aciduria by using flow injection analysis (FIA) method against the conventionally used column-based second-tier approach. **Materials and Methods:** Methylmalonic acid is extracted from 3.8 mm dried blood spot (DBS) using extraction solution containing acetonitrile–water–formic acid (70:30:0.2%). Vortexed for 15 minutes followed by centrifugation at 4000 rpm for 5 minutes. Supernatant was transferred to fresh plate and injected. An isotopically labeled internal standard (d_3-MMA) was used for the quantification. **Results:** Intra- and interassay imprecision for MMA ranged from 4.8% to 5.6%. In this study, we screened around 75 000 newborn samples of which 22 samples showed elevated C3, that is, (C3 > 8 μmol/L) and only two samples showed true positivity for methylmalonic aciduria on second-tier screening for methylmalonic acid. Furthermore, confirmation by the urinary gas chromatography-mass spectrometry (GC-MS) also substantiated the second-tier finding. **Conclusions:** Addition of flow injection analysis approach for the second-tier estimation of methylmalonic acid on the residual blood spot greatly reduced the number of false positive in the primary newborn screening, thereby increasing the specificity of the assay.

098 - High Prevalence of Congenital Adrenal Hyperplasia in the Southeast Mexico

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Objective: Congenital adrenal hyperplasia (CAH) accounts for a significant mortality and morbidity. Homozygous or compound heterozygous mutations in the human CYP21A2 gene provoke the autosomal recessive metabolic disorder CAH (OMIM #201910). In Mexico, the overall frequency is not known, but it has been estimated close to 1:8873 newborns (NBs), which is higher than other countries like Brazil (1:10 300 NBs). The aim of this study is to analyze the birth prevalence of CAH in Yucatan, south of Mexico, through newborn screening. **Methods:** The expanded newborn screening program of Yucatan has analyzed 146 594 NBs samples, from 2008 to 2017. Blood samples were obtained by heel prick and collected in filter paper (Guthrie cards). 17-hydroxyprogesterone blood concentrations were determined through fluoroimmunoassay, using commercial kits. Cutoff value established was 20 nmol/L. All the suspicious NBs were localized in the community for confirmatory tests, and the confirmed cases were treated at the General Hospital of Yucatan. **Results:** From 104 suspected samples, 21 newborns were diagnosed with CAH, with an overall incidence of 1:6980 NBs. Distribution by sex is 13 females and 8 males; 5 patients deceased from adrenal crisis at different ages, the rest 16 alive patients are stable and under treatment. 90% of patients live in endogamic communities in small Mexican villages that are far away from the main cities. **Conclusion:** The birth prevalence of CAH found in this is study in the southeast Mexico is 1:6980 NBs, slightly higher than others reported locally and worldwide.

099 - Potential Cost-Effectiveness of Including Screening for X-linked Adrenoleukodystrophy in the UK National Health Service Newborn Blood Spot Screening Program

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Objective: To model the cost-effectiveness of including screening for X-linked adrenoleukodystrophy (X-ALD) in the UK NHS Newborn Blood Spot Screening Program. **Methods**: A decision tree model with lifetable estimates of outcomes was built. Model structure and parameterization were informed by expert clinical judgment and systematic literature reviews. A public service perspective was used, and lifetime costs and effects were discounted at 3.5%. Outcomes included health, social care and education costs, and quality-adjusted life-years (QALYs). The model assessed screening of boys only and evaluated the impact of improved outcomes from hematopoietic stem cell transplantation in patients with cerebral childhood ALD (CCALD). Sensitivity analyses examined inclusion of girls in screening and utility decrements for non-CCALD patients identified by screening. **Results**: It is estimated that screening 780 000 newborns annually will identify 10 (95%CI 4-17) children with X-ALD, 5 (3-7) will develop CCALD, 3 (2-5) will develop AMN and around 1.5 (0.5-4) will have Addison's only or be asymptomatic. It is estimated that screening may detect 21 (12-34) children with other peroxisomal

disorders who may also have arisen symptomatically. If girls are screened an additional 9 (4-18) cases of X-ALD will be identified. The program is estimated to cost an additional £405 000 (£400-413 000) with savings in lifetime health, social care, and education costs leading to an overall discounted cost saving of £1.4 (0.2, 3.6) million. Patients with CCALD are estimated to gain 9 discounted QALYs giving an overall program benefit of 45 (17-97) QALYs. Each non-CCALD patient would have to experience a utility decrement of 0.583 to negate the benefits to CCALD patients, although this falls to 0.188 per patient per year if girls are also screened. **Conclusion**: Including screening for X-ALD into an existing tandem mass spectrometry based newborn screening program is projected to reduce overall costs and improve outcomes for those with CCALD. The potential disbenefit to those identified with non-CCALD conditions would need to be substantial in order to outweigh the benefit to those with CCALD. Further evidence is required on the additional number of other peroxisomal disorders identified through screening and potential QALY impact of early diagnosis. The favorable economic results are driven by estimated reductions in the social care and education costs though screening.

100 - A Strategy With an Integrative Approach Reinforces Congenital Defect Surveillance in Paraguay

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Introduction: Congenital defects (CD) are structural or functional abnormalities present at birth caused by genetic mutations, environmental factors, or by the interaction of both. In Paraguay, these are the first and second causes of death in neonates and children under 5 years of age. The Program for the Prevention of Birth Defects, inserted into the National Neonatal Detection Program (NDP), initiated in April 2016 the CD Registry in 15 hospitals in order to reduce the underregistration of CD. This strategy which includes questions to obtain more information about DC were added to the NDP in May 2016. An average of 7000 files per month is received from the 1033 sample collection sites in the country. **Objective:** To present the results of this strategy to strengthen CD surveillance integrated to the NDP. **Material and method:** Review and analysis of the NDP database. **Results:** From May 2016 to April 2017, 85,846 newborn files were received. The presence of structural and functional CD was reported in 0.5% (393/85 846), 0.08% (66/85 846) functional CD (congenital hypothyroidism: 30, phenylketonuria: 20 and cystic fibrosis: 16), and in 83% (71 355/85 846) information was collected on structural DC, of which 63% (207/327) was major DC; this included in order of frequency, foot equinovarus, neural tube defects, cleft palate, Down syndrome, and cardiopathies; 37% (120/327) were minor DC, polydactyly, and preauricular nipple. **Conclusions:** The inclusion of questions on CD into the neonatal screening form is a strategy that strengthens its surveillance at the national level, especially in places with no other form of access to information and would otherwise be missed. With this information, there are timely referrals for better care of the newborn with DC and the beginning of family counseling.

101 - Congenital Adrenal Hyperplasia Neonatal Screening in Southern Brazil: The Challenges Until Confirmatory Diagnosis

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Congenital adrenal hyperplasia (CAH) occurs due to enzymatic defects in adrenal steroidogenesis. The 21-hydroxylase enzyme deficiency accounts for 90% to 95% of the cases, triggering accumulation of 17-hydroxyprogesterone (17-OHP). Early diagnosis through neonatal screening (NS) allows adequate treatment and reduction of mortality. Genotype diagnosis is an important confirmatory tool and helps to establish the severity of the disease in confirmed cases and, also help to differentiate false positives from milder forms of the disease. **Objective:** To describe the results of NS for CAH in a public reference center in the state of Rio Grande do Sul, Southern Brazil. **Methods:** Neonates suspected of altering 17-OHP on filter paper were selected using the cutoff levels recommended by the National Neonatal Screening Program for CAH according to the birth weight categories. The classic CAH forms (salt-wasting and simple virilizing) was diagnosed by the increase of 17-OHP confirmed in the retest, by clinical evaluation and by genotype through PCR, mini-sequencing (SNaPshot) and MLPA assays. **Results and Conclusions:** After 24 months, 15 cases of classic CAH were diagnosed of a total of 217 965 neonates screened, with an estimated incidence of 1:14 531. In addition, 7 nonclassical cases, with borderline values of 17-OHP on filter paper, were confirmed by molecular genotyping. The genotype-phenotype correlation was adequate, according literature similar data. The most frequent mutation was IVS2-13A/C> G, followed by V281 L, deletion,

conversion, or rearrangement. The results emphasize the relevance of public NS for CAH and show that the adopted strategy was adequate. The results were similar to those reported by other Brazilian states. Molecular diagnosis brings new perspective in neonatal screening and was fundamental to differentiate false-positive cases from milder forms of the disease, besides allowing adequate genetic counseling to affected families.

102 - Challenges in Neonatal Diagnosis of Cystic Fibrosis: 4 Years of Experience in Southern Brazil

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Introduction: Cystic fibrosis (CF) is a genetic disease. 2017 mutations have been identified in the CFTR gene. Two polymorphic regions, (TG)m and (T)n, located in intron 8 (IVS8) have been shown to affect the synthesis of functional CFTRs. IRT in neonatal screening is one of the indirect indicators of the disease, followed by the sweat test and molecular studies. **Objective:** To describe 4 years results of NBS for CF in Southern Brazil. **Methods:** A cross-sectional descriptive study of all newborns who presented altered IRT results or clinical symptoms screened at Reference Service from June 2012 to May 2016. The sweat test, expanded molecular examination, and clinical evaluation are the confirmatory exams for CF. The genotyping was the extension of base molecular detection of eleven mutations (R1162X, G85E, R117 H, 2789 + 5G> A, G542X, R334 W, W1282X, R553X, 1717-1G> A, 3120-1G> A and G551D) in the CFTR gene in addition to the F508del mutation fragment analysis. The numbers of replicates (TG)m (T)n were also identified by DNA sequencing. **Results:** 435 738 babies were screened. Of these, 2,103 (0.48%) were referred for confirmatory exams with clinical or laboratory suspicion of CF. Among the suspects, 40 newborns (1:10 893) confirmed CF, of which 2 were negative for screening and diagnosed based on clinical suspicion. Deaths were 60 (5.5%) cases before the end of the investigations. The median age of the RNs of the first and second samples was 6.0 and 14 days, respectively. The median IRT in the first sample was 154 and 81.1 ng / mL, and in the second sample was 148 and 33.8 ng / mL between patients and nonpatients. The median time for sweat testing was 34 days and the median chlorine was 109 mEq/L. Of the 40 children with CF, 21 (52.5%) were homozygous for the F508del mutation, 11 (27.5%) were heterozygous, and 3 (7.5%) did not present the mutation. Among the other mutations studied were G542X, 711 + 1G> T, 3120 + 1G> A, R1162X and N1303 K. The most frequently observed alleles for the repeating region (TG)m were TG10 (79%), followed by TG11 (19%) and TG12 (2%) of 21 patients. For the (T)n region, the most observed alleles were T9 (71%), followed by T7 (27%) and T5 (2%). The most frequent genotype in the population was homozygous for TG10T9 (47.6%). **Conclusion:** The implementation of the neonatal screening test contributes to a diagnosis in asymptomatic phases of the disease, allowing an early and adequate therapeutic approach for the RNs in Southern Brazil.

103 - Newborn Screening for Inborn Errors of Metabolism by Tandem Mass Spectrometry in Costa Rica: 11 Years of Experience

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Introduction: Costa Rica uses Tandem mass spectrometry (MSMS) to perform newborn screening for inborn errors of metabolism (IEM) nationwide since year 2006. The goal of this work is to inform about our findings and experience with MSMS screening in these eleven years of continuous work. **Methods:** A total of 782 982 newborns were screened by MSMS from January 2006 to December 2016. We began screening for 16 different metabolic disorders and by 2014 we expanded to 19. Some other conditions were also identified in parallel. **Results:** We identified 124 cases of IEM for an overall incidence of 1:6314. Maple syrup urine disease, along with phenylalanine metabolism disorders, short chain Acyl-CoA dehydrogenase deficiency, and medium chain Acyl-CoA dehydrogenase deficiency (MCAD) were the most common disorders encountered. 46 patients were diagnosed with aminoacidopathies (37%), 42 with fatty acid oxidation disorders (34%), and 36 with organic acidemias (29%). Retrospective testing of family members and older patients with clinical symptoms suggestive of a metabolic disorder led to additional diagnoses not included in this report. **Conclusions:** The overall incidence of IEM seems a bit lower than those reported by other countries; however, we still have some cases from 2016 with unresolved diagnosis. A qualitative evaluation of the most frequent disorders compares to data described in other reports, where phenylalanine disorders are first among aminoacidopathies, while MCAD and 3-Methylcrotonyl-CoA carboxylase deficiencies lead the list for fatty acid oxidation and organic acid disorders,

respectively. We recommend a detailed documentation of the patient condition at the time of sample extraction and a good education on the procedure for sample withdrawing, to avoid both false-negative and false-positives cases. Once established, the main challenges for an MSMS newborn screening laboratory are to maintain a low false positive rate, to constantly improve the quality of the sample and to periodically evaluate cutoffs.

104 - Evaluation of Quality Indicators of The National Newborn Screening Program in Costa Rica (2014 to 2016)

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Introduction: The National Newborn Screening Program (NNSP) in Costa Rica has been working continuously for 27 years, since 1990. For the last 3 years, the Program screened an average of 71,205 newborns per year. Currently, this program detects 29 diseases, including inborn errors of metabolism, hemoglobinopathies, endocrinological defects, and other genetic diseases such as cystic fibrosis. **Objective:** The aim of this work is to evaluate quality indicators related to sampling, processing, and analysis of samples from the NNSP in Costa Rica, during the period 2014 to 2016. **Methods:** The study comprised statistical analysis of data related to the following quality indicators: Coverage Rate (CR), Age at Sample Collection (ASC), Transit Time Trail (TTT), Unsatisfactory Samples (US), and Sample Processing Time (SPT). Statistical analysis comparing the collected data to reference values were performed using the Minitab17 software. **Results:** Results showed that the NNSP has a coverage rate of 97.3%. The ASC showed that 93.5% of samples were taken between the third and seventh day of birth (ideal value > 95%). In 70.2% of the samples, the TTT was less than or up to 4 days (ideal value > 95%). Regarding the US, the analysis showed an average value of 3.8% (acceptable value $\leq 2\%$). The SPT was calculated to be of 47 hours. Finally, entire analysis from sample collection to result report takes a total of 11 days. **Conclusions:** The results highlight the requirement of improvement. Special attention should be taken in account for unsatisfactory samples that have shown a progressive increase throughout the years. Efforts should be made to maintain or improve the coverage rate and sample processing time for the program. The availability of information systems in public health programs make possible to obtain results of indicators of coverage and quality, allowing decision-making to implement strategies for improvement. For example, these results could be support that some policies should be redefined for looking a greater commitment on the part of health authorities of the country, in order to obtain a positive impact on the neonatal screening.

105 - Korle-Bu and Osu Christiansborg Hemoglobin Variants Detected in Costa Rican National Newborn Screening Program (2015-2016)

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Introduction: The inherited disorders of hemoglobin are the most common monogenic diseases with clinically heterogeneous manifestations, caused by pathogenic variants in α-globin and β-globin genes. These variants structurally modify the α-globin or β-globin-chain (hemoglobinopathies) or decrease its synthesis (thalassemia). Some surveys suggest that around 400 000 babies are born each year with a hemoglobin disorder, and approximately 90% of them are from low- or middle-income countries. The establishment of hemoglobinopathies detection through newborn screening programs has permitted an early diagnosis and timely treatment of hemoglobin disorders. Hemoglobin E is considered the second most common pathogenic hemoglobin variant with a mutation in *HBB* gene causing the substitution of glutamic acid for lysine at amino acid position 27 (NM_000518.4: c.79G>A, p.Glu27Lys, HGVS nomenclature). **Methods:** In Costa Rica, the National Newborn Screening Program began in 1990, and it was from 2005 that the detection of hemoglobinopathies was introduced through isoelectrofocusing electrophoresis. In 2014, the electrophoresis technique was replaced by Osu Christiansborg hemoglobin (HPLC). Due to the clinical importance of hemoglobin E, the aim of this study was to confirm through Sanger sequencing of *HBB* gene its presence in all screened babies in our laboratory with a positive result obtained through HPLC during 2015 and 2016. **Results:** During the course of these 2 years, 138 661 babies were screened and 18 were detected to have the hemoglobin E variant through HPLC, all of them showing a chromatogram consistent with FAE phenotype. Sequence analysis detected 6 babies with the variant p.Asp53Asn (c.157G>A) and 12 babies with the variant p.Asp74Asn (c.220G>A), all of them with a heterozygous state. These nonpathogenic variants cause Osu Christiansborg hemoglobin and Korle-Bu hemoglobin, respectively. **Conclusions:** Thanks to the high coverage of our screening program (approximately 98%), these results suggest that the presence of hemoglobin E variant in Costa Rican population is rare. Furthermore, these findings support the need to confirm by other techniques all hemoglobin E results obtained by HPLC in newborn screening.

106 - Establishing Validity of Reference Range Concentrations of Acylcarnitine Species by Tandem Mass Spectrometry in a Cohort of Heathy Neonates in South India: A Pilot Study

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Background: In this study, we aimed at validating reference ranges for acylcarnitine concentrations measured by tandem mass spectroscopy (TMS) in term normal newborns from our population and also comparing it to Region 4 stork (R4 S) data as a gold standard. **Methods:** The study was conducted at Christian Medical College, Vellore, over a period of 6 months when acyl carnitine profile was performed on dried blood spots of healthy term newborns between day 3 and day 7 of life. These data were used to derive our own reference range and were also compared to the values made available by the R4 S program. **Results:** Concentrations of majority of the acylcarnitine species were comparable with the R4 S collaborative project ranges, however they were not identical. **Conclusion:** Diagnosis of inborn errors of metabolism is a complex process and comparing ranges from different centers bears serious limitations considering that there could be a multitude of factors which could lead to variations in values. Extended study of acyl carnitine level in large samples of healthy newborns will help to establish population norms

107-Molecular Diagnosis for Target Metabolic Diseases of Newborn Screening Using a Gene Panel in Japan

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Backgrounds: Newborn screening (NBS) using tandem mass spectrometry has been performed since 2014 in all over Japan, and the target metabolic diseases (TMDs) increased from 6 to at least 19 diseases. Molecular diagnosis for TMDs is not commercially available in Japan and until recently such molecular analyses were mainly performed by pediatricians with "volunteer spirits." To change the situation, we designed and conducted molecular diagnosis for TMDs using a gene panel. **Methods:** We designed a gene panel which consists of more than 60 genes covering the TMDs and the related diseases. This research was financially supported by Japan Agency for Medical Research and Development. DNA was purified from patients' blood at Gifu University, and the gene panel analysis was performed at Kazusa DNA Research Institute using the MiSeq or NextSeq (Illumina®). Sanger sequencing was performed to confirm the detected mutations. Pediatric coauthors in this study are experts responsible to make mutation reports. **Results:** We analyzed 138 patients who were positively screened during 3 years (January 2014 to March 2017) and 44 patients who were diagnosed before that period. The number of patients with TMDs detected by NBS were as follows: Propionic acidemia (35), Hyperphenylalaninemia (19), Methylmalonic acidemia (17), VLCAD deficiency (15), Maple syrup urine disease (13), Methylcrotonylglycinuria (13), Galactosemia (10), primary systemic carnitine deficiency (9), MCAD deficiency (8), Citrullinemia type 1 (7), Glutaric acidemia type 1 (6), CPT2 deficiency (4), Glutaric acidemia type 2 (4), CPS1 deficiency (4), OTC deficiency (4), Multiple carboxylase deficiency (3), and others (11). In most cases, we could find the gene mutations in their corresponding genes and found some common mutations for some TMDs in a Japanese population. **Discussion:** Clinical course and severity may differ among patients in some TMDs. One major factor to determine clinical phenotype is of course genotype. Hence, it is important to follow-up mutation-defined patients to evaluate efficacy of treatment and management. We will individualize clinical guidelines by genotypes in some TMDs in the near future.

108 - Newborn Screening for Hemoglobinopathies in the Public Health System in Southern Brazil From 2004 to 2016

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Introduction: Hemoglobinopathies are genetic disorders resulting from mutations in the genes responsible for globin synthesis and show significant morbidity worldwide. In 2001, the Brazilian Ministry of Health included testing for

hemoglobinopathies in the National Newborn Screening Program (PNTN). **Objective**: To evaluate the prevalence of hemoglobin patterns in newborns screened in the public health system in the Rio Grande do Sul state. **Materials and Methods:** Blood samples of newborns collected by heel prick on filter paper S&S 903 were collected from January 2004 to December 2016. The methodologies used in screening were High performance liquid chromatography (HPLC) and/or isoelectric focusing (IEF). **Results**: A total of 1 379 298 neonates samples were analyzed. Of these, 21 175 (1.53%) presented an abnormal hemoglobinic pattern: 206 cases of sickle cell syndromes (115 Hb FS, 57 Hb FSA, 32 Hb FSC, 01 Hb FSD, and 01 Hb FS/E-Saskatoon) and other hemoglobin (Hb 112 FAH, 05 Hb FCA, 02 Hb FC, 02 Hb FVA, and 01 Hb FCD). Among heterozygotes, 17.037 Hb FAS, 2.717 Hb FAC, 531 Hb FAD, 1 Hb FAE, and 561 carriers of hemoglobin rare variants were presented. For the study of rare variants, 53 DNA samples were obtained for sequencing and were characterized by 31 alpha chain variants (4 Hb Woodville, 1 Hb Chad, 3 Hb Hasharon, 3 Hb G-Philadelphia, 7 Hb G-Pest and 13 Hb Stanleyville II) and 22 beta chain (13 Hb E-Saskatoon, 1 Hb Osu-Christiansborg, 1 Hb Richmond, 1 Hb O-Arab, 1 Hb J-Guantanamo, 1 Hb Shelby, 1 Hb Beckman, 2 Hb Hope and 1 Hb Porto Alegre). The correct newborn screening for hemoglobin allows the early diagnosis in babies and children with sickle cell disorder and the inclusion of the carriers in prevention and treatment programs in addition to the investigation of other family members. The data obtained in this study generate indicators that enable the network of public health services to improve the process of population care, favorably modifying the results of morbidity and mortality, quality of life, and longevity.

109 - 10 Years of Expanded Newborn Screening Program on Southeastern Spain: Impact on Clinical Outcome of Inborn Errors of Metabolism

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Objective: To address the impact of an expanded newborn screening (NBS) program based on tandem mass spectrometry (MS/MS) biochemical diagnosis after 10 years of its implantation. **Methods:** Retrospective study on neonatal screening for inborn errors of metabolism (IEM) in Murcia Region (southeastern Spain) conducted between 2007 and 2017. The NBS Program for IEM is based on heel prick dried blood spot samples obtained from all newborns at 48-72 hours and analyzed by MS/MS Waters Acquity TQD®. Urine second-tier tests were performed to initial positive results before second blood spot samples are required. Clinical Metabolic Monitoring Unit performs patient assessment and evaluation till biochemical diagnostic is confirmed by molecular studies. An implementation of quality indicators defined by Spanish Health Department was applied since 2014 to guarantee early diagnosis and treatment. Clinical outcome and incidence of EIM were compared to historical data from the 10 years before expanded NBS. **Results:** A total of 187 034 newborns were screened. 110 cases of EIM were identified (5.88/10.000 NB). Hyperphenylalaninemia was the most frequent condition (51%) including 16 cases of classic PKU (14%), followed by organic acidemias (15,4%) and fatty acid β-oxidation defects (11,8%). Methylmalonic acidemia (MMA) incidence (0.75/10.000) was higher than described in similar regions, probably due to high levels of immigration/consanguinity. Two false negatives were detected in the first year of NBS program. In most positives, a second sample collection was avoided by applying algorithms based in acylcarnitines relations and secondary metabolites in urine spot. Most false positives were in relation to C3 elevation in vegetarian B12 vitamin-deficient mothers. Comparing with our own historical series, a large number of children appeared affected by alterations of fatty acid β-oxidation defects. The decrease in mortality and percentage of patients with mental retardation or disability, and the lower degree of the same in the affected patients, were the most striking results comparing both series. Conclusions: Expanded NBS increased early diagnoses and prevalence of EIM, but also the survival and quality of life in EIM as well. Compliance of NBS quality indicators guarantee early treatment of patients diagnosed of IEM before 15 days old, especially those diagnosed by MS/MS and consequently, a best disease prognosis.

110 - What Are the Perspectives of Families Who Have Child With Organic Acidemia or Phenylketonuria to Prenatal Diagnosis in Subsequent Pregnancies?

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Introduction: Inherited metabolic diseases are common in our country because of common consanguineous marriages. Genetic counseling for families who have children with metabolic disease is important for early diagnosis in subsequent pregnancies. In recurrent pregnancies, when the fetus was detected as sick on prenatal diagnosis, in terms of the termination of pregnancy, the point of view differs according to the clinical severity or curability of the metabolic disorder in the previous child. **Material and Method:** To evaluate rates of decisions about whether to continue or terminate the pregnancy according to the results of the chorionic villus sampling prenatal diagnosis in new pregnancies, between January 1996 and

January 2016 records of patients with organic acidemia or phenylketonuria were retrospectively screened. **Results:** Fetal phenylketonuria was detected in 18 (24.7%) of 73 chorionic villus specimens in pregnancies following the presence of phenylketonuria. Of these, 3 (16.7%) have decided to terminate their pregnancy. Organic acidemia was detected in 7 (46.7%) of the 15 chorion villus samples taken during the subsequent pregnancies of the families with organic acidemia, and 5 (71.4%) decided to terminate the pregnancy. Termination of pregnancy for children with organic acidemia was 12.5 times higher than phenylketonuria group. **Discussion:** Our findings have shown that in subsequent pregnancies, families absolutely preferred prenatal screened who have children diagnosed with organic acidemia because of more morbidity and mortality. Even if their babies were phenylketonuria, families were usually preferred to give birth instead of ending the pregnancy because of phenylketonuria is a non-fatal disease. There is no consensus on the termination of pregnancies with prenatal diagnosis of phenylketonuria and the indications for termination of pregnancies are controversial.

111 - Molecular Biology Laboratory as an Important Support in the Final Diagnosis of the Diseases Triaged in Newborn Screening in Southern Brazil

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Newborn screening (NBS) is an important public health program. Correct planning and an institutional structure that includes qualified professionals, as well as a broad articulation and cooperation among institutions, are fundamental to the efficiency of a NBS program. Molecular studies increase diagnostic efficacy and allow epidemiological and population data. **Objective:** To characterize the main molecular alterations associated to diseases triaged by the Reference Service of RS state. **Methodology**: After the screening, the samples of patients suspected were submitted to the extraction of nucleic acids to molecular analyzes, following specific protocols. For cystic fibrosis (CF) 11 pairs of oligonucleotides were constructed and a multiplex PCR was standardized. In order to detect 11 point mutations in the *CFTR* gene (1717-1G> A, 2789 + 5G> A, 3120 + 1G> A, G85E, G542X, G551D, R117 H, R334 W, R553X, R1162X and W1282X), SNaPshot protocol was standardized. In addition, standardization of genotyping of the most prevalent mutation (F508del) was also performed by fragment analysis. For hemoglobinopathies, beta globin (HBB) and alpha globin (*HBA1* and *HBA2*) gene sequencing protocols were established, in addition to the diagnosis of the 3.7 kb deletion in the *Alfa Globina* gene. In the CAH the standardization of the PCR technique for amplification of the *CYP21A2* gene was performed. The SNaPshot technique was used to detect 12-point mutations from gene amplicon: P30 L, IVS2-13A/C>G, I172 N, V281 L, Q318X, R356 W, R408C, H62 L, R408C, P453 S, p.Gly110ValfsTer21 and Leu308PhefsTer6. The regions investigated in the *BTD* gene specifically amplify exons 2 and 4, where the mutations 7d3i, G45 R, R79 H, A171 T, V199 M, D444 H, Q456 H and R538C are concentrated. The oligonucleotides used in techniques were created using Bioinformatics tools. **Discussion**: The challenges of NBS confirmatory diagnosis include a physical laboratory structure and highly skilled professionals in molecular techniques. The partnership between SRTN-HMIPV-PMPA, CDCT/SES and UFRGS has made possible the technological development, the provision of services and research related to the screening of these 4 genetic diseases with impact on the reduction of morbimortality and gain of the quality of life. The molecular studies and the correct characterization of genetic diversity makes it possible to establish protocols specific directed to the RS population and to improve the diagnostic efficacy of the NBS.

112 - Neonatal Screening for Biotinidase Deficiency: Experience of the First Six Years in the State of Santa Catarina

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Objective. Newborn screening in public health system for biotinidase deficiency was included in 2010 in Santa Catarina, as the first State in Brazil. We evaluated the coverage, prevalence, and cutoff neonatal screening for biotinidase deficiency in the period from 2010 to 2015. **Methods** A longitudinal study was carried out from 2010 to 2015. Data were obtained from the Lacen Laboratory, health posts, maternities, and at the Infantile Hospital Joana de Gusmão. Biotinidase was determined by fluorometric assay on dried blood spots and plasma. The cutoff point was defined as biotinidase values greater than 70U. If the first sample had a high result, a second sample was requested and if it remained high, it was considered a potential case. The cases were evaluated clinically and biochemically with the measurement of plasma biotinidase. **Results** During the 6 years, 542 148 infants performed newborn screening for biotinidase deficiency. The calculated program coverage already exceeds 90%. A total

of 188 children had altered results and of these, 68 kept their results changed until the first year. In 40 patients, the disease was confirmed by plasma biotinidase activity. 38 patients were classified as partial deficiency of biotinidase and 2 as total. The prevalence in the State of Santa Catarina was 1: 13,553 cases / year. **Conclusion** The high prevalence can be explained in part by the high cutoff value of the newborn screening. This was corroborated by the introduction of plasma activity where the number of confirmed cases was subsequently reduced. The pioneering implantation in Santa Catarina will allow conducting discussions such as the reevaluation of the cutoff value and the need of plasma biotinidase activity in the public health system. We believe that the maintenance of an active surveillance system and the implementation of the Newborn Tracking for Biotinidase Deficiency in other States should contribute to increase the efficiency of this Program and the knowledge of the prevalence of biotinidase deficiency in Brazil.

113-Newborn Screening for Galactosemia: Experience of the First Six Years in the State of Santa Catarina

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Objective: Newborn screening in the public health system for galactosemia was included in 2010 in Santa Catarina, as the first State in Brazil. We aimed to evaluate the coverage, incidence, and cutoff newborn screening for galactosemia in the period from 2010 to 2015. **Methods:** A longitudinal study was carried out from 2010 to 2015. Data were obtained from the Lacen Laboratory, health posts, maternities, and at the Infantile Hospital Joana de Gusmão. Total galactose was measured by oxidase fluorescent method in dried blood spots. The cutoff value was defined as greater than 8 mg/dL. If the first sample had a high result, a second sample was requested and, if it remained high, it was considered a potential case. All cases were evaluated clinically and diagnosed by erythrocyte galactose-1-phosphate uridylyltransferase enzyme activity (GAL1PUT). **Results:** During the 6 years, 542 148 newborns were screened for galactosemia. The calculated program coverage already exceeds 90%. Nine children were classified as probable cases with an average total galactose of 64.3 mg/dL. The mean total galactose dosage in classical galactosemia in the first dried blood spots was 125.7 mg/dL and 15.36 mg/dL in variants. The values GAL1PUT had a mean 1.16 μmol/h/gHb in classical and 13.1 μmol/h/gHb variant (normal value range 37-66). Of the nine cases, 4 were classified as classical galactosemia. Only 2 cases had molecular analysis, patient 1 S135L/G175D (c.404C>T/ c.524G>A) and patient 2 S135L/F171 S (c.404C>T/c.512T>C). The cumulative incidence was 1:50,251 live births. **Conclusion:** We suggest that the 8 mg/dL cutoff of the galactose dosage should be increased to 10 or 12 mg/dL to reduce the number of unnecessary samples recollected. This is the first report of the incidence of galactosemia followed regularly in Brazil with 1:50 251 live births. The pioneering implementation in Santa Catarina allowed to conduct discussions if this program of newborn screening for galactosemia meets the criteria of public health conditions in Brazil. Galactosemia is rare disease but potentially fatal if not treated early.

114 - Prevalence of Congenital Hypothyroidism During the Years 2007 to 2016, in Arauca, Colombia

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Introduction: A public health policy in Colombia is to perform newborn screening for the early diagnosis of congenital hypothyroidism (HC), avoiding consequences such as mental retardation and cretinism. The objective of the study is to compare the prevalence of HC during the years 2007 to 2011 and 2012 to 2016, in the Department of Arauca, Colombia. **Materials and Methods:** Samples of umbilical cord blood, collected on filter paper, were processed during the years 2007 to 2011 a total of 12 389 samples, and during the years 2012 to 2016, a total of 11 206 samples. Neonatal TSH hormone was quantified using the UMELISA TSH Neonatal Ultramicroelisa technique, with a cutoff value of 15 mIU/L. The samples were collected in the Hospitals of the municipalities of the department of Arauca. Cases that were elevated were immediately reported to local health institutions for the location, confirmation and timely handling of the hypothyroid. The quality of the Laboratory is ensured by an SAC (Quality Assurance System) software, provided by the Immunoassay Center and External Performance Evaluation by the National Health Institute. **Results:** During the years 2007 to 2011, a total of 12 389 newborns were screened, 51 were high, 4 newborns were confirmed as hypothyroids, with a prevalence of 0.032%. During the years 2012 to 2016, a total of 11 206 newborns were shifted, 45 were high, confirming as hypothyroid 0 (newborn), with a prevalence of 0%. The average calling later time was 5 days, and the average age of early diagnosis was at 15 days old. In the evaluation of the external control, the results were satisfactory, reflecting a good analytical performance in the laboratory. **Conclusions:** Comparing the prevalence of HC during the years 2007 to 2011, that was 0.032%, it decreased to 0% in the period 2012 to 2016. In the last 5 years, promotion and prevention campaigns for HC have been strengthened for pregnant women in the Department of Arauca. The diagnosis of confirmed cases for HC was performed on children before the age of 1 month, and all of them are treated timely and followed avoiding mental retardation.

115 - Prevalence of Metabolopathies and Hemoglobinopathies, Identified in the Health Services of the Mexican Armed Forces From May 2012 to July 2016

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Objective: Define the prevalence of metabolic pathologies and hemoglobinopathies of the expanded newborn screening program (ENS), which is performed on newborns (NBs) in the Mexican Armed Forces (MAF) Health Services from May 2012 to July 2016. **Methods:** Descriptive statistics indicated in percentages, averages and standard deviation were used for the analysis of results. The variables were analyzed: age, time of sampling, proportion of samples performed between 2th and 5th day of life, and the ENS report. The combined incidence of metabolic birth defects was calculated as the rate as follows: (number of cases identified positive / total number of infants screened) $\times$ 10 000. All the NBs that were screened in the health facilities that are beneficiaries of social security of the MAF were included in the study, from May 29, 2012, to July 18, 2016, all children under 30 days of age, of any sex and gestational age. The samples were analyzed by MS/MS, immunofluorometric assay (AutoDELFIA/GSP), isoelectric focusing and HPLC techniques in the laboratory of "Tamiz Mas de Químicos Maldonado." The ENS of the MAF Health Services covers five groups of metabolic diseases, all endorsed by the American Academy of Pediatrics, the American College of Medical Genetics and recommended in Official Mexican Standard NOM-034-SSA2-2013 for the prevention and control of birth defects: endocrinopathies, aminoacidopathies, fatty acid oxidation defects, carbohydrate metabolism disorders, and hemoglobinopathies. **Results:** 35 539 samples were analyzed, which were taken in 89 medical facilities of the MAF, which are distributed in the 32 states of Mexico. Samples were performed on average at 5 ($\pm$ 3) days of NB life, 66% were taken at optimal time. The average age of mothers at the time of pregnancy was 26.2 years ($\pm$ 6.3), with a BMI of 24.8 kg/m^2 ($\pm$ 4.4), the average birth weight was 3.1. ($\pm$ 0.48) kg. Pregnancies, 53% were vaginal delivery and 47% by cesarean section. A total of 88 positive cases: 32 congenital hypothyroidism, 9 congenital adrenal hyperplasia, 3 organic acidemias, 2 Phenylketonuria, 4 cystic fibrosis, 4 hemoglobinopathies, and 34 deficiency of G6PD. The combined prevalence of metabolic birth defects was 24.7 $\times$ 10 000. **Conclusions:** In the MAF, the prevalence of metabolic birth defects was 1/404 newborns. This study is the first of its kind in Mexico and the first with a representative sample of all geographic areas.

116 - 14 Years of Newborn Screening Program for Congenital Hypothyroidism in the Instituto Nacional Materno Perinatal, Lima-Peru

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Introduction: The newborn screening program for congenital hypothyroidism had started in the Instituto Nacional Materno Perinatal in 2003 and is mandatory in Peru by Law 29885. **Objective:** To determine the incidence of congenital hypothyroidism. **Methodology:** Period 2003-2016. 206 558 dried blood spots samples were collected from newborns until February 2016; they were analyzed by ELISA and subsequently by time-resolved fluorometry. Cutoff value: TSH $\geq$ 10 uIU/mL. Diagnosis was confirmed by TSH, T3, T4 level in serum. **Results:** 206,558 newborns. 90 cases diagnosed. Incidence 4.4/10,000 live births. TSH average: 79.45 uIU/mL (10.00 to 793.52). Gestational age: 26.9 weeks (16 to 39). Male: 29.21% Female: 70.79%. Birth weight: 3424 gm. (1430 to 4500) Birth length: 49.8 cm (40 to 51.5). Coverage by percentage vs. cases: 2003: 1478 (31%), 1; 2004: 13 596 (67.50%), 4; 2005: 1 629 391 (48%), 9; 2006: 15 416 (93.60%), 7; 2007: 16 326 (95.95%), 6; 2008: 17 684 (89.93%), 8; 2009: 18 033 (98.16%),11; 2010: 16 149 (95.72%), 9; 2011: 14 224 (93.60%), 4; 2012: 14 797 (92.08%), 7; 2013: 14 555 (85.17%), 8; 2014: 13 766 (74.43%), 7; 2015: 17 173 (79.09%), 1; and 2016: 17 068 (77.37%), 7. **Conclusion:** Congenital Hypothyroidism Incidence: 4.4/10 000 live births (90 cases) and coverage 76.29%. Thyroid ultrasound and cardiology 100%. Congenital heart disease 7%, physical growth is for 94% of cases within 10 to 90 percentile. Neurological monitoring 85%, otoacoustic emissions a 65%, tiroidocintigrafia were performed in 47%, psychological 58%, and genetic 30%.

117- Evaluation of Neonatal CF Research Strategies in the Province of Santa Fe. 21 Years of Experience

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Introduction: Cystic fibrosis (CF) is a genetic disease, with autosomal recessive, multisystemic and lethal inheritance, more frequent in Caucasians. It is caused by mutations in the

CFTR gene. Today 2157 mutations are known, but not all are disease causing. Santa Fe incorporated CF in its panel of neonatal research (PN) in 1995, Provincial Law N° 11.319. **Objectives:** a) To describe the changes in the different analytical methodologies and applied diagnostic algorithms that optimized the process and quality indicators. B) Report incidence of the disease. C) Coverage ratio. **Materials and Methods:** From 1995 to 1998 the detection of trypsin immunoreactive in neonatal research (PN) was performed by DELFIA method, time delay immunofluorescence, cutoff point (pc) 70 ng/mL, TIR strategy / Sweat test, Iontophoresis method (Gibson and Cook) / clinical diagnosis in the CF Unit. The methodology was changed to immunoassay using an ELISA technique developed by MP Biomedicals. The TIR (pc: 150 ng / ml /) / TIR diagnostic algorithm 20-25 days of life (pc: 130 ng / ml) / TS / Medical diagnosis / molecular biology (BM) according to medical request, derived outside the province. In 2012, the BM algorithm was incorporated into the CEMAR of Rosario, and finally in 2013, three modifications were made to the 3 M mutation, DF508, G542X and N1303 K. (a) Any TIR greater than 200 ng / mL is forwarded to the CF Unit for simultaneous measurement of TS and BM; (b) the pc of the second card is decreased to 100 ng / mL; and (c) the panel of mutations is extended to 32. Training was conducted to improve sampling. **Results:** The recall rate of 0.95% decreased to 0.26%, the rejected sample rate from 0.40% to 0.32%, the genetic index to cover 66.45% of the mutated alleles at 75%. Of the most frequent alleles (63% 2 alleles, 37% 1 allele) and the presumptive biochemical diagnostic time from 60 $\pm$ 15 days to 30 $\pm$ 15 days. The coverage rate from 58% in 2005 to more than 99% in 2016 and the incidence 1 in 7006 children, over 245 193 patients surveyed. **Conclusion:** Systematic changes in analytical methodologies and the evolution of diagnostic algorithms improved the indicators and allowed to know the alleles involved in majority of cases, thus collaborating with the diagnosis in the pre-symptomatic stage.

118 - Evaluation of Homogeneity of Control Materials for Phenylalanine and TSH in Dried Blood Spots

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Introduction: Homogeneity is one of the main requirements that control materials (CM) provided by External Quality Assurance Schemes (EQAS) for Newborn Screening (NBS) must fulfill. Homogeneity must be guaranteed by the EQAS in order to avoid a contribution of the CM variability to the measurements variability, thus allowing an objective results interpretation. **Objective**: The aim of this work is to present the results of the evaluation conducted to demonstrate the homogeneity of CM for Phenylalanine (Phe) and TSH prepared by the EQAS for NBS (PEEC-PN) of the Argentine Biochemical Foundation, based on a protocol described by the Newborn Screening Quality Assurance Program—Centers for Disease Control and Prevention. **Materials and Methods**: CM were prepared using human whole blood; HBV, HCV, and HIV nonreactive; hematocrit adjusted to 50%; and impregnated on filter paper Ahlstrom Grade 226 (70 µL/spot). Enrichments were made adding a concentrated solution of Phe [L-Phe Sigma P-2126] and serum from adult patients with high levels of TSH. The selected CM lots for the evaluation were # 197 for TSH and # 199 for Phe. For the study, nine cards corresponding to each one of both lots were selected along the lot of production, beginning with the card # 1 and ending with the last card of the lot (# 210). All the selected cards were analyzed in duplicate in the same run and run 5 times. Measurements were made using AutoDELFIA Neonatal hTSH for TSH and an in house Fluorometric method for Phe. Outliers were excluded considering the 99% QC limits. The averages of the 5 runs for each card were plotted against the card number, the shift across the lot (SAL) was calculated multiplying the slope of the best fit line by the number of cards, the residual deviation (RD) was established taking the square root of the average of the variation of each card, and a comparison between RD's regarding the absolute values of SAL's was made in order to define if the lots were homogeneous ([SAL] < RD) or not ([SAL] > RD). **Results and Conclusions:** The results obtained were as follows: (1) Phe: mean = 5.7 mg/dl, RD = 0.457, [SAL] = 0.072; (2) TSH: mean = 34.0 µU/mL, RD = 3.545, [SAL] = 1.172. Given that both SALs were smaller than the corresponding RDs, both lots were considered homogeneous allowing establishing that the Phe and TSH variation observed in the PEEC-PN is a true reflection of the analytical measurements variation.

119 - Neonatal Thyroid Stimulating Hormone: Introducing a Critical Cutoff to Improve Laboratory Services

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Background: In most of the Asian countries, neonates are discharged within 24 to 48 hours of birth, while screening for congenital hypothyroidism (CH) is recommended after 48 hours. So, to add value to these services, we included neonatal TSH in our critical informing list. This audit was performed to determine a critical cut-off value of TSH results. **Methods:** A retrospective cohort analysis of TSH results was conducted at the section of Chemical Pathology, Department of Pathology and Laboratory Medicine over 2 years period, from January 2015 to December 2016. Serum samples for TSH are analyzed at an automated analyzer ADVIA Centaur (Siemens Diagnostics, US) using chemiluminescence immunoassay technique. Initially, TSH cutoff was defined after literature search and consensus of pediatric endocrinologist and results higher than the defined cutoff was taken as abnormal. Attending physicians

or parent/guardians (in case of outside referrals) were informed if their TSH results were abnormal. In January 2017, these patients were then contacted to take clinical history regarding CH on a predefined questionnaire. **Results:** After literature search >20 μIU/mL were taken as critical limits for TSH in consensus with pediatric endocrinologist. Total 27 407 tests were performed over 2 year's period, 41% (n = 180) had a value of >20 μIU/mL. Repeat TSH was performed in 26.6% (n = 48) and 8% (n = 9) of these had TSH levels >20 μIU/mL. While free thyroxine (FT4) was tested in 18.3% (n = 33); and it was low in only 9% (n = 6) of these neonates. Based on repeat TSH and FT4 testing CH was diagnosed in 12 subjects only and lowest initial TSH noted in these subjects was 21.3μIU/mL. Clinical history of CH was available for 73.91% (n = 327), symptoms of CH at the time of birth were present only in 1.4% (n = 6) neonates, 6.4% (n = 21) of these were on treatment for CH and lowest TSH levels noted in this cohort was 20.8 μIU/mL. **Conclusion:** Based on these findings, we recommend a cutoff value of >20 μIU/mL. TSH levels should be used as a critical for CH screening and results higher than this should be timely informed to physicians to prevent delays in management.

120 - 17 Years of Experience of Newborn Screening Program: Mendoza, Argentina

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Introduction: Early detection and diagnosis of presymptomatic congenital disorders, treatment and follow up of the affected children with systematic and continuous evaluation are the pillars of a Newborn Screening (NS) Program. We aim to present the results and quality indicators of our NS Program. **Methods:** 1999-2009: neonatal samples were processed to determine TSH and phenylalanine (Phe) using Perkin Elmer (PE) reagents. Since 2010, two stages were followed:·(1) Samples were processed to determine TSH, Phe, 17-OHProgesterone, total galactose, and Biotinidase activity using SUMA reagents.· (2) Samples with "uncertain or positive" results on the previous stage were processed using the PE reagents. Immunoreactive trypsinogen (IRT) to screen for Cystic Fibrosis was determined only by PE reagent. NS data and indicators were analyzed using specially designed software. **Results:** 1999-2009: 200 004 newborns. 2010-2016: 154 048 newborns. Indicators, mean: -Child's age (CA) when collecting the dried blood spot samples: 3 days. -Transit time trail: 4 days. -CA at screening result report: 9 days. -CA at results delivery: 12 days. -Diagnostic confirmation and treatment initiation: 15 days. -Samples with incomplete data: 0,5%. -Rejected samples: 0,1%. -Recall rate: 2,02%. -Recalled newborns: 99% were located. -Coverage: 99% from public hospitals. -NS Program, 17 years: 177 children with congenital hypothyroidism (incidence (I) = 1/2000), 5 phenylketonuria-15 persistent hyperphenylalaninemia (I = 1/17 703), 15 congenital adrenal hyperplasia (I = 1/10 270), and 21 cystic fibrosis (I = 1/7336). **Conclusion:** Prevention of disability and other consequences of diseases is possible with strong interdisciplinary work and optimizing NS Program indicators.

121 - Evaluation of IRT/IRT Strategy for Cystic Fibrosis Early Detection in a Hospital of High Complexity, Mendoza Argentina

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Introduction: Different strategies are used in cystic fibrosis (CF) neonatal screening (NS); one of them is the IRT/IRT. It is very important that the stages of NS, diagnostic confirmation, treatment, and follow-up of patients can be carried out in the same hospital. **Objectives:** To evaluate indicators and diagnostic performance of the CF detection system. Methods: IRT/IRT strategy, DELFIA-Perkin Elmer® method. Cutoff values: ·IRT ≥ 70,0 ng/mL, newborns (NBs) until 7 days of life ·IRT ≥ 60,0 ng/mL, NBs 8 to 30 days. Positive results were reported to: -CE.P.E.I.I.'s Social Work Area for recitation -CF Center for clinical evaluation and subsequent referral to the Hospital's Biochemistry Department for diagnosis by sweat test and/or molecular studies. General indicators and performance of the test were evaluated, MedCalc®v16.8. **Results:** 2010-2016: 155 662 evaluated NBs CF: 21 children; incidence: 1:7412; first and second IRT, values mean/median: 160/129 ng/mL and 157/140 ng/mL; first and second IRT, child's age, mean/median: 4/2 days and 18/16 days ·621 NBs presented first IRT ≥ 70.0 ng/mL -recall rate: 0,40%. 33 deceased NBs and 51 non-localized NBs were excluded from the analysis. They worked with the data corresponding to 537 NBs, IRT ≥ 70.0 ng/mL: ·1st IRT: -values, mean/median: 111/97 ng/mL -child's age, mean/median: 5/3 days -sensitivity (S) = 100% -specificity (E) = 99.67% -positive predictive value (PV(+)) = 3.91% -Cutoff > 70 ng/mL, analysis by ROC curve. Of 537 NBs with first positive IRT, only 99 NBs presented second IRT ≥ 60.0 ng/mL: false positive, 78 NBs and CF, 21 NBs. ·Second IRT: -values, mean/median: 124/103 -child's age, mean/median: 19 days ·IRT/IRT strategy: -S = 100% -E = 99.95% -PV(+) = 21.21%. **Conclusion:** From the cost-effectiveness perspective, taking into account the presented indicators and system performance, is considered to IRT/IRT is a valid strategy for CF NS. Interdisciplinary work within the Hospital Dr. H. Notti, with direct participation of

the Department of Biochemistry and the Provincial Programs of NS and CF, allowed to make the diagnosis, treatment, and follow-up of patients detected.

122 - High Incidence of Congenital Hypothyroidism in the State of Yucatan, Mexico

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Objective: Congenital hypothyroidism (CH) is a common pediatric endocrine disorder that can cause mental retardation. Early diagnosis and treatment must be done in order to prevent irreparable brain damage and growth retardation. The aim of this work is to present the birth prevalence of CH in the State of Yucatan, Mexico. **Methods:** From December 2008 to March 2017, a total of 146 594 newborns (NBs) attended at the medical units of the Ministry of Health of Yucatan, Mexico were screened. Five drops of blood were taken through heel prick and collected in standard Guthrie cards; thyroid stimulant hormone (TSH) was quantified by time-resolved fluorometry. All samples with TSH above 7 µUI/L were considered as positive, requiring immediate localization and confirmatory tests; an expert pediatric endocrinologist group made final diagnosis at the public pediatric hospital. CH birth prevalence rate was calculated as the number of confirmed cases per 100 000 screened NBs. **Results:** From 146 594 screened NBs, 247 samples were suspected (1:577 NBs). All suspected NBs were localized, and 121 cases showed normal serum thyroid profiles and were classified as false positives (0.082%); the remaining 126 were classified as confirmed CH cases (8.6 per 10 000 NBs = 1:1163 NBS), with a female sex predominance of 2.3:1 (87 female/38 male). The average age of onset of treatment was 16 days. **Conclusions:** CH birth prevalence in Yucatan, Mexico (8.6 per 10 000 NBs) is higher than that previously reported. Although the increase may be due to the fact that the screening strategy has substantially improved the detection of the disease, the high incidence found in our study must be explained. The CH frequency found by us is similar to the recently reported by other authors for Hispanic population (7.12 per 10 000 NBs) (1). Genetic, immunological, environmental, and micronutrients factors have not been investigated thoroughly. Our study does not exclude transitory CH cases, something that surely positively influenced the high incidence observed. CH in the Southeast of Mexico is a frequent birth defect, thus early detection and further research into underlying causes must be a priority. (1) Feuchtbaum L, Carter J, Dowray S, Currier RJ, Lorey F. Birth prevalence of disorders detectable through newborn screening by race/ethnicity. *Genet Med*. 2012;14(11):937-945.

123 - National Newborn Screening Program for a Better Indonesia

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Screening program for newborn screening in Indonesia started in 2000 as a pilot project exclusively for Congenital Hypothyroidism. The program was partially funded by IAEA, but the lack of support and interest on the program made it short lived and was not adopted as a national program. Since 2012, however, with a growing interest from medical practitioners for a better diagnosis and treatment of metabolic disorders on newborns, the Ministry of Health started a National program for Congenital Hypothyroidism in Indonesia. **Objective of this study:** To estimate the prevalence of Congenital Hypothyroidism and perform early treatment on newborns in Indonesia. **Materials and Methods:** Since 2012, training and workshop were performed in many provinces all over Indonesia to support the dried blood spot (DBS) collection. Afterward, a total of 67 484 samples were collected using DBS and collected from provinces all over Indonesia to make sure that it can better represent the diverse ethnicity of Indonesian population. Then it was send to the Cipto Mangunkusumo Hospital as the National Referral Laboratory. From there, we started to also grow small pilot projects for screening of PKU, MSUD, CAH, and G6PD. All tests but MSUD were performed using Fluorometric method, and only MSUD test were performed using Elisa method. **Results:** From 67 484 samples screened for Congenital Hypothyroidism, 92 babies have a high TSH level of which 43 were recalled and retested. From the other 49 samples, some were retested at local provinces but the rest were lost because the local health care workers were unable to reach out to the remaining suspected patients. From these, 11 samples were reported to have a positive Congenital Hypothyroidism. **Conclusion:** Since Indonesia is an archipelago country comprises of thousands of islands, it creates a difficult problem for health-care workers to collect all newborn samples and to follow up on the results. Moreover, child labors are not always performed by medical doctors as rural areas have limited doctors. Thus, labors are commonly performed by local traditional healthcare workers with no sufficient knowledge regarding metabolic disorder. To better remedy this, the Ministry of Health is creating better guidelines (including videos on how to collect

samples), more educational workshops for both health-care workers and expectant mothers, and local training to improve local health-care providers.

124 - Newborn Screening for Fabry Disease in Tuscany and Umbria: Importance of Family Studies

Elisabetta Pasquini, Giancarlo La Marca, Amelia Morrone, Marta Daniotti, Giulia Forni, Lorenzo Ferri, Duccio Malesci, Sabrina Malvagia, Silvia Funghini, Emanuela Scolamiero, and Maria Alice Donati

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Introduction: New treatments and improved strategies for screening test on dried blood spot (DBS) have led to the development of several pilot newborn screening program (NBS) for some lysosomal storage disorders (LSDs). In November 2014, a prospective pilot project for Pompe (PD), Fabry (FD), and Mucopolysaccharidosis type I (MPS I) has been introduced into the routine NBS of Tuscany and Umbria. FD is X-linked disease, caused by alpha-galactosidase A (α-Gal A) deficiency due to mutations in the galactosidase alpha (*GLA*) gene. The spectrum of clinical presentations is wide and includes the severe classic male phenotype, later onset variant phenotypes, and variable clinical presentations in female patients ranging from asymptomatic to severe classic phenotype. Disease progression may result in renal, cardiac, and neurological disease with severe morbidity and reduced life expectancy. Enzyme replacement therapy and chaperone therapy are available. Early detection allows higher treatment efficacy preventing development of irreversible organ damages. **Objective**: To estimate the prevalence of FD among newborn population, to allow early diagnosis avoiding patients' diagnostic odyssey, and to extend the study to the at-risk family members providing a better genotype–phenotype correlation. **Methods**: α-Gal A enzyme assay was carried out on the same DBS used for expanded NBS. Newborns with low α-Gal A enzyme activity (Triplex reaction/Single reaction) (≤2.38 μmol/L/h) were retested and analyzed by *GLA* gene sequencing. **Results:** 52 592 newborns were screened. 19 resulted positive to the α-Gal A enzyme assay, 14/19 were confirmed by α-Gal A enzyme assay on leukocytes and by GLA gene sequencing (PPV% 72.2). **Genotyping:** We identified 2 known mutations related to classic FD and 3 known mutations reported in late onset FD. 2 further mutations were reported but of unknown significance and 1 variant was novel. Pedigree analysis: all mothers were heterozygous; in two families, the grandfathers died prematurely without diagnosis presenting cardiomyopathy and/or renal failure, another grandfather presented late onset renal involvement. **Conclusions:** The prevalence of FD in Tuscany/Umbria is 1:3757. All patients identified by NBS are in long-term follow-up. Pedigree construction is a useful tool to help physicians for diagnosing FD patients between at risk family members, particularly heterozygous females, to provide accurate genetic counselling and early treatment.

125 - Newborn Screening for Methylmalonic Acidemias in Emilia-Romagna (Italy): A 4-Year Study

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Background: In 2013, we introduced the second-tier test on dried blood spot for free methylmalonic/propionic (MMA/3OHP) acids in patients with high blood propionyl-carnitine (C3) in our Newborn Screening (NBS) program. It was performed immediately for cases with C3 levels >6.58 mmol/L and once a week for cases with C3 3-6.58 mmol/L. **Aim:** The aim of the study is to evaluate the incidence of isolated methylmalonic acidemia (MMA acidemia) detected by NBS in the Emilia Romagna region in the last 4 years. Patients and methods: Between 2013 and 2016, we screened 153 948 newborns, of which 121 cases showed elevated C3 and a positive second-tier test for MMA. Each patient underwent confirmation studies including a second DBS, plasma vitamin B12, homocysteine, acylcarnitine, urinary organic acids, and molecular analysis in selected cases. Patients were divided into 2 groups according to their C3 levels: >6.58 mmol/L for Group 1 and 3-6.58 mmol/L for group 2. **Results:** In group 1 (13 cases), 6 cases (46%) showed MMA acidemia: 1 patient (NBS: C3 7.02) was homozygous for the known p.Arg403* *MUT* gene mutation, one patient (NBS C3 6.76) was compound heterozygous for the known p.Arg91Lysfs*14 and p.Arg161Gln *MMACHC* mutations, 4 cases (30%) had MMA acidemia due to maternal vitamin B12 deficiency. In group 2 (108 cases), 27 cases (25%) showed MMA acidemia: one patient (NBS C3 3.08) was compound heterozygous for the novel p.Gly215Ala and the known p.Leu736Phe *MUT* variants, another case (NBS C 3: 4.88) with high levels of urinary MMA (759 mmol/mol creat) and inconstant small excretion of malonic acid (10 mmol/mol creat) resulted homozygous for the known p.Glu490Asp *ACSF3* mutation by next generation sequencing of 12 MMA-related genes; 25 cases (23%) were affected by MMA acidemia due to maternal vitamin B12 deficiency. **Conclusion:** In our region the incidence of isolated MMA acidemia is 1:38.500; the second-tier test was useful for the detection of 2 cases with isolated MMA acidemia among patients with only slightly elevated C3. The high percentage of MMA acidemias due to maternal vitamin B12

deficiency suggests the importance of measuring the vitamin B12 levels in mothers during pregnancy.

126 - Total Parenteral Nutrition Effect on Maple Syrup Urine Disease Newborn Screening Recall Rate

María Laura Sueiro, Federico José Torres, Mercedes Hunt, Claudio Ariel Castillo, Adriana Oneto, Hernán Eiroa, Ana Chiesa, Gladys Guarrera, Gustavo Maccallini, and Claudio Aranda

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Introduction: Maple syrup urine disease (MSUD) is a congenital metabolic disorder affecting branched-chain amino acids leucine (Leu), isoleucine, and valine (Val) metabolism. Introduction of tandem mass spectrometry into newborn screening (NBS) programs allowed testing for these diseases with relative ease by determining levels of Leu and Val on dried blood spot (DBS). Several studies suggest that amino acid-rich solutions could lead to an increase of false positive rates while screening newborns under total parenteral nutrition (TPN). **Objective:** Assess the effect of TPN on MSUD newborn screening recall rate in our population. **Materials and Methods:** A total of 48 410 MSUD newborn screening results, obtained between April 2015 and April 2017, were retrospectively analyzed, of which 207 were false-positive results. Of these, as recorded on their sample card, 111 were under TPN, 131 were preterm infants (< 37 weeks of gestation) and 138 had low birth weight (LBW) (< 2500 g). 203 of recalled patients were hospitalized and only 4 on their homes. Results with a Leu and/or Val value above their cutoff in a first analysis and with both tests under their cutoff values in a subsequent analysis (new sample) were considered as false positives results. Statistical analysis was performed using the IBM SPSS Statistics 23 software. Chi-Squared test was used for hypothesis testing. **Results**: The proportion of recited patients under TPN (53.6%) showed no difference with those not under TPN (46.4%) ($P = .297$), while we observed a higher proportion of preterm (63.3%) versus term infants (36.7%) ($P < .001$) and of LBW patients (66.7%) versus normal birth weight patients (33.3%) ($P < .001$). Among newborns with TPN reported we observed a higher proportion of preterm (73.9%) over term infants (26.1%) ($p < 0.001$) and of low birth weight (80.2%) over normal birth weight newborns (19.8%) ($p < 0.001$). Among those with no reported TPN, proportion of preterm (51%) and term (49%) infants were comparable ($P = .838$), as was the proportion of normal birth weight (49%) and LBW (51%) infants ($P = .838$). **Discussion**: The fact that the majority of patients are hospitalized facilitates the taking of a second sample. According to our study, the effect of total parenteral nutrition seems significative only for the group of preterm and low birth weight infants. Suspension of parenteral feeding prior to sample recollection could reduce the false-positive rate in these particular populations.

127 - Spectrum of Metabolic Disorders via Expanded Newborn Screening by MSMS at a Tertiary Healthcare Centre of North India

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Background: The utilization of MSMS in expanded screening of metabolic disorders helps in amending innate course of diseases by reducing morbidity & mortality. Aim of this study was to decipher the spectrum of metabolic disorders screening in Delhi State. **Method:** Heal prick blood spot of 200 000 newborns infants were taken after 24 h of birth, on 903 S card. Analysis of 11 amino acids and 29 acylcarnitine by LCMSMS (3200MD QTrap), percentile evaluation by R-4 Stork. **Results:** 52 in 200 000 identified positive in expanded screening, with second-tier confirmation, revealed 3 cases of tyrosinemia-II, 1-MSUD. 3-propionic academia, 3 citrullinemia, 2 of Carnitine palmitoyltransferasetype-1,7 and 6 cases of Cbl c, d & Cbl a, b deficiency, 5 of glutaric aciduria,6 cases of FILA, 10 of mitochondriopathy & 1 each case of β-Ketothial def., Isovaleric academia, Arginemia, IBG, Alkaptonuria & VLCAD, respectively. **Conclusion:** Study shows incidence 1 in every 3846 live births, higher than incidence rate in other populations, due to high degree of consanguinity, epigenetic factor, and interbreeding. With 19.92 births/1000 population/year, high incidence results onto a very large population. Expanded screening not only represents importance of valuable preventive medicine in early diagnosis & initiate treatment at an early stage of life but also lays down ground supports to develop uniform core screening program for every newborn across the India.

128 - 6-Pyruvoyl-Tetrahydropterin Synthase Deficiency in Mexico: A Call to Action

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6-Pyruvoyl-tetrahydropterin synthase (PTPS) is involved in BH4 de novo biosynthesis, and its deficiency (PTPSD, MIM *612719) is characterized by hyperphenylalaninemia and deficit of central monoamine neurotransmitters. It is widely known that neonatal differential diagnosis of hyperphenylalaninemia should include pterin profile determination in order to discard defects on BH4 metabolism. Herein we describe the clinical characteristics and mutational spectrum of PTPSD patients, as well as the difficulties for their diagnosis and treatment in an underdeveloped country. **Material and methods**: Analyses of the files of PTPSD patients diagnosed by molecular analysis of *PTS* gene at the National Institute of Pediatrics from Mexico. **Results:** Five PTPSD patients (including 2 siblings) have been lately diagnosed, 4/5 presented hyperphenylalaninemia in the newborn screening, and all of them were considered as phenylalanine hydroxylase deficiency (PKU) and consequently treated with phenylalanine restricted diet but with bad outcome. All patients presented severe early neurological manifestations with dystonia, seizures, and extrapyramidal signs. Mean age at PTPSD diagnosis was 1.4 months, the main reason of the diagnostic delay was the difficulty to perform pterin profile because this methodology is not of easy access in our Country. Another major difficulty in these cases was to obtain the specific treatment with, L-dopa, 5-hydroxytryptophan and sapropterin, because this last drug is not covered by all health public insurance systems, so only 2 patients had access to treatment. **Conclusion**: PTPSD and other defects of BH4 metabolism must always be considered in the differential diagnosis of patients with hyperphenylalaninemia. This is a call to action for underdeveloped countries that are beginning their PKU newborn screening programs that should contemplate this situation, and implement pterin profile in order to achieve early diagnosis.

129-Establishment and Clinical Validation of a Cutoff Value for Newborn Screening for Isovaleric Acidemia and Glutaric Acidemia Type I

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Introduction: Newborn screening is a preventive healthcare program whose main goal is early detection, diagnosis, and intervention of inborn errors of metabolism that may otherwise produce serious clinical consequences or even death. Establishing a proper cutoff is a key factor when introducing a new disorder into the screening program. **Objective:** To characterize, in our population, the distribution of values of isovalerilcarnitine (C5) and glutarilcarnitine (C5DC+ C6-OH), biochemical markers of isovaleric acidemia (IVA), and glutaric acidemia type 1 (GA-1), respectively, and to establish and clinically validate a cutoff value for the screening of these disorders. **Materials and Methods:** 2740 dry blood samples of apparently healthy breast-fed newborns, obtained between 2-5 days of age, were tested for C5 and C5 DC by tandem mass spectrometry on an API3200 (ABSciex) using MassChrom Non-derivatized kit (ChromSystems). Mean ($\bar{x}$), standard deviation (SD) and different percentile values were calculated. Results were compared to those provided by the collaborative international database Region 4 Stork (R4 S) up to April 2017. Cutoff value was considered clinically validated if it fell within the percentile 99 (P99) of normal population and percentile 5 (P5) of the confirmed positive cases (405 for IVA and 539 for GA-1) and within the percentile 25 (P25) and percentile 75 (P75) of the distribution of cutoff values reported for these disorders. **Results:** *Distribution of Values* **C5** $\bar{x} = 0.12$ μM, SD = 0.08 μM, P99 = 0.30 μM, P99.7 = 0.50, P99.8 = 0.55, P99.9 = 0.67 μM. **C5 DC** $\bar{x} = 0{,}20$ μM, SD = 0.08 μM, P99 = 0.44 μM, P99.7 = 0.50, P99.8 = 0.55, P99.9 = 0.57 μM. *Clinical validation:* Isovaleric acidemia Selected cutoff: 0.67 μM(P99.9) P99–P5 range = 0.38 μM–1.13 μM P25–P75 range for cutoff values = 0.49 μM–1.00 μM Glutaric Acidemia Type I: Selected cutoff: 0.50 μM (P99.7). P99–P5 range = 0.10μM–0.28 μM; P25–P75 range for cut off values = 0.25 μM–0.43 μM **Conclusions:** We characterized the distribution of C5 and C5 DC values in our population and successfully validated the selected cut-off for IVA, allowing its implementation in a near future. We failed to achieve clinical validation of GA-1 cutoff. Despite P99 falling within the P25-P75 range for reported cutoff values, it is above P5 of reported true positives cases, not fully meeting the validation criteria. This may be due to the fact that true positive C5 DC values reported in the R4 S database seem not to be discriminated between derivatized and underivatized methodologies.

130 - Impact of the Use of the SUMA Technology in the Program of Congenital Hypothyroidism in the Colombian Health System

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Introduction: Congenital hypothyroidism (HC) is the most common endocrine disease and the most common cause of preventable mental retardation. Since 2000, Colombia has begun the mass screening of newborns for the prevention of HC, for which one of the vanguard technologies in this program has been SUMA Technology. **Objective:** To show the impact

of the SUMA technology on the Congenital Hypothyroidism Program in the Colombian Health System. **Methods:** A retrospective, longitudinal study of all newborns that were study by the UMELISA Neonatal TSH of the SUMA technology was perform in the period from 2004 to 2015, using data from the National Network of laboratories. **Results:** A total of 573 712 cases were studied, representing 6.8% of all births in the country, which allowed the identification of 130 newborns with elevated TSH levels, indicating that after confirmation, these children begin a therapeutic regimen that guarantees better life quality. **Conclusions:** SUMA technology has played a very important role in the development of the neonatal sieve for TSH in Colombian territory. A significant number of births have been sieved through technology, a valuable contribution in Colombian children, due to the ease of management of the technology, its quality and the commitment to provide coverage in all departments of the country.

131 - Seven-Plex MS/MS Method to Measure I2 S, NAGLU, GALNS, GLB1, ARSB, GUSB, and TPP1 Enzyme Activities in DBS

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The mucopolysaccharidoses (MPS) family of lysosomal storage disorders (LSDs) is caused by defects in the metabolic breakdown of glycosaminoglycans (GAGs). This work demonstrates a novel MS/MS assay that simultaneously monitors the activity of seven different MPS enzymes. Using a single 3.2 mm dried blood spot (DBS) punch and incubation cocktail, our assay has the ability to identify samples with low enzyme activities for I2 S (MPS II), NAGLU (MPS IIIB), GALNS (MPS IVA), GLB1 (MPS IVB), ARSB (MPS VI), GUSB (MPS VII), and TPP1 (CLN2). The seven-plex is incubated overnight in the presence of incubation cocktail at 37°C followed by a post-incubation, fully automated workup that is less than 30 minutes per plate. Sample-to-sample time using MS/MS analysis can be as low as 2 minutes, which allows the possibility to obtain more than 5000 results per day if desired. Method performance studies show good linearity for each enzyme in their respective activity range. Furthermore, a study consisting of several hundred presumed healthy neonates, confirmed low I2S/NAGLU/GALNS/GLB1/ARSB/GUSB/TPP1 activity and CDC control DBS showed excellent resolution and clear distinctions between the different enzyme activity levels.

132 - Glutaric Aciduria Type-I Missed by Newborn Screening: Report of Three Cases

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Background: Glutaric aciduria type I (GA-1) is a rare autosomal-recessive disorder of the degradation of the amino acids lysine and tryptophan caused by mutations of the *GCDH* gene encoding glutaryl-CoA-dehydrogenase. Affected patients typically present in infancy or early childhood with progressive macrocephaly and an acute encephalopathic crisis often caused by a catabolic state, e.g., a febrile viral illness. Accumulation of toxic metabolites may lead to irreversible damage of the basal ganglia and severe permanent dystonic cerebral palsy. Newborn screening (NBS) for this condition is done in Austria since 2004 and in Switzerland since November 2014 based on elevated levels of glutarylcarnitine (C5 DC) in dried blood spots (DBS). **Case Reports**: Here we describe three patients, being born in Switzerland and Austria, in whom the diagnosis of GA-1 was missed by newborn screening. They were finally diagnosed at the ages of nine months, four and 10 years, respectively, after presenting signs and symptoms that were typical for this disorder (metabolic deterioration, encephalopathic crises, basal ganglia changes on MRI, movement disorder) and led to targeted metabolic and genetic screening. **Results**: Glutarylcarnitine (C5 DC) in DBS was normal in the NBS cards of all 3 patients. It was also normal at the time of diagnosis, and during follow-up. Even after a carnitine load, C5 DC stayed normal. The concentrations of the marker metabolites, glutaric acid and 3-hydroxy glutaric acid in urine, were only slightly elevated. The diagnosis was confirmed by mutation analysis of the *GCDH* gene. Both Austrian patients were compound heterozygous for p.Arg257Glu and p.Met405Val. The Swiss patient was compound heterozygous for p.Gly241Val and p.Gly390Ala. **Conclusions**: Normal C5 DC in NBS cannot totally exclude GA-1. The so-called non-excretors can have normal C5 DC even after a carnitine load. In case of a clinical suspicion of GA-1, a complete workup is necessary, even if the child had a normal NBS result.

133 - Training Courses and Update for Primary Health Care Professionals

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Introduction: The Center for Education and Social Support (CEAPS) of the Center for Action and Research in Support Diagnostics (Nupad), conducts Training and Updating Courses about the Guthrie test and neonatal screening diseases, including phenylketonuria (PKU), since 1998. The main public is health professionals from the State of Minas Gerais who work in primary care in Basic Health Units (BHU). **Objective**: To describe the flux of training for professionals in primary health care. **Results:** The CEAPS contacts the health manager who indicates health professionals—involved in collecting the neonatal heel prick test and/or accompanying patients diagnosed with PKU—to participate in the training course with classes on sample blood collection and screening. There have already been 242 courses and more than 12 thousand trained professionals. Only in 2015, 553 professionals were trained. After 2015, due to lack of resources from the Government, the face-to-face courses were suspended. Therefore, CEAPS has proposed, with the multidisciplinary team that attends to patients with PKU, the creation of video-lessons and distance courses for training professionals. Video-lessons and courses will target health and education professionals with relevant topics on disease, treatment and biopsychosocial implications. **Conclusion:** Education/training/updating in health is indispensable for all professionals, regardless of the area in which they work. With the accelerated production of knowledge, distance education emerges as a way of survival and professional development, as well as breaking the barriers of time displacement and flexibility, resource savings and student autonomy of the rhythm of studies. Thus, it is evident the importance of the training of health professionals favoring a greater understanding of the disease by the professional and, consequently, through its performance, adherence to treatment by the patients/family and the improvement in the management of patients with PKU.

134 - Pilot Newborn Screening Program for Treatable Lysosomal Diseases in Brazil: Study Design for Biochemical Diagnosis

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Lysosomal storage disorders (LSDs) are inherited conditions usually caused by a deficiency in a specific lysosomal enzyme. This deficiency usually causes the storage of substrates, leading to a progressive and most times severe problems in many organs and systems. Most LSDs are inherited in an autosomal recessive manner, with few exceptions which are X-linked. At present, there is no cure for LSDs. Today, bone marrow transplantation (BMT) and mainly enzyme replacement therapy (ERT) are the usually available treatment options for patients with selected LSDs. As some LSDs can be treated and as there is evidence that a better result can be expected in cases treated early, a program of neonatal screening is being considered for these diseases. Enzyme assays for Gaucher, Fabry, Pompe, MPS I, MPS II, and MPS VI, and also quitotriosidase as potential biomarker were added to the standard screening panel (phenylketonuria, congenital hypothyroidism, hemoglobin disorders, cystic fibrosis, biotinidase deficiency and congenital adrenal hyperplasia). This project, in its first stage, adapted the standard fluorometric enzyme assays to micromethods, reducing the cost of reagents and mainly of the specific expensive substrates. In the second step, the study is evaluating operational issues (feasibility, costs, false-positive rate, and other aspects) related to the inclusion of the lysosomal panel in the newborn screening program and provide valuable information to the newborn screening operators in Brazil.

135 - Reproductive Options, a Secondary Benefit of Newborn Screening (NBS) for X-Linked Adrenoleukodystrophy (X-ALD): A Model and Lessons From the New York State NBS Program at a Single Institution

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On January 1, 2014, NYS started NBS for X-ALD. All confirmed cases are followed using a protocol (published somewhere) including neurology and endocrinology exams and imaging (brain MRI) and labs (C26:0, endocrine). According to the protocol confirmatory testing includes the proband and the mother. If the mother is positive, testing other siblings is then discussed. After confirmation, the mother and the siblings are enrolled in the protocol. For male siblings, brain MRIs with contrast and the possibility of the use of Lorenzo's oil are considered. A search for a compatible BMT/HSCT donor is also addressed. A secondary benefit of the NBS has emerged as discussing reproductive options. Few instances of prenatal testing have been published and all were known familial cases. Conversely, since NBS is new no information is available. Hence, we are proposing a model to address reproductive options to reduce recurrence risk. A discussion about X-linked inheritance, risk of transmission, different risks if the transmitting parent is a male or female, and if the affected proband is male or female are discussed. Strategies for risk reduction or management include not to have children,

adoption, egg or sperm donor, prenatal diagnosis, and no intervention. For prenatal diagnosis, the techniques discuss include chorionic villous sampling, amniocentesis, and preimplantation genetic diagnosis. For each of them, the degree of reduction varies and is addressed. At our institution, we have used the model in 3 families since NBS started. Family 1 was found to have a newborn, his older brother, and their mother affected. Family 2 has an infant and his mother affected. Family 3 was found to have a newborn, an older brother and their mother, all affected. All affected family members are currently follow according protocol. For family 1 and 2 reproductive options have been discussed. Family 1 has opted not to have more children. Family 2 is considering further children, but no decision has been taken yet. Family 3, so far, has not considered to have a discussion about reproductive issues. In conclusion, although NBS has recently started and the experience is short, at our center we have implemented a model to discuss reproductive options that seems to be effective. Further cases, longer follow-up and share experience from other centers will give more input into the validity of this model in the context of NBS for X-ALD

136 - Molecular studies in patients with Congenital Adrenal Hyperplasia in Newborn Screening Service

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Objective: To carry out the molecular examination in the CYP21A2 gene in a patient with altered results in newborn screening for Congenital Adrenal Hyperplasia (CAH), in other words, high values of 17-hydroxyprogesterone (17-OHP). Thus, this study aims to improve the diagnostic conclusion, in addition to reducing the follow-up time of false-positive individuals. **Methods:** Four patients with neonatal 17-OHP levels above 20 ng / mL, evaluated by the Newborn Screening Service of the Federal District, were selected for the molecular study. The examination was performed from the isolation of the active gene by Nested-PCR. Then, the Automated Sanger Sequencing was performed to investigate point mutations, and the MLPA (Multiplex ligation-dependent probe amplification), for investigation of large gene rearrangements, such as deletions and conversions. **Results:** Relevant mutations were found in the four patients evaluated. The mutations found were I2 Splice/I2 Splice, I2 Splice/p.I172 N, E6Cluster (the mutations I236 N, V237E and M239 K found in tandem forming a cluster) and a total deletion of the gene region encompassing the CYP21A2 gene. Conclusion: This study allows the better follow up on patients diagnosed with CAH and the exclusion of the false-positive hypothesis. Furthermore, it allows the standardization of the molecular test in the Newborn Screening Service of the Federal District. It is also concluded that the association between the Automated Sanger Sequencing and the MLPA methodologies are fundamental for the molecular diagnosis, and enable greater accuracy and efficiency than other techniques listed in the literature.

137 - Comparative Study Between Three Methods of Analysis in Newborn Screening for Hemoglobinopathies

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The diagnosis of hemoglobinopathies is based on the separation of fractions of hemoglobin (Hb). The most common conventional methodologies used in Newborn Screening (NS) are isoelectric focusing (IEF), high performance liquid chromatography (HPLC), and capillary electrophoresis (CE). These methods can be used alone or in case of positive or doubtful results can be associated for better sensitivity and specificity. Among other methodologies, in the last decades the HPLC method has provided an automated and robust method with fast identification of the Hb, used in many laboratories of NS around the world. Recently, the Primus Ultra2 Genesys (Trinity Biotech) equipment was introduced in to the market, with fast and accurate analyses using two types of assay in HPLC method: the fast screening program (HPLC-Quick), and the High Resolution (HPLC-High) used to confirm the results. The objective of this study was to analyze and compare three methods, HPLC (Primus Ultra2 Genesys - Trinity Biotech), EC (Capillary Neonat Hemoglobin) and IEF (Perkin Elmer) in the routine of NS for hemoglobinopathies in the Laboratory of APAE DE SÃO PAULO. In this study, were randomly selected 258 samples of dry blood in filter paper from laboratory routine for analysis in all these three methods. Comparing the HPLC-Quick and EC methods with the IEF data, we obtained a great correspondence for both methods, regarding the normal Hb (FA / AF / AA) pattern, that is, of the 63 hemoglobins detected by the IEF, 80.95% to 95.24% presented the same result in HPLC-High and HPLC-Quick. The hypothesis test between the EC, HPLC - High and Quick methods for Hbs F, A, S and C showed values of $P < .001$. Considering the results obtained, we found that the HPLC-High method presents a larger diagnostic range, it can be considered a more precise method and its better use for the detection of variant Hbs. Based on the EC method, the HPLC-High and HPLC-Quick methods were more

accurate than the latter, although they showed good correlation between methods.

138 - International Collaborative Newborn Screening Pilot Program

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Objectives: Determining positive cases of congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), cystic fibrosis (FC), phenylketonuria (PKU), and galactosemia(GAL) on dried blood samples (DBS) on filter paper. **Methodology:** A basic panel of Newborn Screening was performed by Medicine students from San Carlos University, randomly in healthy babies that received medical attention in the CAIMIs (for its acronym in Spanish, Maternal-Infantile Integral Attention Centers) in five departments of the country, including Escuintla, Santa Rosa, Jutiapa, Alta Verapaz, and Quetzaltenango. Babies included had between 2 and 30 days of life when screened. Samples were sent to the Swiss Newborn Screening in Zurich by express courier. And results were giving to the Physician to give the integral medical attention to the babies that tested positive. **Results and Discussion:** A total of 400 samples were obtained from 58% of males and 42% females healthy babies, of who 99% had breast-fed and had normal birth weight. From the 400 tests, only 18 (4.5%) tested positive, 10 (2.5%) CF, 4 (1%) PKU, 2 (0.5%) CH, and 1 case of CAH and GAL, respectively. **Conclusions:** The international collaboration and the interinstitutional network can speed the process for implementing a Newborn Screening Program, collecting 400 samples during May and June 2016. Babies that tested positive were scheduled for follow-up with genetic clinic at La Obras Sociales del Hermano Pedro

139 - Alleatory Screening for Aminoacidopathyes and Disorders in Acyl Carnitines in a Newborn Population from Guatemala

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Objective: Guatemala a mystic country, where mayan and ladino people live in the same territory. But both with no knowledge of what a metabolic screening plan is, this expands the horizons for multiple investigations and the use of translational medicine to help in creating programs to aware people about the importance of diagnosing inborn errors of metabolism at early stages. **Methods:** Here in 5 of the 22 departments that Guatemala has, the current study investigated 400 neonates, between 2 and 28 days, for low or high values in the quantification of amino acids and acyl carnitines in each of the samples that where taken with blood drops in a filter card, and processed by tandem mass spectrometry using cutoff points in the Guatemalan population. **Summary:** We had 4 samples with phenylalanine above the 99.9 percentile, 2 samples with phenylalanine/tyrosine above percentile 99.9, 26 samples with citruline above percentile 99, 5 samples with Gln/Lys above percentile 99, 4 samples with Glu above percentile 95, 5 samples with Gly above percentile 99, 5 samples with Val above percentile 95, 4 samples with Met above percentile 95, 5 samples with Orn above percentile 95, 5 samples with Pro above percentile 95, 4 samples with Serine above 99 percentile, 4 samples with Tyr above percentile 99, 4 samples with C0 above percentile 99, 59 samples with C5 below percentile 0.1, 5 samples with C5 above percentile 95, 1 sample with C8 below percentile 0.1, and 5 samples with C8 above percentile 95. **Conclusions:** It is for the best of Guatemalan people to have a strong screening program that can detect, in early stages, aminoacidopathies and abnormalities in acyl carnitines so we can prevent the management of highly complicated and invalidating diseases as well as to reduce the economic and labor impact these can cause, because they do exist we have found positive cases and we need the help of countries that have their established program to educate us in how to start.

140 - Clinical Profile and Outcomes of Individuals With Elevated Methylmalonylcarnitine/ Hydroxyisovalerylcarnitine Ratio in Newborn Screening Test

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Tandem mass spectroscopy (MS/MS) was first instituted in the public newborn screening (NBS) test in Brazil in 2010, in the Federal District, as a result of state's legislation. From a single bloodspot, this method enables the early detection of more than 20 inborn errors of metabolism, particularly organic acidemias, amino acid and fatty acid oxidation disorders. One of the advantages of MS/MS is that allows the detection of a large number of analytes in a single assay. Meanwhile primary

analytes are well established for a number of diseases, secondary analytes are utilized to improve sensitivity and tend to vary between programs, since the selection of both is dependent on the preference of the individual NBS program. One of these optional analytes is the methylmalonylcarnitine–hydroxyisovalerylcarnitine ratio (C4DC/C5OH) that may be abnormal in several organic acidemias. **Objective:** To evaluate the clinical profile and outcomes of individuals with elevated C4DC/C5OH in NBS. **Methods:** Medical records from all individuals with elevated C4DC/C5OH in NBS tests performed between January 2015 and December 2016, were analyzed. **Results:** Of 88.726 NBS tests performed between the study's period, 69 individuals had elevated C4DC/C5OH; 78.26% as isolated form and 21.74% associated with other analytes in abnormal range. Of these 69, 66.67% were male and 33.33% were female; 79.41% were born at term and 77.94% were adequate for gestational age. Twenty-seven (39.13%) had normalized the results without any medical intervention: 85,19% up to 6 months and 14.81% up to 1-year-old. Thirty-three (47.83%) persisted with the alteration and 9 (13.04%) lost follow-up. Among those who persisted with the abnormality, 2 had the diagnosis of organic acidemia (Beta-ketothiolase Deficiency and 3-Methylcrotonyl-CoA Carboxylase Deficiency, respectively) and 8 had cobalamin deficiency. Conclusion: the elevation of C4DC/C5OH was more prevalent in male babies, born at term and adequate for gestational age. Among those who normalized, the majority were up to 6 months. We found two organic acidemias, with isolated elevation of C4DC/C5OH and 8 cobalamin deficiencies. We did not analyze the maternal cobalamin status, which would be interesting to relate to alterations in NBS test and for nutritional recommendations. Finally, we need to expand our study to all the NBS tests performed since the implementation of MS/MS in NBS program to assess the costs and benefits of C4DC/C5OH analysis.

141 - Clinical Outcomes of Individuals with Hypergalactosemia in Newborn Screening Test Accompanied in the Public Health System in the Federal District, Brazil

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Galactosemia is a genetic disorder due to deficiency in one of three enzymes related to galactose metabolism: galactose-1-phosphate uridylyl transferase (GALT), galactokinase, and galactose epimerase. The most prevalent forms of galactosemia are those related to GALT deficiency and can be classified as classic galactosemia, clinical and biochemical variant galactosemia. Classic galactosemia, if untreated, can be a life-threatening disease characterized by feeding difficult, failure-to-thrive, hepatic damage, bleeding, and *E. coli* sepsis. Newborn Screening test (NBST) for galactosemia is available in the Public Health System in the Federal District (SES-DF), Brazil, since 2012. **Objective:** to evaluate the clinical outcomes of individuals with hypergalactosemia in NBST accompanied in SES-DF, Brazil. **Methods:** Medical records from individuals with hypergalactosemia in NBST accompanied in SES-DF between January 2012 and April 2017 were analyzed. **Results:** During the study's period, 236 042 NBST for galactosemia were performed. Of them, 54 (0.023%) met laboratory's criteria to be referred for clinical evaluation. Another 3 patients are accompanied in our service, 2 for hypergalactosemia in NBST from an external laboratory and a third one coming from another Brazilian state. Erythrocyte GALT enzyme activity was normal in 33.3%, 47.4% had low to moderate decrease on enzymatic activity and 19.3% had undetectable or very low levels of GALT activity and were classified as classic galactosemia. Dietary restriction of lactose was instituted for all patients with abnormal GALT activity and for those with normal activity of this enzyme who persisted with hypergalactosemia. Poor sucking was seen in 7%, failure-to-thrive in 3.5%, jaundice in 33.3%, bleeding in 5.3%, sepsis in 1 individual, cataracts in 2/42, neurodevelopmental delay in 2/56, speech problems in 5/41, abnormalities in motor function in 1/54, and psychomotor agitation in 4/52. **Conclusion:** NBST for galactosemia is challenging due to the numerous pathogenic variants in genes encoding enzymes related to galactose metabolism, determining a great clinical and biochemical variability. Here we observed that, despite early dietary intervention, some individuals presented clinical manifestations of the disease. Finally, we recognize the necessity of genetic testing of individuals with elevated total blood galactose to identify pathogenic variants in our population, for adequate treatment, prognosis, and genetic counseling.

142 - Tandem Mass Spectroscopy Profile in Newborn Screening Test of Premature Brazilian Patients of the Federal District and Their Clinical Outcome

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Newborn Screening (NBS) is recognized as an important population health initiative, involving the systematic testing of asymptomatic individuals for a specific condition to detect early stages of inborn errors of metabolism (IEM) and other congenital disorders. The NBS service of Federal District is a pioneer in performing the expanded screening test using Tandem Mass Spectroscopy (MS/MS) technology in Public Health Service in Brazil. One abnormal finding in MS/MS can be translated into several different metabolic diseases, and a bunch of factors can modify the result of this exam, such as gestational age, weight, need for parenteral nutrition (PTN), vitamins deficiency. **Objective:** To describe abnormal analytes detected by MS/MS in premature newborns of Federal District, as well as their clinical outcome. **Methods:** A cross-sectional study, based on the analysis of medical records of all children with alterations in MS/MS on NBS test, born in the Federal District between January 2015 and December 2016. **Results:** During the analyzed period, 88.726 NBS tests were performed and 267 individuals were referred to our service for presenting abnormal results on MS/MS in two or more samples. Of these, 47.56% were premature, 66% of which were using PTN. The analytes usually found out of range were arginine, methionine, free carnitine, octanoylcarnitine–decanoylcarnitine ratio, tyrosine, and phenylalanine–tyrosine ratio. More than one alteration was seen in 72.44%. From the total of premature newborns, 67.72% (86/127) normalized the results up to 6 months of age and were classified as having transient alterations; 10.24% lost the follow-up, still, 14.96% remain under investigation. Five (3.94%) individuals were diagnosed as having a specific IEM. The remaining five patients died before the tests were repeated, and none of these deaths could be related to IEM. **Conclusion:** Our results are concordant with those of literature, where is established that prematurity increases the probability of multiples alterations, and the use of PTN can quadruple the rate of false positives in NBS test. We observed that the majority of the premature newborns normalized the alterations in MS/MS up to 6 months, which indicates that the follow-up performed in our service is effective for the monitoring of these children. We should evaluate the best timing to collect the sample in those individuals using PTN in an attempt to reduce the false-positives rates.

143 - IRT/IRT Strategy for Early Cystic Fibrosis Detection: Importance of Newborn's Day of Life and Methodological Performance

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Introduction: Different strategies are used in cystic fibrosis (CF) neonatal screening (NS); one of them is IRT/IRT. To define PN, it is important to consider: 1-Days of life (DL) of the newborns (NBs) at the time of sampling for the first and second IRT 2-Development of the method at decision levels. **Objectives:** Establish: -DL of the NBs for the 1st and 2nd IRT -Performance of the method in concentrations close to the cut levels. Methods: IRT/IRT strategy, DELFIA-Perkin Elmer® method. Cutoff values: ·IRT $\geq$ 70,0 ng/mL, NBs until 7 DL ·IRT $\geq$ 60,0 ng/mL, NBs 8 to 30 DL. Positive results were reported to CE.P.E.I.I.'s Social Work Area for recitation. -DL of the NBs for 1st IRT and 2nd IRT were established. The performance of the analytical system was evaluated (EP_{15}-A_3 CLSI; ISO 15189:2012): -using commercial control materials; concentration 65,2 ng/mL and -6 PEEC surveys, 2016-2017: reference parameters, median participating laboratories $\pm$1.5 SD. The defined quality requirement was: Eta = 25%. **Results:** 2010-2016: 155 662 NBs evaluated, DL of NBs, mean-median = 3/2 1-For 537 NBs •First IRT elevated, DL mean/median = 5/3 •Second IRT DL mean/median = 19. Only 99 NBs had second IRT elevated; 78 false positives and 21 CF (mean/median = 18/16 DL). 2-Perfomance of the method, concentration 60-70 ng/mL:·Precision, CV%: repeatability/intralaboratory = 3.23/6.19 lower than CV% of de manufacturer = 6.50/8.30. CVintralaboratory% = 6.19 lower than EAa% = 6.25 -SD of the laboratory = 3.87ng/mL lower than the value of verification = 9.08. Manufacturer's specifications were verified. •Veracity: -Interval verification ng/mL = 62.23-68.17; includes mean obtained = 62.39ng/mL. Bias% = 4.31 lower than ESa% = 12.5. Verification accepted from the statistical/clinical point of view. •Total error% = 16.69 less than ETa% = 25. •External quality control, PEEC: concentration range, ng/mL, 11-338.10. All submitted results were within the median $\pm$1.5 SD. Bias% = 6.44 with respect to peer group. •Uncertainty: -components associated with Bias%/random error%: b = 7.53/S = 8.05. Expanded uncertainty% = 22.04. Conclusion: It is considered adequate: -DL of the NBs for first and especially second IRT -Performance of the measurement procedure in concentrations close to cutoff values. In these conditions, from a cost-effectiveness perspective, IRT/IRT is considered a valid strategy for the early detection of CF in our program.

144-Clinical Review of Fatty Acids β-Oxidation Diseases Through Extended Newborn Screening in South West of Spain (West Andalusia)

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Introduction: Fatty acid β-oxidation disorders are a group of autosomal recessive inheritance diseases that affect the metabolic pathway of energy production, especially important in situations of prolonged fasting and metabolic stress (fever and prolonged exercise). Traditionally, these diseases have been

considered the main cause of sudden infant death of metabolic origin. Specifically, the deficiency of medium-chain acyl-coA dehydrogenase (MCAD), a more frequent defect of mitochondrial fatty acid β-oxidation, was associated with mortality of 20% to 25%. The expanded neonatal screening programs (ENBS) have allowed the early detection of certain pathologies, anticipating medical intervention aimed at reducing the morbidity and mortality associated with a spontaneous debut of the disease. **Objectives:** To analyze the epidemiological and clinical characteristics of patients diagnosed with FAOD in Andalusia in 2009 and 2010 and in the western region of the community between 2011 and 2016. To study the genotype of patients diagnosed with FAOD in Andalusia in 2009 and 2010 and in the western region of the community between 2011 and 2016. Analyze the impact of the extended newborn screening program for endocrine-metabolic diseases of the Junta de Andalucía on the natural course and morbidity and mortality associated with this group of diseases. Analyze the risk of decompensation of patients with MCAD fed exclusively breast milk during the neonatal stage compared to patients who were fed formula with initiation. In order to reach these objectives, we will analyze the results obtained by the Unit of Metabolopathies, Nutrition and Child Dietetics of the University Hospital Virgen del Rocío (Seville) in the management of patients diagnosed of FAOD in Western Andalusia between 2009 and 2016, and compare them with the published data on these diseases before and after the implementation of the screening programs. **Results:** In this paper, we present data on 28 patients diagnosed with FAOD ENBS in the period between 2009-2016 in Andalusia, the date on which the ENBS was implemented in our Community, allowing the early start of treatment and providing a diagnosis to our patients and their families. 64% were MCAD, 18% short-chain acyl-CoA dehydrogenase deficiency (SCAD), 7% long chain (VLCAD), 7% deficiency carnitine palmitoyl transferase 1 (CPT-1), and 3.5% primary deficit of carnitine. **Conclusions:** The prevalence of FAOS is underestimated. Most frequent mutation is c.985A>G.

145 - Results of an Expanded Newborn Screening Program in the Health Services of the Mexican Secretariat of National Defense

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Objective: In 2013, the Ministry of Health of Mexico issued a new regulation which establishes as mandatory the expanded newborn screening in Mexico, therefore the health services of the Secretariat of National Defense (Secretaría de la Defensa Nacional, SEDENA) decided to implement a new program for the detection of congenital metabolic diseases. **Methods:** All newborns (NBs) attended at the medical units of the Mexican Army were screened. Blood samples were obtained by heel puncture, deposited on filter paper cards and analyzed through AutoDELFIA® / GSP®, mass tandem spectrometry, electrophoresis, isoelectric focusing or high-resolution liquid chromatography. A retrospective analysis of the database of this neonatal screening program of the health services of the Mexican Army was carried out. **Results:** From December 2013 to March 2017, and with the participation of 38 military medical units distributed in 24 of the 32 Mexican States; 28,275 NBs were screened. A total of 292 samples were considered suspected cases (recall rate of 1.03%), 75 cases were confirmed (1: 377 NBs), with 213 false positive cases (0.75%). The detected diseases were: 29 cases of glucose 6 -phosphate dehydrogenase deficiency (1: 975 NBs); 25 cases of congenital hypothyroidism (1: 1131 NBs); 8 patients with congenital adrenal hyperplasia (1: 3535 NBs); 5 patient with Hemoglobinopathies (2 sickle cell, 2 Hbs/beta Thalassemia, 1 beta Thalassemia) (1: 7068 NBs); 2 cases of hyperphenylalaninemia (2: 8938 NBs) and 3 patient with organic acidemias (1: 9425 NBs; isovaleric acidemia, isolated 3-methylcrotonyl CoA Carboxylase and propionic acidemia). In addition, 4 cases of transient hyperthyrotropinemia (1: 7068 NBs) were detected, which are not considered in the global prevalence. All the affected children were evaluated and began treatment before the 20 days of life. **Conclusion:** The studied population showed a high prevalence of birth defects (1: 377 NBs), being the thyroid defects and glucose 6-phosphate dehydrogenase deficiency the most frequent.

146 - The Importance of Newborn Screening in the Diagnosis of Congenital Adrenal Hyperplasia (CAH)—The Brasilia experience

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Objective: To evaluate the benefit of initiating early treatment of patients diagnosed with CAH. **Methods:** Retrospective analysis of patients diagnosed with CAH. **Results:** After the onset of extend Newborn Screening in Brasília, 15 patients were diagnosed with CAH. Seven patients are males and 8 females, with a ratio of 1:1. The most common enzyme deficiency was 21-hydroxylase (21-OH), corresponding to 86% (13/15 patients), with 2 patients being diagnosed with 3 beta-hydroxysteroid dehydrogenase deficiency (3BHSD). The most prevalent form of CAH was the salt loser, corresponding to 93% (14/15 patients). The mean time of diagnosis and treatment of all patients was 24.6 days; however, when we separated the patients with early collection recommended by the program, and with a diagnosis of 21-OH deficiency (9/15), the mean was 19.6 days, with 1 case of false negative. None of the patients who had an early collection presented a salt loss crisis. Of the 3 patients who performed late collection, the onset of treatment was also late (mean of 30.6 days), one of which had the simple virilizing form and two presented a salt-losing crisis, requiring hospitalization, in which one of them was necessary care at the intensive care unit. The two patients with 3BHSD deficiency are males and had alteration on external genitalia. All patients with disturbance of sexual differentiation had an early karyotype and had a defined genetic sex. Currently, 14 patients are followed up at the CAH ambulatory, which is a reference in Brasilia. One patient died, with no definite cause. All patients currently being followed-up presented a growth adequate for age and sex and none presented advancement of bone age. **Conclusion:** The early diagnosis and treatment of patients with CAH prevented an adrenal crisis, characterized by loss of salt, which could lead to death and decreased health-care costs. A major challenge for the National Newborn Screening Program is to reduce the number of children not screened. The coverage at the Federal District, in public health, is 100%. The CAH ambulatory works in the form of free demand, it reduces the waiting time for care of children with altered results. It is hoped that with the improvements of the service, the onset treatment will be even shorter.

147 - Five Years of Expanded Newborn Screening Program at Brazil's Capital

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Brazil has a newborn screening program since 2001. This federal program is funded by the government, and therefore the entire Brazilian population has access to exams for phenylketonuria, congenital hypothyroidism, sickle cell disease, cystic fibrosis, congenital adrenal hyperplasia, and biotinidase deficiency. The Federal District (the country's capital) has its own newborn screening program, which is funded by local resources without charges for the directly benefited population. This program expanded the disease's panel to 30 since 2012, including also galactosemia, congenital toxoplasmosis, G6PD deficiency, and 21 inborn errors of metabolism. The Federal District is the only State in South America that provides such service for the population. The aim of this study is to share this experience of an extended newborn screening program in a development country. In the last 5 years were collected samples of 223 836 newborns. The procedure recommended is heel prick which is done prior to discharge in local hospitals or birthing centers so it's possible to ensure the screen to all newborns. The Federal District's primary care units can also collect the sample if it is necessary. The samples have been sent to the laboratory in two or three days. From samples to results, the process takes more seven days and the first visit to the specialist occurs in average at the twentieth day of life. In the last five years were screened 8 phenylketonurias, 83 congenital hypothyroidism, 129 SS sickle cell disease, 8 SC sickle cell disease, 13 cystic fibrosis, 12 congenital adrenal hyperplasia, 17 biotinidase deficiency, 14 classic galactosemia, 93 congenital toxoplasmosis, and 86 inborn errors of metabolism. G6PD deficiency is in 4% of the population. The Federal District's health-care services also provide the appropriate treatment for each disease which ensures the best possible outcome for the babies. The significant number of children treated in an early stage of their diseases justifies the Federal District government investments, what should be expanded to the whole country.

148 - Implementation of Newborn Screening Tests According With Institutional Needs in Mexico

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In Mexico, there are approximately 2.2 million births per year, 1.7 million of these births are served in public health care, and the rest in private health care. The health-care institutions offer different newborn screening programs, which are adapted to the economic and incidence needs of each institution. In most of these institutions the most recurrent tests are, 17OH-progesterone, TSH, PKU, galactose, biotinidase, and recently IRT. Ensure that newborn screening tests meet the specific needs of each health-care institutions, to fit their disease

prevention programs. Institutional diversity in Mexico requires that newborn screening services be tailored to the needs of each institution. In the public sector, where the majority of births occur, there are specific newborn screening programs that cover the whole country; however, in the private health care, the test profiles are very varied. Our company has efficiently implemented newborn screening tests in various institutions at all country, in the public and private health-care services, which responds to the diverse needs of neonatal screening programs.

149 - Expanded Newborn Screening Program in the Health Services of the Mexican Navy: A 5 Year Experience

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Objective: The main goal of newborn screening is timely detection and intervention for genetic disorders that may otherwise produce serious clinical consequences. Nowadays newborn screening is part of the health-care system of a high number of countries and institutions. Since 2012, an expanded newborn screening program was implemented for the beneficiaries of the health services of the Secretariat of the Navy - Mexican Navy (Secretaría de Marina-Armada de México, SEMAR). The aim of this study is to describe the birth prevalence of congenital defects detected by the mentioned program and to analyze the main performance indicators. **Methods:** From July 2012 to March 2017, blood samples from the heel were taken in Medical Units of the Mexican Navy, located in 18 states of Mexico. All samples were analyzed by time-resolved immunofluorometric assay (AutoDELFIA/GSP), tandem mass spectrometry, and isoelectric focusing or high-performance liquid chromatography techniques. The number and type of congenital diseases were evaluated. **Results:** A total of 12 847 newborns were screened; 69.02% of the samples were taken between the 3-5 days of life, and 2.6% of the samples were considered inadequate. A total of 179 samples were considered as suspected cases and all these newborns were located and retested. Thirty-nine cases were confirmed, with a false-positive rate of 0.98%. The detected diseases were 19 glucose 6-phosphate dehydrogenase deficiency, 9 congenital hypothyroidism, 7 congenital adrenal hyperplasia, 1 hemoglobinopathy, and 1 case of 3-methylcrotonyl-CoA carboxylase deficiency. All the cases arrived to the appropriate medical protocol evaluation before 16 days of life. All affected families received genetic/reproductive professional counseling. **Conclusions:** In the studied population, the birth prevalence of metabolic defects was 1 / 329 newborns. The expanded newborn screening program allowed their early identification, with the aim of preventing disability and death.

150 - Newborn Screening Program on the Social Security Health Insurance of Peru: Period 2008-2016

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The universal newborn screening (NBS) program in Peru was created by law N°29885 in June 2012, with congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), phenylketonuria (PKU), cystic fibrosis (CF), hearing loss, and congenital cataract as the condition included. Currently, the NBS program at the public sector is in phase of implementation. The Social Security Health Insurance of Peru (EsSalud) attends approximately one-third of total population of the country. Experience in NBS program in EsSalud began in October 2002 for CH and CAH began at Hospital Edgardo Rebagliati Martins of Lima, spreading nationwide since January 2008. In the same year, the screening for PKU and GAL began only at Edgardo Rebagliati Hospital and his affiliated medical centers, spreading nationwide since September 2011. **Objective:** To describe the results of Newborn Screening Program at the Social Security Health Insurance of Peru, during 2008-2016. **Methods:** Dried blood spot (DBS) samples of newborns from the Social Security Hospitals all over the country are sent to the Mother-Child Laboratory at Edgardo Rebagliati National Hospital, where are processed. It was used enzymatic colorimetric method to determine Phenylalanine (Phe) and galactose levels, Thyroid stimulating hormone (TSH) level was determined by enzyme-linked immunosorbent assay (ELISA) and 17α-hydroxyprogesterone (17-OHP) by enzyme immunoassay (EIA). The established cut-off was TSH: 10 μUI/mL, 17-OHP: 10 ng/mL, Phe: 3 mg/dl, galactose: 7 mg/dL. It was necessary a second assay in a positive case. **Results:** NBS was performed to 799 467 neonates, with an average coverage of 91%. A total of 294 positive cases were detected, including 235 cases of hypothyroidism (0.311 × 1000), 33 congenital adrenal hyperplasia (0.045 × 1000), 12 PKU (0.021 × 1000) and 15 galactosemia (0.027 × 1000). The incidence of positive cases for congenital hypothyroidism was 1/3211 live births, congenital adrenal hyperplasia 1/22 207, PKU 1/46 970 and galactosemia 1/37 576. **Conclusions**: The Social Security

Health Insurance of Peru (EsSalud) has the first experience of an NBS national program in the country detecting congenital hypothyroidism, congenital adrenal hyperplasia, phenylketonuria and galactosemia, with a high coverage of neonates. The incidences are similar to those reported in other countries of the region.

151 - Experience in Newborn Screening in the Maternity Clinic Rafael Calvo Cartagena. Colombia During the Years 2005-2016

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Introduction: The Maternidad Rafael Calvo Clinic is the second-level public institution with more deliveries in Cartagena and Bolívar. A newborn screening program was established. On August 17, 2005, we started the Newborn TSH program, developing it within internal controls and National and International. **Objectives:** To determine how many tests were performed between 2005 and 2016 and to establish the number of positive cases in the period. **Methodology:** Observational, descriptive, and retrospective. **Materials and Methods:** Neonatal umbilical cord TSH from August 17, 2005, to December 31, 2016. ELISA with Tecnosuma fluorescence was used. **Results:** In August 2005, 2224 newborns were screened. In 2005 2224, 2006: 4866, 2007: 6536, 2008: 7694, 2009: 7986, 2010: 6866, 2011: 9257, 2012: 826, 2013: 10 333 2014: 8760, 2015: 7249, 2016: 7247. There was an increase in coverage due to the awareness of the importance of taking the sample and that the Institution assumed the cost of screening patients without social security. Colombia normalized the compulsory nature of the test as an integral part of the delivery and mandatory notification. A total of 87 278 newborns were screened. Being the year 2013, the year with more siftings and 2006 the year with less sifting. Positive Cases Per Year: In 2005, 2006 0 cases, 2007 2 cases $P = .00030$, % 0.030, 2008 0 cases, 2009 0 cases, 2010 1 case $P = .00014$, % 0.014, 2011 4 cases $P = .00043$, % 0.043, 2012 12 cases $P = .00145$, % 0.145, 2013 3% 0.068. Cases $P = .00029$% 0.029, 2014 15 cases $P = .00171$ % 0.171, 2015 10 cases $P = .00137$% 0.013 and 2016 5 cases $P = .00068$. For a total of 52 cases with a prevalence of 0.000595, % 0.0595. The year where more positive cases were found was 2014 with 15 cases. The years with no positive cases were: 2005, 2006, 2008, and 2009. Demonstrating the low prevalence of positive cases in the Institution. Conclusions: The newborn screening program has achieved neonatal umbilical cord TSH to 87,258 neonates of the Institution, achieving 100% coverage. During this period of time, 52 positive cases were found. Congenital hypothyroidism is not a frequent disease in our region, since its incidence is too low, but due to the magnitude of it, identifying a single case is invaluable.

D) Dietetics and Nutrition (152 to 207)

152 - The Effects of Low Protein Products Availability on Growth Parameters and Metabolic Control in Selected Amino Acid Metabolism Disorders Patients

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In Saudi Arabia, a diet for life policy has been adopted in the management of amino acid metabolism disorders for years. However, low protein products—which are one of the important treatment tools—were not available up until several years ago. Our aim was to measure the compliance and quality of life in patients affected with these disorders following in the metabolic nutrition clinic at King Faisal Specialist Hospital & Research Centre (KFSH&RC), Riyadh, Saudi Arabia. **Methods:** using a nonrandomized retrospective/prospective study utilizing the growth parameters, biochemical data, and questionnaires with the patients and their family. A total of $n = 180$ patients affected with selected amino acid metabolism disorders were enrolled. Some were excluded $n = 82$ for various reasons. Sample analyzed were: phenylketonuria (PKU) (44), Maple Syrup Urine Disease (MSUD) (30), tyrosinemia (TYR) (17), and homocystinuria (HCU) (7). Data were obtained using (COMPLE) Microsoft Access which was designed by the metabolic nutrition clinic at KFSH&RC, Riyadh. Student's paired *t*-test were used to investigate relations. **Results:** The main findings were the improvement in the tandem mass spectrometry (TMS) levels of some of the selected amino acid metabolism disorders pre and post the usage of low protein products. In PKU patients, the TMS PHE level post were significantly decreased (P value <.0001). This was also the case in MSUD patients with significant decrease in Leucine/Isoleucine levels (P value .0008) but not in Valine levels (P value .1148) as 36.7% of them received Valine supplements while enrolled in the study. **Conclusion:** Low protein products availability were successful in improving selected amino acid metabolism disorder biochemical outcomes. However, due to compliance issues and impracticality of the diet, the results were not significant in all enrolled patients.

153 - Parent Perceptions of Liberalizing Fat Intake in Children With Very Long-Chain Acyl-CoA Dehydrogenase Deficiency

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Background: Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) causes loss-of-function within the mitochondrial β-oxidation pathway. Treatment generally involves

the restriction of long-chain triglycerides and reduced fasting times. Newborn screening has led to an increase in VLCADD diagnoses, but most babies diagnosed are asymptomatic. It is unknown whether asymptomatic VLCADD patients require dietary restriction of fats. When liberalization of previous restrictions is advised it appears that asymptomatic VLCADD patients often continue with a restrictive low-fat diet. **The aim** of this study was to evaluate the thoughts and understanding of the use of a liberalized fat intake of parents of children with asymptomatic VLCADD. **Method:** Parents of children with VLCADD who attended Sydney Children's Hospital and followed a low-fat diet from diagnosis were recruited. Participants completed an interview-administrated patient questionnaire. Questions were structured under four conceptual themes. **Results:** Six participants (100% response rate) completed the questionnaire. The following themes were reviewed 1) Social Impact: All parents felt positive about liberalizing their child's low-fat diet as they could increase variety of foods and attending social events was less stressful. 2) Support: All parents felt well supported by the metabolic team and despite feeling anxious about stopping the dietary treatment they "trusted the professionals". 3) Education: One family was given practical advice on introducing higher fat foods and found the process less stressful and introduced a greater range of foods. Five families were given no recommendations but told the child can "eat everything". These families delayed introducing high fat foods. 4) Efficacy of therapy: Five participants were concerned about causing harm by liberating the diet. Two of these reported that they would prefer to keep to the low-fat diet because they believed "it's more beneficial in the long-term" and felt they were "actively doing something to benefit my child". **Conclusion:** This study shows that parents are somewhat willing to follow a liberalized diet if their child has asymptomatic VLCADD but remain anxious about doing so. They trust their medical team but require extensive support and a comprehensive step-by-step education plan on how to liberate their child's restrictive diet. It may be that more discussion of the possibility of later diet liberalization is needed soon after diagnosis.

154 - The Effect of Ketogenic Diet on Hematological Parameters of Patients With Intractable Epilepsy

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Aim: Ketogenic diet (KD), which is high in fat and low in carbohydrates, mimics the metabolic state of starvation and is used therapeutically for pharmacoresistant epilepsy. The aim of this study was to evaluate the hematological parameters of patients receiving KD for at least 1 year due to drug resistant epilepsy. **Methods:** A total of 53 patients treated with KD for at least 1 year were enrolled. Patients who previously diagnosed as nutritional anemia and treated with iron, vitamin B12 or folic acid were excluded from the study. Complete blood counts, serum iron, total iron-binding capacity, transferrin saturation, ferritin, folic acid, B12 levels were measured at baseline and at post-treatment months 6, and 12. The changes in the parameters were analyzed with the repeated-measures ANOVA (post-hoc Bonferroni correction) or Friedman test, as appropriate. **Results:** 23 (43.4%) patients were females. The mean age of the group was 7.4 $\pm$ 4.4 years (2-18 years). There were no statistically significant differences between median serum iron (71, 70 and 71 μg/dL, respectively), median serum ferritin (30.6, 37.2 and 46.9 ng/mL, respectively), median B12 level (604, 576.5 and 627 pg/mL) and mean serum folic acid (17.1 $\pm$ 6.9, 20.2 $\pm$ 5.3 and 17.6 $\pm$ 6.6 ng/mL, respectively) levels of patients at baseline, post treatment 6th and 12th months ($P > .05$). Mean hematocrit (39.8 $\pm$ 3.4, 39.6 $\pm$ 2.96%) and hemoglobin (13.1 $\pm$ 1.2 and 13.2 $\pm$ 1.0 g/dL) levels in the 6th and 12th months of KD treatment were found to be higher than baseline levels (38.1 $\pm$ 3.3%; 12.6 $\pm$ 1.2 mg/dL), ($P < .0001$; P :.001). Also, median corpuscular volume (MCV) levels were higher in the 6th and 12th months of KD treatment (87.2; 87.3 fL) compare to initial level (84.1 fL) (P :0.001). Anemia was detected none of the patients and the erythrocyte transfusion was not performed during the KD treatment. **Conclusion:** In this study, improvement on hemoglobin, hematocrit, MCV levels in patient treated with KD was detected. Moreover, no side effect of KD on hematological parameters was revealed. Our study includes 1-year follow-up of results. Long-term studies are warranted to confirm our findings.

155 - Propionic Acidemia and Twin Pregnancy: A Case Study

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Background: Propionic acidemia (PA) is an autosomal recessively inherited metabolic disease. It is a rare disorder of 1: 50 000 to 1 in 100 000 births. It is caused by a deficiency in the enzyme propionyl CoA carboxylase. Propionyl CoA is produced in the metabolism of amino acids valine, isoleucine, threonine and methionine and odd chain fatty acids. Deficiency results in an accumulation of organic acids including propionic acid which are toxic to tissues. Patients risk decompensation, severe acidosis and hyperammonemia. In pregnancy, women need to be managed closely to prevent metabolic decompensation. **Case report:** A 36-year-old woman diagnosed at 9 months with (PA), under long-term metabolic follow-up conceived through IVF. Prior to pregnancy she had good metabolic control with a single decompensation in late childhood due to relaxation of her low protein diet. She had an episode of decompensation following embryo transfer as part of her IVF

procedure. Prior to pregnancy she weighed 59.3 kg and had a BMI of 21.7. Her management consisted of minimal dietary protein restriction and L-carnitine. **Results:** Dietetic treatment during pregnancy involved 40 calories per kg, a low protein diet providing 1.1 g/kg protein in the first, second and 1.2 g/kg protein in the third trimester. This included a medical protein supplement (free of valine, isoleucine, threonine and methionine). Additional vitamins and minerals, a glucose polymer and L-carnitine were also given. Total weight gain during pregnancy was 25.2 kg. Twin girls were delivered at 33 weeks gestation by caesarean section, during which she was given 10% dextrose iv. They weighed 1.55 kg and 1.34 kg. Dietary protein intake was slowly reintroduced over 2-week period postpartum. The iv dextrose continued until oral intake was sufficient. Her oral emergency regimen of glucose polymer, low protein diet and medical protein supplement were continued for 2 weeks postpartum. The patient subsequently returned to her pre-pregnancy low protein diet and medications. The twins tested negative for PA. **Discussion**: There is limited literature on the management and pregnancy outcomes for women with PA. Close monitoring by the multidisciplinary team is needed to ensure metabolic stability and normal growth and development of the fetus. This is the first reported case of twin pregnancy in PA.

156 - Do Amino Acid Medical Foods Negatively Impact Skeletal Health in Phenylketonuria?

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Background: Reduced bone mineral density (BMD) Z-scores and fractures are complications of treated phenylketonuria (PKU). A diet containing amino acids compared with glycomacropeptide reduces bone size and strength in wild type and PKU mice (PLoS ONE 7(9): e45165, 2012). Human PKU shows reduced bone strength in relation to muscle force and reduced cortical thickness using peripheral quantitative computed tomography (*J Inherit Metab Dis* 40:219-226, 2017). **Objective:** We tested the hypothesis that amino acid medical foods (AA-MF) provide a high dietary acid load, subsequently increasing urinary excretion of renal net acid, calcium, magnesium and sulfate compared to glycomacropeptide medical foods (GMP-MF). **Design:** In a crossover design, 8 subjects with PKU (16-35y) provided food records and 24-hr urine samples after consuming a low-Phe diet combined with AA-MF and GMP-MF. We analyzed amino acid and mineral content and calculated potential renal acid load (PRAL) of AA-MF and GMP-MF. BMD was determined using DXA (n = 15). **Results:** AA-MF provided 1.5-2.5-fold higher PRAL and resulted in 3-fold greater renal net acid excretion compared to GMP-MF ($P = .002$). Dietary protein, calcium, and magnesium intake were similar between treatments. GMP-MF significantly reduced urinary excretion of calcium by 40% ($P = .01$), and magnesium by 30% ($P = .029$). Urinary calcium excretion with AA-MF negatively correlated with L1-L4 BMD (g/cm^2; $P = .02$; $r = -0.79$). Consistent with significantly higher dietary intake of sulfur-containing Met and Cys with AA-MF compared to GMP-MF, urinary excretion of sulfate was higher with AA-MF ($P = .0008$). Protein intake from AA-MF was negatively correlated with total femur Z-scores ($P = .048$; $r = -0.58$). In conjunction with higher intake of AA-MF, males had lower BMD Z-scores for total body ($P = .01$), and total femur ($P = .08$) compared to females. Serum Vitamin D and markers of bone turnover were similar between treatments. Blood Phe concentrations were not correlated with urinary calcium or magnesium excretion. **Conclusion:** This is the first intervention study to assess the impact of medical foods differing in protein source and PRAL on skeletal health in PKU. Compared to GMP-MF, AA-MF increase dietary acid load, subsequently increasing urinary excretion of bone-related minerals, and likely negatively affecting skeletal health in individuals with PKU. J Nutr Metab 2017; https://www.hindawi.com/journals/jnme/ Funding, FDA R01-FD003711 & NIH.

157 - In Methylmalonic Acidemia and Propionic Acidemia Does the Removal of Precursor Free Amino Acid Supplementation Affect Long-Term Metabolic Stability, Plasma Amino Acid Status and Growth?

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Introduction: Use of precursor-free amino acids (PFAA) in propionic acidemia (PA) and methylmalonic acidemia (MMA) is controversial. Theoretically they improve protein status but biochemical and clinical signs of protein deficiency are documented. Furthermore, patients are at risk of hyperammonemia, which is associated with higher protein intakes. Recent guidelines suggest they should only be prescribed if natural protein intake is less than WHO/FAO/UNU 2007 safe levels of protein intake (SPI). **Aim:** To report the nutritional outcome, quantitative amino acid profiles and growth after discontinuing the use of PFAA for 12 months in children with PA/MMA. **Subjects:** 6 girls, 6 boys, [8 PA, 4 MMA] commenced PFAA at median age of 0.1 y (0.1-5 y) but they were discontinued at a median age 7.4 y (2-15.5 y) for 12 months' duration. All children were tube-feed dependent (gastrostomy n = 10, nasogastric n = 2) and on carnitine. One child received sodium benzoate associated with raised ammonia. **Results:** Natural protein (g/kg/day) intake improved without PFAA from a median (range) of 0.9 (0.5 -1.1) to 1.05 (0.8 -1.2) ($P = .002$); and subjects were more likely to meet SPI (compared with SPI:

natural protein intake with PFAA was a median [range] of 98% [55-126%] vs without PFAA, 114% [87-138%]). When using PFAA, 42% (n = 5) children failed to meet SPI for natural protein compared to 8% (n = 1) without PFAA. Plasma essential amino acids (except isoleucine) remained within reference range with and without PFAA. Almost two thirds of plasma isoleucine concentrations were below the lower reference range with and without PFAA. Median [range] isoleucine level with PFAA was 33 μmol/L [28-46] and without PFAA 37 μmol/L [24-100]. There was no change in energy intake or height and weight z scores (with PFAA; median [range] weight was 0.6 [−1.6 to 2.8] and height was −0.7 [−2.8 to 1], without PFAA weight was 0.9 [−0.9 to 2] and height was −0.7 [−2.9 to 1.2]). The number of hospital admission days with metabolic decompensations did not increase without PFAA and remained at 3 days (range 1 to 86) over 12 months. **Conclusions:** Removing PFAA improved natural protein intake, almost all children met SPI. Plasma isoleucine levels remained low and this was not explained by use of ammonia scavenging drugs. Growth and metabolic stability were unaffected. There appeared few advantages to using PFAA in MMA/PA and their exclusion simplified feeding plans.

158 - Dietary Management of Galactosemia With Galactose Restricted Diet

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Type I galactosemia is an inborn error resulting from mutations on both alleles of the *GALT* gene which leads to absence or deficiency of galactose-1-phosphate uridyltransferase (GALT). Galactose-1-phosphate accumulates within cells, and surplus galactose is reduced to galactitol or oxidized to galactonate. It is characterized in its early stages by hepatocellular damage (jaundice, hepatomegaly, and abnormal liver function tests), food intolerance (vomiting, diarrhea, and poor feeding) and failure to thrive. Patients with this condition have substantial motor, cognitive, and psychiatric impairments despite dietary treatment. Classical galactosemia is frequently associated with Q188 R, S135 L, and K285 N mutations and N314D is associated with Duarte galactosemia and is wide spread among various worldwide populations. The objectives of this study are to evaluate the presence of urinary galactitol to monitor the soy formula diet of patients with galactosemia. The present study aims to detect urinary galactitol by quantitative Benedict's test with galactose restricted diet based on soy formula. Unexpectedly, galactitol was found in the urine of all patients receiving a galactose-restricted diet (soy formula), and postulated that this sugar alcohol has its origin in part from endogenously produced galactose. There are still dilemmas about the management of galactosemia with galactose restricted diet and patients suffer from psychomotor abnormalities with hypogonadism in women irrespective of dietary management.

159 - Metabolomic Insights Into Lipid Metabolism in Phenylketonuria: Essential Fatty Acids, Cholesterol, Carnitine, and Choline

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Objective: We utilized RBC fatty acid profiles and metabolomics to assess lipid metabolism in adults with PKU consuming amino acid medical foods. **Design:** Fatty acid profiles in RBC membranes isolated from fasting blood samples were determined at Kennedy Krieger Institute; n = 25 PKU and 143 controls. Metabolomics analyses of fasting plasma samples were conducted by Metabolon Inc; 10 PKU (5 variant, 5 classical) and 15 controls. RBC fatty acid levels, %: **n-6 pathway**: Compared to controls, PKU subjects had significantly higher levels of linoleic acid (18:2n6), γ-linolenic (18:3n6), and dihomo-γ-linolenic acid (20:3n6) but similar levels of arachidonic acid (20:4n6). **n-3 pathway**: Compared to controls, PKU subjects showed elevation in α-linolenic acid (18:3n3), no change for docosapentaenoic acid (22:5n3), but significantly lower eicosapentaenoic acid (20:5n3) and docosahexaenoic acid (DHA, 22:6n3). DHA levels more than 1 SD below the mean control level were observed in 20 of 25 PKU subjects who consumed medical food without added DHA. Consistent with a pro-inflammatory phenotype and elevation in cytokines (IL-1β, IFN-γ, IL-6), the ratio of total n-3/n-6 fatty acids was lower. However, consistent with compensatory anti-inflammatory response, a lower ratio of 20:4n6/20:3n6 occurred in PKU vs controls ($P < .001$). **Metabolomics:** Phe metabolites were similar. Variants consumed more cholesterol than classical PKU subjects, (mg/d, Classical: 25, Variants: 72, $P = .02$), and had higher plasma cholesterol levels compared with classical PKU. Variant PKU showed higher plasma levels of 3-hydroxy-3-methylglutarate compared with classical PKU and controls. Regardless of carnitine intake, plasma levels of carnitine and acylcarnitines were similar in PKU subjects and controls. Deoxycarnitine, indicative of endogenous carnitine synthesis, was significantly lower in PKU subjects compared to controls. Despite low choline intake, plasma levels of choline and phosphatidylcholine species were similar in PKU subjects and controls. **Conclusions**: Supplementation with DHA is needed in PKU as adequate intake of 18:3n3 is not sufficient. Variant compared with classical PKU shows higher plasma cholesterol and its precursor, suggesting greater endogenous cholesterol synthesis. Carnitine

supplementation does not affect plasma carnitine levels, which is similar to controls, despite lower endogenous carnitine synthesis in PKU subjects. **Funding**: R01 FD003711

160 - Dietetic Management of Infants Screened Positive for Glutaric Aciduria Type 1 (GA1) on Expanded Newborn Screening (ENBS)—A single Centre's Experience

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Background: Treatment for patients diagnosed by ENBS with GA1 is: carnitine, low LYS, low TRP diet, LYS free, low TRP amino acid supplement (AAS), and emergency regimen. Low lysine diet is monitored by measuring quantitative plasma amino acids. The objective was to review plasma lysine results and dietary LYS intake of our ENBS cohort and compare to published UK and European guidelines. **Methods**: A retrospective review of patients diagnosed from 2012 to April 2017; n = 3 female, n = 2 males. Current age range is 13 m to 4 y 7 m. Data was collected on low LYS diet, plasma amino acids and growth. **Results**: At screening all were well, one premature infant in hospital. Diagnosis confirmed from 10-18d (median 13d) of age, confirmatory blood-spot glutaryl carnitine ranged: 0.28-4.31 μmol/L. Low LYS diet was commenced age 13-19 d (median 15 d). Plasma amino acids were measured 2-4 monthly at routine outpatient appointments, sample timings varied. From age at start of treatment to 6 m diet intake: natural protein 1.1 -1.7g/kg/d (median 1.6g/kg/d), lysine 88-153mg/kg/d (median 113mg/kg/d) from breast-feeds or infant formula and LYS-free, low TRP AAS 0.8-2.31 g/kg/d (median 1.7g/kg/d). Plasma LYS levels ranged 56-220 μmol/L (median 87 μmol/L). 8/14 results were below reference range 56-99 μmol/L (median 79 μmol/L). Each patient had at least one low plasma LYS. For 7/8 results LYS intake was above recommended intake of 100mg/kg/d and 4 of those above 114 mg/kg/d. From age 7-12 m diet intake was: natural protein 0.44 -1.65g/kg/d (median 1.32 g/kg/d), lysine 17.4-116.2mg/kg/d (median 77 mg/kg/d) and LYS-free, low TRP AAS 0.87 -1.7 g/kg/d (median 1.3 g/kg/d). Plasma LYS levels ranged 37-193 μmol/L (median 107 μmol/L). 5/11 results (three patients) were below reference range 37-96 μmol/L (median 54 μmol/L). For 4/11 results LYS intake was lower than recommended and 1/11 above recommended intake. All had normal growth. **Conclusion**: In the <6 m age-group, 7/14 and 7-12 m age group (1/11) of plasma LYS results were low, despite diet providing above recommended LYS intake. In the 7-12 m age-group, another 4/11 results were low but LYS intake was below recommended intake. Growth was normal despite some low LYS levels. Plasma LYS monitoring in the <6 m age-group is essential to guide adequate LYS intake and should be maintained within the normal reference range. Ideally sample timing should be standardized.

161 - Is Diagnosing Patients With Organic Acidurias and Aminoacidopathies Enough? Conundrums of a Low Middle Income Country

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Objective: To determine the factors responsible for nontreatment of inherited metabolic disorders (IMDs) requiring "Food for Special Medical Purposes" (FSMPs) in Pakistan. **Method:** A survey was conducted by Departments of Pediatrics & Child Health and Pathology & Lab Medicine, AKU from Jan 2013 to December 2014. Patients were categorized in to three groups, Group A: patients on FSMPs treatment, Group B: patients started on FSMPs but discontinued and Group C: patients not started on treatment. Patient diagnosed with diseases requiring FSMPs were contacted on telephone to collect details of treatment advised by the primary physicians and mortality assessed. **Results**: Total 86 patients were diagnosed with IMDs; 51% (n = 44) of these required FSMPs. Seven patients were excluded due to non-availability of information. Out of the total 37, 12 patients belonged to Group A of which 8 are alive [Methylmalonic Acidurias (MMA) n = 3, Maple Syrup Urine Disease (MSUD) n = 2 and one each of Urea Cycle Disorder (UCD), Cystathionine Beta Synthase Deficiency (CBS) and Isovaleric Acidemia (IVA)] and 4 have expired [MSUD n = 3, MMA n = 1]. Two patients belonged to Group B [both had Propionic Acidemia (PPA)], one of them was alive. Group C had 23 patients, out of which 18 are alive [CBS n = 4, Phenylketonuria n = 4, MMA n = 3, Glutaric Aciduria type 1 n = 3, UCD n = 2, one each of MSUD and IVA] and 5 have expired [UCD n = 2, one each of PPA, CBS, and MSUD]. Total 25 patients were in groups B and C. Non-affordability was the leading cause leading to non-treatment in 15 patients followed by lack of advice given by pediatricians in 8 patients. Out of the total 37 patients 21 were under the care of metabolic physician and 16 were followed by general pediatricians. Survival rate was higher in patients under care of metabolic physician as compared to general pediatricians; 85.7% and 56.2% respectively. **Conclusion**: In Pakistan health is mostly paid through out-of-pocket by patients. Non-affordability was the major factor observed causing both non-initiation and discontinuation of treatment. The FSMPs are imported in Pakistan under the same Harmonized System code as routine infant formulas, thus an accumulative tax of 67.7% is applied on this life-saving treatment required by the IMD patients. Advocacy at government level for the reduction of the tax on the FSMPs by the health care providers is critical for the sustainability of treatment for IMD patients needing FSMPs.

162 - Glycogen Storage Disease Type Ib. Nutritional Outcome Post Liver Transplant

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Background: Liver transplant is an effective treatment option for diet treated glycogen storage disease (GSD) type Ib. It improves metabolic control, normalizes fasting tolerance although neutropenia persists. There is little nutritional information about children with GSD type Ib post liver transplant. We analyzed the nutritional outcome in 6 children with GSD type Ib pre-and post-transplant. **Methods:** A retrospective, longitudinal study examined survival, complications, nutrition, feeding methods, feeding problems and anthropometry in 2 boys and 4 girls all British Pakistani ethnicity. **Results:** The median transplant age was 4.5 years (3-7 years) with a median follow-up post-transplant of 3.6 years (1-9 years). Although 100% survived the transplant procedure, complications occurred and included: perianal abscess (n = 1), respiratory infections (n = 2), and rejection (n = 2). Pre-transplant, all children were tube-fed dependent with a negligible oral intake. Post-transplant, the median time to cessation of tube feeding was 6 months (1 month to 9 months) and one child remained on tube feeds. 50% (n = 3) required nutritional supplementation. Post-transplant, feeding difficulties included retching, inability to self-feed, diarrhea, and food refusal and were a continuation of problems pre-transplant. No children required emergency feeds post-liver transplant. Faltering growth occurred post-transplant. Pre-transplant, the mean z-score for BMI was 1.33 (SDS ±1.28); for height -1.38 (SDS ±0.89); and for weight 0.13, (SDS ±1.49). 12 months' post-transplant, the mean z-score for BMI was 0.53 (SDS ±1.33); for height -1.18 (SDS ±0.53); and for weight -0.28, (SDS ±0.99). Current assessment (median 3.6 years), mean BMI z-score was 0.53 (SDS ±0.43); height -1.45 (SDS ±1.1); weight -0.48 (SDS ±0.59). **Conclusion:** Post-transplant, GSD Type 1b children were prescribed a normal diet without emergency feeds but poor growth persisted. Poor nutritional status and abnormal feeding patterns were prevalent. Facilitating a successful transition onto a normal diet, ensuring adequate nutritional intake and preventing faltering growth caused parental anxiety. Regular long-term nutritional support from a multidisciplinary team with knowledge of IMD is essential to support the transition from tube feeding to oral feeding post transplantation and should be an integral part of all post-transplant care plans.

163 - Maternal Depression and Anxiety of Patients Under Restricted Diet and Having Risk of Metabolic Decompensation

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Objective: Diet restriction and coma attacks constitute substantial risk factors for psychological disorders of the parents. The aim of this study was to investigate the presence of depression and/or anxiety in mothers of patients who have different disorders of inborn errors of metabolism (IEM) under treatment of special restrictive diet and who have risk of metabolic decompensation. **Methods:** This cross-sectional study was conducted between December 2016 and April 2017. Mothers of 83 patients with disorders of IEM who were receiving restrictive diet and 38 healthy controls completed the self-filled questionnaires. In patient group, 38 children had disorders having high risk of encephalopathy or metabolic decompensation (urea cycle disorders, organic acidurias, maple syrup urine disease, hereditary fructose intolerance and fatty acid oxidation disorder) (group 1). Remaining 45 patients were having disorders of IEM treated with restricted diet but having no risk of metabolic decompensation (phenylketonuria, galactosemia and nonketotic hyperglycinemia) (group 2). Beck Depression Inventory (BDI) and State-Trait Anxiety Inventory (STAI) S and STAI-T were used to assess the maternal depression and anxiety. Data were expressed as median [25-75 percentiles]. **Results:** There were no significant differences between the groups regarding the children's age and gender, and mother's age and the educational level. Depression and anxiety scores of mothers of group 1 and group 2 were (group 1: BDI: 17 [11-26], STAI-S: 44.5 [33-54], STAI-T: 49 [41-52] and group 2: BDI: 12 [7-19], STAI-S: 41 [36-45], STAI-T: 45 [40-49], respectively) significantly higher than healthy controls (BDI: 5 [3-8], STAI-S: 34 [27-40], STAI-T: 39 [35-44]) ($P < .05$). Moreover, BDI values were significantly higher in mothers of group 1 than group 2 ($P = .036$). On the other hand, STAI-T and STAI-S values were not significantly different between the two patient groups. Two mothers in group 1 were taking antidepressant medication for treating their depression. **Discussion:** High prevalence of depression and anxiety in mothers of children receiving restricted diet and having risk of metabolic decompensation could affect the quality of caring. Therefore, better outcomes in the progress of the disorder needs health professional's special attention in reducing the psychological distress of the mothers.

164 - The Effect of Home Visiting to the Ketogenic Diet Compliance in Multi-Drug Resistant Epilepsy

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Objective: There is growing evidence that ketone bodies derived from fatty acid oxidation and produced during fasting or consumption of high-fat diets can exert broad neuroprotective effects. Therefore, ketogenic diet which include high fat, adequate protein, and restricted carbohydrates can be an effective treatment option in refractory seizures. The aim of this therapy is to stop seizures or decrease the use of antiepileptic medicines. But sometimes the patients and their families don't follow this diet modification well. Therefore, we experimented home-visiting to increase the compliance to the diet. **Method:** Eight patients, who received ketogenic diet because of drug-resistant epilepsy, were included in this study. Home visiting was started at following week of the ketogenic diet, and made by a nutrition nurse and a dietitian. These visits were repeated once a month, and the patients were called once a week. The mistakes were corrected during these visits. Statistical analyses were made by SPSS 19 version. **Result:** We found positive correlation between the diet applying performance of the mothers and the diet compliance of the children ($P = .033$). Five patients experienced reduction in seizure frequency. One patient became seizure-free. There was no positive feedback for the diet in a patient due to noncompliance. There were no changes in electroencephalography in a patient who has not have seizures. **Conclusion:** The noncompliance to the ketogenic diet of the patients can be decreased by the collaboration and follow-up at frequent intervals.

165 - Nutritional Status and Protein Energy Ratio in Patients with Phenylketonuria

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Objective: To analyze the relation between protein to energy (P: E) ratio and nutritional status in patients with phenylketonuria (PKU). **Methods:** Retrospective analysis of 45 PKU patients. Assessment of calorie and protein content was performed using a 24-hour dietary recall method and analyzed using the Metabolic Pro program®. P: E ratio was calculated for each dietary recall of every patient and expressed as gram protein/100 kcal. Anthropometric measurements (weight, height) were obtained and BMI was converted to Z score, patients were stratified into 3 groups: undernourished (< -1), normal (-1 to 1) and overweight (> 1). A correlation analysis was performed. **Results:** From the 45 patients 467 dietary recalls were collected (minimum 1, maximum 36 per patient, 10.3 media) and paired with BMI Z score. Analyzing the P: E ratio in each group we found that in the normal group 91.2% (291/323) had a ratio of 2-4 g of protein/100 kcal, meanwhile only 1.5%(5/323) had a P: E ratio > 4. On the overweight group 45% (33/74) had a P: E ratio above 4, and just one patient in this group had a ratio below 2. On the undernourished group 21% (15/70) had a P: E ratio below 2, and 12/70 (17%) above 4. No correlation was found between BMI Z score and P: E ratio, only a slightly positive tendency ($R^2 = 0.069$) between these two factors. **Conclusion:** Most of the patients (91.2%) with normal BMI Z Score, had a P: E ratio between 2-4. No clear relation with P: E ratio was found on the undernourished group. On the other hand, on the overweight group almost half had a ratio above 4. These results suggest that not only protein intake should be calculated but also its relation with energy, because it appears to be related with nutritional status. Diets for PKU patients need to be well balanced in protein and energy to maintain an adequate BMI Z score.

166 - Does the Additional Phenylalanine in GMP-AA Protein Substitute Lead to Destabilization of Blood Phenylananine Concentrations Compared to Conventional Amino Acid Protein Substitutes?

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Introduction: Caseinoglycomacropeptide (CGMP) is an alternative protein substitute to conventional Phe-free L-amino acids (L-AA) in PKU. Commercial CGMP (GMP-AA) developed for PKU is based on a low phenylalanine (Phe) peptide, supplemented with tyrosine (tyr), tryptophan, histidine, leucine and arginine. It is associated with improved protein utilization, bone density, and better taste. However, it contains a residual amount of Phe (36 mg per 20 g protein equivalent), and its long-term efficacy has not been demonstrated in children. **Aim:** To study the long-term effect of GMP-AA vs L-AA on blood Phe/Tyr control in children with PKU over 24 months' duration. **Methods:** In a longitudinal, parallel study, 36 children with PKU, with a median age 9.2 y (5.1-16.9 y; 21 boys 15 girls) were recruited. 25 were on GMP-AA and 11 L-AA. Median protein equivalent in both groups was 60 g/d (50-80 g) and the total protein equivalent provided by GMP-AA was a median of 75% (33-100). Median prescribed natural protein

intake was 5 g/d (3-30). In the GMP-AA group, there was no adjustment to compensate for the GMP-AA Phe content (it provided an additional median Phe intake of 81mg/day [36-108 mg]). Weekly blood Phe concentrations were recorded over 12 months (n = 36) and 24 months (n = 20). Children were divided into 2 age-groups: 5-12 y (n = 29) and 13-18 y (n = 11) (4 children crossed age-groups). **Results:** For children ≤12 y, studied for 12 m, median blood Phe (range) concentrations were: GMP-AA group (n = 23), 285 (160-450) μmol/L and in the L-AA group (n = 6), 280 (200-465) μmol/L. At 24 m, in the GMP-AA group (n = 8), blood Phe was 285 (200-345) μmol/L and L-AA group, (n = 4) was 370 (240-560]. In patients aged ≥13y, studied for 12 m, in the GMP-AA group (n = 2), blood Phe was 412 (340-485) μmol/L and L-AA group (n = 5) was 435 (360-600) μmol/L. At 24 m follow-up, in the GMP-AA group (n = 4), blood Phe was 430 (300-930) μmol/L and in the L-AA group (n = 4) Phe was 428 (270-720) μmol/L. No significance difference was found between or within groups for Phe or Tyr concentrations. **Conclusions.** Blood Phe and Tyr concentrations remained stable when a median of 75% of total protein equivalent intake was given as GMP-AA over the 24-m study. No dietary adjustments were made for the additional Phe from GMP-AA. In PKU, long term prospective data on the use of GMP-AA when it is given as the sole source of protein substitute is still necessary in large groups of children to study impact on blood Phe control.

167 - Low Protein Mobile and Web-Based Diet Applications: What's Out There?

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Background and objective: Mobile apps are an important educational resource in IMD and are increasingly being developed for low protein disorders, commonly PKU. Many of the problems in acquiring instant access to the timely protein content and suitability of foods for inclusion in a low protein diet could be overcome by access to a mobile low protein diet app available on mobile phones, tablets and computers. Our objective was to review the availability and suitability of apps for IMD low protein diets. **Methods:** An online search was conducted in September 2016 on Apple iTunes (iOS) and Google Play (Android) for currently available apps suitable for protein metabolic disorders, using the search terms "low protein diet", phenylketonuria, PKU, and "metabolic diet". **Results:** 22 apps were identified (18 PKU specific, 4 for a range of conditions) from 11 countries (10 USA, 2 Netherlands, 2 Italy, and 1 UK, Canada, Germany, France, Spain, Turkey, Chile, Slovenia). Only 4 were developed by health professionals/organizations, the remainder by commercial companies (who produce dietary products) (n = 5), parents/patients with the condition (n = 3) or independent/unknown individuals. 5 were available in >1 language. 18 were available free of charge, one required a subscription to fund the national PKU service, and 3 varied in price from £0.79-£4.99. Only 5 were available on both iOS and Android although 1 was not accessible on the UK App store and 3 were web-based requiring internet access to function. Food databases tend to reflect local diets and are less suitable internationally due to differing guidelines for allocation of dietary protein. The majority focused on protein calculators (n = 16), low protein recipes (n = 7) and monitoring intake/blood results (n = 5) rather than the suitability of foods for inclusion in the diet. None were inclusively: developed by experts, available on both iOS an Android, free, and able to answer user questions on food suitability. **Conclusion:** Few available metabolic diet apps are developed by clinical dietitians, based on scientific expert consensus, are free from commercial bias/conflicts of interest and regulated in terms of content. It is important that clinical IMD dietitians work together to produce national apps that can be used as a resource by patients/caregivers based on consistent, unbiased information reflecting national and international professional guidelines.

168 - UK BIMDG Dietetic Consensus Guidelines on Low Protein Labeling Interpretation and Food Allocation for Patients and Caregivers With PKU

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Background/objective: In PKU, dietitians do not always agree on the interpretation of how different protein containing foods should be allocated in a low phenylalanine (PHE) diet. This has led to variable advice being given to patients/caregivers, causing confusion exacerbated by recent labelling legislation changes. The UK BIMDG (British Inherited Metabolic Disease Group) dietitians, using DELPHI consensus, developed and systematically reviewed a series of 16 written guidelines to help with interpreting food labels and determining how to incorporate certain food groups into a low PHE diet. **Methods:** In November 2015, BIMDG dietitians (n = 70) were asked to complete a multiple-choice questionnaire about allocation of food items and interpretation of food label protein analysis for 14 food products. 49 dietitians (70%) responded and based on majority answers, structured guidelines were developed for interpreting protein from food labels and allocating fruit and vegetables in PKU. Over 18-months, using Delphi methodology, the guidelines were reviewed, refined and adapted with facilitator documenting discussions until a final, clear majority was attained for each statement. **Results:** Guidelines covered controversial dietary topics: terminology for exchange-free foods; a practical "scale" for guiding precision of protein

counting from nutritional content on food labels; definition of exchange-free foods in general and specifically for fruits and vegetables, spices, fats/oils, soya sauce, cooking sauces, sweets, and vegetable crisps. Dietetic responses were divided into pediatric and adult groups. After the first discussions, there was majority consensus (≥88%) by pediatric dietitians (n = 29 respondents) for 14/16 guidelines. For 2 remaining guidelines (soya sauce and fruit/vegetables with PHE content between 76-100 mg/100 g), a further 2 structured discussions were required, with a final majority consensus of 72% (n = 26/36) agreement for the soya sauce and 64% (n = 16/25) for the fruit and vegetables guidelines. 75% of adult dietitians agreed with the guidelines but 48% suggested separate maternal PKU guidelines were required. The overall guidelines were endorsed by the UK NSPKU. Conclusions: The development of consensus dietetic guidelines should help harmonize advice given to patients with PKU across the UK. It is important to monitor acceptance and adherence to these guidelines by dietitians and patients and their families. *On behalf of contributing BIMDG dietitians

169 - Dietary Management of Early Onset Lysosomal Acid Lipase Deficiency (LAL-D/Wolman Disease): A Case Report

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Introduction: Wolman disease (early onset LAL-D), presents in infancy with persistent vomiting, diarrhea, hepatosplenomegaly, adrenal calcification and liver failure. There are few cases reported and information is limited about dietary treatment on enzyme replacement therapy (ERT). **Aim:** To report the dietary management of a 2.5 y old girl with Wolman disease treated with ERT. **Case report:** A full-term, exclusively breast-fed, female infant, of Egyptian origin with consanguineous parents, was diagnosed with Wolman disease at 20 d of age. She presented with fat malabsorption, bile stained vomiting, diarrhea, abdominal distention, and faltering growth (z score: weight -5.14; length - 2.5). ERT (recombinant human lysosomal acid lipase) was started at age 2 m. She commenced a formula feed containing fat with 84% medium chain triglycerides (MCT) and 16% long chain triglycerides (LCT) via a nasogastric tube (NGT). The vomiting and diarrhea improved. By 1 y of age, her weight improved (z score: weight -1.23; length 2.23) while the mid upper arm circumference (MUAC) z score was -3.5. As the enzyme appeared to be working in the liver and spleen, she was gradually liberalized to a normal fat diet with no NGT feeds. However, she developed persistent diarrhea and her growth deteriorated. Z score for weight was -3.44 and height -2.69 within 3 m of starting a normal diet. At age 18 m, she started a modular MCT feed using hydrolyzed whey as the protein source and low-fat meals (providing 1.5 g fat/100 g meal). Weight z score (-2.4) improved and stool losses reduced. At age 21 m, she remained on a low-fat diet with bolus MCT formula feeds (16% LCT, 84% MCT providing 73% of energy requirements). At age 2 y, she deteriorated clinically with increased diarrhea and faltering growth (z score: weight -0.8; height -2.91). A modular MCT feed was prescribed (1.3 g fat/kg/d, LCT <1%) based on complete amino acid mixture (3 g/kg/d protein), additional CHO, vitamins, minerals and added essential fatty acids. Diarrhea resolved and growth improved (MUAC z score -0.05, weight z score -0.06; height z score -1.99). **Conclusions:** This case illustrates that intensive dietary management with a MCT based modular feed and severe restriction of LCT helps maintain growth and ameliorate gastrointestinal symptoms. More long-term case studies are required to improve the understanding of the intestinal malabsorption and enteropathy in Wolman disease and enable consensus dietary management guidelines.

170 - Growth of Children With Phenylketonuria Taking a Semi-Solid Weaning Protein Substitute is Comparable to Control Children in the First 2 Years of Life

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Objective: Infants with PKU from weaning age usually require additional protein equivalent from a phenylalanine (Phe)-free protein substitute to meet protein requirements. In the UK, practice is to introduce a Phe-free weaning protein substitute (WPS) as a semi-solid consistency and administer it from a spoon. 5 g of protein substitute powder usually contains 2 g protein equivalent but only 16 kcals. The amount of WPS is gradually increased and volume of Phe-free infant formula reduced. There is concern that the low energy density of WPS will not compensate for the energy content of infant protein substitute and this may impact on growth and weight gain. In a longitudinal, prospective, controlled study, the growth of infants with PKU weaning on a WPS (containing protein equivalent 2g/5 g powder) was compared with control infants. **Methods:** 20 PKU (14 male) and 20 control (12 male) infants matched for mother's educational level and birth order were reviewed monthly from weaning commencement to 12 m of age, then at 15 m, 18 m, and 24 m. Body weight, length, and BMI were recorded at each review and z-scores calculated. **Results**: Median age for diagnosis of PKU was 10 days (range: 2-16 days). Median age of weaning was 4.4 m (range: 3.2-6.6

m) for PKU and 5.1 m (range: 3.7-6.5 m) for controls. Prescribed total protein intake was 3g/kg/day and median natural protein intake was 5g/day. There was no significant difference between PKU and control group for median z-scores for all growth parameters with both groups within normal range at all ages. Median z-scores for weight at weaning, 12 m and 24 m were: PKU group, 0.29, 0.37, and 0.45; and control group, 0.08, 0.52, and 0.61; length: PKU group, 0.98, 0.89, and 0.52; and control group 0.17, 0.33, and 0.65; and BMI: PKU group, −0.98, −0.37, and 0.19; and control group, 0.13, 0.35, and 0.35. No children with PKU had z-scores below -2 at 24 m of age. There were some gender differences. Boys with PKU had lower median BMI z-scores than control boys; girls with PKU had length z-scores higher than control girls; and girls with PKU had higher weight and BMI z-scores than boys with PKU. **Conclusion**: Despite a low Phe diet and Phe-free protein substitutes, children with PKU taking a low-energy WPS had comparable growth patterns to control children during their early years with all growth parameters within normal range for age. There is a trend for boys with PKU to grow at a slower rate than control boys and girls with PKU.

171 - Dietary Management of a Premature Infant With Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCADD)

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Introduction: Medium Chain Acyl CoA Dehydrogenase deficiency (MCADD) is an autosomal recessive disorder of fatty acid β-oxidation (FAO). Preterm formulas have higher amounts of medium chain triglycerides (MCT) (containing 18% fat as MCT) to promote growth and aid fatty acid utilization. Although in MCADD there are guidelines for the dietary management of term babies, no consensus on dietary advice exists for the management of preterm infants who receive a higher MCT intake from breast milk or preterm formula than term infants. **Aim:** To report the dietary management of a low birth weight preterm baby diagnosed with MCADD. **Case report:** A preterm baby boy was induced at 33 weeks gestation (birth weight 1.22 kg; z score: weight: -2.0; length -3.4) because of poor fetal growth. Due to a previous sibling death he was tested on day 2 of life and diagnosed on day 5. Initially he was given a preterm formula (18% fat as MCT) and maternal expressed breast milk (EBM) (containing 14% fat as MCT). He was fed orally via a nasogastric tube every 2 hours. On day 5, the pre-term formula was stopped due to concerns about the MCT content of the preterm formula and he was given a standard infant formula (10% fat as MCT) and EBM. His feeding plan consisted of 50% breast milk and 50% standard infant formula. Weight gain at 37 weeks gestational age was poor (weight z-score -2.9). At 41 weeks gestational age, he was discharged from hospital with no other clinical concerns and was transferred to a high energy infant formula (12% fat as MCT). He stayed on this regimen exclusively for 2 weeks whilst his weight gain showed some improvement (weight z-score -2.3). Our case remained well despite the temporary use of 12% MCT feed and frequent change of feeds. At 43 weeks gestational age, he remained on standard infant formula and was weaned at 6 months of age. Long term follow-up at 4 years of age, showed his height was on the 0.4th centile (z-scores weight -3.00, height -2.54). **Conclusions:** The safety of using a formula with higher concentrations of MCT fat in preterm infants with MCADD is unknown. This preterm infant was managed on a combination of standard infant formula and breast milk, and although he remained well, his growth was an issue. Collaborative reports of experience from multiple centers are required to describe the dietary management of preterm infants with MCADD. Addressing this issue within the existing guidelines for MCADD would be helpful.

172 - Help! My Home is Making Me Sick

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Background: In inherited metabolic disorders (IMD), poor socio-economic and housing conditions may affect caregiver ability to adhere to treatments and potentially lead to poor metabolic control although this has not been systematically studied. Here we present two case studies; one child with propionic acidemia (PA) and one child with maple syrup urine disease (MSUD) both with housing and social issues where a IMD support worker advised and assisted families with socioeconomic issues. **Case report 1:** A 3 y girl with PA, of Arabic origin, from poor socioeconomic status had a history of recurrent metabolic decompensations and subsequent hospital admissions. Both parents spoke limited English. The support worker inspected and evaluated the home and suggested this may be a likely trigger for recurrent illness. There were long term social and housing issues. Due to the damp, cramped and overall unsuitable conditions, the home was deemed unsafe for the child to return. The support worker made short term arrangements to re-house the family while long term solutions were pursued. Multi-disciplinary meetings were arranged to resolve the housing issues; these meetings were attended by both medical and the IMD support worker. A suitable home was found resulting in better treatment and a recent liver transplant. **Case report 2:** A 1 y girl with MSUD, established on dietary treatment attended the emergency department with vomiting. The family, of Pakistani origin, not only had long-term housing issues but also the parents were subject to immigration control. The IMD support worker inspected and

evaluated home conditions and it was concluded that unsatisfactory and unsanitary communal living conditions were the trigger for this acute episode. It was decided that on medical grounds this vulnerable girl could not return to the overcrowded communal setting and a permanent solution was required. Working with the local housing teams and the UK 'Home Office' the family was re-housed in suitable sanitary conditions and given long term stay in the UK. **Conclusions**: In both cases, we found that a pro-active approach was vital and the IMD support worker was critical for a successful outcome. The IMD support worker helped to educate and communicate with multi-disciplinary agencies about the seriousness of IMD conditions and the need for immediate action. Taking time to help resolve social and housing has long term clinical benefits.

173 - 3 Month Audit Data of 24 Hour Dietetic On-Call Service in Inherited Metabolic Disease

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Background: Children with some inherited metabolic disorders (IMD) are at risk of acute metabolic decompensation unless a timely and effective emergency regimen is commenced and diligently supervised. Dietary modification plays a key role in helping prevent metabolic decompensation and possibly avoid hospital admissions. For many years, we have provided an out of hours' dietetic service to support IMD families in a pediatric IMD service, but this has not been formally evaluated. The necessity for this service may be questioned. **Aim**: To audit the frequency of usage and characterize the type of calls received in a 24 hour on-call metabolic dietetic service. **Methods**: All calls received out of standard working hours were recorded on a database. The Dietitian in receipt of the call recorded the time, purpose and the outcome of the calls between October to December 2016. **Results**: A total of 104 calls were received for 92 days and were equally distributed between the 3 months. Almost half of the calls (n = 47, 45%) were related to poor feed tolerance with vomiting and diarrhea or poor/inadequate feeding. Caregiver queries to check overnight feeds/following days feeding plan (n = 20, 19%) were the second highest number of calls. Hospital queries from evening/night shift nurses related to inpatient enteral feeding plans occurred almost weekly (n = 11, 11%). Telephone calls about poor feeding (n = 8, 8%) were predominantly related to children who were orally fed and had a fasting intolerance. The remaining calls requested information only or required sign posting to another service. Of the 87 (84%) calls requiring immediate dietetic action, the trained IMD Dietitian resolved 89% (n = 77) of the problems satisfactorily be telephone advice. Of the (n = 47) calls received for poor feed tolerance or poor feeding, 37 (79%) of patients were maintained safely at home by using dietary management, typically with the emergency feeding plan, the remaining calls were not for immediate action. At all times, Dietitians liaised with IMD physicians of ongoing issues with patients and no adverse events because of this service were reported. **Conclusion**: The IMD "out of hours" dietetic service was commonly used by caregivers and hospital professionals. It safely prevented many hospital admissions in children with less severe intercurrent illnesses with feed intolerance or poor feeding. Such a service should be conducted closely with physicians.

174 - International Practices in the Dietary Management of Fructose 1-6 Biphosphatase Deficiency

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Background: The dietary management of fructose 1,6 bisphosphatase (FBPase) deficiency aims to prevent hypoglycemia and lactic acidosis by controlling intake of fructose and sucrose and avoiding prolonged fasting. It is unclear if dietary strategies such as the use of uncooked corn starch (UCCS) are effective. **Objectives:** To assess international differences in dietary management of FBPase deficiency. **Methods:** A cross-sectional web-based questionnaire (13 questions) was sent to all members of the SSIEM. **Results:** 36 centers (15 countries) reported the dietary management of 126 patients with FBPase deficiency. Patients' age at questionnaire completion was: 1-10y, 45% (n = 57), 11-16y, 22% (n = 28), and >16y, 33% (n = 41). Diagnostic age was: <1 y, 34% (n = 43); 1-10y, 59% (n = 74); 11-16y, 3% (n = 4); and >16y, 4% (n = 5). 25% (n = 9) of centers (67% [n = 6] from Northern Europe), did not advocate dietary restrictions when patients were well. However, in the other 27 centers (75%), more adult patients were prescribed dietary restrictions (age 1-10 y, 67% (n = 38/57), 11-16 y, 68% (n = 17/25) and >16 y, 85% (n = 35/41). Dietary restrictions were: sugary food avoidance only (n = 7 centers, 19%); fruit, vegetable and sweet restrictions (n = 13, 36%); and restricted sweets and fruit only (n = 4, 11%). A further 3 centers gave individualized dietary advice. Only 3 centers quantified fructose restriction. The patients reported to have normal fasting tolerance increased with age, from 30% (1-10y), to 36% (11-16y), and 58% (>16y). A total of 20 centers (56%) routinely provided UCCS for overnight fasting in 47 patients (37%). The median amount of UCCS prescribed by age category was 1.3g/kg/dose (n = 30) in 1-10y; 1.3 g/kg/dose (n = 7) 11-16y; and 1 g/kg/dose (n = 10) in >16y with an overall median dose of 1 g/kg/dose regardless of fasting tolerance. In patients given UCCS (data available in 36

patients), 13 (36%) had a fasting tolerance <10 h and 23 (64%) had a fasting tolerance >10 h. All centers advocated an emergency regimen mainly based on glucose polymer for illness management. **Conclusions:** When well, dietary advice varied widely from normal diet to low sucrose and fructose diets, with almost all adults on dietary restriction. UCCS was used by some centers, even when fasting tolerance exceeded 10 h. International guidelines are necessary to help direct dietary management in this condition.

175 - Dietary Management of Maternal Phenylketonuria With Glycomacropeptide: A Case Report

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Background: In maternal PKU, the traditional protein substitutes (PS) are provided by phenylalanine (PHE)-free L-amino acids (AA), but glycomacropeptide-based protein substitute (GMP) is an alternative consideration. **Objective:** To describe the first Portuguese Maternal Phenylketonuria (MPKU) managed with GMP. **Case report:** A 31-year-old MPKU female with classical PKU (mutations P281 L / P281 L), diagnosed by newborn screening, with a lifelong history of poor metabolic control. She has a history of partial bicornuate uterus and she had a previous miscarriage in the first trimester. Preconception, her median blood PHE was 7.7 mg/dL but throughout pregnancy the median reduced to 4.3 mg/dL. GMP provided 30 g/day protein equivalent (46 mg/day PHE). Total protein equivalent from PS increased from 58 to 86 g/day during pregnancy but AA provided all additional protein equivalent intake. Both GMP and AA were well tolerated with no morning sickness. Normal morphologic evaluation and adequate fetal growth with cephalic biometry near the 5th percentile was determined. Maternal weight gain during pregnancy was normal (15 kg). The infant was born at 39.3 weeks with 2570 g (3rd percentile), with a length of 47.5 cm (10th percentile) and a head circumference (HC) of 31.5 cm (1st percentile). In the neonatal period, the infant had craniofacial dimorphism with metopic suture prominence. Father also had bitemporal narrowing. At 3 months HC was on the 50th percentile. By 12 months of age, the infant's weight (15th percentile), length (50th percentile) and HC (50th percentile) were normal although bitemporal narrowing persisted. **Conclusions:** This is the first Portuguese case reporting the use of GMP in MPKU. Its PHE content did not adversely affect control although it only provided part of the PS intake. Some intrauterine development delay occurred in the last trimester, although we do not think this was related to MPKU syndrome. GMP's nutritional composition is different from conventional AA, and no data is available on the effect of different amino acid profiling in MPKU. More data is essential to examine the impact of using GMP on morning sickness severity and aversion, maternal weight gain, blood amino acid concentrations and variability of blood PHE concentrations.

176 - Dietary Management in Pyridoxine-Dependent Epilepsy (PDE) due to Antiquitin Deficiency: A Single Center Experience

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Objective: Despite treatment with pyridoxine, 75-80% of PDE patients have developmental delay. PDE consortium guidelines (www.PDE.online.org) recommend a trial of restricted lysine diet (RLD) to investigate if a reduction of lysine intermediates improves outcome. Our objective was to describe implementing RLD in patients of different ages, their biochemistry and clinical outcomes. **Methods**: Prospective longitudinal data was collected on genetically confirmed PDE patients from commencement of RLD (2015-2017) including: pyridoxine dose, growth, developmental assessments, and biochemistry. Dietary data was compared to PDE consortium guidelines and biochemical parameters to age-dependent reference ranges. **Results**: Fourteen patients (10 ethnic backgrounds) from 12 families were identified. Ten commenced RLD, age range 6 wks to 13.8y (4 < 6 m, 3 > 13 y), median time on diet 4 m, range 2 wks to 20 m. Two were yet to start diet and two opted for arginine treatment. On commencing RLD seizures were well controlled on pyridoxine. All <1y (n = 4) had normal developmental assessments and all others >2y (n = 6) had FSIQ below average (range 45-55) and uneven developmental profiles. Pre-RLD dietary lysine intake exceeded PDE guidelines by 130-312%, median 190%. Pre-RLD plasma lysine and tryptophan (n = 10/10) and arginine (n = 9/10) were in normal reference range and urine α-AASA concentration elevated in all (range 9-287 mmol α-AASA/mol Cr). RLD was taught as a series of education sessions. Diet comprised lysine intake and lysine free, tryptophan low l-amino acid (LFAA) supplement equating to age based PDE guidelines. Lysine was provided by protein exchanges: cereals/vegetables 1 g protein = 40 mg lysine (67%-75% intake), dairy products 1 g protein = 70 mg lysine (25%-33% intake). Compliance with LFAA supplements was good. Manufactured low protein foods and milk substitutes were used by most (n = 9). LFAA supplement and diet

provided vitamins and minerals. Growth was normal (n = 7) or overweight (n = 3) and on RLD continued to track the same. Urine α-AASA reduced by 24-92% once on RLD but remained above upper limit of normal. Plasma lysine decreased in all, and if below normal reference range dietary lysine was increased. **Conclusion**: RLD with LFAA supplements was well tolerated, safe and achievable even when commenced beyond infancy. Reduction in urine α-AASA and plasma lysine was observed in all patients. Further assessment of neurodevelopmental progress is required to show long term benefit.

177 - Ensuring Optimal Nutritional Management for Adult Inpatients With Inborn Errors of Metabolism

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Background: For unwell patients with Inborn Errors of Metabolism (IEM), successful recovery depends on prompt treatment using medications and individualized diet therapy aimed at metabolic stabilization.[1] Current best practice recommendations indicate that at hospital admission, IEM patients should be referred urgently to a metabolic team, including a metabolic dietitian.[2,3] This project aims to ensure accurate nutritional management and reduce clinical incidents for IEM patients during hospital admission. **Methods:** A retrospective chart audit was conducted to investigate referral to the metabolic dietitian and adequate nutrition provision during admission. A literature review on referral processes for metabolic dietitians and foodservice provision for IEM inpatients was undertaken. Benchmarking surveys were disseminated across metabolic centers in Australian and New Zealand to identify hospital processes for dietitian referrals, meals and formula provision. The outcomes of the literature review and benchmarking surveys translated into recommendations to improve patient care at Mater Group Brisbane. An electronic clinical alert was implemented for all IEM patients urging referral to the metabolic team upon admission. Metabolic sick day nutrition plans were implemented via an "alerts" tab in the patients' electronic record, providing recommendations for diet therapy and formula recipes. **Results:** No best practice was identified for referral pathways or food provision for IEM during hospital admissions. Both the literature and benchmarking recommended the need for urgent referral to the metabolic team. Results from a 6-month retrospective chart audit identified that 47% of IEM inpatients were not referred for dietetic assessment or received unsuitable meals and formula. After implementation of the electronic clinical alert, 100% of IEM inpatients were assessed by the metabolic dietitian within 24 hours of admission or the next working day. Sick day nutrition plans and metabolic formula recipes are being accessed after hours. This has reduced the number of associated nutrition related clinical incidents affecting this patient group. **Conclusion:** Implementation of an electronic clinical alert for IEM inpatients is effective in notifying the metabolic dietitian of IEM admissions and ensuring accurate nutritional management. Electronic alerts for urgent referral to metabolic teams can improve nutritional management in metabolic specialist centers across the world.

178 - Retrospective Audit of the Dietary Management of Current Patients with Glutaric Aciduria Type 1 at a Single Metabolic Center

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Glutaric Aciduria type 1 (GA1) (glutaryl-CoA dehydrogenase deficiency) affects the catabolism of L-lysine, L-hydroxylysine and L-tryptophan. Untreated individuals typically experience acute encephalopathic crisis during the first 6 years of life. In line with SSIEM guidelines, dietary management of GA1 at the Genetic Metabolic Disorders Service (GMDS) Sydney includes regular review, aggressive emergency treatment, and a lysine restricted diet supplemented with lysine-free, tryptophan-reduced amino acid mixture (AAM) and L-carnitine. Emergency and diet plans are individualized. Protein counting or limiting high protein food, is used as a simpler way to reduce lysine, based on plasma lysine levels. **Objective:** Review the dietary prescription and dystonia of patients with GA1 who are currently managed by GMDS. **Method:** Diagnosis, anthropometry, prescribed protein (PP) and AAM, emergency plan, and dystonia were collected from medical records. PP intake was calculated from diet prescription or history and compared with Australian Recommended Dietary Intake (RDI). **Results:** Fifteen patients met inclusion criteria (5 male; median age 7.8 years, range 1.25-17.2). Two patients were excluded; 1 transferred and 1 deceased. Four patients presented clinically and 11 patients were diagnosed after positive Newborn Screening (NBS). Weight range: 8 healthy, 4 overweight, 3 underweight. Seven had evident dystonia (including all clinical presentations). PP was specified as grams of natural protein (n = 11) or serves of high protein foods (n = 4) per day. Median natural protein intake was 1.1g/kg (range 0.5 -1.5g/kg); median 115% RDI (range 65%-330%). AAM was consumed by 9 patients (1 diagnosed clinically). All NBS positive patients commenced AAM, 3 patients ceased at 3, 5, and 12 years due to poor compliance. Median prescribed protein equivalent (PE) from AAM was 0.9g/kg (range 0.5 -1.5g/kg); median 104% RDI (range 46%-165%). Median total PP including natural and PE was 1.85g/kg (range 0.5-3 g/kg) or 185%

RDI (range 65%-330%). Patients consuming AAM when well were prescribed same or higher dose when unwell with extra energy; median 1.3 g/kg PE (range 1.2 -1.5 g/kg) or 145% RDI (range 106-185%). **Conclusion:** There is a wide range of PP and PE intake in our cohort, dependent on tolerance and adherence. Improved outcome with early treatment is demonstrated. However, despite early diagnosis and individualized diet and emergency plans, this management is not always successful in preventing dystonia.

179 - Feeding Outcomes Pre and Post Liver Transplant in Pediatric Patients with Urea Cycle Disorders and Organic Acidemias

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Background: Liver Transplant (LT) is a treatment option for severe forms of urea cycle disorders (UCDs) and organic acidemias (OAs). Restriction of protein and the use of supplementary tube feeding (TF) pre-transplant is often necessary to maintain metabolic stability and minimize the accumulation of toxic metabolites. Although formal protein restriction is relaxed post-transplant, long-term feeding outcomes and protein intakes are not widely documented. **Objective**: To audit feeding route and protein intakes pre- and post-transplant in pediatric patients with UCDs and OAs. **Method**: Retrospective case review of transplanted patients with UCDs or OAs from a single center in the UK from 2011-2017. **Results**: Eight patients (7 male) were transplanted; Propionic acidemia (3), Carbamoyl phosphate synthetase I deficiency (1), citrullinemia (4). Seven patients presented clinically in the neonatal period, 1 was diagnosed prenatally due to positive family history. Frequent metabolic decompensation was the indication for transplant in 7 and elective in 1. Median age at transplant was 1.84 years (range = 0.84-7.0yrs). Pre-transplant, median protein restriction was 1.22 g/kg/day (range = 0.9 -1.3g/kg/day). 50% of patients were prescribed amino acid (AA) supplements which contributed a median of 26% to total protein intake (range = 19-31%). Six of 8 (75%) patients required supplementary TF, which provided a median of 96% (range = 82%-100%) of estimated average requirement (EAR) for energy. All patients had an emergency regimen (ER) in place for illness. Median follow-up post-transplant was 1.97 years (range = 0.03-6.0yrs). All patients had normal graft function and none had experienced metabolic decompensation. Median protein intake was 2.02 g/kg/day (range = 1.13-2.8 g/kg/day), none required AA supplements. Of the six patients who required supplementary TF pre-transplant; four (50%) continued to require TF, 2 (25%) required high calorie oral nutrition supplements (ONS) to maintain nutritional status. TF or ONS provided a median of 79% (range = 23-100%) of EAR for energy. Seven of 8 patients (88%) self-selected a low protein diet and all continued with an ER in place for illness. **Conclusion**: A significant number of post-transplant patients self-select a low protein diet and continue to require supplementary nutritional support. Close dietetic supervision should remain an essential component of post-transplant care in pediatric patients with UCDs and OAs.

180 - Follow-Up of the Nutritional Treatment of a Patient With Biochemical and Molecular Diagnosis of 3 MCC: Case Report

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Introduction: The 3 MCC deficiency is a disorder in leucine catabolism, which can be clinically asymptomatic or development of ketoacidosis, hypoglycemia, hyperammonemia, and coma. It is a genetic disease with autosomal recessive inheritance, with two loci associated: MCCC1 (3q27.1) and MCCC2 (5q12-q13) from whom the clinical characteristic of disease depends on pathogenicity of genetic variants. Long-term treatment is aimed at limiting protein intake to avoid accumulation of organic acids and allow normal growth. **Objective:** to present the follow up of the nutritional treatment of a patient with biochemical and molecular diagnosis of 3 MCC. **Materials and Methods:** Male 1 month 3 weeks of age, weight 4,230 g, height 55.5 cm (P / T, T / E, P / E: P°50) with abnormal neonatal metabolic screening in 2 consecutive samples, with C5OH (3-OH-isovalerylcarnitine) 3.92 µmol / L and 3.95 µmol / L (normal <1 µmol / L) respectively. Free carnitine = 15.02 µmol / L (normal> 3 µmol / L); Total carnitine = 40.18 µmol / L (normal> 10 µmol / L). The urine organic acids showed increased levels in 3-hydroxy-isovaleric and 3-methyl crotonyl glycine excretion. Molecular analysis of the MCCC2 gene revealed a heterozygous state with a pathogenic variant (c.1065A> T) and a variant of uncertain significance (c .129 + 3A> G), located in a splicing site that could be of clinical impact. Under this panorama, we decided to start with a limited diet in leucine at 120 mg / kg, 120 cal / kg, 3.5 g protein / kg, using leucine free formula (IValex 1) 14 measures and 6 measures of Similac and with feeding started complementary at 4 months. **Results:** Currently 10 months of life, asymptomatic, with adequate psychomotor development, good muscle tone, healthy skin, and no particular odors. Weight: 9,100 g (P / E: P° 50-75), size: 70.5 cm (T / E: P°50), PC: 47 cm (P° 50), Urine organic acids, profile of acylcarnitines

and plasma amino acids are normal; still continuing in control of leucine in the diet. **Conclusions**: In this case to have the biochemical and molecular diagnosis of 3-MCC deficiency permitted the timely treatment giving a better quality of life to the patient.

181 - Innovative Model for Delivering Online Genetic Metabolic Nutrition Education: A Pilot Project (Phase I)

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Background: Electronic Genetic Nutrition Academy (eGNA) is the first academically oriented online genetic metabolic nutrition education program. Phase I of our 3-phase project promotes application-based clinical and research via case conference and journal club sessions. The methods and outcomes of the first eGNA sessions are reported. **Objective:** eGNA is designed to increase clinical knowledge about medical nutrition therapy (MNT) for inherited metabolic disorders (IMDs) among genetic metabolic dietitians. **Methods**: The 2017 eGNA program focuses on MNT education for urea cycle disorders (UCDs) and includes 3 eGNA case conferences and journal club sessions. Attendees participate via live web-conferencing, article evidence analyses, and online discussion fora facilitated by national experts. Upon completion of pre-test, post-test, and course evaluation for each session, participants will receive a certificate for up to 4 continuing education units (CEUs). Data were captured from web conferencing stat log, registration log, answers to pre- and post-tests and course evaluations. Descriptive analysis will be performed and statistical comparisons with t-test and ANOVA will be used to compare pre- and post-tests across sessions using SPSS. An additional session will be held before the ICIEM conference, and results will be updated. **Results**: The first eGNA case conference held on February 1, 2017, focused on Arginase I Deficiency (ASD1). Of 135 interested queries, 56 (42%) attended the live webinar. Of the 56 attendees, 75% completed the pre-test, post-test, and course evaluation to earn 1 CEU. The majority of attendees were registered dietitians (n = 39, 68%) from medical institutions (n = 42, 75%) residing in North America (n = 53, 94%). Only 34% of attendees had experience with a patient with ASD1. Post-test scores improved by 24% ($P > .0039$). Overall course evaluation was generally positive; although 55% of respondents reported difficulties with registration and site access. One person completed the journal club evidence analysis to acquire 1 additional CEU. No discussion fora participation occurred. **Conclusions**: The results illustrate the success of a web-based learning tool for education on IMDs. High professional turnout and interest support the value of eGNA. Data collected from this pilot launch is important to further improving course features as well as expand the eGNA curriculum to include other platforms of web-based clinical learning.

182 - Improvement of Nutritional Intake for a Complex MMA Patient Following Implementation of Australia's First Hospital Room Service Choice on Demand: A Case Study

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Background: In 2016, Mater Hospital Brisbane became the first public hospital in Australia to implement room service choice on demand (RSCoD) as their hospital foodservice model. This involved transition from a traditional order in advance 7-day cycle, set meal time model (TM), to an integrated order on demand room service model with a la carte style menu. During this transition, a patient with Methyl Malonic Aciduria (MMA), complicated by gastrointestinal track surgery and renal failure had 8 acute hospital admissions. This case study aimed to compare the energy and protein intake for a complex MMA inpatient pre- and post-implementation of the RSCoD foodservice model. **Methods**: A retrospective audit was conducted by the metabolic dietitian of intake data for a patient with MMA, during 4 acute hospital admissions pre- and 4 acute admissions post-implementation of RSCoD. Food intake records were obtained from CBORD® electronic menu management system and from patient recall as documented by the dietitian in the medical chart, and used to estimate energy and protein intake. The intake of protein (g) and energy (kJ) for each admission were collated and averaged for each period pre- and post-RSCoD implementation. A validated foodservice patient satisfaction survey using a Likert scale from 1 to 5 to rate elements of the foodservice [1] was also completed by the patient pre- and post- implementation of RSCoD. **Results**: The patients' intake during admissions with TM foodservice averaged 52% (range 32%- 63%) of estimated requirements for energy and 28% (range 0%-45%) for protein. After the implementation of RSCoD, average intake improved to 75% (range 50%-100%) of estimated requirements for energy and 58% (range 36%-90%) for protein. Survey results revealed that the patient's satisfaction for food quality with RSCoD improved from 1.8 to 3.8 and overall satisfaction improved from 3.0 to 4.0. **Conclusion**: Implementation of RSCoD improved the nutritional intake (protein and energy) and overall food satisfaction for a complex MMA inpatient. This foodservice model in hospital settings may be an innovative method of improving nutritional outcomes for patients with Inborn Errors of Metabolism.

183 - Challenges of Initiating Low Protein Diets in Toddlers: A Patient With Arginase Deficiency

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Background: Arginase deficiency is a rare urea cycle disorder that results in elevated plasma arginine levels. Without treatment, patients may present with reduced growth, seizures, progressive spasticity, and developmental regression. **Case report:** A 2-year 5-month-old girl of nonconsanguineous parents was diagnosed with arginase deficiency (plasma arginine 616 μmol/L, normal range 49-129 μmol/L) via cascade screening after her sibling was diagnosed by newborn screening. Her newborn screen showed normal blood Arginine (105 μmol/L, normal newborn screen <115 μmol/L) and clinically she had normal growth and development. Diagnosis was confirmed by enzyme (Arginase activity 0.3μmol/h/mg Hb, control range 2-7 μmol/h/mg Hb) and mutation analysis (compound heterozygote c.206A>T and c.837C>A). Initial dietary assessment documented a predominantly lacto-ovo vegetarian diet with a wide variety of foods consumed. Protein intake was approximately 3.3gm/kg/d, energy and micronutrients intake was adequate. Initial dietary intervention included restriction of a natural protein intake to 1.5 g/kg/d and supplementation with a protein-free energy and micronutrient-containing formula and essential amino acid supplement (EAA) at 0.5 g/kg/d, to optimize her protein and nutritional intake. However, compliance with these supplements was poor. Normal growth pattern continued with a natural protein intake of approximately 1.4 g/kg/d. Target plasma Arginine levels (<500 μmol/L) were achieved, and other plasma amino acids remained within normal range yet her micronutrient intake was suboptimal, and she showed a reduction in some nutritional marker including blood ferritin, iron and zinc over time. **Conclusions:** 1. Whilst adequate plasma Arginine and normal growth can be achieved on a low natural protein diet without essential amino acid, energy and micronutrient intake may be low and present a significant risk of deficiencies (specifically iron, zinc, calcium, selenium and vitamin B12) in the longer term. 2. Plasma amino acids and dietary intake must be carefully monitored. 3. Enforcing a restricted diet may result in emergence of new behavioral feeding difficulties, including food refusal, which further compromises the patients' nutritional management and general well-being.

184 - A Severe Hypertriglyceridemia in Berardinelli-Seip Congenital Lipodystrophy—Nutritional Management

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Background: Berardinelli-Seip syndrome or congenital generalized lipodystrophy (BSCL) type 2, is a rare autosomal recessive disease, characterized by near-absence of fat tissue and no functional adipocytes. Ingested lipids are stored in other tissues, including muscle and liver. They also develop insulin resistance and, approximately 25% to 35% develop diabetes mellitus between ages 15 and 20 years. Serum concentration of triglycerides (TG) can be markedly elevated and is sometimes associated with hypercholesterolemia. Clinical management includes restriction of fat intake between 20% and 30% of total dietary energy to maintain normal serum TG. **Case report:** 28 days old female with serum TG levels 3459 mg/dL, hyperglycemia, intra uterine growth restriction, hypotonic and low birth weight (P > 3). After 24 h fasting, TG dropped to 1589 mg/dL. She started special formula extremely low in fat (<0.1 g/100 ml) 93 kcal/kg/day. She achieved the lowest TG levels (175 mg/dL). She started essential fatty acid supply and MCT oil based formula, progressively. At fat intake of 11% of total dietary energy, TG increased to 668 mg/dL. At 10 months old, the identification of two allelic pathogenic variants in BSCL2 gene, established diagnosis of BSCL. At 14 months old, she achieved the 50th percentile for height and for weight. We started n-3 polyunsaturated fatty acids supplement derived from fish oils and started gradually to reduce the caloric value of her diet. At the moment, she is 17 months-old, her diet consists of 115 kcal/kg/day; 4.7 g/kg/day protein; 1.8 g/kg/day fat; 20 g/kg/day carbohydrates with low absorption rates. Medium serum levels are TG 550 $\pm$ 300 mg/dL; Total cholesterol 196 $\pm$ 34 mg/dL; LDL cholesterol 121 $\pm$ 47 mg/dL; HDL cholesterol24 $\pm$ 4 mg/dL; TGP 98 $\pm$ 33 U/L. **Discussion:** Recommended restriction of total fat intake between 20% and 30% of total dietary energy, was markedly insufficient to control TG levels. In our case we needed to reduce to 11% and even so was not possible to achieve TG reference values. We started n-3 polyunsaturated fatty acids derived from fish oils supplement to help to improve those levels. We were able to improve her high insulin levels by increased the calories provided by protein and lower the carbohydrates. In fact, we managed to achieve percentile 50th for both height and weight. Now, our goal is slowly continuing the reduction of total dietary energy intake and delay pharmacological therapies.

185 - Energy and Nutrient Intakes in PKU Patients in Relative to the Turkey Dietary Guidelines

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Background and objective: Because the nature of the dietary treatment in phenylketonuria (PKU), it is important to evaluate daily energy and nutrient intakes regularly. The aim of this study was to assess energy and nutrient intakes in relative to Turkey Dietary Guideline in PKU. **Methods:** Two hundred and twenty-two patients (107 male, 115 female) aged 10.8 months to 31.9 years (median age = 9.9 years) with phenylketonuria were recruited. Dietary energy and protein intakes in 222 patients and vitamin B_{12}, calcium (Ca), zinc (Zn) and iron (Fe) intakes in 67 patients (median age = 10 years, range 1.2 to 27.9 years) who did not use vitamin and mineral supplement were analyzed from a parent-reported 24-hour food recall. The percentages of energy and protein intakes were compared according to the Turkey Dietary Guidelines. If the percentage of energy and nutrient intakes were below 67%, between 67% to 133%, and above 133%, they were referred as inadequate, adequate and excessive intakes, respectively. **Results:** The percentages of mean inadequate energy and protein intakes in relative to the guideline were 47.7% and 20.3%, respectively. The percentage of patients who had high energy intake was 2.7%. The percentage of inadequate vitamin B_{12}, Ca, Zn, and Fe intakes were 28.4%, 53.7%, 19.4%, and 11.9%, respectively. The mean energy and nutrient intakes was found to be adequate (energy = %72.8 ± 28.2, protein = %95.3 ± 35.6, vitamin B_{12} = %83.9 ± 43.8, Ca = %70.4 ± 32.8, Zn = %137.5 ± 96.2 and Fe = %127.9 ± 56.2). There was negative correlation between age and dietary energy ($r = -0.505$, $P < .001$), protein ($r = -0.550$, $P < .001$), vitamin B_{12} ($r = -0.466$, $P < .001$), Ca ($r = -0.394$, $P < 0.01$), and Zn ($r = -0.618$, $P < .001$) intakes. There was a significant positive correlation between the amount of protein equivalent from protein substitute and the percentage of vitamin B_{12} ($r = 0.643$, $P < .001$), Ca ($r = 0.479$, $P < .001$) and Fe ($r = .472$, $P < .001$). **Conclusion:** Adherence to diet diminishes with age because of the difficulties of finding suitable low protein foods especially at school or work environment and diet itself. Inadequate consumption of special low protein foods and protein substitutes leads to insufficient energy, protein, vitamin B_{12}, Ca, Zn and Fe intake. Alternative products and formulas should be developed in order to improve dietary adherence especially in adult PKU patients and the compliance of the diets should be ensured by increasing the follow-up frequency of the patients.

186 - The Contribution of Food Groups to the Daily Energy, Protein, and Phenylalanine Intakes in Patients With PKU

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Background and objective: The nature, nurture, and culture of the patient, as well as inconvenience, cost, and availability of dietary treatment in phenylketonuria (PKU) affect the ability of the patient and caregiver to comply. It is important to evaluate daily energy and nutrient intakes as well as the contribution of food groups in PKU. This study aimed to assess the contribution of food groups to the daily energy, protein and phenylalanine (phe) intakes in PKU. **Methods:** Two hundred and twenty-two patients (107 male, 115 female) with PKU were recruited. The median age was 9.9 years (10.8 months to 31.9 years). All patients followed a strict low-phe diet comprising: (i) a dietary phe allocation using a 15 mg phe exchange system; (ii) a phe-free protein substitute; and (iii) special low-protein foods permitted in usual quantities. Exclusion criteria included: not on dietary treatment, on breast milk, BH4 or LNAA, pregnant, 6 months or younger and lost to follow-up. Energy, protein and phe intakes were analyzed from a parent-reported 24-hour food recall and the contribution of food groups to the daily energy, protein and phe intakes were calculated. **Results:** The contribution of food groups to the total energy intakes were low protein special products (36.7%), oils and fats (14.8%), fruits (9.5%), sugars (7.8%), protein substitutes (6.8%), olives (5.9%), cereals (5.6%), vegetables (4.6%), potatoes (3.8%), and normal bread (0.8%). The contribution of food groups to the total daily protein intakes were protein substitutes (62.7%), vegetables (13.8%), fruits (5.4%), potatoes (4.6%), cereals (4.0%), low protein special products (3.0%), olives (1.8%), normal bread (1.2%) and milk and dairy products (0.8%). The contribution of food groups to the phe intakes were vegetables (35.8%), cereals (15.1%), potatoes (14.9%), fruits (9.5%), olives (6.0%), normal bread (4.3%), milk and dairy products (2.7%), and low protein special products (3.6%). The contribution of spices and yeast to protein and phe intakes were negligible (for spices 0.3% and 0.7%, respectively and for yeast 0.7% and 2.4%, respectively). The contribution of nuts and seeds to protein and phe intakes were found 0.5% and 1.9%, respectively. **Conclusion:** Energy, protein and phe of PKU patients need to be assessed carefully using dietary intake records and food frequency questionnaires. Protein and phe containing food sources and their effects on blood phe levels should be evaluated on a regular basis.

187 - Production of Enteral Feeds for Inpatients With Inborn Errors of Metabolism in a Pediatric Hospital

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Garrahan Hospital is a public referral center (534 total beds) for complex pediatric diseases, including inborn errors of

metabolism (IEM) that require a special individualized diet. Around 1400 enteral feeds for inpatients are prepared every day by trained personnel in a Formula Room under the supervision of specialized dietitians. The aim of this study was to describe the production of enteral feeds for inpatients with IEM during the last year in our center. **Material and Methods**: Review of all individualized enteral feeds recipes indicated by metabolic dietitians for inpatients with IEM, prepared in the Formula Room of Garrahan Hospital, from May 2016 to April 2017.The following variables were evaluated: total amount of formula feeds prepared and its distribution by: type of IEM, number and type of ingredients in each feed and frequency of adjustment of indication. Data was analyzed with IBM SPSS Statistic version 20. A comparison of the time dedicated for each individualized preparation vs regular standardized preparations was estimated by direct observation of the preparation process. **Results:** 189 individualized enteral feeds recipes from 27 inpatients were analyzed. 3414 enteral feeds for inpatients with IEM were produced in a year. Urea Cycle Disorder was the most frequent pathology (27%); followed by organic acidemias (23.8%), glutaric acidemia type 1(21.7%) and maple syrup urine disease (15.3%). A great diversity of ingredient combinations was found: 43.4% of the units were prepared with medical food + standard formula + module; 27.5% medical food + standard formula and 15.3% only medical food. An elevated frequency of adjustment of indication was found: 53.4% every 24 and 48 hours. Only 3.7% of the indications remained stable for more than a week. It was observed trained personnel took five more times to complete the production of an individualized feed, versus a standard one. **Conclusions:** IEM patients have very precise and dynamic dietetic indications due to the diversity of pathologies and individual tolerance. Inpatients with IEM requiring a special individualized diet, need not only a multidisciplinary team but also a fully equipped Formula Room with trained personnel, under the supervision of specialized dietitians, for the safe preparation of their enteral feeds under exacting standards of accuracy.

188 - Micronutrient Status in Patients With Phenylketonuria: The Role of Protein Substitutes Intake

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Background: Diet is the cornerstone of treatment in PKU and protein substitutes (PS) are used to prevent protein deficiency and to optimize metabolic control. PKU patients may be at risk for micronutrient deficiencies, although excessive intakes for some nutrients have been described. **Objectives**: To investigate micronutrient status of PKU patients under follow-up at CGM, and to understand the influence of PS intake. **Methods**: 90 PKU patients aged 16.7 ± 7.4y (57.1% males; 29% Classical PKU) were included in this study, based on the following inclusion criteria: early-diagnosed, under a natural protein-NP restricted diet, supplemented with PS, who completed the annual nutritional status evaluation in 2014. Data on micronutrient status [*iron, selenium, zinc, calcium, Vitamin (Vit) B12, Vit D and folic acid*] was recorded. Also, PS (g/kg), NP (g/kg), total protein (TP g/kg) and micronutrient intakes (total micronutrient intake–TMI and micronutrient intake obtained from PS) were collected from dietary records. **Results**: Median TP intake was 1.5[1.28;1.83], with PS and NP intakes of 1.03 [0.63;1.30] and 0.58 [0.33;0.97], respectively. PS/TP ratio was 0.74 [0.41;0.88]. TMI was 20.5 ± 5.1 mg; 42.3 ± 20.2 µg; 17.8 ± 4.9 mg; 1410.4[1141.4;1539.7] mg; 3. 5[2.8;4.0] µg; 11.3 ± 3.7 µg and 492.3 ± 119.4 µg for *iron, selenium, zinc, calcium, Vit B12, Vit D* and *folic acid*, respectively. *Folic acid, Vit B12* and *zinc* intakes were above DRI in ≥90% of the patients, with *zinc* and *folic acid* exceeding tolerable upper intake levels in 9% and 6% of the sample, respectively. PS contributed >50% to micronutrient intake. Median/average levels of micronutrients were within reference ranges (RR), except for calcium and *Vit B12*, which were above (2.4 ± 0.1 mmol/L and 664.1[480.5;875.1]pg/mL). *Calcium, Vit B12 and folic acid* were above RR in 63%, 51% and 39% of the patients, respectively. *Vit D* was below RR in 40% of the patients. *Vit B12, iron, zinc and selenium* were below RR in 1%, 3%, 9% and 20% of the patients, respectively. Positive correlations were found between *folic acid, selenium, Vit B12, Vit D* and PS intake ($r = 0.512$, $P < .01$; $r = 0.296$, $P < .01$; $r = .304$, $P < .01$; $r = .351$, $P < .01$, respectively). **Conclusions**: Micronutrients levels were within RR on the majority of the patients (except for *calcium* and *Vit B12*). Our results underline the large contribution of PS to the micronutrients intake. The chronic excessive status of some micronutrients, especially *folic acid*, should deserve our attention.

189 - Home Visit Program in Patients with PKU: Does It Affect Blood Phenylalanine Levels?

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Background and objective: Home visits done by registered dietitians play a vital role in providing nutrition education in

chronic diseases. Data regarding the efficacy of home visits on blood phe level are limited in phenylketonuria (PKU). The aim of this retrospective study was to evaluate home visit program (HVP) carried out by a dietitian on blood phenylalanine (phe) levels in a group of PKU patients. **Methods:** Eighty patients (31 male, 49 female) aged 2.4 to 19.7 years (median = 9 years) who were directed to HVP by Hacettepe University Metabolism Unit were included. Mean of the blood phe levels for the previous 3-year period and during HVP were obtained retrospectively. Patients were classified as within or above the center's blood phe guidelines according to the age groups (target phe levels <360 μmol/L for patients aged ≤10 years, <720 μmol/L for patients aged 11 to 16 years and <900 μmol/L for patients aged >16 years). Mean blood phe levels for the previous 3-year period were compared with the first 6-month period (n = 62), 7 to 12-month period (n = 57) and 13 to 24-month period (n = 35) blood phe levels of HVP. **Results:** The median length of HVP was 17.5 months (1.6-24 months). The mean blood phe levels for the previous 3-year period was 553 ± 228 μmol/L (range = 132-1098 μmol/L). Fifty-one subjects (63.8%) had a mean blood phe level above and 29 subjects (36.3%) within the center's guideline for the previous 3 years. There were no significant changes in blood phe levels during the HVP in well controlled patients (previous 3 years = 387 ± 157 μmol/L, first 6 months = 328 ± 182 μmol/L, 7 to 12 months = 394 ± 210 μmol/L and 13 to 24 months = 524 ± 275 μmol/L). Although there was a statistically significant decrease in blood phe levels during the first 6 months of the program compared to previous 3-year period in patients whose phe levels were high (previous 3 years = 648 ± 207 μmol/L and first 6 months = 546 ± 336 μmol/L, $P < .05$), no significant change was observed during 7 to 12 months and 13 to 24 of the program (7 to 12 months = 530 ± 290 μmol/L and 13 to 24 months = 559 ± 296 μmol/L, $P > .05$). **Conclusion:** PKU requires lifelong dietary treatment and it is important to observe the patients at their home settings. Intense and continuing education can help to ensure that patients and their families understand the diet, improve the motivation and generate a positive attitude towards the diet, eventually resulting in the achievement of blood phe control.

190 - Phenylalanine and Protein Intakes in Patients With PKU: Do They Adhere to Dietary Recommendations?

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Background and objective: Poor adherence to diet is a commonly reported problem in phenylketonuria (PKU). The aim of this study was to evaluate dietary phenylalanine (phe), total, natural and protein equivalent intakes from PS and compare with the recommended amounts for each individual. **Methods:** Two hundred and twenty-two patients (107 male, 115 female) with PKU were recruited. The median age was 9.9 years (10.8 months to 31.9 years). All patients followed a strict low-phe diet. Exclusion criteria included: not on dietary treatment, on breast milk, BH4 or LNAA, pregnant, 6 months or younger and lost to follow-up. Phe and protein intakes were analyzed from a parent-reported 24-hour food recall. Recommended dietary phe (mg/day), total protein (g/day), natural protein (g/day) and protein equivalent amount from protein substitutes (PS) (g/day) were compared to actual phe (mg/day), total protein (g/day), natural protein (g/day) and protein equivalent intakes from PS (g/day). The last blood phe levels (on the same day with the food recall day) were analyzed. **Results:** It was found that 82.4% (n = 183) of the patients' dietary total protein, 80.2% of the patients' (n = 178) natural protein and 11.7% (n = 26) of the patients' protein equivalent intakes from PS was lower than recommended. The mean of inadequate total protein intake was 7.6 ± 6.5 g/day, natural protein intake was 6.7 ± 4.5 g/day and protein equivalent intake from PS was 9.5 ± 11.3 g/day. Seven patients (3.2%) did not take their PS. Patients consumed significantly less total (31.1 ± 9.6 g/day) ($P < .001$), natural (11.6 ± 5.0 g/day) ($P < .001$) and protein equivalent from PS (19.5 ± 8.1 g/day) ($P < .05$) than recommended (36.9 ± 8.9 g/day, 16.5 ± 5.2 g/day, and 20.4 ± 7.5, respectively). The mean contribution of PS to total protein intake was 61% ± 17%. Sixty-nine patients' dietary phe intakes were above recommendations (31.1%). The mean of excess phe intake was 108 ± 141 mg/day. Similarly, mean dietary phe intake (406 ± 210 mg/day) was significantly lower than recommendations (506 ± 172 mg/day) ($P < .001$). It was not found a significant correlation between the last blood phe level and the amount of protein equivalent intake from PS in patients who did not follow recommended PS dosage. **Conclusion:** Phe and protein intakes of PKU patients need to be assessed carefully using dietary intake records and food frequency questionnaires. Patients should be informed on the importance of PS consumption on a regular basis.

191 - Do Dietary Energy and Protein Intakes Affect on Anthropometric Measurements in PKU Children?

Ozlem Yilmaz[1], Berrak Bilginer Gürbüz[2], Yilmaz Yildiz[2], Emine Goksoy[2], Ali Dursun[2], Serap Sivri[2], Ayşegül Tokatli[2], Turgay Coskun[2], and Hülya Gökmen Özel[1]

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Background and objective: Phenylalanine (phe) restricted diet prescribed in patients with phenylketonuria (PKU) permits

normal growth and development. Only a few studies have been performed on possible consequences of this diet on the physical growth. The aim of this study was to assess the relation between dietary energy and protein intake with some anthropometric measurements. **Methods:** Two hundred and four children (98 male, 106 female) with PKU were recruited. The median age was 9.1 years (10.8 months to 18.9 years). All patients followed a low-phe diet comprising: (i) a dietary phe allocation using a 15 mg phe exchange system; (ii) a phe-free protein substitute; and (iii) special low-protein foods permitted in usual quantities. Exclusion criteria included: not on dietary treatment, on breast-milk, BH4 or LNAA and lost to follow-up, pregnant, 6 months or younger, 19 years and older. Body weight and height were measured. Body mass index (BMIZ) for age (n = 204), weight for age (WAZ) (n = 117), height for age (HAZ) (n = 204) z scores were calculated using WHO Anthro and Anthro Plus Programs. Energy and protein intakes were analyzed from a parent-reported 24-hour food recall. The percentages of energy and protein intakes were expressed in relative to the Turkey Dietary Guidelines. **Results:** It was found that 23.9% and 31.9% of patients were overweight or obese according to WAZ (>+1SD) and BMIZ (>+1SD) respectively and 10.8% were stunted (HAZ ≤−2SD). The percentage of patients whose BMIZ score ≤−2SD was 4.4%. Mean WAZ, HAZ and BMIZ scores were -0.03 ± 1.34, -0.66 ± 1.15 and 0.35 ± 1.37, respectively. The percentage of mean energy intake in relative to the guideline was $74.6\% \pm 28.3\%$ and mean protein intake in relative to the guideline was $98.2\% \pm 35.2\%$. A significant positive correlation was found between protein intake percentage (in relative to the guideline) and HAZ ($r = 0.289$, $P = .000$). There was no relationship between percentage of energy intake and z scores of BMI, weight for age and height for age. **Conclusion:** Overconsumption of low protein special foods leads to obesity while inadequate consumption of protein substitute leads to inadequate protein and micronutrient intake which will cause to decrease in height velocity. Every patient should be monitored carefully regarding low protein special products and protein substitutes in each visit.

192 - Phenylalanine Intake from Free Foods in Patients with PKU: Do They Contribute Significant Amount to Daily Phenylalanine Intake?

Hülya Gökmen Özel[1], Ozlem Yilmaz[1], Berrak Bilginer Gürbüz[2], Yilmaz Yildiz[2], Emine Goksoy[2], Ali Dursun[2], Serap Sivri[2], Ayşegül Tokatli[2], and Turgay Coskun[2]

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Background and objective: It is common practice in Turkey to count all fruits, vegetables, low protein special products and other foods naturally low in protein in patients with phenylketonuria (PKU). The aim of this study was to evaluate dietary phenylalanine (phe) intake from free foods and the effects of their intake on blood phe levels in a group of PKU patients. **Methods:** Two hundred and twenty-two patients (107 male, 115 female) aged 10.8 months to 31.9 years (median age = 9.9 years) with PKU were recruited. All patients followed a strict low-phe diet. Exclusion criteria included: not on dietary treatment, on breast milk, BH4 or LNAA, pregnant, 6 months or younger and lost to follow-up. Phe intakes from free foods and their contribution to the daily phe intake were analyzed from a parent-reported 24-hour food recall. Free foods were defined as follows: for vegetables and fruits ≤75 mg Phe/100 g, for low protein special products <20 mg Phe/100 g, foods containing very small amount of protein (sweets, jam, honey, cornstarch) and other miscellaneous foods such as spices, yeast. The last blood phe levels (on the same day with the food recall day) were analyzed. **Results:** The mean contribution of free foods to the daily total phe intake was $46.3 \pm 26.3\%$. The highest contribution to the daily total phe intake was free vegetables (25.9%), olives (6.7%), free fruits (6.2%), low protein special products (3.5%) and yeast (2.8%), respectively. The lowest contribution to the daily phe intake was spices (%0.02) and sweety foods (sweets, jam, honey) and cornstarch (1.2%), respectively. Mean phe intake from free foods was 162 ± 92 mg/day. Mean phe intake from free vegetables, olives, free fruits, low protein special products and yeast was 91.9 ± 74.4 mg/day, 25.4 ± 25.8 mg/day, 21 ± 22.5 mg/day, 10.5 ± 10.6 mg/day and 9.4 ± 16.4 mg/day, respectively. Mean phe intake from spices and sweety foods was very low (0.06 ± 0.85 mg/day and 4.0 ± 6.1 mg/day). There was a significant positive correlation between the latest blood phe level and daily total phe intake from free foods ($r = 0.246$, $P < .001$), phe intake from free vegetables ($r = 0.197$, $P < .01$), olives ($r = 0.252$, $P < .001$) and free fruits ($r = 0.155$, $P < .05$). **Conclusion:** It is important to systematically assess the effect of unrestricted consumption of vegetables and fruits containing 75 mg/100 g and less phe, low protein special products, and other foods containing very small amount of protein on blood phe levels in PKU.

193 - Types of Protein Substitutes in PKU: Do Patients Still Prefer High Volume, High Energy Drinks?

Hülya Gökmen Özel[1], Ozlem Yilmaz[1], Berrak Bilginer Gürbüz[2], Yilmaz Yildiz[2], Emine Goksoy[2], Ali Dursun[2], Serap Sivri[2], Ayşegül Tokatli[2], and Turgay Coskun[2]

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Background and objective: It is recommended that all children treated for phenylketonuria (PKU) receive a protein

substitute (PS). PS can be taken as a drink, paste, added to food, eaten like a chocolate bar, or as tablets. In this study, the aim was to evaluate the type of PS preferred and their contribution to energy, macronutrient and phenylalanine (phe) intakes in a group of PKU patients. **Methods:** Two hundred and twenty-two patients (107 male, 115 female) aged 10.8 months to 31.9 years (median age = 9.9 years) with PKU were recruited. All patients followed a strict low-phe diet comprising: (i) a dietary phe allocation using a 15 mg phe exchange system; (ii) a phe-free protein substitute; and (iii) special low-protein foods permitted in usual quantities. Exclusion criteria included: not on dietary treatment, on breast-milk, BH4 or LNAA, pregnant, 6 months or younger, and lost to follow-up. Types of PS and their contribution to energy, macronutrient, and phe intakes were analyzed from a parent-reported 24-hour food recall. **Results:** It was found that 9.5% (n = 21) of the patients consumed powder, 24.3% ready to drink PS. More than half of the patients (62.6%) preferred high volume, high energy drinks (powder PS mixed with sugar, cornstarch, oil, fruit juice, low protein milk or ordinary biscuits). Only one patient consumed ready to drink PS mixed with low protein milk and sugar. Seven patients (3.2%) did not take their PS every day. The lowest energy content among PS consumed was powder formulations (mean $\pm$ SD = 94.4 $\pm$ 34.5 kcal/day). Ready to drink preparations supplied 130.1 $\pm$ 40.5 kcal/day. High volume, high energy drinks provided 3-fold higher energy compared to ready to drink PS (mean $\pm$ SD = 392.4 $\pm$ 216.7 kcal/day) ($p<0.001$). Mean carbohydrate and fat content of high volume, high energy drinks were significantly higher (52.4 $\pm$ 34.7 g/day and 10.9 $\pm$ 13.2 g/day, respectively) compared to ready to drink PS (9.7 $\pm$ 3.0 g/day and 0.2 $\pm$ 0.4 g/day, respectively), because of added sugar and oil ($P < .001$). Although powder and ready to drink formulations did not contain phe, high volume, high energy drinks supplied 18.8 $\pm$ 47.1 mg/day phe, due to phe contain foods such as fruit juice, ordinary biscuits, cornstarch or low protein milk. **Conclusion:** Palatability is the major difficulty with protein substitutes for PKU patients. Fruit juices, flavorings and added sugar, oil, and cornstarch can help to improve the palatability, but energy and macronutrient content of PS can increase.

194 - Overweight and Obesity Prevalence in Children With Medium-Chain acyl-CoA Dehydrogenase Deficiency From a single UK Centre Compared to the UK Population

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Background: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most common fatty acid oxidation disorder in the UK with an incidence of 1: 10 000. It has been part of the Newborn screening program since 2004 in our hospital and 2009 in the UK. Management involves education to ensure safe fasting times and an emergency regimen to prevent metabolic decompensation during illness. MCADD patients may be at risk of being overweight or obese as a result of parental anxiety around frequent feeding.[1,2] The prevalence of overweight and obesity in the general population continues to be monitored annually by Public Health England. **Aim:** To compare the prevalence of overweight and obesity in MCADD patients from a single UK center to the UK population using data from the Health Survey England report (HSE 2015). **Method:** Retrospective review of anthropometric data at the most recent follow up appointment for all pediatric MCADD patients from a single center. The World Health Organization classification (2006 & 2007) Body Mass index (BMI) cut-offs were used to identify adiposity. Patients between 2 and 15 years of age were included in order to compare with a similar dataset from HSE 2015. **Results:** 59 MCADD patients aged 2-15 years (median age 7.3, 56% male) were identified. No significant difference in mean BMI was noted between our patients and HSE 2015 control dataset (18.32 kg/m2 [study data] vs 18.2 kg/m2 [control data] $P = .85$). Overall 32% (n = 19/59) of our patients were either overweight (14%, n = 8/59) or obese (19%, n = 11/59) and this was shown not to have statistical difference to the control data. However, a statistically significant increase in the proportion of obese boys was observed—our patients showed an obesity prevalence in boys of 27% (9/33) versus control dataset prevalence of 15% ($P = .05$). **Conclusion:** There is no difference in mean BMI between our MCADD patients and the general UK population. However, the prevalence of obesity in boys was found to be increased.

195 - Dietary Compliance During BH4 Loading Test in Patients With Phenylketonuria

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Background: In Phenylketonuria (PKU), prior to BH4 treatment, a loading test (BH4-LT) is usually necessary to determine responsiveness. The Portuguese Society of Metabolic Disorders advocate a 72 h BH4-LT. Concurrently with a

BH4-LT, a strict dietary protocol should be followed, but adherence with this is rarely reported. **Objective:** To compare prescribed natural protein (NP, g/kg), Phenylalanine (Phe, mg/kg), L-amino acid supplements (AA, g/kg) and total protein (TP, g/kg) with reported dietary intake during 72 h BH4-LT's. **Patients and Methods:** 78 PKU patients (4-48 y; 20.9 ± 9.1 y; 51.3% females; 41% classical PKU, 49% mild PKU, 4% hyperphenylalaninemia and 6% late diagnosed) who had a BH4-LT between March 2015 and January 2017 were studied. Potential BH4 responsiveness was considered with a blood [Phe] reduction ≥ 30%. Anthropometry, NP, Phe intake, AA and TP prescriptions was documented. A 3-day diet diary was used to calculate mean daily nutritional intake during BH4-LT and to compare with diet prescription. **Results:** Prescribed NP and Phe were similar with reported nutritional intakes during BH4-LT (0.80 ± 0.46 vs. 0.77 ± 0.44 g/kg, $P = .106$; 38.13 ± 22.74 vs. 36.73 ± 21.37 mg/kg, $P = .116$). In contrast, reported AA and TP intakes were significantly lower compared with dietary prescription (1.01 ± 0.37 vs. 1.05 ± 0.35 g/kg, $P = .006$; 1.64 ± 0.49 vs. 1.71 ± 0.49 g/kg, $p = 0.003$). Potential BH4 responders (N = 33) reported Phe and NP intakes in accordance with dietary prescription (43.10 ± 24.41 vs. 43.37 ± 24.26 mg/kg, $P = .922$; 0.90 ± 0.50 vs. 0.91 ± 0.49 g/kg, $P = .721$, respectively), while nonresponders (N = 45) reported lower Phe and TP intakes compared with diet prescriptions (32.05 ± 17.69 vs. 34.30 ± 21.00 mg/kg, $P = 0.048$; 1.57 ± 0.50 vs. 1.66 ± 0.50, $P = .004$, respectively). 57 (73.1%) of 78 patients reported ingestion of nonprescribed food items: e.g. soft drinks (47.4%), cakes and sweet desserts (26.3%) and potato chips (24.6%). **Conclusion:** Our results demonstrated incomplete dietary adherence with prescribed dietary protocols during BH4-LT. It is important to fully monitor and support patients during BH4-LT to ensure entire consumption of prescribed NP, TP, and AA in order to aid accuracy of outcome with BH4-LT.

196 - Metabolic Control in Patients With Phenylketonuria (PKU): Impact of Phenylalanine Titration for BH4 Loading Test

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Background: In PKU, the policy of the Portuguese Society for Metabolic Disorders is to identify all potential BH4 responders, using a loading test (LT). To do this, phenylalanine (Phe)/natural protein (NP) intake were increased to elevate blood [Phe] to >480 μmol/L before LT. We were concerned that a short-term increase in NP intake may have a long-term effect on blood Phe control, particularly in non-responders. **Objective:** To verify the impact of Phe and NP titration on metabolic control post-LT in PKU patients. **Patients and Methods:** 58 early treated PKU patients (4-34 y; 19.6 ± 8.2 y; 50% females; 48.3% classical PKU, 51.7% mild PKU) that completed a LT in 2015 were studied. At the baseline (2010-2013) patients were exclusively diet treated. Phe and NP titration was started in 2014. LTs were concluded in 2015. In 2016 patients were either exclusively diet treated (N = 49) or prescribed BH4 treatment (N = 9). Anthropometry and NP (g/kg), protein equivalent (PE) (g/kg) and total protein (g/kg) intakes were recorded at last appointment of each year (2013-2016). All blood Phe measurements done in 2010-2016, for each patient, were used to calculate median blood Phe and measurements within target range (%) (when blood [Phe] ≤6 or ≤8 mg/dL with age < or ≥12 y, respectively), at the 4 consecutive study periods. **Results:** During the 4 study periods, mean (SD) NP (g/kg) intake was similar [0.58 (0.3); 0.53 (0.3); 0.50 (0.3); 0.55 (0.3), respectively] but with a trend to a lower PE intake [1.72 (0.4); 1.49 (0.3); 1.46 (0.4); 1.41 (0.3), respectively]. Median blood Phe in 2010-2013 was lower than in 2016 (6.80 [4.70-10.30] vs. 7.91 [6.53-11.11]; $P < .001$). Blood Phe measurements within target range (median %) was higher in 2010-2013 vs. 2016 (64 [28-85] vs. 45 [0-66]; $P < .001$). A statistically significant positive correlation was found between % of blood Phe measurements within target range in the same patients in 2010-2013 and 2016 ($\rho = 0,.2$; $P < .001$). The median of differences of % of blood Phe measurements within target range between 2016 and 2010-2013 was not statistically different in BH4 treated vs. diet treated patients: −1 [−46; 22] vs. −17 [−33;0]; $P = .408$. **Conclusion:** Although worsening blood Phe control may occur over time, our results suggest that transient Phe titration may adversely affect long term blood Phe control. Studies examining the long-term effect of LT and protein intake, including a review of length and strategy of preparation for LT are necessary.

197 - Ketogenic Diet in Children With Drug-Resistant Epilepsy, GLUT-1 Deficiency syndrome and Pyruvate Dehydrogenase Complex Deficiency

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Objective: Ketogenic diet (KD) is a kind of diet that include low-carbohydrate, high-fat, and variable-protein, mimics fasting without causing significant catabolism. The KD is a safe, effective and tolerable treatment option in childhood drug-resistant epilepsy, GLUT-1 deficiency and pyruvate dehydrogenase complex deficiency (PDHCD). We evaluate the efficacy and tolerability of the KD in patients who get diagnosis with drug-resistant epilepsy, GLUT-1 deficiency syndrome and PDHCD in childhood. **Case reports:** In drug-resistant epilepsy: Ten patients, followed up by drug-resistant epilepsy, aged between 18 months and 9 years, were treated with KD for 3-12 months (mean 8 months) in the last one year, were evaluated. After KD, two patients (20%) had become seizure free, four (40%) had a 75% to 99% decrease in seizures, two (20%) had a 50% to 74% decrease, and the remaining two children (20%) had a <50% decrease. The numbers of antiepileptic drug treatments were reduced in three patients and KD was well tolerated in all patients. No patients had treatment failure due to complications of diet. **In GLUT-1 deficiency syndrome:** A 3-years-old female who had generalized seizures that started at nine months of age, admitted with ataxia and frequent falls. Her language and personal-social developments were normal. She had ataxic gait and acquired microcephaly on her examination. Brain MR imaging and metabolic examinations were normal. In CSF examination, glucose was 30 mg/dL, simultaneous serum glucose was 86 mg/dL (CSF / serum glucose: 0.36). A heterozygous mutation in the SLC2A1 gene was detected. She was diagnosed with GLUT-1 deficiency syndrome and KD was started. Ataxic gait was improved in early stage. **In PDHCD:** A 19-year-old male who had respiratory failure, lethargy and lactic acidosis in neonatal period, was diagnosed with PDHCD at 4 years old. His language, motor and personal-social developments were delayed. The KD was started at 6 years old. It was noticed that he recovered in mental skills (reading and speaking) and motor skills (walking without support) after the treatment of the KD. **Conclusion:** The KD is an effective, safe and well tolerated treatment option in childhood drug-resistant epilepsy. Early treatment of the KD may contribute positively to the prognosis. In addition, KD is the first choice of treatment in GLUT-1 deficiency syndrome to be effective on seizures and probably ataxia and growth retardation, in PDHCD on all motor and mental skills.

198 - The European Phenylketonuria (PKU) Guidelines and the Challenges on Management Practices in Portugal

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Background: Phenylketonuria (PKU) management practices differ between and within countries. In 2007, the Portuguese Society for Metabolic Disorders (SPDM) approved the Portuguese Consensus (PC) for the nutritional treatment of PKU. The recently published Key European PKU Guidelines (EPG) aimed to improve patient care and harmonize treatment protocols in European Countries. **Objective:** To understand how the EPG will be accepted and implemented in Portuguese treatment centers. **Methods:** A 21-question electronic questionnaire was prepared under the agreement of the PKU Think Tank of SPDM. A link to the questionnaire was sent to all SPDM members (135 professionals working in the field of Metabolic Disorders). The questionnaire highlighted the ten key recommendations of EPG, comparing each statement with the information previously published on the PC. Responses were compiled and descriptive analyses performed. **Results:** Twenty-five of one hundred thirty-five professionals responded to the questionnaire, and over half of the respondents (56%) were nutritionists/dieticians. At least one questionnaire from each of the 10 national treatment centers was obtained. Only the EPG recommendation regarding target phenylalanine (Phe) concentrations between 120-360 μmol/L for patients < 12 years received 100% consensus, with all responders accepting the cut-off values. The greatest concern was the recommendation regarding upper target blood Phe concentration for patients aged ≥12 years, with 48% considering that further discussion was needed before acceptance/rejection of this recommendation. Concerning the EPG recommendation about diagnosis and classification of Phe hydroxylase deficiency, almost one third (32%) failed to agree and preferred to keep the classification proposed by the PC. Although there had been no discussion in the PC, 76% agreed with the EPG recommendation about actions to be implemented when Phe values in patients < 12 years are out of range for a determinate time period. In general, responders accepted most of the recommendations, independently of already implementing these or not in their treatment centers. All professionals agreed that an open discussion was necessary to review the PC, a challenge imposed by the new EPG. **Conclusion:** EPG received overall good acceptance but there was divided opinion on a few key recommendations which require further discussion before being implemented in the Portuguese treatment centers.

199 - The Incidence of Overweight and Obesity in Irish Children With Phenylketonuria (PKU) Compared With the Growing Up in Ireland Study

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Background: Phenylketonuria (PKU) is an inherited metabolic disorder. Dietary treatment for PKU is a lifelong low natural protein diet supplemented with synthetic protein. In 2009 a study at NCIMD indicated an increasing prevalence of overweight and obesity in the PKU population. As a result of these findings, routine BMI monitoring was implemented and dietary changes were made as appropriate. **Objective:** To look at the incidence of overweight and obesity in the PKU population compared with previous figures in 2009 and with the general pediatric population; using the Growing up in Ireland study (GUI) data. **Methodology:** A retrospective chart review of 44 Irish children with PKU aged 3, 5, 9, and 13 in 2015 was carried out. The most recent weight and height were collected. Their BMI was calculated and they were classified as normal weight, overweight or obese using the International Obesity Task Force (IOTF) age and sex specific cut-offs. The data was then compared against the NCIMD 2009 PKU dataset (n = 42) and the general pediatric population using the GUI Study data. **Results:** There was an overall reduction of 9% (P = .455) in the prevalence of overweight and obesity from 2009 to 2015 in the PKU population. Broken into age groups; the 3-year-olds had a 35.9% reduction (P = .115), 5-year-olds 2.2% increase (P = .698), 9-year-olds 13.1% increase (P = .359) and the 13-year-olds 15.3% decrease (P = .645). There is a 2.2% difference in prevalence of overweight and obesity between the PKU children in 2015 and the general pediatric population from the GUI study; 26.5% versus 24.3% (P = .520) with no statistical significance. **Conclusion:** The implementation of BMI monitoring charts has had a positive impact on the prevalence of overweight and obesity in children with PKU as shown by the overall decrease in overweight and obesity from 2009 to 2015. This prevalence of overweight and obesity in PKU patients from 2015 is comparable to that of the general pediatric population. Health-care professionals have the opportunity to educate and empower parents and children with PKU in clinic and in group sessions on understanding what is a healthy BMI, to help them to focus on healthy eating within the constraints of a low protein diet, and to promote physical activity to help further reduce the prevalence of overweight and obesity in this population.

200 - Nutritional Status in BH4 Treated Patients With Phenylketonuria: Preliminary Data From TNSPKU Project

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Background: In BH4-responsive PKU patients, drug treatment can decrease both severity of the natural protein (NP) restriction and protein substitute (PS) requirement. Few studies have examined the impact of BH4 treatment on overall nutritional status. **Objective:** In PKU patients, to compare the metabolic control, nutritional intake, anthropometry, body composition, blood pressure and biochemistry at two consecutive annual nutritional status evaluations (ANSE): whilst on diet treatment only (ANSE-1) and BH4 treatment (ANSE-2). **Patients and Methods:** 13 PKU patients (10-30 y; 19.0 $\pm$ 6.8 y; 9 females; 1 HPA, 3 classical PKU, and 9 mild PKU) with a BH4 loading test of 52.4 $\pm$ 15.1% (blood [Phe] reduction) were studied. Median blood [Phe] (mg/dl) and [Tyr] (μmol/L) were calculated in the previous year on diet only (ANSE-1) and between BH4 start until ANSE-2 (10.2 $\pm$ 5.2 months). NP (g/d), Phe (mg/d), protein equivalent from PS (PE, g/d) and total protein (TP, g/d) were recorded at both ANSE. Blood lipids, glucose/insulin and micronutrients were evaluated. **Results:** Median blood [Phe] reduced (7.2 [5.2-8.1] vs. 5.8 [4.6-6.0]; p = 0,033) while blood [Tyr] remained unchanged (61.9 [54.6-64.5] vs. 57.5 [48.0-60.5]; p = 0,294) in patients on BH4. NP (g/d), Phe (mg/d) and TP (g/d) intakes increased on BH4 treatment (25 [22-39] vs. 41 [38-61], p = 0,002; 1173 [980-1879] vs. 1959 [1792-2853], P < .001; 70 $\pm$ 26 vs. 84 $\pm$ 23, P = .022; respectively) while PE intakes remained similar (g/d) (38 [28-50] vs. 35 [28-38], P = .161). Three of 13 (23.1%) patients remained overweight at both ANSE. Although waist circumference was similar at both ANSE (77.1 $\pm$ 11.8 vs. 78.7 $\pm$ 11.1, P = .094), body fat % significantly increased from ANSE-1 to ANSE-2 (18.4 [15.6-29.3] vs. 25.3 [18.1-31.9], P = .016). Systolic and diastolic blood pressure increased from ANSE-1 to ANSE-2 (103.6 $\pm$ 11.1 vs. 109.7 $\pm$ 10.9, P = .031; 55.2 $\pm$ 12.0 vs. 62.6 $\pm$ 7.8, P = .035), although within targets. All biochemical markers remained unchanged, except vitamin B12 which increased [749.9 $\pm$ 399.9 vs. 904.4-482.6,

$P = .036$] in ANSE-2. In 5/13 patients, blood Zinc reduced below reference range at ANSE-2. **Conclusions:** With BH4 treatment there was little adverse nutritional biochemical impact, probably related to NP increase and continued PS therapy. However, increased fat mass % and blood pressure, with lower blood Zn concentrations underlines the need for continuing nutritional surveillance in patients on BH4 treatment.

201 - Intravenous Sources of Medium Chain Triglycerides for Critically Ill Patients With Fatty Acid Oxidation Disorders

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Objective: Treatment of critically ill patients with long chain fatty acid oxidation disorders (FAOD) with intravenous (IV) sources of medium chain triglycerides (MCT). **Methods:** Three critically ill patients with long chain FAODs were admitted in metabolic crisis unable to receive enteral nutrition. Patient A was a 19-month-old female with a presumed long chain FAOD admitted in metabolic crisis with cardiomyopathy and ventricular tachycardia. The patient had an affected older female sibling that was deceased. The patient was later found to have TANGO2 gene variants. Patient B was a 10-month-old female with long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency admitted in metabolic crisis and heart failure secondary to dilated cardiomyopathy. Patients A and B had worsening cardiac function and were considered for cardiac transplantation. Patient C was an 18-year-old male with trifunctional protein (TFP) deficiency, status post heart transplantation, admitted in metabolic crisis with severe rhabdomyolysis associated with intercurrent illness. Based on the critical nature of their condition and inability to tolerate enteral nutrition, all three patients received Lipofundin® MCT/LCT 20%, an IV source of MCT available in Europe. Lipofundin was obtained with approval of an emergency Investigational New Drug application (eIND) from the Food and Drug Administration (FDA) and Institutional Review Board (IRB). Lipofundin® provided 50% MCT and 50% long chain triglycerides (LCT). During a subsequent admission, Patient C developed a bowel obstruction preventing enteral feeds. Smoflipid®, an IV source of MCT approved by the FDA in 2016, was administered to Patient C. Smoflipid® provided 30% MCT and 70% LCT. Lipofundin® and Smoflipid® were administered with the goal of <10% of calories from LCT until enteral feeds were tolerated. **Results:** Lipofundin® and Smoflipid® were well tolerated with no observed adverse reactions. Patients improved or remained metabolically stable while receiving these products. The initial shipments of Lipofundin® were held by United States Customs, delaying use for several weeks in critically ill patients. Smoflipid® was readily available and did not require eIND or IRB applications. The limitation of Smoflipid® was the 30% MCT content compared to 50% MCT content of Lipofundin®. **Conclusion:** Use of Lipofundin® and Smoflipid® in long chain FAOD patients was well tolerated with no adverse effects.

202 - Growth Evaluation and Biochemical Profile of Patients With Glycogen Storage Disease Type I Under Treatment With Uncooked Corn Starch Therapy: Retrospective Study

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Aim: To evaluate growth, biochemical profile and compliance of children and adolescents with type I glycogenosis (GSDI) under treatment with uncooked corn starch therapy attended at a Reference Center for Inborn Errors of Metabolism (CREIM). **Method:** A retrospective, descriptive and analytical study was carried out based on data from charts arranged in a collection form for data standardization. The following were evaluated: date of birth, sex, family history, age at onset of symptoms, initial clinical manifestations of the disease, age at diagnosis, age at start of treatment, biochemical tests, weight and height. Anthropometric indicators were analyzed according to the classification of the World Health Organization (WHO). Results: Eleven patients were included in the study. Four were males and seven females. The median age of diagnosis and initiation of treatment was seven months (ranged from 3.9 to 150 months). In the anthropometric evaluation, short stature was present in 63.6% of the patients in the diagnosis period (height-for-age) and 72.7% were eutrophic, according to the BMI-for-age. In the final evaluation, 45% were still short stature and 82% were eutrophic. There was no statistically significant change for lactate and glycemia levels, however, there was a significant reduction in the triglycerides ($P < .05$). **Conclusion:** The uncooked corn starch therapy had a positive effect on growth and biochemical control. It is a simple and inexpensive method that alters the prognosis of the disease. Dietary treatment, besides being indispensable for a disease, can improve the anthropometric parameters, since it had a good adhesion.

203 - Body Composition in Patients with Urea Cycle Disorders on Protein Restricted Diet

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Objective: The hypothesis of this study is that a suboptimal protein intake, due to poor assumption with diet or to the use of drugs reducing branched chain amino acid (BCAA) plasma concentrations may affect the body composition of patients with urea cycle disorders (UCD). We hypothesize that these patients may have a reduced lean mass compared to the healthy population. We then verified if the parameters of body composition correlated in UCD patients with the plasma concentrations of BCAA and the ratio between synthetic essential amino acids (EA) and natural proteins (g) and total dietary protein intake. **Methods:** 17 subjects of both sexes (10 females) were recruited, including 8 children (ages 7- 18) and 9 adults (ages 18 - 65) with different UCD who underwent dual-energy X-ray absorptiometry (DXA). Three standard biometric indexes were derived from DXA: Fat Mass Index (FMI), Lean Body Mass Index (LBMI in kg/m^2), and FAT percentage (FAT %). A multiple linear regression analysis included as predictors for each of these three parameters: plasma concentrations of valine, isoleucine and leucine (μmol/L), total protein intake (g) and the ratio synthetic amino acids over natural proteins (g) intake. **Results:** the multiple linear regression provided a correlation between each of the three parameters FMI, LBMI and FAT % and the five explanatory variables considered (adj. $R^2 = 0.94, 0.79, 0.67$ for pediatric patients and 0.82, 0.74, 0.64 for the adults, respectively). Some patients showed a reduced lean mass compared to reference values for the healthy population. It is also noted that the patients supplemented with EA (6 of 8 children and 5 of 9 adults) had no significant benefits on lean body mass compared to patients without supplementation. **Conclusion:** We conclude that our UCD patients seemed to have a reduced lean mass compared to the reference values of healthy population; it might be due to protein-restricted diet. Furthermore, EA supplemented patients showed no significant benefits on lean body mass compared to patients without supplementation. It may suggest that the dose administered is not enough or that the real problem is the total intake of proteins (and relative nitrogen). Surprisingly in our study the fat mass correlates positively with the protein parameters. This is difficult to explain and future studies are needed to clarify this aspect.

204 - Acute Effect of a Phenylalanine-Free Amino Acid Mixture on the Glycidic Metabolism in the Rat

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Background: Phenylalanine (Phe)–free amino acid mixtures (AAM) constitute one of the protein substitutes used to treat patients with Phenylketonuria. Notwithstanding, their metabolic effects are not deeply studied. **Objective:** We aimed to compare, in rats, the acute metabolic effects of free amino acids (free A.A.) present in AAM with intact protein (iProtein). **Methods**: Male Wistar rats received a bolus of free A.A. (n = 7) or iProtein as albumin (n = 7), with equivalent nitrogen amounts. Plasma glucose and insulin levels were measured at 0 and 15, 30, 60, and 120 min after gavage. Pancreas and gut were collected for analysis by immunohistochemistry (insulin, ki67 and GLP-1 receptor) and Western blotting (GLP-1), respectively. **Results**: Free A.A. improved glucose tolerance by decreasing blood glucose area under the curve. iProtein maintained higher levels of insulin at 120 min, accompanied by significantly higher pancreatic insulin content. We observed an increase in Langerhans islet area and a tendency to express Ki67 in rats fed with free A.A. A decreased expression of GLP-1 and its receptor was observed in the gut after 120 min in the free A.A. treatment group. **Conclusion**: This study suggests that free A.A. from AAM exhibit metabolic effects that differ from iProtein, particularly on glycidic metabolism.

205 - Dietary Treatment in Patients with HIBCH and ECHS1 Defects: Clinical and Biochemical Response to Low-Valine Diet

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Introduction: HIBCH and ECHS1 are recently described defects of valine catabolism with early onset and progressive encephalopathy suggestive of Leigh syndrome. Buildup of toxic acryloyl- and methacrylyl-CoA metabolites may contribute to the neuronal damage. Their derivatives, S-(2-carboxypropyl)cysteine (SCPC) and its carnitine ester SCPC-C are diagnostic in both conditions, while 4-OH-Isobutyrylcarnitine (C4OH) is a marker for HIBCH defect only. A low valine diet

was associated with clinical improvement in one HIBCH patient. **Aim:** To report outcomes in 2 HIBCH and 3 ECHS1 patients treated with valine restricted diet. Patients 1 and 2 (HIBCH siblings) have been reported (MGM. 2015;115:161). Dietary treatment started at ages 13 and 14 y respectively. Patients 3 and 4 (ECHS1 siblings) carry a novel heterozygous paternal variant, c.832G>A (p.Ala278Thr). Low enzyme activity and ECHS1 protein levels in fibroblasts and increased SCPC and SCPC-C confirmed the disease. Patient 3 presented in infancy while his older brother (patient 4) has a milder phenotype. Diet was initiated at 6 and 13 y respectively. Patient 5 (ECHS1) is compound heterozygous for a novel c.849-852del (p.Lys284Profs*31) variant and a previously reported c.713C>T (p.Ala238Val) mutation. He presented in infancy and diet was initiated at 3 y. **Diet:** Intact protein was decreased to maintain fasting valine values at low-normal range. Patients 1, 2, 3 and 5 are G-tube fed. Their total protein intake was maintained using a BCAA free formula (Ketonex, Abbott ®) while calories were adjusted with protein free formulas. For patient 4 who eats orally, protein intake was decreased and calories maintained with a protein free formula, but Ketonex was not used. **Results:** In patients 1 and 2, dietary valine content was reduced to 673 mg/day (70%) over 19 months. All marker metabolites decreased during treatment in both patients: C4OH 24-38%, SCPS 59-60%, and SCPC-C 25-70%. Patient 3 was severely affected, and malnourished. His valine intake was reduced to 900 mg/day (50%). In patient 4 intact protein decreased to 37 g/day (~26%). No significant changes in SCPC and SCPC-C levels were seen in patients 3 or 4 over the 19-mo follow-up period. In patient 5 valine intake decreased to 601 mg/day (75%) over 6 months. SCPC and SCPC-C decreased 32 and 47%, respectively. All 5 patients demonstrated clinical improvements (alertness, interaction, speech, muscle strength) without side effects or nutritional deficiencies.

206 - Clinical characterization, metabolic and nutritional profile of patients with Maple Syrup Urine Disease accompanied at a Center of Reference in Inborn Errors of Metabolism

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Objective: To characterize the clinical, metabolic and nutritional profile of patients with Maple Syrup Urine Disease followed at a Brazilian Reference Center for Inborn Errors of Metabolism. Methods: Cross-sectional Retrospective study using data from the follow-up collected in medical records. Results: Nine patients, 55.5% (n = 5) females and 44.4% (n = 4) males, median age at diagnosis was 1.1 months, ranging from 0.2 to 70.1 months of life. The median of age in patient's first assessment in our service was 3 months, ranging from 0.5 to 69.7 months. The median of symptoms onset was observed with 5 days, ranging from 2 to 10 days, which the most frequent symptoms were: lethargy, poor feeding, neurological crying and irritability. Leucine serum levels median at the moment of diagnostic was of 1670 µmol/L, ranging from 1143 to 2336 µmol/L. The median of serum leucine during periods of metabolic stability was 74.5 µmol/L, ranging from 11 to 151 µmol/L, without clinical complications. It was observed high serum levels of leucine during occurrence of infectious events, being the most reported in our study: pneumonia, candidiasis and bronchiolitis, or some reported cases of no diet compliance, which median serum leucine levels was of 462 µmol/L, ranging from 235 to 1514 µmol/L . We observed high serum level of leucine at diagnosis when compared to the treatment levels ($P < .0001$). At the moment of diagnostic, 66.6% of the patients presented a predominance of low/very low height and 55.5% presented a low/very low weight for age. Of those, 37.5% presented significative improvement in weight for age and 50% in height for age. **Conclusions:** The importance of an early diagnosis of the disease aims to minimize the risk of severe decompensation, with subsequent neurological impairment, as well as the importance of a good follow-up with treatment adherence is fundamental to control the amino acid levels of branched-chain amino acids in plasma, as well as assure an adequate weight and height development.

207 - Influence of a Phe-Restricted Diet on Growth Development From Birth to Age 18 Years in Patients With PKU: A Multicenter European Study

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Background and objectives: There is concern that a severe phenylalanine (Phe)-restricted diet may adversely affect normal growth (height) of patients with PKU. This study aims to investigate the longitudinal relationship of protein intake (natural protein and Phe-free L-amino acid supplement) with growth development from birth to young adulthood. This has not been studied previously. **Methods:** Anthropometric measurements and dietary protein prescription were collated annually from dietetic/medical records from 182 patients with PKU from 8 metabolic centres in 8 countries. Infants were diagnosed by newborn screening and followed up from age 0 to 18 years. Development of height (meter) over time was regressed on the time-variant process of natural protein intake (g/kg/day) by applying a parallel process model (i.e. a longitudinal structural equation model). Effects of blood Phe-levels (mg/dL) at diagnosis before therapy, classification of PKU (classical vs. mild), gender, as well as length at birth and amount of natural protein at 1 year of age were also assessed in this model. Valid data for this longitudinal statistical analysis was available for 120 patients. **Results:** Patients growth in height varied at birth and over time and showed a quadratic shape with a particular steep increase in the first year of life and a gradual levelling thereafter. Absolute natural protein intake (NP: gram/day) under treatment increased over time following a cubic shape. Variation in intake in NP was increased in particular during the first 2 years of treatment and after the age of 12 years. The model regression estimates revealed that with each additional gram of NP intake on average patient's height increased by 1.1 cm ($p < 0.006$). This was independent of blood Phe-levels at diagnosis, classification of PKU, gender and length at birth. Preliminary analyses also considering the amount of Phe-free-L-amino acid supplement over time in a similar parallel process model did not show a significant effect on height development. **Conclusion:** Natural protein intake over time is an important factor for development in height. This suggests that it is essential to maximise natural protein intake to tolerance in all children with PKU. However, this finding should be replicated in further longitudinal studies with larger sample sizes.

E) New Metabolic Disease Groups (208 to 218)

208 - SOPH Syndrome Causes Recurrent Acute Liver Failure With Onset in Infancy

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SOPH syndrome (MIM 614800) is a clinically rare disease characterized by severe postnatal growth retardation, aging, cutis laxa, osteoporosis, optic atrophy, neutrophil karyotype abnormality, and normal intelligence. It is a disease caused by biallelic mutations in the neuroblastoma amplified sequence (NBAS) gene and inherited by an autosomal recessive fashion. Here we report a girl had SOPH syndrome with RALF starting in infancy. Our patient was a 1.5 years old girl with recurrent acute liver dysfunction, complicated with febrile infections. After our physical examination, head circumference 42.5 cm, unclosed bone joints, anterior fontanel 5*7 cm, wide and uplift forehead, protruding deep eyes, low ear, small jaw, and short limbs were observed. Blood routine showed blood cell count was normal, but blood smears showed the Pelger-Huët anomaly of granulocytes. Liver function showed elevated ALT (up to 663U/L) and AST (up to 218U/L). After several days of parenteral application of lipids and glucose infusion, her ALT and AST was decreased to 189 U/L and 140 U/L, respectively. Her birth weight and length were 1650 g (<3rd centile) and 40 cm (<3 rd centile), respectively. She presented at 1 month of life with fever, cough, and elevated alanine (up to 95U/L) and aspartate transaminases (up to 200U/L), after treated with antibiotic and hepatic protective drugs in several days, both ALT and AST didn't decrease significantly. She presented several similar episodes in the first 6 months of life, always triggered by fever. At the age of 7 months, she was diagnosed with SOPH syndrome according to gene detection (two heterozygous mutations were found in the NBAS gene: C.6496_6497insA, p.S2166Ffs*2 (maternal), unreported yet; c.5741C> T, p.R1914H(paternal), known mutations). Acute liver failure (ALF) in infancy and childhood is a life-threatening emergency and in about 50% the etiology remains unknown. It is reported that biallelic mutations in NBAS were confirmed as a new molecular cause of RALF with onset in infancy. So, in our clinical works, genetic diagnosis plays an indispensable role in patients with etiology unclear RALF.

209 - Aminoacylase 1 Deficiency in an Infant With Congenital Anomalies and a Spinal Tumor

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Aminoacylase 1 (ACY1) deficiency (OMIM # 609924) is a rare autosomal recessive disorder characterized by nonspecific psychomotor delay, neurodevelopmental regression, seizures, or normal clinical features. There have been 9 reported cases to date (Engelke 2008). It is largely heterogeneous in clinical presentation and only recently have been described as an inborn error of metabolism (Sass et al 2006). Aminoacylase 1 is a zinc binding enzyme encoded by the ACY1 gene, found on the short arm of chromosome 3. It hydrolyzes N-acetyl amino acids into free amino acid and acetic acid; the deficiency of which leads to accumulation of N-acetyl amino acids in the urine. Most mutations of the ACY1 gene result in complete loss of enzyme activity (Sommer et al 2011). We report a patient with atypical features of ACY1 deficiency, diagnosed by urinary excretion of large amounts of N acetylated amino acids and confirmed by genetic mutation analysis. She was born from parents of a consanguineous marriage, with central

hypotonia, hypertelorism, bilateral cleft lip and palate, multiple muscular ventricular septal defects requiring antifailure medications, and nasogastric tube feeding to achieve adequate nutrition. At age 10 weeks, she presented with neuroregression, right sided limb weakness, and a rapidly progressive right cervical mass. A guided biopsy of the mass showed a malignant primitive neuroectodermal (PNET) tumor. She developed worsening neurologic and respiratory compromise and died at the age of 14 weeks. Molecular genetic analysis demonstrated a missense homozygous mutation c.827 G > A (p.Arg276His) of the ACY1 gene. Parental genetic testing is pending. Parental urinary organic acid analyses showed her father to be an excretor of N-acetyl amino acids. He was otherwise asymptomatic with no history of cognitive, neurological, or muscular impairments. It is still unclear if ACY1 deficiency is a metabolic disorder caused by an enzyme deficiency culminating in a heterogeneous clinical phenotype or simply a biochemical abnormality. In 2006, Sass et al reported 4 individuals of variable presentation with ACY 1 mutations, functional ACY1 deficiency, and excretion of N acetylated amino acids and argued that ACY1 deficiency may be a modifying factor later in life. Observation of individuals with ACY1 deficiency along with family studies is important in order to reduce erroneous diagnosis, improve management, and prognostication.

210 - Understanding the Functional Impact of Movement Disorders in Glucose Transporter Type 1 Deficiency Syndrome (Glut1 DS)

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Glut1 DS is a rare, genetic, metabolic disorder characterized by impaired glucose transport across the blood–brain barrier, leading to a state of cerebral energy deficiency. Glut1 DS symptoms include seizures, developmental delay, paroxysmal movement disorders, and nonmotor manifestations. Limited information detailing the burden of Glut1 DS movement disorders exists. This study was conducted to explore the patient experience of Glut1 DS movement disorders and their functional impacts. A 1-day study visit (n = 7) and telephone interviews (n = 10) were conducted with Glut1 DS patients and caregivers. This included semistructured clinical interviews about symptoms and impacts, questionnaires (SF-10/SF-12v2), tests of walking capacity (12 Minute Walk Test (12MWT)) and fine/gross motor function (BOT-2), and clinician-rated scales of ataxia and abnormal involuntary movements. Actigraphy was assessed for $\leq$10 days after the study visit. Videos of movement disorders were submitted for review and comment by 2 expert clinicians. Ataxia, dysarthria, dystonia, and chorea/myoclonus were the most frequently reported movement disorders. Ataxia and dysarthria were primarily described as persistent manifestations, whereas dystonia and myoclonus/chorea were typically paroxysmal. Movement disorders were reported to affect walking, balance, coordination, exercise, and activities of daily living (ADLs) as well as social, emotional, and psychological impacts. Mean SF-10 Physical Summary Scores reflected impaired physical health secondary to movement disorders with scores $\sim$3SD below normative values. Performance based tests revealed decreased walking capacity, with a mean 6-minute walk distance of 69% predicted during the 12MWT. Fine and gross motor function were not consistently impaired based on BOT-2 scores. Clinician-rated scales showed a minimal level of ataxia and abnormal movements at the time of testing. Of note, no subjects experienced paroxysmal symptoms during assessments. Actigraphy analysis suggested significantly less daytime activity compared to controls. Patients with Glut1 DS experience a wide range of debilitating movement disorders affecting physical function, ADLs, and HRQoL. The variable and often unpredictable nature of paroxysmal movement disorders in Glut1 DS has a significant effect on those living with Glut1 DS. This evidence expands on the limited information on the burden of movement disorders and disabling impacts experienced by patients with Glut1 DS.

211 - Severe Metabolic Abnormalities Observed in Patients with Confirmed Diagnosis of Congenital Generalized Lipodystrophy Including AGPAT2 and BSCL2 Mutations

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Objective: Lipodystrophy syndromes (LD) are rare diseases, clinically heterogeneous, congenital or acquired, and can be life threatening. The underlying pathogenesis of generalized lipodystrophy is the irreversible widespread loss of adipose tissue leading to low leptin levels. This analysis examined the clinical characteristics of patients with congenital generalized lipodystrophy (CGL) and a subset of patients with confirmed AGPAT2 and BSCL2 mutations. **Methods:** This is a retrospective analysis of patients with CGL, first diagnosed at Hospital Universitário Walter Cantídio in Brazil. Sanger sequencing was conducted in a subset of patients to identify AGPAT2 and BSCL2 mutations. Triglycerides (TG), A1c, fasting glucose (FG), AST, and ALT were collected at diagnosis. **Results:** Between 2002 and 2017, 21 patients were diagnosed with CGL. The mean $\pm$ SD age was 7.8 $\pm$ 10.6 years and 62% of patients

were female. Patients (n = 21) had mean serum leptin levels (n = 20) of 1.2 ± 0.6 ng/mL, mean TG of 562.5 ± 611.1 mg/dL, A1c (n = 18) of 6.8 ± 2.5%, FG of 127.7 ± 85.3 mg/dL, AST of 44.2 ± 33.7 U/L, and ALT of 55.2 U/L ± 37.5 U/L. Of the 21 patients, AGPAT2 mutation was identified in 4 patients (mean age 6.0 ± 10.0 years; 75% female), and BSCL2 mutation was identified in 7 (mean age 1.3 ± 1.3 years; 57% female). Similar serum leptin levels were observed in patients with AGPAT2 or BSCL2 mutations (n = 4; 1.1 ± 0.3 ng/mL, n = 6; 1.1 ± 0.2 ng/mL, respectively). Similar A1c levels were observed in patients with AGPAT2 and BSCL2 mutations (n = 3, 6.5 ± 2.8%; n = 6, 6.0 ± 1.4%, respectively) as well as FG (108.0 ± 43.0 mg/dL, 102.4 ± 55.7 mg/dL, respectively). Conversely, mean TG were notably higher (n = 4; 912.3 ± 1002.8 mg/dL) in patients with an AGPAT2 mutation than in those with a BSCL2 mutation (n = 7; 572.4 ± 637.7 mg/dL). Patients with a BSCL2 mutation had higher AST (41.9 ± 26.3 U/L) and ALT (59.7 ± 48.9 U/L) values when compared to those with an AGPAT2 mutation (AST of 24.5 ± 8.2 U/L; ALT of 39.8 ± 20.1 U/L). In contrast to patients with an AGPAT2 mutation, all patients with a BSCL2 mutation reported loss of mechanical fat. **Conclusion:** Severe metabolic abnormalities were evident in all patients irrespective of an identified AGPAT2 or BSCL2 mutation, in addition to observed low leptin levels. Further research is needed to fully understand the differential clinical characteristics of patients with identified mutations leading to the CGL phenotype.

212 - Can Leptin Replacement Alter the Course of Disease in Patients With Acquired Generalized Lipodystrophy?

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Objective: Lipodystrophy syndromes (LD) are rare diseases, clinically heterogeneous, inherited or acquired, and often life-threatening disorders. The underlying pathogenesis of generalized lipodystrophy is the irreversible, widespread loss of adipose tissue, leading to low leptin levels. This retrospective analysis aimed to examine the impact of leptin replacement (metreleptin [ML]) therapy on disease progression in a pediatric patient with acquired generalized lipodystrophy (AGL). **Methods:** This pediatric patient (male, 11 years old) with AGL was followed for 10 years at Hospital de Niños Pedro de Elizalde in Argentina. Yearly triglycerides (TG), hemoglobin A1c (A1c), AST, and ALT were collected for 6 years before the initiation of ML and 4 years after ML therapy. In the 10-year observation period, the number of hospitalizations were also collected. **Results:** At the age of 11, this patient presented with panniculitis in the face which progressed to generalized lipodystrophy. Prior to ML therapy, he had 7 hospitalizations of which 6 were for infectious diseases (including 3 for cellulitis) and experienced worsening of metabolic control. Despite more than 500 units/day of insulin and fenofibrate therapy, A1c was 13% and TG were 1099 mg/dL. Within 1 year of initiating ML therapy, all antidiabetic medications were discontinued and A1c remained below 6.5%. During 4 years of observation after initiating ML, no hospitalizations were reported, and A1c (ranged from 4.9 to 5.8%) and TG (ranged from 36 to 84 mg/dL) remained controlled. AST and ALT values were elevated prior to the start of ML but decreased from 103 U/L to 41 U/L and 170 U/L to 56 U/L, respectively, by the end of the 4-year follow-up period after ML therapy. **Conclusions:** In this retrospective analysis, leptin replacement therapy with ML restored this patient's A1c and TG to normal levels. Improvements in liver function were also observed. Additional studies are needed to fully understand how ML potentially modifies the course of disease in generalized lipodystrophy.

213 - Hereditary Spastic Paraplegia Type 15 as a Rare Metabolic Disorder of Cellular Trafficking—2 New Cases

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Hereditary spastic paraplegias (HSPs) are a heterogeneous group of inherited neurological disorders with the cardinal feature of a length-dependent axonopathy of corticospinal motor neurons. They are now mainly classified by their mapped genetic loci, SPG1 to SPG71. Mutations in *ZFYVE26* gene are described as being responsible for SPG15. The majority of *ZFYVE26* mutations are of loss of function. Different cellular pathogenic mechanisms have been suggested for SPG15. Some evidences suggested that SPG15 might be caused by impaired cellular trafficking. We identified two new families with members harboring mutations in the *ZFYVE26* gene. Case 1, female, 37 years old, presented with symptoms since the age of 14 years that included progressive weakness of the lower limbs, spasticity, cramps, atrophy of the limbs (predominantly distal), no paresthesia, very dry skin, no seizures. Cerebral and cerebellar MRI was normal at the beginning of the disease, but later showed cerebral and cerebellar atrophy. The disease has progressed with degeneration of main functions: the gait was lost (she is now using a wheelchair), she has dysarthria, she can no longer use her hands, she has difficulties in writing, and she presents with muscular atrophy. Her two sisters (one of them is deceased) present/presented with the same symptomatology. Molecular genetic testing showed two heterozygous variants in the *ZFYVE26* gene: c.2114dup (p.Glu706*) in exon 11 and c.5621+1G>A in intron 29. Case 2, female, 19 years old, from a consanguineous family, possessed cardinal features of HSP

such as muscle weakness, gait disturbance, unsteady gait, fasciculation and myopathy starting from age of 17 years. Now she is no longer able to walk, but with normal intelligence. Molecular evaluation identified homozygous variant in the *ZFYVE26* gene, c.2639T>C (p.Leu880Pro). Spastic paraparesis can be one of the multiple presentations of inborn errors of metabolism in children and adults and in some cases the symptom spastic paraparesis remains the only symptom for years; therefore, these metabolic causes should be included in the general diagnostic approach to sporadic spastic paraparesis. Genetic analysis of HSP genes represents the only way for diagnosis. Genetic counseling and prenatal and presymptomatic testing become possible options.

214 - Disturbances in the Respiratory Chain Complexes as a Possible Cause of Mitochondrial Dysfunction in Chronic Fatigue Syndrome

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a debilitating illness with yet unknown etiology, making diagnosis and treatment challenging. Research in ME/CFS is hampered by heterogeneity in clinical expression and diagnostic criteria. Recent research has shown mitochondrial dysfunction with mitochondrial energy deficiency and oxidative stress in ME/CFS patients. We hypothesize that this mitochondrial dysfunction is caused by a vicious cycle of overproduction of reactive oxygen species, lipid peroxidation of the inner mitochondrial membranes, and damage to the respiratory chain complexes. We have collected peripheral blood mononuclear cells (PBMCs) and skin fibroblasts from six unrelated patients diagnosed with a severe form of ME/CFS. All patients are Caucasian females, 30-50 years old and with onset of symptoms after an infection or another immunogenic trigger. The aim of this study is to further investigate mitochondrial function and their role in ME/CFS, using PBMCs and an *ex vivo* model of skin fibroblasts. Mitochondrial function will be assessed using the extracellular flux analyzer (SeaHorse XFe-96), an instrument that enables real-time measurement of two central pathways to generate ATP in living cells: glycolysis and oxidative phosphorylation. Preliminary results on PBMCs from the six patients showed an enhanced metabolism, especially an enhanced glycolytic activity towards lactate production. Additionally, mitochondrial proton leak is increased. This could indicate a metabolic shift caused by oxidatively damaged mitochondrial membranes that affect mitochondrial respiration efficiency, supporting our hypothesis of mitochondrial dysfunction in ME/CFS. Besides the mitochondrial function assessment, lipid peroxidation will be quantified with image cytometry using the BODIPY® 581/591 undecanoic acid probe, which fluorescence upon lipid oxidation. Finally, the integrity of the respiratory chain complexes will be validated by blue native polyacrylamide gel electrophoresis. To follow up on our hypothesis, we will investigate whether we can improve mitochondrial function in cells harvested in our ME/CFS-patients by treatment with SS-31 peptides, a well-known cell-permeable antioxidant peptide that reduces intracellular free radicals and inhibits lipid peroxidation. Mitochondrial membrane integrity and function will be analyzed before and after treatment of skin fibroblasts with SS-31 peptides.

215 - Heterogeneous Clinical Spectrum of DNAJC12-Deficient Hyperphenylalaninemia: From Attention Deficit to Severe Dystonia and Intellectual Disability

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Background: Mutations in *DNAJC12*, encoding a co-chaperone of the heat-shock protein 70 (HSP70) family, which interacts with phenylalanine, tyrosine and tryptophan hydroxylase, were recently described to lead to hyperphenylalaninemia (HPA), central biogenic amines deficiency, dystonia and intellectual disability (see also abstract from Schiff et al). **Methods**: Patients with HPA that were excluded for phenylalanine hydroxylase deficiency or defects in tetrahydrobiopterin (BH4) cofactor metabolism, were screened for *DNAJC12* variants using Sanger sequencing. **Results**: We describe 5 additional patients with HPA from 3 unrelated families with homozygosity or compound heterozygosity in *DNAJC12* with 3 novel variants. In 2 subjects, neurotransmitter metabolites in CSF were analyzed showing low 5HIAA and HVA concentrations (currently on BH4 treatment only), while all 5 cases presented with much milder neurological symptoms than in the previously reported patients. All patients responded to oral BH4 challenge (20 mg/kg) by lowering blood phenylalanine levels down to normal. **Conclusions**: DNAJC12 deficiency appears to result in a more heterogeneous neurocognitive phenotype then initially described with minor symptoms in these 5 patients. While early identification and institution of treatment with BH4 and neurotransmitter precursors (L-dopa/

carbidopa and 5-hydroxytryptophan) is crucial to ensure optimal neurological outcome in DNAJC12-deficient patients with a severe phenotype, optimal treatment for patients with a milder phenotype remains to be defined.

216 - Further Characterization of TBCK-Related Phenotype: Two Sibs With Severe Hypotonia, Intellectual Disability, and Epilepsy

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Introduction: Biallelic *TBCK* mutations were recently described as the cause of a severe congenital neurodevelopmental disorder with profound developmental delay, severe hypotonia, minimal motor and language acquisitions, often associated with epilepsy and brain anomalies.15 cases were reported. We describe one additional family with two affected sibs. **Clinical report:** *Subject 1:* female born at term, diagnosed since birth with hypotonia, distal hypertonia, nystagmus, feeding difficulties and stridor. Brain ultrasound performed at 2 days of life revealed subependimary cysts. At 5 months (M) seizures emerge and developmental delay was evident. At 18 M, during a muscle biopsy, a severe apnea episode occurred with subsequent permanent need of non-invasive ventilation. At 3 years (Y) feeding difficulties led to gastrostomy. She did not develop any language or active movements and died at the age of 9Y from respiratory insufficiency. *Subject 2*: brother, after a normal pregnancy and birth, presented in the first months of life with developmental delay, severe hypotonia with hyporeflexia and tongue fasciculation, irritability, abnormal ocular movements and strabismus. Brain MRI (18 M) showed brain atrophy, incomplete corpus callosum, widening of brain ventricles, periatrial gliosis and reduced volume of white matter. At 21 M, Growing skills II evaluation revealed a global developmental quotient of 1-3 M. EEG performed at 14 M disclosed an encephalopathic pattern and at 30 M, for epilepsy suspicion, showed paroxysmal activity, controlled with levetiracetam. Since 8Y he had 3 episodes of sudden respiratory failure that recovered after symptomatic therapy. He is now 8Y8 M and has profound intellectual disability (ID), severe hypotonia without any eye contact or language. Array Comparative Genomic Hybridization (CGH)and extensive neurometabolic work-up were inconclusive. Remote consanguinity was a possibility and Sanger sequencing of 23 genes in the larger common homozygous regions failed to identify potential pathogenic variants. Subsequent whole-exome sequencing in both sibs identified compound heterozygosity for the *TBCK* variants: c.1439delT (p.L480 fs) and a multi-exon deletion. **Discussion:** The phenotype described in this family fits well the clinical spectrum of the recently described *TBCK*-related ID autosomal recessive syndrome. TBCK is involved in the mTOR pathway and these pathogenic mutations were associated with its inactivation, in contrast to classic mTOR disorders.

217 - Phenotypic and Molecular Characterization of a Third Patient With Primary COQ10 Deficiency Caused by COQ7 Mutations

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Background: Whole exome sequencing (WES) has become the preferred diagnostic tool for identification of the molecular basis of rare genetic conditions. Primary coenzyme Q10 (CoQ10) deficiency is caused by mutations in genes encoding for proteins involved in ubiquinone biosynthesis. These conditions pose a diagnostic challenge due to their rarity and phenotypic heterogeneity. **Objectives**: to investigate the molecular basis of a mitochondrial condition in a 15-year-old male and characterize his clinical phenotype. **Methods:** We used whole exome sequencing (WES) to evaluate a proband with clinical findings suggestive of a mitochondrial disorder. Clinical data was obtained by parental interviews, chart review and direct patient assessment. **Results:** Clinical characteristics of the proband include hypotonia, intellectual disability, ADHD, autistic traits, bilateral sensorineural hearing loss, elevated lactate in blood and cerebrospinal fluid, and past history of oligohydramnios, lung hypoplasia, and feeding difficulties. The proband's family history was notable for 2 male siblings who died on first day of life secondary to lung hypoplasia. His brain MRI showed generalized mild periventricular matter changes in a leukodystrophy distribution and a gliotic region at the left temporal lobe consistent with a prior infarct. WES revealed 2 compound heterozygous missense genetic variants in the *COQ7* gene; a maternally inherited c.446A>G (p.Tyr149Cys) and a paternally inherited c.161G>A (p.Arg54Gln) variants. Neither of these variants has been previously described as either pathogenic or benign, nor are they observed in large population cohorts. We compared the clinical phenotype in the proband to two previously reported cases of primary CoQ10 deficiency due to *COQ7* mutations. **Conclusions:** We report a third patient with primary CoQ10 deficiency caused by compound heterozygous missense variants in *COQ7*. The phenotype associated with *COQ7* mutations is characterized by prenatal onset lung hypoplasia secondary to oligohydramnios. Postnatal manifestations include hypotonia, feeding difficulties, hearing loss, developmental delay/intellectual disability,

and neurobehavioral abnormalities. Molecular and clinical characterization of additional patients with *COQ7* mutations is necessary to better understand the phenotypic spectrum of this condition and potential genotype–phenotype correlations, and to assess the response to treatment.

218 - Severe Scoliosis in a Colombian Patient With Juvenile Hypophosphatasia: Case Report

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Hypophosphatasia (HPP), (OMIM 146300, 241500, 241510) is a rare genetic pathology characterized by deficiency in bone and dental mineralization with an approximate incidence of 1: 100 000 to 1: 300 000 for severe forms and being larger for forms Moderate and mild, the latter probably underdiagnosed. It is caused by deficiency in non-specific alkaline phosphatase bone activity (TNSALP) due to mutations in the ALPL gene. To date, six clinical forms have been described according to the age at onset of symptoms and severity: perinatal lethal, perinatal benign, infantile, juvenile, adult and odontohypophosphatasia. Its clinical expressiveness is highly variable from in utero death to spontaneous loss of teeth without bone involvement. The diagnosis is based on the measurement of serum alkaline phosphatase (FA) and the sequencing of the ALPL gen. We describe the case of a Colombian patient with juvenile onset hypophosphatasia in which her main manifestation was the presence of severe scoliosis.

F) Phenylketonuria; General (219 to 280)

219 - The Effect of Growth Hormone Therapy on Plasma Phenylalanine Level in Patient with Phenylketonuria: Case Report

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Introduction: Phenylketonuria (PKU) is a congenital disorder of the phenylalanine (Phe) metabolism caused by mutations in the liver enzyme, phenylalanine hydroxylase, encoded by the PAH gene. The dietary treatment of PKU is based on the restriction of Phe intake in order to maintain blood Phe concentrations within the recommended range. Guidelines advise that children with PKU are regularly monitored for growth, hematological and biochemical markers. **Case:** A 4-year-old girl with poor controlled classic PKU (c.115-117delTTC and c.754C>T) was followed up in our center. In 3.5 years old of age, height growth was revealed as 4 cm per year. After inadequate response to growth hormone stimulation test, growth hormone therapy (somatropin, Humatrope®, 35 mcg/kg/day, sc) was initiated. In the 6 months prior to starting growth hormone, growth velocity increased to 10 cm/year. While, the mean phenylalanine value in the last year was found to be 800 $\pm$ 106 μmol/L (744-864 μmol/L), after the growth hormone treatment the mean phenylalanine level in the last 6 months reduced to 442 $\pm$ 199 μmol/L (175-696 μmol/L) with the same diet chart (20mg/kg/day phenylalanine and 1 gr/kg/day protein). **Conclusion:** Although, it is difficult to determine the dietary adherence of patients with PKU, interrogation of nutritional status and assessment of anthropometry are keys to identifying reasons of elevated serum phenylalanine levels and abnormal growth patterns. We hypothesized that the anabolic effect of growth hormone causes to decrease in serum phenylalanine level of patient. Long-term, studies are needed to identify the effect of growth hormone in patient with PKU.

220 - Investigation of the Factors That Affecting Adherence to Dietary Treatment in Patients With Phenylketonuria

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Objective: We aim to determine the difficulties of the patients and their families about managing the dietary treatment of classical phenylketonuria (PKU) and to develop methods for improving adherence to diet. **Methods**: The mothers of 76 children with classical PKU (1-18 years old) requested to fill a questionnaire that evaluate their sociodemographic data, knowledge, experiences and behaviors about the disease and diet. The mothers were also asked to fill the Short Form-36 (SF-36) scale that evaluate their health-related quality of life. Seventy-one aged-matched women were selected for control group and were asked to complete SF-36 scale and a questionnaire that evaluate their social demographic data. **Results**: The mean rate of strictly adherence to diet was 28.9% in the whole group (40% under 12 years-old vs 7.7% up 12 years-old groups, $P = .002$). There was no correlation between the distance of the patient's house to the hospital and the mean blood phenylalanine level during the last year ($r = -0.165$, $P = .154$). Regular outpatient visits of the metabolism unit were related with the higher adherence to diet (38.0 vs 11.5%, respectively, $P = .017$). Receiving social support of the mother from family members or friends was positively affecting diet compliance (38.5 vs 8.3%, respectively, $P = .007$). Lower physical health component summary scale (PCS) of the SF-36 of the mothers was related with higher last year phenylalanine levels of the patients ($r = -0.229$, $P < .05$). In logistic regression analysis, the main factors that affect the adherence to diet were frequency of using amino acid mixtures in daily basis (>3 vs <3 times in a day, OR: 15.24, 95% CI: 3.02–

76.95, $P = .001$), the age of patient (<12 vs >12 years-old, OR: 10.22, 95% CI: 1.93-54.15, $P = .006$) and receiving social support of the mothers (receive vs not receive, OR: 7.76, 95% CI: 1.49-40.31, $P = .015$). **Conclusion**: It is concluded that individualized patient-centered approaches should be developed in order to strengthen the dietary adherence of patients with classical PKU by considering all factors that directly affect the patients' adherence to diet. Bearing in mind that adherence to diet is particularly affected in a negative manner by aging and social support makes a positive contribution, a comprehensive biopsychosocial approach should be implemented toward the patients and families.

221 - Degradation of Tyrosine by Intestinal Microbes Contributes to Reduced Bioavailability of Tyrosine from Amino Acid Compared With Glycomacropeptide Medical Foods in Phenylketonuria

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Background: Deficiencies of the monoamine neurotransmitters, such as dopamine synthesized from tyrosine (Tyr) and serotonin synthesized from tryptophan (Trp), are of concern in PKU. Glycomacropeptide (GMP) demonstrates prebiotic properties and increases intestinal production of short chain fatty acids. **Objective:** Our objective was to utilize metabolomics analysis to assess monoamine metabolites in early-treated adolescent and adult subjects with PKU consuming amino acid medical foods (AA-MF) and glycomacropeptide medical foods (GMP-MF). **Methods:** Subjects with PKU consumed a low-Phe diet combined with AA-MF and GMP-MF for 3 weeks each in a randomized, controlled, crossover study. Metabolomic analysis was conducted by Metabolon, Inc. on plasma (n = 18) and urine (n = 9) samples. Catecholamines and 6-sulfatoxymelatonin were measured in 24-hr urine samples. **Results:** Intake of Tyr and Trp was ~50% higher with AA-MF, and AA-MF were consumed in larger quantities, less frequently during the day compared with GMP-MF. Performance on neuropsychological tests (CANTAB and D-KEFS) and concentrations of neurotransmitters derived from Tyr and Trp were not significantly different with AA- MF or GMP-MF. Plasma serotonin levels of gut origin were higher in subjects with variant compared with classical PKU. Metabolomics analysis identified higher levels of microbiome-derived compounds synthesized from Tyr, such as tyramine and phenol sulfate, and higher levels of compounds synthesized from Trp, such as quinolinic acid, in the kynurenine pathway with ingestion of AA-MF compared with GMP-MF. **Conclusions:** The Tyr from AA-MF is less bioavailable due, in part, to greater degradation by intestinal microbes compared with the Tyr from prebiotic GMP-MF. Routine Tyr supplementation in an attempt to increase catecholamine neurotransmitters may have negative effects on the intestinal microbiota. Research is needed to understand how changes in the intestinal microbiota affect health for individuals with PKU. Supported by FDA Office of Orphan Products Development and NIH. Manuscript available in Mol Genet Metab DOI: 10.1016/j.ymgme.2017.04.003

222 - Patient Survey on PKU Treatment

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Phenylketonuria (PKU) is a rare metabolic disorder characterized by impaired conversion of phenylalanine (Phe) to tyrosine. If left untreated, the resultant accumulation of excess blood Phe can cause physiological, neurological, and intellectual disabilities. The National PKU Alliance (NPKUA) conducted a survey of its membership to assess current health status and interest in new treatments for PKU. Of the 625 survey respondents, less than half (46.7%) reported blood Phe within (120-360 μmol/L)—the range recommended by the American College of Medical Genetics and Genomics (ACMG). The survey results also showed that younger (≤ 18 years) subjects were about 3-times as successful in keeping their blood Phe concentrations within the recommended clinical range compared with adults. Blood Phe over 360 μmol/L was reported in one-quarter (25.5%) of ≤ 18-year-old subjects and almost two-thirds (61.5%) of > 18-year-old subjects. A little more than half (51.7%) of respondents reported having difficulty in managing their PKU, including the maintenance of a Phe-restricted diet. Subjects with PKU desire new treatments that would allow them to increase their intake of natural protein, discontinue or reduce their intake of Phe-free medical food and low-Phe foods, improve their mental health (including depression and anxiety), and reduce blood Phe concentrations. Respondents preferred oral administration of any newly developed therapies and, in general, disliked therapeutic injections. Injections at home were preferred over injections at a clinic. Payers, government agencies, clinicians, and industry partners should consider patient input when developing and approving new therapies and treatments for PKU.

223 - International PKU Patient Registry

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The implementation of an outcomes oriented patient registry for phenylketonuria (PKU) expands our knowledge basis on the variability of the clinical phenotype, identifies unmet needs and guides the scientific community in the development of novel therapies. The NPKUA Patient Centered Outcome Registry, launched in January 2017 is a global registry for patients with

PKU. The registry will collect information from families/participants who are affected by PKU and who are interested in participating in future research. The registry utilizes a web-based interface (https://pku.iamrare.org) to maximize accessibility to participating families and clinics at a global scale. Registry participants will automatically be enrolled in the Global Rare Disease Registry (GRDR), and their de-identified information aggregated with information from other rare diseases. The purpose of the NORD Platform & GRDR is to enable analyses of data across many rare diseases and to facilitate clinical trials and other studies. Third parties may seek access to data in the PKU Patient Registry. Third parties may include, but are not limited to, researchers or companies conducting retrospective studies or conducting research and/or clinical trials on new therapies and will only be granted access to registry information upon review and approval of the Registry Advisory Board. Utilizing a survey format, the PKU Patient Registry collects data on long-term metabolic control and management, co-morbidities and co-medication, general health and life style, social-economic factors and PKU genotypes. Access to Phenylhydroxylase gene sequencing is facilitated through collaboration with Baby Genes (www.babygenes.net). Registry enrollment within 3 months of launch exceeded 500 with participants from the US and Canada. Marketing efforts utilizing social media and partners in industry and the clinical setting proved to be successful with a favorable response from the PKU community.

224 - PKU Knows No Borders

Norman Adams[1] and Christine Brown[2]

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PKU knows no borders. PKU patient advocacy organizations representing Australia, Canada, Europe, and the United States came together last year and agreed it is time to make history in PKU by forming a global association of PKU organizations representing the diverse population of our world-wide community. This initial group sees the formation of such an organization as a critical need in the PKU community as newborn screening expands, pressure for access to medical foods and other treatments increases and to more efficiently handle requests for assistance from many parts of the world. This new global umbrella organization would have the following priorities: 1. Mentor new countries and offer them guidance, best practices, and support on how to form an engaged patient/family group in their country/jurisdiction that is sensitive to their experiences, culture, and language(s). 2. Create a global platform for advocacy that includes access to newborn screening, clinical care, and treatments. 3. Foster collaboration among researchers, scientists, and clinicians to move the basic science and research forward to inform access to current treatment and accelerate new knowledge discovery of PKU and future treatments. An organizing meeting for the international group will be held July 2017 with PKU patient organizations from Canada, the United States, the United Kingdom, Australia, Germany, Turkey, China, Brazil, Mexico, Argentia and Chile. We would like to announce the formation of this group at ICIEM.

225 - Phenylalanine Hydroxylase: Molecular Study Implementation in Chile

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Background: Phenylketonuria (PKU, OMIM 261600) is an autosomal recessive disease, caused by mutations in Phenylalanine Hydroxylase (*PAH*) gene situated in chromosome 12q22-q24.2. This gene has 13 exons. Up to date 970 mutations have been described. **Objective:** Characterize Chilean PKU genotype. **Methods:** 71 PKU subjects to study the *PAH* gene by restriction fragment length polymorphism (RFLP) and sequencing techniques to identify the genotype, previous exon and intron amplification by PCR technique. **Results:** 26 mutations were identified, in 134 of the 142 alleles studied (94.4%), completing the analysis in 88.7% of the subjects, 11.3% had one allele identified. 84.5% of the sample was heterozygous. Exon 7 included the majority of mutations (23%). 50% of mutations were missense. Most frequent mutations were IVS10-11G>A, p.Ex5del, and p.Val388Met. **Conclusions:** The most frequent mutations in Chilean PKU patients can be identified by RLFP and MLPA, which lowers the costs of genetic analysis.

226 - What is the Optimal Dosage of LNAA Combined With a Semi-Free Diet for Patients With PKU?

Kirsten Ahring[1], Jens Rikardt Andersen[2], and Lisbeth Birk Moller[1]

[1]Kennedy Centre, Copenhagen University Hospital, Glostrup, Denmark
[2]Copenhagen University, Copenhagen, Denmark

Background: Phenylketonuria (PKU) is a congenital, hereditary metabolic disorder in which blood levels of phenylalanine (Phe) is extremely high because of lack of the enzyme phenylalanine hydroxylase (PAH). Without treatment, this results in severe mental retardation, microcephaly, epilepsy and other neurological symptoms caused by severe high cerebral concentrations of Phe and/or low cerebral concentrations of other Large Neutral Amino Acids (LNAA). Supplement of LNAA in adult patients has in some studies shown to be capable of some degree of lowering blood Phe level, while the influence

of cerebral Phe content has been greatest. **Objective:** The purpose of the study was to investigate the effect of short-term treatment with two different products containing LNAA in varying amounts in relation to lower blood (and thus brain) levels of Phe in adult patients with PKU. Furthermore, it was investigated whether variation in product and dosage affects general wellbeing in this patient group without change of habitual diet. **Methods:** The study was a prospective, randomized, double-blind cross over study with a total of four consecutive periods of three weeks. Twelve patients aged 20-43 years tested two different LNAA tablets (Prekunil and Neophe), both preparations in two different doses. Patients received blood tests for analysis of amino acid profile and toxicological blood samples at baseline and at the end of each period, a total of five times. At the end of each period the participants filled out an SF36 form and a 3-day dietary record. In addition, the patients did blood tests at home every day the last week of each period, a total of seven times. These blood samples were analyzed for plasma Phe and - tyrosine. **Results:** There was no significant difference between the four treatment groups, either with respect to blood Phe, amino acid profiles or general wellbeing **Conclusions:** High dose of LNAA did not influence the Phe level significantly but had an enhancing effect on tyrosine. Twenty-five % of the participants reported that they felt markedly better on high dose Neophe

227 - What is the optimal dosage of Large Neutral Amino Acid when combined with a semi-free diet for patients with Phenylketonuria?

Kirsten Ahring[1], Jens Rikardt Andersen[2], and Lisbeth Birk Moller[1]

[1]Kennedy Centre, Copenhagen University Hospital, Glostrup, Denmark
[2]Copenhagen University, Copenhagen, Denmark

Background: Phenylketonuria (PKU) is a congenital, hereditary metabolic disorder in which blood levels of phenylalanine (Phe) is extremely high because of lack of the enzyme phenylalanine hydroxylase (PAH). Without treatment, this results in severe mental retardation, microcephaly, epilepsy and other neurological symptoms caused by severe high cerebral concentrations of Phe and/or low cerebral concentrations of other Large Neutral Amino Acids (LNAA). Supplement of LNAA in adult patients has in some studies shown to be capable of some degree of lowering blood Phe level, while the influence of cerebral Phe content has been greatest. **Objective:** The purpose of the study was to investigate the effect of short-term treatment with two different products containing LNAA in varying amounts in relation to lower blood (and thus brain) levels of Phe in adult patients with PKU. Furthermore, it was investigated whether variation in product and dosage affects general wellbeing in this patient group without change of habitual diet. **Methods:** The study was a prospective, randomized, double-blind cross over study with a total of four consecutive periods of three weeks. Twelve patients aged 20-43 years tested two different LNAA tablets (Prekunil and Neophe), both preparations in two different doses. Patients received blood tests for analysis of amino acid profile and toxicological blood samples at baseline and at the end of each period, a total of five times. At the end of each period the participants filled out an SF36 form and a 3-day dietary record. In addition, the patients did blood tests at home every day the last week of each period, a total of seven times. These blood samples were analyzed for plasma Phe and - tyrosine. **Results:** There was no significant difference between the four treatment groups, either with respect to blood Phe, amino acid profiles or general wellbeing **Conclusions:** High dose of LNAA did not influence the Phe level significantly but had an enhancing effect on tyrosine. Twenty-five % of the participants reported that they felt markedly better on high dose Neophe.

228 - Post-Prandial Amino Acid Profile in Children with Phenylketonuria Taking CGMP-AA or Phe-Free Protein Substitutes

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Introduction: The rate of delivery of amino acids into the systemic circulation affects their physiological utilization. Low phenylalanine caseinomacropeptide (CGMP-AA) a peptide based protein substitute, supplemented with L-amino acids is an alternative to conventional Phe-free L-amino acid supplements (AA) in phenylketonuria (PKU). It is suggested that CGMP-AA slows the rate of amino acid absorption compared with AA. **Aim:** To measure the pre- and post-prandial amino acid concentrations in a group of children with PKU taking either AA or CGMP-AA. **Methods:** 33 children (19 boys and 14 girls with a median age of 9.4 y, range 5-16y) were studied. 20 were taking CGMP-AA and 13 AA, as their protein substitute. Two capillary blood samples for quantitative amino acids were collected: (1) morning fasted sample (PAA) and (2) 2-hour postprandial (PPAA) sample, after ingestion of 20 g of protein equivalent from either CGMP-AA or AA and a low phenylalanine breakfast. **Results:** There was no significant difference in PAA and PPAA for total amino acids (total median [range] PAA with CGMP-AA 2354 mg [1889-3014], and AA 2460 mg [1684-2972]; PPAA concentrations with CGMP-AA were 3900 mg [2181-5383] and AA 3484 mg [2677-4755]. Total branched chain amino acids, essential amino acids and large neutral amino acids (excluding phenylalanine) were also

not significantly different for PAA and PPAA between the two formulations. However, PPAA (but not PAA) were statistically higher with CGMP-AA for isoleucine, threonine and methionine (median [range] PPAA isoleucine: CGMP-AA, 170 mg [97-270]; AA, 128 mg [72-201] [p = 0.0019]; threonine: CGMP-AA, 309 [197-614]; AA, 217 [122-358] [p = 0.0002]; methionine: CGMP-AA, 39 mg [24-82]; AA, 30 mg [24-410] [p = 0.004]). PAA and PPAA concentrations of phenylalanine, tyrosine and arginine for CGMP-AA and AA were not statistically different. **Conclusions:** Elevated concentrations of PPAA amino acids that are naturally high in CGMP-AA (threonine, isoleucine and methionine) were observed and overall CGMP-AA did not appear to slow the rate of amino acid absorption. It is important to gain better understanding of the absorption kinetics of CGMP-AA compared with AA by conducting detailed human studies.

229 - Blood Phenylalanine Reduction Corrects Monoamine Neurotransmitter Deficiencies and Improves Behavioral Performance but has no Effect Upon Cognitive Disability in Adult Mice With Phenylketonuria

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Central nervous system (CNS) deficiencies of the monoamine neurotransmitters dopamine and serotonin have been implicated in the pathophysiology of neuropsychiatric dysfunction in human phenylketonuria (PKU). In this study, we confirmed the occurrence of brain dopamine and serotonin deficiencies in association with severe behavioral alterations and cognitive impairments in hyperphenylalaninemic C57BL/6-*Pah*$^{enu2/enu2}$ mice, a model of human PKU. Phenylalanine-reducing treatments, including either dietary phenylalanine restriction or liver-directed gene therapy, initiated during adulthood were associated with increased brain monoamine content along with improvements in nesting behavior but without a change in the severe cognitive impairments exhibited by these mice in the water maze. Following the behavioral and cognitive testing, the mice were euthanized for biochemical analyses. Brains of *Pah*$^{enu2/enu2}$ brain showed significant reductions in the protein abundance and maximally stimulated activities of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), the rate limiting enzymes catalyzing dopamine and serotonin synthesis respectively, in comparison to those seen in wild type animals. Phenylalanine-reducing treatments initiated during adulthood did not affect brain TH or TPH content or maximal activity. Despite this apparent fixed deficit in striatal TH and TPH activities, initiation of phenylalanine-reducing treatments yielded substantial correction of brain monoamine neurotransmitter content suggesting that phenylalanine-mediated competitive inhibition of already constitutively reduced TH and TPH activities is the primary cause of brain monoamine deficiency in *Pah*^{enu2} mouse brain. We propose that CNS monoamine deficiency may be the cause of the reversible adverse behavioral effects associated with chronic hyperphenylalaninemia in *Pah*^{enu2} mice, although phenylalanine-reducing treatments initiated during adulthood are unable to correct the neuropathology and attendant cognitive deficits that develop during juvenile life in late-treated *Pah*^{enu2} mice.

230 - Treatment Adherence During Childhood in Individuals With Phenylketonuria: Early Signs of Treatment Discontinuation

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Introduction: Phenylketonuria (PKU) is an autosomal recessive disorder characterized by a deficiency in phenylalanine (Phe) hydroxylase activity. Early diagnosis and continuous treatment with a low Phe diet prevents severe neurological and cognitive impairment. **Aims:** 1. Analyze how treatment adherence evolves through infancy, childhood, and early adolescence in individuals with PKU. 2. Identify early signs of treatment discontinuation. **Methodology:** This longitudinal, retrospective study included 75 children diagnosed through newborn screening, ages 7 to 13 years. Data on blood Phe concentration, number of blood samples sent, proportion of samples with Phe concentrations over the recommended range, and number of visits to the metabolism clinic were recorded. Logistic regression analysis was used to identify the variables that predict treatment discontinuation before 13 years of age. **Results:** A progressive increase in mean blood Phe concentrations with age was identified. The greatest increase occurred between the first and second years of life. By age ten, mean Phe blood concentration of the group was above the recommended range. The proportion of samples with Phe concentrations over the recommended range also increased with age, from an average of 13% during the first year of life to 67% in early adolescence. Sixty-eight percent of the children attended the outpatient clinic and sent samples from birth to the time of the study. Individuals who discontinued follow-up showed significantly higher mean blood Phe concentrations (360 vs. 220.9 μmol/L; $P = .004$) and the proportion of samples over the recommended range (37% vs. 12% $P = .002$) was significantly higher during the second year of life. Mean age for children

who discontinued treatment was 5.5 years of age. Blood Phe concentration values at 12 to 23 months of age and at 6 to 8 years of age significantly predicted treatment discontinuation before 13 years of age. Conclusion: Treatment adherence in PKU diminishes with age. Early signs of treatment discontinuation can be identified during the second year of life, allowing preventive interventions in high risk groups.

231 - Hyperphenylalaninemias, Anthropometric Evaluation, and Body Composition of a Group of Patients, Havana, Cuba

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Hyperphenylalaninemias are caused by total or partial deficiency of the enzyme Phenylalanine hydroxylase. They comprise several conditions that differ from one to another, both clinically and biochemically, with classical Phenylketonuria being the most common entity. It is considered the most frequent metabolic disease with appearance after the neonatal stage. Symptoms usually occur after 6 months, presenting retarded psychomotor development. The diagnosis before a month of life allows to establish the appropriate therapy and to prevent neurological deficits. The nutritional evaluation is part of the treatment and is performed monthly in the consultation through clinical, anthropometric, biochemical and dietary indicators. **Objectives:** To perform the anthropometric and body composition evaluation in a group of patients with Hyperphenylalaninemias. Identify patients with poor nutrition. **Methodology:** An observational, descriptive cross-sectional study was performed. **Population under study:** Patients with hyperphenylalaninemias treated at the national reference clinic of the Pediatric Hospital of Centro Habana in December 2016. For the anthropometric evaluation, the following measurements were taken: weight, height, brachial circumference, triceps folds; Subscapular; Suprailiac and brachial. Body Mass Index (BMI), Muscle Area (AM), Fat Area (AG), and Summation of folds (SP) were calculated. Reference standards and cutoff points were used for the Cuban population up to 19 years. The data were processed in the SPSS program. **Results:** We evaluated 12 patients from 3 and 18 years old, of both genders. According to the BMI: normal 75.0%, overweight 16.7%, obese 8.3%; weight / size: normal 66.7%, overweight 25%, obese 8.3%; weight / age: normal 75%, high 25%; size / age: normal 91.7%, high 8.3%. body composition. AM: normal 100%; AG: normal 58.3%, high 41.7%; SP: normal 66.7%, high 33.3%. All are determined phenylalanine and tyrosine and indicates diet therapy with its special formula. **Conclusions:** Most patients had an adequate nutritional status. Patients with obesity or risk of obesity were identified, which is a consequence of the consumption of free allowable foods, which are rich in simple sugars and oils.

232 - Clinical Characteristics of the Adult Population With Phenylketonuria Attended at the University Hospital Virgen del Rocio

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Introduction: Phenylketonuria (PKU) is an inborn error of the phenylalanine metabolism with an estimated frequency in Europe of 1/10000 newborns. Accumulation of phenylalanine with consequences that if not treated promptly can cause neurocognitive impairment, seizures, aberrant behavior, and psychiatric symptoms, among others. For this, we consider of great importance knowing the clinical characteristics and degree of control of patients with this pathology treated in our unit. **Materials and Methods:** Descriptive cross-sectional study including all patients >18 years with PKU attended in our Unit from 2013-16 (excluding those with benign hyperphenylalaninemia). The following items were analyzed: sex, current age, age at diagnosis, severity, late diagnosis, genotype, control degree according to treatment type, pregnancies and comorbidities. **Results:** We studied 45 patients with a mean age of 32[21-55] years, being 55.6%(25) of them women. 86.7%(39) have a diagnosis of classical PKU, 11.1% (5) moderate and only 2.2%(1) have mild PKU. Among patients with classical PKU, 33.3% (13/39) had a late diagnosis. Talking about co-morbidities, overweight/obesity was present in 33.3% of patients, dyslipidemia in 17.8%, hypertension in 8.9%, osteopenia/osteoporosis in 22.2%, vitamin D deficiency in 15.2%, B12 deficit in 13.3% and folic acid deficit in 11.1%. We found 51 different mutations, being the most common: IVS10nt-11g> a (10 patients), I65T(6), V388M(6) and IVS4nt + 5g>1(6). Regarding the age at diagnosis, 67.4% (29) were diagnosed in the first month of life, 2.3% (1) between 1-3 months, 4.7% (2) between 3 months and 1 year, 23.3% (10) between 1-5 years and only 2.3% (1) with >10 years. Currently, 84.4% (38) of the patients have nutritional treatment and 15.6% (7) have sapropterin. 53.3% (24) of patients have phenylalanine levels <10 mg/dL, 40% (18) between 10-20 mg/dL and only 6.7% (3) >20 mg/dL. Within patients with nutritional treatment, 47.4% (18/38) have phenylalanine level <10 mg/dL as opposed to 100%(7) of patients with sapropterin. Finally, there were 5 pregnancies, 2 ended with voluntary interruption and one child was affected with

maternal PKU syndrome. **Conclusions:** Although most patients had an initial diagnosis of classical PKU, an early diagnosis in a considerable percentage has allowed us to achieve adequate control of phenylalaninemia with nutritional treatment and sapropterin at least in half of the patients.

233 - Mechanistic Aspects of Phenylketonuric Bone Disease

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Phenylalanine hydroxylase (PAH)-deficient phenylketonuria (PKU) is a treatable amino acidopathy that originally motivated newborn screening. Among the least investigated aspects of PKU is the bone phenotype. Human and rodent studies have established decreased bone mass to be observed in PKU which was originally attributed to dietary intervention; however, recent investigations indicate the bone phenotype is part of the disease pathology. The mechanism of PKU bone disease is poorly characterized but a definitive correlation with phenylalanine (PHE) management is not fully established. While PAH expression has been described in bone, it is minor compared to that observed in liver and kidney. We hypothesized that bone cells in the Pah^{enu2} mouse might have a direct bone defect unrelated to PHE levels based on findings that mesenchymal stem cells (MSC) or osteoblasts express PAH ($p = 0.04$), which thus might prevent effects of PHE on MSC differentiation and bone formation. The Pah^{enu2} mouse was assessed by static and dynamic histomorphometry; which showed bone density in PKU mice decreased 33% relative to the C57BL/6 background strain ($P = .03$, $n = 4$); bone volume/total volume was similarly decreased; trabecular thickness was unchanged while trabecular spacing increased ($P = .05$). On dynamic histomorphometry, the labeled surface did not change but mineral apposition decreased by 20%, $p < 0.001$. These data suggest a mineralization defect. We tested this hypothesis by isolating MSC from C57BL/6 and Pah^{enu2} mice, and subsequently inducing in vitro bone differentiation. MSC from both C57BL/6 and Pah^{enu2} formed normal bone nodules when cultured in standard bone differentiation medium containing ascorbate and beta glycerol phosphate. However, addition of 1000 μM PHE to differentiation media prevented MSC from both C57BL/6 and Pah^{enu2} from forming bone. We conclude that the PKU bone phenotype is mediated by PHE-directed interference with MSC maturation, and that the low-level endogenous PAH is insufficient to protect developing bone. At least in vitro, where sustained 1000 μM PHE is practical, the bone effect correlates directly with PHE levels, independently of MSC being derived from C57BL/6 or homozygous Pah^{enu2} animals. That MSC from wild type animals behave identically to MSC from Pah^{enu2} when cultured in media containing PHE, provides evidence that PHE toxicity is a mediator of PKU bone disease.

234 - Consistent Epigenetic Findings Across Human PKU, Mouse PKU, and Mouse MPKU

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Phenylalanine hydroxylase deficient phenylketonuria (PKU) is a rare, treatable amino acidopathy. The maternal PKU syndrome (MPKU) is a somatic embryopathy whereby in utero PHE toxicity affects the offspring of PKU-affected women. Despite >50 years of investigation, little has been characterized regarding PHE toxicity and pathological mechanisms wherein neurologic phenotypes are realized in either PKU or MPKU. Based on unequivocal data demonstrating epigenomic dysfunction owing to chemically diverse toxic exposures, we investigated patterns of DNA methylation as a response to PHE intoxication in human PKU, mouse PKU, and mouse MPKU. In homozygous Pah^{enu2} mice investigations were performed in brain tissue of hyperphenylalaninemic and PHE restricted animals. Using Pah^{enu2} to model MPKU, investigation was performed in heterozygous E18 offspring of either hyperphenylalaninemic or PHE controlled females. Human PKU was investigated in mixed leukocytes of classical PKU patients that were either exceedingly well controlled or therapy noncompliant. Methylated DNA immunoprecipitation and genomic sequencing were applied to assess patterns of DNA methylation. Consistent among all systems is significantly increased aberrant DNA methylation in the context of uncontrolled hyperphenylalaninemia and reduced but not fully eliminated aberrant methylation when PHE is well managed. Non-coding RNA genes and particularly microRNA genes are frequent targets of aberrant methylation across all systems. A common target is the microRNA gene cluster within the imprinted Dlk1-Dio3 locus. MiRNA gene demethylation causes upregulation of their expression which has downstream impact upon the expression of protein coding genes having neurologic function. Other consistent elements include genes involved in glutaminergic neuron function. Even in untreated classical PKU, improvement is realized after establishing PHE control which suggests a reversible mechanism characteristic of epigenetic systems. We posit that epigenetic mechanisms underlie elements of neurologic dysfunction brought on by PHE toxicity. PKU management has singularly focused upon PHE reduction but never the consequence(s) of PHE intoxication. We suggest neurologic aspects of both PKU and MPKU may be treatable by repurposing existing epigenome targeting drugs.

235 - A Pig Model of PAH-Deficient Phenylketonuria

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Phenylalanine hydroxylase deficient phenylketonuria (PKU) is the paradigm for a treatable inborn error of metabolism which motivated prospective newborn screening. The *Pah*^{enu2} mouse is the standard PKU animal model possessing the c.835T>C (p.F263 S) *Pah* mutation and hyperphenylalaninemia consistent with classical disease. While *Pah*^{enu2} biochemically models human disease, clinical manifestations of human PKU are poorly recapitulated. To create a PKU model with greater clinical relevance to human disease, CRISPR/Cas9 gene editing was applied to create a PKU pig model. Candidate guide RNAs (gRNA), for deletion of exon six in the pig *PAH* gene, were optimized in pig: rodent somatic cell hybrids. PCR demonstrated efficient editing (deletion, inversion) of the target region. An optimized pair of gRNAs and mRNA encoding Cas9 were injected into pig zygotes that were subsequently cultured *in vitro* prior to implantation into surrogate sows from which two F1 female animals (116 -1 and 116-2) were born. The F1 animals were characterized by molecular, biochemical, and clinical means. PCR studies confirmed that animal 116 -1 contained two edited PAH alleles, one with the expected 1.2 kb deletion of exon 6, while the second allele contained a 4.1 kb deletion encompassing exons 6 and 7. Animal 116-2 had a single edited allele with the expected 1.2 kb exon 6 deletion. Biochemically 116 -1 demonstrated blood hyperphenylalaninemia of 2,063 μM, urine phenylalanine of 12.24 mM/gram creatinine, and excretion of organic acid metabolites (N-acetylphenylalanine, phenyllactate, phenylpyruvate) consistent with PKU. Animal 116-2 has blood PHE in the normal range (114 μM), undetectable PHE in urine, and excreted no PKU associated organic acids. Clinically 116 -1 is hypopigmented and growth retarded while the carrier 116-2 has normal growth and pigmentation. These animals represent the first large animal model of PAH deficient PKU, and will provide a model to study PKU pathophysiology and serve as a preclinical model to assess potential therapeutic interventions.

236 - Daily Life, Dietary Practices, and Health Conditions of Adult PKU Patients: A Multi-Center Cross-Sectional Study

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Background: Only few data on dietary management of adult PKU patients are published. Therefore, the aim of this study was the assessment of living conditions, dietary practices and health conditions of adult PKU patients. **Methods:** 164 PKU patients ≥18 years of age from 7 German metabolic centers received the access code for an online survey (LimeSurvey), containing a total of 91 questions, including a standardized questionnaire on depression. Data on living conditions and health conditions were compared to published data on the German average population. **Results:** 125 patients completed the questionnaire (82 females/43 males). With 48%, the proportion of single households is higher than the German average. The number of children per patient (0.4) is lower than the German average. The educational level is similar to the German average. 49% of the patients work full time. 57% of the patients manage diet on their own. 85% adhere to a low-protein diet: 10% of the patients adhere to a phenylalanine (phe) restricted diet with strong calculation of phe intake, 49% to a phe restricted diet without calculation of phe intake, 3% to a vegan diet, and 23% to a vegetarian diet. 48% of the participants consume low-protein foods almost daily, 79% never eat fish, 59% do not eat eggs, and 27% no meat. 84% of the patients take amino acid mixtures regularly, 77% also on vacation. 70% of the participants feel better with diet, and 49% never interrupted the diet. 88% have regular contact to a metabolic medical center, 95% check their phe-level at least once a year. BMI is comparable to German average (26 kg/m^2). With 37%, the prevalence for depression is increased in adult PKU patients. Further health problems are allergies (37%), skin problems (31%), increased sleep requirement (34%), and concentration difficulties (31%). Only one case of a malignant tumor was reported, which is less frequent than in the average population. 83% of the patients rate their health condition at least as good, and 90% consider their quality of life as good. 62% consider no or nearly no restrictions due to PKU. 35% have regular contact with other PKU-patients, 37% take part in patients' support groups. **Conclusion:** These data show that adult PKU patients are socially well integrated. Adherence to diet is very high, and restrictions in daily life due to diet are considered low. However, these data may be

biased as participation in the survey may be skewed towards highly motivated patients.

237 - Blood Phenylalanine Control in Phenylketonuria in One UK Centre: Audit From 2010 to 2016

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Background: In phenylketonuria (PKU), in 2002, the blood phenylalanine (Phe) control was reported over 6 years from 1994-2000 in 4 centers: 3 UK, 1 Australian [1]. Patients aged <10y had 70% of blood Phe within target range but this decreased to 30% in patients >15y. Treatment strategies and dietary products have improved in recent years. In 2017, the new European PKU guidelines [2] suggested lower blood Phe target concentrations (age 0-12y, 120-360µmol/L; and age >13y, 120-600µmol/L). **Objectives:** To evaluate metabolic control, as measured by blood Phe concentration of patients with PKU from Birmingham Children's Hospital and to compare these with European PKU guidelines and the 2002 audit results. **Methods:** We analyzed 7 years' blood Phe concentrations between 2010 to 2016, inclusively from 98 patients (53 boys, 45 girls), diagnosed by newborn screening with PKU. Data was categorized into 3 age ranges: 0-6y, 7-12y and 13-18y. Patients had a median age of 4y (1-12) at the beginning of the study. 90 patients were treated by low Phe diet and 8 by low Phe diet and sapropterin. **Results:** Median blood Phe concentrations increased from 195 µmol/L in 0-6y, 295 µmol/L in 7-12 y, and 500 µmol/L in 13-18 y. For each year of age, blood Phe increased by a median of 23 µmol/L per annum (20 µmol/L in 0-6 y, 18µmol/L in 7-12 y and 26 µmol/L in 13-18 y patients). There was no difference between females and males (median blood PHE levels were: 0-6 y female 190µmol/L vs. male 200µmol/L; 7-12 y female 290 µmol/L vs. male 300µmol/L; 13-18 y female 520µmol/L vs. male 480µmol/L). The median % of blood Phe concentrations within target range were age 0-6 y, 88% (0-100); 7-12 y, 71% (0-100), and 13-18 y, 79% (0-100). Only one child aged 0-6y did not have any Phe levels within target. There were no gender difference for % blood levels within target range in children ≤12y, although in >12, 85% females vs. 74% males had blood levels in target range. The annual median number of home blood spot samples decreased from 46 (8-115) in 0-6y, to 32 (4-99) in 7-12y, and 27 (3-61) in 13-18y. **Conclusions:** Although median blood Phe concentrations consistently increased with age, most patients remained in good metabolic control. Overall, blood Phe results were better than the 2002 audit despite a lower blood Phe target range. We continue to strive for optimal metabolic control. [1] Walter et al, *Lancet*. 2002; 360(9326):55-7. [2] Van Spronsen et al, *Lancet Diabetes Endocrinol*. 2017. pii: S2213-8587(16)30320-5.

238 - Oxidative Stress in Cuban Patients With Phenylketonuria

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Oxidative stress has been thought to be involved in the pathogenesis of several inborn errors of metabolism including phenylketonuria (PKU). The aim of this study was to investigate some redox status biomarkers in patient with PKU treated with a diet restricted of phenylalanine. We performed an observational case–control study. We included a total of 13 patients of both genders and 16 healthy children as controls from Havana city. Informed consent was obtained from all parents of children who participated in this study. Plasmatic levels of malondialdehyde, advanced oxidation protein products, total thiols and intraerythrocytic enzymatic activities of Cu/Zn Superoxide Dismutase and Catalase were determined by spectrophotometric methods. It was found high plasmatic levels of total thiols in PKU patients compared to controls. The activity of antioxidant enzymes tested the ratio of Cu/Zn superoxide dismutase to catalase and oxidative damage markers in PKU patients were not different from the control group. The increase in thiols groups concentrations and the low levels of oxidized products found suggest the existence of mild oxidative conditions in PKU patients with a restrictive diet.

239 - Studying the Effect of Large Neutral Amino Acid Supplements on Oxidative Stress

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Objective: Oxidative stress is may be considered to be responsible for the mental retardation in phenylketonuria patients. Phenylalanine (Phe) reduces antioxidant defense and promotes oxidative stress by causing increase in reactive oxygen species and reactive nitrogen species. Our study aims to investigate the effect of different treatments (amino acid mixture/Large neutral amino acid (LNAA) supplements), which are applied to patients with late diagnosis, on the oxidative stress that arises

in patients. To the best of our knowledge, this is the first study to investigate the effect of LNAA supplements on oxidative stress. **Method**: Twenty patients with classical phenylketonuria who were diagnosed late were included in the study. Patients included in the study were classified into two groups: patients under Phe-restricted diet and using amino acid mixtures, which do not contain Phe (Group-I) (mean age 13.8 ± 2.8), and patients taking LNAA supplements (Group-II) (mean age: 14.8 ± 3.8). Healthy controls (Control) (mean age 13.6 ± 4.8) with ages consistent with the ages of the experimental groups was included. A p value less than 0.05 was considered statistically significant. **Results**: Glutathione peroxidase (GSHPx) is lower in Group II than the control group ($P = .022$). Coenzyme Q10 (Q10) is lower in Group I than the control group and it is significantly higher in Group II than Group I ($P = .0001$, $P = .028$, respectively). No significant differences were detected in total antioxidant status (TAS), total oxidant status (TOS), Paraoxonase-1 (PON1) and L-carnitine levels. **Conclusion**: Reduced levels of GSHPx in patients can be due to the fact that LNAA supplements do not contain selenium, which is a cofactor of GSHPx. Increased synthesis of Q10 due to high levels of vitamin B6 in LNAA supplements can be the cause of increased Q10 levels in patients. Although it lacks L-carnitine in LNAA supplements, high levels of lysine in LNAA supplements might have increased L-carnitine synthesis, causing increased L-carnitine levels in patients. Nutritional deficiencies in patients taking LNAA supplements in addition to dietary therapy should be closely monitored by a nutritionist specialized in metabolic diseases. If required, a combination of dietary therapy, LNAA supplements, and amino acid mixtures should be used to make up for vitamin-mineral deficiencies.

240 - Chilean Experience in the Conventional Treatment of Children with Classic PKU Diagnosed by Newborn Screening Program: 24 years of Experience

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Introduction: Phenylketonuria (PKU) is a metabolic disease characterized by increased plasma levels of phenylalanine (FA) which causes mental retardation. Through the National Neonatal Screening Programme for PKU 248 cases were diagnosed classical PKU, 46% of the patients are older than 10 years old, this represents important challenges regarding adherence to treatment and PKU maternal syndrome prevention. **Methodology:** The treatment is based on restricting intake of FA, providing formula without FA, medical and nutritional assessment, psychometric (Weschsler Bayley Scale) and biochemical evaluations (MS / MS). **Results:** Average age of diagnosis: 17.6 ± 9.5 days, with initial values of FA: 18.5 ± 8.9 mg / dL and Tyrosine: 1.2 ± 0.7 mg / dL. Current age range is 1 month to 24 years. The average diet provides: 15.5 ± 10 mg / kg / day, 2.1 ± 0.7 g Prot / kg / day (80% of the formula), 1330 ± 441 kcal / day. 71% of PKU patients maintain blood FA values under 6 mg / dL (good metabolic control), with significant correlation ($P < .01$) with IQ. The 89% of PKU patients have normal IQ, 9% borderline and 2% boundary slight delay. 61% have normal nutritional status, 19% are overweight, 15% are obese and 5% are underweight. **Conclusion:** Diagnosed PKU children's neonatal growth and development is within normal ranges when FA levels are maintained under 6 mg/dl during follow-up.

241 - Hyperphenylalaninemia due to Dihydropteridine reductase (DHPR) Deficiency: Experience in Diagnosis and Management in the Social Security of Peru

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Introduction: Dihydropteridine reductase (DHPR) deficiency (OMIM #261630) is a severe form of hyperphenylalaninemia (HPA) produced by impaired regeneration of tetrahydrobiopterin (BH4), which leads to decreased levels of neurotransmitters (dopamine, serotonin) and folate in cerebrospinal fluid, and causes neurological symptoms such as psychomotor delay, hypotonia, seizures, abnormal movements, hypersalivation, and swallowing difficultness (Orphanet, 2014). **Objective:** To describe a peruvian case of DHPR deficiency. **Methods:** We present a two-years-old patient from Piura-Peru (DOB 07-01-2015), referred to the genetics department with a Non-PKU Hyperphenylalaninemia (non-PKU HPA) diagnosis. **Results:** The patient is the third child from healthy, non-consanguineous parents. He had an older brother, who died at 15 years old, diagnosed with "cerebral palsy," and a healthy sister. He was born by cesarean section at the 38th week of pregnancy, at birth he weighted 2650gr (p13), measured 47 cm (p17) long, and had a head circumference of 34.5 cm (p65). Apgar score was 9^1-10^5. At the 8th day of being born, jaundice was noted and he received phototherapy for one day. At 4 months of age, he started physical therapy due to developmental delay. At 9 months of age, his weight and height was below the 3 rd percentile. Physical examination revealed relative macrocephaly and marked muscular hypotonia. He did not achieve cephalic control nor sitting. The phenylalanine level measured by Tandem Mass Spectrometry was 359 μmol/L and by high-performance liquid chromatography was 193 μM. The enzymatic activity of DHPR was undetectable and the levels of biopterin were relatively high compared to those of neopterin. Both findings were compatible with DHPR deficiency. Patient began folinic acid supplementation in addition to

neurotransmitter precursors (5-hydroxytryptophan and levodopa/carbidopa) and a phenylalanine-restricted diet at age 1. At 19 months old, his weight was 8.9 kg (p1), measured 80 cm (p7) tall and had a head circumference of 49 cm (p53). He improved his cephalic and thoracic control, and has improved movement with hands and legs. **Conclusion:** This is the first experience in diagnosis and management of a patient with DHPR deficiency at Edgardo Rebagliati National Hospital, the main hospital of the Social Security in Peru. With adequate treatment, the clinical evolution has been good and our goal is to stabilize the patient and prevent further neurocognitive deterioration.

242 - A Pharmaceutical Technology Applied to Medical Food Engineered to Allow Physiological Absorption of Amino Acids (AA) for Phenylketonuria

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A pharmaceutical technology has been applied to a mix of amino acids (AAs) with the double aim of fully masking their taste, odor and eliminating aftertaste as well as of prolonging their release in the gut, allowing a physiological absorption. A pre-clinical study investigated the kinetics of two AA mixes engineered (engP-1 and engP-2) to allow a prolonged and gradual release of the AAs compared to the same mix of free (nonengineered; n-engP) AAs and to casein. In a cross-over design, 4 groups of female pigs (2 animals/group) received the 4 oral products (engP-1, engP-2, n-engP, and casein) in single dose, with wash-out periods $\geq$ 48 h. The administered dose was of 0.8 g AA/kg body weight. Blood sampling was obtained before (0.75, 0.50, and 0.25 hours) and after (0.25, 0.50, 0.75, 1, 1.25, 1.50, 2, 2.50, 3, 4, and 5 hours) each administration. AA concentrations were determined by a validated HPLC-MS/MS method. The following parameters were calculated: area under the concentration/time curve ($AUC_{0\text{-last}}$), peak concentration (C_{max}), time to C_{max} (T_{max}) and last measured concentration (C_{last}). C_{max} of the measured AAs (n = 13) in engP-1 and engP-2 resulted lower, 17% and 18% respectively, compared to the n-engP. This reduction in C_{max} was conspicuously higher and statistically significant for essential AAs (EAAs; $P < .01$), large neutral AAs (LNAAs; $P < .01$) and branched chain AAs (BCAAs; $P < .01$), indicating that the applied technology is able to reduce the typical absorption peak of free AAs, allowing to obtain products with C_{max} values very similar to casein, a reference food protein. As expected, $AUC_{0\text{-last}}$ of engP-1 and engP-2 was not statistically different from the $AUC_{0\text{-last}}$ of n-engP. AUC data indicate that the amount of absorbed AAs in engP-1 and engP-2 is comparable to that of n-engP, although complete AA absorption should be observed over >5 hours. The dissolution tests of all AA mixes showed a good in-vitro/in-vivo relationship. The technology conferred taste-, odor-, and aftertaste-free properties after initial in-house tests. Tests in healthy volunteers and patients are planned. Application of a pharmaceutical technology to AA mixes for PKU can bring new and important organoleptic improvements theoretically conducive to optimal adherence to dietary treatment as well as potentially leading to clinically relevant benefits based on a more physiological absorption of AAs.

243 - Adolescents in the PKU Clinic

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Phenylketonuria (PKU) is an inherited autosomal recessive disease. Metabolic control can be difficult to achieve especially for adolescents. Literature discusses the importance to meet the growing independence of young patients and increasingly adult behavior, as the risk for adolescents to 'drop out' of the medical care system if the patient is not understood. Transition from child to adult health care is a particular vulnerable period for young patients with inborn metabolic diseases as PKU. In Denmark, there is only one clinic that offers the very specialized knowledge about PKU. Since 1967 Center for PKU has handled the treatment and follow up visits of the patients in order to stay mentally well functioning by learning and guidance the individuals in the very restricted lifelong low protein diet. In order to understand the adolescents with PKU in Center for PKU, twenty-seven questionnaires were sent by email to PKU patients aged between seventeen and twenty-one years in age. Seven adolescents with PKU (4 females and 3 males) answered the questionnaire. Questions as: "Do the professionals talk about things that you find of your interest?" and "is it an advantage or disadvantage that you meet the same environment and staff as when you were a child and that there is a knowledge of your childhood?" In a combination with observations in the clinic the results points to that experience and knowledge about the patients' childhood and life amongst the employees in the clinic is crucial to maintain the important relationship between the professionals and the adolescents. If not, the patients express less feeling of safety and continuity. Furthermore, they express a feeling of neglect when they meet organizational changes and many different professionals in the clinic. In conclusion, PKU adolescents do not find it beneficial and necessary to make a transition to an adult clinic in order to be met and understood as an adolescent.

244 - Case Report: Study of the Clinical Significance of the 168 + 19 T> C Mutation

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Introduction: Phenylketonuria (PKU) is an inherited error of metabolism caused by a deficiency in the enzyme

phenylalanine hydroxylase. This enzyme is codified in PHA gene found on chromosome 12, and a mutation results in an increase of phenylalanine and decrease of tyrosine in blood. The mutational spectrum of the PHA gene is large, and have been identified a wide variation of genotype distributed along its 13 exons. The identification of PHA genotype provides the information to give to the family genetic counseling, genotype-phenotype correlation and in some cases treatment adjustments. **Objective:** Confirm the pathogenicity of 168+19T>C mutation. **Materials and Methods:** Molecular genetic analysis was performed in a family to confirm PKU diagnosis in a child detected through Newborn Screening. Genomic DNA was obtained from samples of whole blood on Whatman S&S 903 filter paper. DNA was amplified by polymerase chain reaction (PCR) technique, using *PAH* gene primers. The PCR products were purified and sequenced by ABI 310 (Applied Biosystems). After DNA analysis, 168+5G>C and 168+19T>C mutations were detected in the newborn in homozygosis. Moreover, *PAH* gene was amplified in both parents, using primers for exons 2 in order to find the same mutations as the patient. **Results**: Mutations 168+5G>C and 168+19T>C were present in the mother, both in heterozygosis. The father was heterozygote for 168+5G>C and homozygote for 168+19T>C. Phenylalanine concentration was analyzed in both parents by tandem mass spectrometry showing normal values: 34 μmol/L and 52 μmol/L, respectively, also with normal phenylalanine and tyrosine relation. **Discussion and Conclusion:** In accordance to the results found in parent's molecular analysis, is possible to confirm that the mutations in the PKU child are in different alleles. The father's profile shows normal values for phenylalanine as well as normal phenotype with 168+19T>C mutation in homozygosis. The clinical significance of this mutation is unknown in HGMD data base. So, with these evidence, we could conclude that the mutation 168+19T>C is not pathogenic for PKU.

245 - A Tool for Allelic and Genotype-Based Prediction of Metabolic Phenotypes in PKU

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Background: Since the genotype determines the activity of phenylalanine hydroxylase (PAH) and thus the metabolic phenotype, there is growing evidence of genotype–phenotype correlation. More recent studies have confirmed a significant relationship between allelic phenotype, enzyme activity, and BH_4 responsiveness. The nature of the variants or the known effect of the variants on PAH structure can reliably predict the enzyme activity in a number of genotypes but not all. The statistical and analytic power of large gene variant databases can be used to explore the relationship between genotype and phenotype in PKU. **Methods**: A total of 9484 PAH-deficient patients with a full genotype and metabolic phenotype from the BIOPKU database has been linked with the PAH locus-specific database (both at www.biopku.org). 588 out of 993 variants in PAHvdb have information on the phenotype from BIOPKU and 250/588 variants were classified as null variants with no residual enzyme activity. Based on the assumption that an observed metabolic phenotype depends on the maximum residual PAH activity of both variants, we develop an iterative algorithm to flag each variant as a classic PKU, mild PKU, MHP or unknown and assigned an allelic phenotype value (APV). **Results:** The algorithm based on frequencies of metabolic phenotypes of unknown alleles combined with null variants (functional hemizygosity) and with already flagged variants, generated 10 possible combinations and the metabolic phenotype was predicted by the maximum enzyme activity of the two alleles. Depending on the algorithm-specific classification error rate, up to 50% of variants could be flagged as classic, mild or MHPA variants, with up to 70% correct classified cases in BIOPKU. Our model makes only a prediction when there were at least 4 cases with known metabolic phenotype. More than 50% of all genotypes reported to occur however in only one patient, and therefore do not allow prediction so far. **Conclusions:** Our data-driven model combines information about expert ratings and frequencies of metabolic phenotypes to assign APV. APV can be used to predict a metabolic phenotype with a reasonable sensitivity. Because our model depends on the observed frequencies of variants, up to 50% of combination of variants could not be flagged due to sparseness. This, however, allowed us to avoid imprecise predictions for genotypes with less frequent variants, and therefore possibly unfounded clinical predictions.

246 - Maternal Phenylketonuria and Offspring Outcomes: A Single Centre Experience

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Phenylketonuria (PKU) is an autosomal recessive inborn error of amino acid metabolism. Elevated phenylalanine (Phe) levels in a pregnant woman with PKU may result in "Maternal PKU Syndrome (MPKU)," an embryopathy whose clinical phenotypes include spontaneous abortion, preterm labor, intrauterine growth retardation-IUGR, small for gestational age-SGA, microcephaly, psychomotor delay, facial dysmorphism, cardiopathy and associated malformations, regardless fetus' genetic PKU status. While PKU newborns can now be treated by early dietary and/or pharmacological intervention, damage caused to the fetus by mother's high Phe levels is irreversible. More favorable outcomes are observed when Phe levels are maintained within "safe ranges" prior to conception or promptly as

an unplanned pregnancy is recognized. Newborn screening program for PKU was introduced in Italy by law in 1992; its success resulted in a large number of women with PKU now of childbearing age at risk of having children with MPKU. Purpose of this study was to describe a female PKU group in charge at our Metabolic Department and to evaluate their obstetric and neonatal complications' incidence. 24 PKU women for a total number of 39 pregnancies were followed: 15 were diagnosed by newborn screening, 2 late (> 1 y.o.) diagnosed and 7 after labor. Among all 39 pregnancies, it was described: spontaneous abortion (15.3%), IUGR (2.5%), and premature labor (2.5%). Within the total of 33 newborns, we found microcephaly (24.2%), SGA (6%), facial dysmorphisms (9%), cardiopathy (6%), labiopalatal cleft (3%), and psychomotor delay (18.1%). Pregnancies of women with Phe values consistently <240 μmol/L (Group A) compared to ones with >240 μmol/L (Group B) showed a statistically significant difference referred to outcomes, with significantly higher neonatal complications in Group B ($P = .0013$). This was confirmed considering both obstetric complications as a whole ($P = .010$) or spontaneous abortion isolated ($P = .027$). Concluding, beneficial effects of newborn screening must not be overshadowed by possible birth defects due to maternal PKU. Prompt identification of PKU females of childbearing age combined with intensive patient counseling throughout preadolescence, adolescence, and adulthood is mandatory, optimizing care to ensure benefits for the next generation.

247 - Finger Prick to Finger Tip: Novel Use of Technology to Improve Patient Communication in PKU

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Objective: The metabolic dietetic team deal with approximately 120 weekly Phenylalanine (Phe) levels. The related phone calls to communicate results take an average of 10 minutes. An audit of 500 Phenylalanine levels highlighted that 52% of the results being communicated were within the target range. This project aimed to reduce the amount to dietetic time on telephone calls. **Method:** Collaboration between ICT, metabolic laboratory, Data Protection Commission, and metabolic dietitians enabled the development of the PKU texting system. Following a successful pilot study, the system was offered to all PKU patients >2 years. When the Phe has been analyzed and authorized on the laboratory system, the demographics are matched with the patient mobile phone number. Text messages are then validated and sent by the dietitian via a web portal using Defero SMS texting service. There are four different messages in use: (1) Phe level if within target range; (2) Phe level out of range, please contact dietitian; (3) Sample insufficient. Please repeat; (4) Results delayed. **Results**: 231patients/families currently using the texting system. A Patient Survey Monkey (n = 46 and 16% response rate) showed that 87% rated the texting system as either very good or excellent. 94% agreed that it was time saving. 84% felt there was no influence on dietary compliance. When financial implications and the impact on dietetic time over 21 months were analyzed it was found that the savings on texting versus phone calls was 3275 euro, while the dietetic time saved was 580 hours. A retrospective study was carried out to review whether or not the implementation of the texting system had any effect on Phe levels and the percentage within target range. The frequency of sampling remained unchanged and the target range remained unchanged at 52%. **Conclusion:** Collaboration between three departments in the hospital allowed the successful implementation of technology to enhance patient care. Results are received more timely. There is no evidence two years post implementation that the system has had an effect on either the Phe levels in terms of recommended range or frequency of sampling. Patient feedback has been very positive.

248 - Development of Genetically Engineered E. coli Nissle Strains for the Treatment of Phenylketonuria and Maple Syrup Urine Disease

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The fields of synthetic biology and microbiome research expanded greatly over the last decade and enables the development of new therapeutic strategies, using engineered microbes that operate from within the gut. Phenylketonuria (PKU) and maple syrup urine disease (MSUD), two inborn errors of metabolism disorders (IEMs) where a toxic substrate is present in the intestinal lumen, are potential therapeutic targets for this approach. PKU, caused by a defect in phenylalanine hydroxylase (PAH) activity, is characterized by the accumulation of systemic phenylalanine (Phe) that can lead to severe neurological deficits unless patients are placed on a strict low-Phe diet. As an alternative treatment, *Escherichia coli* Nissle (EcN), a well-characterized probiotic, was genetically modified to efficiently import and degrade Phe (SYN-PKU). The coupled expression of a Phe transporter with a Phe ammonia lyase (PAL) allows rapid conversion of Phe into trans-cinnamic acid (TCA) in vitro. Experiments conducted in the *enu2−/−* PKU mouse model showed that the oral administration of SYN-PKU is able to blunt an increase in blood Phe level triggered by subcutaneous Phe injection by 49% compared to controls. To support further the development of

SYN-PKU, hippuric acid (HA) was examined as a urine biomarker of in vivo activity. Analysis of *enu2−/−* mice dosed orally with TCA demonstrated that 88% of gavaged TCA was excreted in the urine as HA, and *enu2−/−* mice treated with SYN-PKU showed greatly elevated urine HA levels, concomitant with a significant decrease in their blood Phe level. In addition to SYN-PKU, a second EcN strain was genetically engineered to rapidly import and degrade branched-chain amino acids (BCAAs) for the treatment of MSUD (SYN-MSUD). MSUD is caused by a defect in branched-chain ketoacid dehydrogenase activity leading to the toxic accumulation of BCAAs and their ketoacid derivatives. The controlled expression in SYN-MSUD of two BCAA transporters and three BCAA-degrading enzymes result in the efficient degradation of BCAAs into branched-chain alcohols. In a mouse model of MSUD, the oral delivery of SYN-MSUD suppressed the increase in blood BCAAs level induced by a high-protein challenge and prevented the associated moribund phenotype, as measured by locomotor activity. In conclusion, the in vivo therapeutic effects observed with SYN-PKU and SYN-MSUD support the further evaluation of engineered microbes as promising approaches to treat serious IEMs.

249 - Phenylketonuria and Calcium Consumption: Case Report

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The Phenylketonuria is the metabolism's innate error most found in the brazilian population and it is related to the metabolism of the amino acid phenylalanine. Thanks to a restrictive diet, some foods that are sources of calcium, like milk, for example, are removed from the patient's diet, potentially causing diseases of bone demineralization. Because of this, the goal of this report was to evaluate calcium consumption of this patient. After nine months of study it can be noted a diminution of sérico calcium however it is judged necessary more studies about the relationship of consumption of this mineral and dietary restriction as a consequence of phenylketonuria. The study was conducted with a student answered in outpatient chronic patients of the Hospital Infantil Joana de Gusmão, classical phenylketonuria, diagnosed in the newborn screening, 7-year-old male, a resident of the city of Florianópolis, State of Santa Catarina. An only child, living with parents who are cousins. Queries are performed according to Protocol for patients with phenylketonuria. Lack of adherence to treatment and low consumption of food sources can lead to a lack of calcium, a mineral with extensive participation in the growth and development of children and adolescents with Phenylketonuria. On this concern, this study shows that low calcium consumption can interfere significantly in bone metabolism but should be conducted more studies in children with this innate error of metabolism.

250 - Short-Term Biological Variance of Phenylalanine in Patients With Phenylketonuria

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Control of blood phenylalanine (PHE) levels is the primary goal in managing phenylketonuria (PKU). Both the overall exposure to PHE and the variation in PHE are thought to contribute to long-term neurocognitive outcome. The objective of this study was to measure the diurnal variation of PHE in patients $\geq$4 years of age and to examine the interaction of dietary patterns with PHE levels. Four groups were studied: The first two groups were patients with PKU (at least 1 documented PHE level $\geq$600 μM) who were treated with diet alone. These were divided into "High PHE" (n = 6) and "Target PHE" (n = 12) groups based on blood PHE levels on the day of the study ($\geq$600 μM or <600 μM, respectively). The "Sapropterin" group (n = 9) was PKU patients treated with diet plus sapropterin dihydrochloride (KUVAN® BioMarin Pharmaceutical Inc., San Rafael, CA). Finally, the "Control" group (n = 5) was patients with benign / mild hyperphenylalaninemia (PHE levels <600 μM with no diet or sapropterin therapy). After an overnight fast, preprandial and 1- and 2-hour postprandial samples were collected for each meal over 24 hours. Plasma was analyzed for amino acids and complete food records were analyzed. The maximum, mean, and standard deviation of PHE levels were calculated. Significance was calculated by two-way single factor ANOVA with post hoc Man-Whitney *U* test. The mean PHE levels were 1077, 306, 377, and 370 μM in the High PHE, Target PHE, Sapropterin, and Control groups, respectively. The High PHE group was significantly higher versus the other 3 groups ($P < .0001$), with the same pattern seen for the maximum PHE. The standard deviation of PHE was not significantly different between the groups. Intact protein intake was significantly lower in the High PHE and Target PHE groups compared to Control ($P < .0001$), while Sapropterin was not significantly different from Control. The most common diurnal PHE pattern was a high level in the morning with a decrease over 12 hours followed by a rise over night. PHE and PHE/Tyrosine ratio usually fell post-prandially, especially if PHE-free formula was consumed, and generally no "spikes" in PHE were seen post-protein intake. In conclusion, intact protein intake was similar between Sapropterin and Control groups, indicating a more "natural" diet in the Sapropterin group. PHE levels tend to fall throughout the day when eating and rise when fasting, which is an important consideration for timing of monitoring samples.

251 - Amino Acid Level Correlations Between Tandem Mass Spectrometry and Ultra-Performance Liquid Chromatography and the Clinical Relevance for Phenylketonuria Management

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Management of phenylketonuria (PKU) and other metabolic disorders includes repeated measurement of analytes in blood to monitor and adjust therapy. Plasma or serum are common sample types, but their collection generally requires trained personnel and often requires a patient to travel to a blood collection center or laboratory. Whole blood collected as dried blood spots (DBS) on filter paper are commonly used in newborn screening, but because collection requires very little training and shipping to a central laboratory is inexpensive, this sample type can also be used for home monitoring. In our PKU clinic, plasma samples for amino acids (phenylalanine, in particular) are routinely measured by ultra-performance liquid chromatography (UPLC). Other samples are collected at home as DBS and these are shipped to our regional newborn screening laboratory, which offers amino acid analysis of monitoring samples by tandem mass spectrometry (TMS). The different analytical methodologies may contribute to possible bias between plasma and DBS sample types. Monitoring serial analyte levels in one PKU patient may include both sample types, therefore it is important to know about any bias when comparing results. 450 venous samples were collected on 32 patients with PKU. Each sample was collected in a syringe which was used to spot whole blood onto filter paper and then fill heparinized tubes for plasma separation. DBS samples were analyzed by a Waters Acquity TMS using flow injection and stable isotope dilution quantitation of a limited number of amino acids. Plasma samples were analyzed using MassTrac Amino Acid Analysis on a Waters Acquity UPLC. 9 amino acids were measured by both methods (alanine, arginine, citrulline, glycine, methionine, ornithine, phenylalanine, tyrosine, valine), while "leucines" (combined measurement of isobaric leucine and isoleucine on TMS) was compared to the sum of leucine and isoleucine (individually measured by UPLC). Phenylalanine showed a high correlation ($R^2 = 0.96$) but DBS values were 15% lower than plasma (slope 0.85). Arginine and ornithine had poor correlation ($R^2<0.20$). Most other amino acids showed good correlation (R^2 0.70-0.88) but DBS consistently showed lower levels than plasma (ranging from 55% to 16% lower). These results show that DBS amino acid measurement by TMS has a negative bias compared to plasma measurement by UPLC. Caution should be exercised when comparing serial phenylalanine measurements using different methodologies.

252 - Trends in Overweight and Obesity Among Adolescent Classical Phenylketonuria Patients Between 2007 and 2017

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Objective: Excess body weight is increasing in phenylketonuria (PKU) patients in the last decade. The first aim of this study was to detect the prevalence of overweight and obesity in adolescent classical PKU patients. The second aim was to compare this prevalence with the previous five (2012) and ten (2007) years. **Methods**: Data of 32 adolescent PKU patients from 2007 (group I), 37 from 2012 (group II) and 48 from 2017 (group III) were evaluated retrospectively. The body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Overweight and obesity was defined as a BMI between 85 and 95th percentiles, and exceeding the 95th percentile, respectively, for the patients age and sex. Data were expressed as median [25-75 percentiles]. **Results**: Median age of the patients were 13.5 [12.5-16.7], 16.0 [13.0-17.5] and 14.5 [12.5-17.0] years, of group I, group II and group III, respectively ($P = .376$). All patients were on Phe-restricted diet. Median BMI was significantly higher in group III (BMI: 21.85 kg/m^2 [18.45-24.7]) than group I (18.45 kg/m^2 [16.8-19.8], $P = .03$) and group II (19.3 kg/m^2 [17.5-21.5], $P = .03$). There was no difference between group I and II patients regarding BMI ($P = .22$). The prevalence of excess body weight (overweight plus obesity) was significantly higher in group III (18 patients, 37.5%) than group I (3 patients, 9.4%) and group II (5 patients, 13.5%), $P = 0.006$ and $P = .012$, respectively. As expected, there was no difference between group I and II ($P = .440$). **Discussion**: The prevalence of overweight and obesity in adolescent PKU patients has increased over ten years, especially in the last five years following the same trend as the general adolescent population. Along with the sedentary lifestyle, restriction of natural protein sources and excess consumption of Phe-restricted special products and also carbohydrates are the main causes of excess weight gain in classical PKU patients.

253 - DNAJC12 Deficiency: A Novel Treatable Cause of Hyperphenylalaninemia, Central Biogenic Amines Deficiency, Dystonia, and Intellectual Disability: The First Six Patients

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Objective: Phenylketonuria (PKU, phenylalanine hydroxylase deficiency), an inborn error of metabolism, can be detected through newborn screening for hyperphenylalaninemia (HPA). Most individuals with HPA harbor mutations in the gene encoding phenylalanine hydroxylase (PAH), and a small proportion (2%) exhibit tetrahydrobiopterin (BH4) deficiency with additional neurotransmitter (dopamine and serotonin) deficiency. Here we report six individuals from four unrelated families with HPA who exhibited progressive neurodevelopmental delay, dystonia and a unique profile of neurotransmitter deficiencies without mutations in *PAH* or BH4 metabolism disorders related genes. **Methods**: In these six affected individuals, whole-exome sequencing was performed after exclusion of mutations in the known genes associated with HPA (*PAH, GCH1, PTS, QDPR* and *PCBD1*) or biogenic amine neurotransmitter defects (*SPR, TH, DDC, DAT* and *VMAT2*). **Results**: Biallelic mutations in *DNAJC12*, which encodes a heat shock co-chaperone family member that interacts with phenylalanine, tyrosine and tryptophan hydroxylases respectively catalyzing the BH4-activated conversion of phenylalanine into tyrosine, tyrosine into L-dopa, the precursor of dopamine and tryptophan into 5-hydroxytryptophan, the precursor of serotonin. The DNAJC12 protein was undetectable in fibroblasts from the individuals with null mutations. PAH enzyme activity was reduced in the presence of *DNAJC12* mutations. Early treatment with BH4 and/or neurotransmitter precursors had dramatic beneficial effects and resulted in the prevention of neurodevelopmental delay especially in the one individual treated before symptom onset who remained asymptomatic at 2 years of age. **Conclusion**: DNAJC12 deficiency is a novel preventable and treatable cause of intellectual disability that should be considered in the early differential diagnosis when screening results are positive for HPA. Sequencing of DNAJC12 may resolve any uncertainty and should be considered in all children with unresolved HPA. **Reference**: Anikster et al. (2017). *Am. J. Hum. Genet.* 100:257-266.

254 - Mutations of Phenylalanine Hydroxylase Gene Detected in 536 Patients From Southeastern Part of Turkey

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Background: Phenylketonuria (PKU) is the most common inborn error of amino acid metabolism, which was firstly included into the neonatal screening in Turkey, due to its devastating-irreversible effect on central nervous system. Up to date 991 variants are reported in phenylalanine hydroxylase (*PAH*) gene. **Objectives:** With this study, we aimed to assess the genotype-phenotype correlation of patients from the Southeastern part of Turkey. **Methods:** Non-PKU-hyperphenylalaninemia (HPA) defines the blood phenylalanine level: 120-360 µmol/l, mild PKU: 360-600 µmol/l, moderate PKU: 600-1200 µmol/l, and classic PKU $\geq$ 1200 µmol/l. *PAH* gene sequence analysis was performed by using MiSeq next generation sequencing platform. **Results:** The distribution of 536 PKU patients are; Hyperphenylalaninemia: 32.5%, mild-PKU: 12.1%, moderate-PKU: 25.5%, and classic-PKU: 29.9%. 460 patients were diagnosed with newborn screening and 76 of them were late diagnosed. With this study, we identified 17 different mutations which are not reported before. The patients have either compound heterozygous (222) or homozygous (314) mutations. The most frequent homozygous mutations are; [IVS10-11G>A];[IVS10-11G>A] (32.8%), [p.R261Q];[R261Q] (13.7%), [IVS4+5G>T];[IVS4+5G>T] (8%), [p.A300 S];[A300 S] (5.1%) and [p.P281 L];[P281 L] (4.8%). The phenotypes of homozygous patients are; HPA: 16.6%, mild-PKU: 11.5%, moderate-PKU: 32.2% and classic-PKU: 39.7%. The most frequent compound heterozygous mutations are; [IVS10-11G>A];[p.A403 V] (4%), [IVS10-11G>A];[IVS4+5G>T] (3.6%), [p.E178G];[IVS10-11G>A] (2.2%), [p.E178G];[IVS10-11G>A] (2.2%), [p.A300 S];[A403 V] (2.2%) and [IVS10-11G>A];[p.T380 M] (2.2%). The phenotypes of compound heterozygous patients are; HPA: 55.2%, mild-PKU: 12.7%, moderate-PKU: 16.3%, and classic-PKU: 15.8%. **Conclusions:** As both the prevalence of PKU (1/6500) and the frequency of consanguineous marriages is high in Turkey, we

need a national data for both treatment and genetic counseling. This study is an important step for this aim and adds 17 new mutations to the literature.

255 - Evaluation of the Relation Between the Physical Activity Practice and the Body Fat Percentage in Phenylketonuria Adolescents

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Introduction: An increase in the number of overweight and obese individuals has been observed in phenylketonurics, but little is known about the practice of physical activity in this population. **Objective**: To verify the existence of an association between the percentage of body fat and the level of physical activity of phenylketonuric adolescents. **Methods**: This is a cross-sectional study carried out with phenylketonuric patients between 10 and incomplete 20 years, early diagnosed and treated at the Special Genetic Service of the Hospital das Clínicas of the Federal University of Minas Gerais. All participants were submitted to the test of electrical bioimpedance to determine the body fat percentage and answered the PAQ - C questionnaire to assess the level of physical activity. Individuals who scored less than 3 points in the PAQ-C were considered sedentary, and those with scores greater than or equal to 3 were classified as physically active. The Pearson correlation test and Mann Whitney test were applied with significance level of 5%. **Results**: From 92 participants, 51 were male (55.4%) and 89 were considered sedentary (96.7%). The mean percentage of body fat in the group was 18.2 ± 8.8 and the median of the points in the physical activity questionnaire was 1.92 (interquartile range 1.63-2.23). No statistical difference was observed between the percentage of body fat of the active and sedentary individuals ($P = .114$). There was a moderate negative correlation between the points obtained in PAQ-C and the participants' age ($r = -0.311$; $P = .003$). Males are more active ($P = .008$), but there was no difference between the time spent in sedentary activities by boys and girls ($P = .963$). **Discussion**: The stigma of the disease, the low executive function of phenylketonuric and the lower social interaction can lead to a decrease in motivation and participation in physical activity, justifying the high prevalence of sedentarism in this population. The insufficient number of individuals considered as physically active may have contributed to the absence of difference of body fat percentage according to the physical activity classification. There is a tendency to reduction of the physical activity practice with advancing age. **Conclusion**: Phenylketonuric adolescents are mostly sedentary and there was no difference in the body fat percentage according to the level of physical activity practiced by these individuals.

256 - Pedagogue Attributions in the Care of Patients With Phenylketonuria

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Introduction: The pedagogical service of the Newborn Screening Program of Minas Gerais (PTN-MG) is responsible for the activities and monitoring of educational actions and for the pedagogical orientation, especially in the initial period of the schooling process. The pedagogue acts on demand of the family and/or, spontaneously, when it is known that a patient is about to start school life, when he or she encounters some learning difficulties or even in the face of the challenge of the peculiarity of the disease in the school context. The work is done together with a nutritionist when it is necessary to adjust the school meals. **Objective:** To describe the flow of the pedagogue's role in the care of patients with phenylketonuria (PKU) in PTN-MG. **Results:** In general, the pedagogical workflow is based on: 1) Conversation between the pedagogue and the family about the importance of school attendance, insecurities, peculiarities and health risks, ignorance of education professionals about the disease and adherence treatment in the school environment. 2) Delivery of the pedagogical and medical report for the family to deliver at the school. 3) Pedagogical contact with director/teacher about the peculiarities of the disease and the student with PKU. 4) Request the contact of the nutritionist responsible for the school meal. 5) Sending of the menu by the school or by the responsible nutritionist. 6) Adequacy and resubmission of the menu suitable for the school by the PTN-MG nutritionist. 7) Follow-up of cases. Since 2014, 82 reports have been sent for insertion and/or follow-up from the student in Basic Education. In addition, 3 reports have already been made for PKU undergraduates requesting an increase in the time for testing, which is part of the Inclusive Education perspective. **Conclusion:** The work of the pedagogue is fundamental for monitoring the development and for the insertion of the student with PKU in the school environment. In addition, the pedagogue presents and discusses the peculiarities of this student in the course of his or her school life with all the education professionals involved with

it, making the school routine more natural. It is very important that the pedagogue works together to form a multidisciplinary team of care for the PKU patient.

257 - Pregnancy in Women With Phenylketonuria in Minas Gerais, Brazil

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Introduction: Women with phenylketonuria (PKU) or non-phenylketonuric hyperphenylalaninemia (HP) may have children with microcephaly, intellectual disability, cardiovascular system malformations, and other congenital anomalies related to the teratogenicity of elevated levels of phenylalanine (phe) during pregnancy (Maternal PKU Syndrome). Phe concentrations recommended during this period are between 120-360 µmol /L (2-6 mg/dL). **Objective:** To describe the follow-up of pregnant women with PKU or with HP of the State Program of Newborn Screening of Minas Gerais (PTN-MG). **Results:** Until 2017, eight patients became pregnant (nine pregnancies), of which seven were women with classic PKU and one with HP. Currently there are eight pregnancies completed and one pregnancy in progress. Blood Phe dosages are performed weekly. Geneticist, gynecologist/obstetrician, and nutritionist do the follow-up. Prenatal care is also performed in Primary Health Care (SUS). The mean age of women in early pregnancy was 17.7 ($\pm$ 1.22) years. The mean values of Phe were: pregnant 1 (481.15 $\pm$ 204.82 µmol/L), pregnant 2 (1st gestation: 505.07 $\pm$ 177.83 µmol/L) and second gestation: (746.39 $\pm$ 261, 12 µmol/L), pregnant woman 3 (657.93 $\pm$ 107.06 µmol/L), pregnant woman 4 (307.19 $\pm$ 176.18 µmol L), pregnant woman 5 (601.42 $\pm$ 119.47 µmol/L), pregnant woman 6 (821.71 $\pm$ 131.01 µmol/L), pregnant woman 7 (144.73 $\pm$ 30.02 µmol/L), pregnant woman 8 (975.87 $\pm$ 343.54 µmol/L). Two patients had spontaneous abortions, at 11 and 16 weeks. Six children were born, one with microcephaly and two with low birth weight. **Conclusion:** The aim of the treatment is to control the dietary intake of Phe, to maintain the amino acid blood concentrations within the recommended limits during the gestation and at the same time to provide adequate nutrition to the pregnant woman, the adequate growth and development of the baby. Adherence to the diet and amino acid mixture is very difficult, which was evident since most of the patients did not reach the adequate levels of Phe. The follow-up of the born child is still very short to make some diagnosis of deficiencies caused by maternal PKU, and a definitive conclusion is not possible, although some developmental problems have already been observed. It is important to invest in this area, especially in controlling Phe blood levels immediately before and throughout pregnancy.

258 - Comparison Between Casein Glycomacropeptide (CGMP-20) and Free Synthetic Amino Acids (AA) After a Standardized Meal on Selected Biomarkers in Phenylketonuria (PKU) Patients

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Background: Phenylketonuria (PKU) is a metabolic disorder with elevated blood levels of phenylalanine (Phe) due to deficiency of the enzyme phenylalanine hydroxylase. Casein glycomacropeptide (CGMP) is a natural peptide, released in whey during cheese making by the action of the enzyme chymosin. CGMP in its pure form does not contain Phe and this makes it nutritionally suitable as part of the diet for patients with PKU when supplemented with tyrosine, tryptophan, histidine, arginine, methionine, lysine and leucine. We investigated the influence of the following biomarkers when comparing the absorption of CGMP versus synthetic, free amino acids (AA): Ghrelin, glucose, insulin, Peptide-Tyrosine-Tyrosine (PYY), Cholecystokinin (CCK), Glucagon-like-peptide-1 (GLP-1) and Blood Urea Nitrogen (BUN). Lacprodan® CGMP-20 is the name of the product used in this study. **Methods:** Patients (7 females, 1 male, age 15-48 (mean 33.25 + Standard deviation (SD) 11.21), weight 47-85 kg (mean 72.8 + SD 15.9) were received in the clinic in the morning, fasting. They had been selected from the Kennedy Centre database from the following criteria: well treated from birth, age >15, and classical PKU. All patients had 4 identical visits in randomized order and had a new drink mixture (DM 1-4) at every visit, containing CGMP or AA. The four DM were designed as follows: DM1:100% CGMP, DM2:100% AA (=DM1), DM3:78.4% CGMP+19.6% AA, DM4:100% AA (=DM3). Ghrelin was measured at time 0 (fasting) right before eating the meal), 15, 30, 60, 120, and 240 minutes after the meal. The rest of the biomarkers were measured at time 0 and 240 minutes. **Results:** None of the seven nutritional parameters showed a significant change from baseline (time 0) to 240 min: DM1: Ghrelin ($P = .454$), glucose ($P = .334$), insulin ($P = .563$), PYY ($P = .981$), CCK ($P = .523$), GLP-1

($P = .871$), and BUN ($P = .260$). DM2: Ghrelin ($P = .180$), glucose ($P = .935$), insulin ($P = .726$), PYY ($P = 1.000$), CCK ($P = .910$), GLP-1 ($P = 0.420$) and BUN ($P = .800$). DM3: Ghrelin ($P = .473$), glucose ($P = .071$), insulin ($P = .484$), PYY ($P = .382$), CCK ($P = .620$), GLP-1 ($P = .131$) and BUN ($P = .937$). DM4 Ghrelin ($P = .986$), glucose ($P = .906$), insulin ($P = .537$), PYY ($P = .985$), CCK ($P = .297$), GLP-1 ($P = .068$) and BUN ($P = .991$). **Conclusion:** None of the selected biomarkers did significantly change from time 0 to 240 min after a meal. Thus, CGMP may be useful as a supplement in the PKU diet.

259 - Compliance in Children Aged 4-12 Years With Classical PKU, When Current Recommendations of Phenylalanine Levels are Reduced to Follow European Guidelines

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Background: PKU is an inborn error of the phenylalanine metabolism that requires lifelong dietary treatment. February 1, 2017, we reduced our recommended phenylalanine level to follow the European guidelines published in 2016.

Ultimo April 2016, the patients received a newsletter that the recommendations for phenylalanine levels would be changed. In a patient meeting in June 2016, all patients were once more informed of the changes according to the European Guidelines. January 2017 the patients again received a letter explaining the limits. Furthermore, some patients were informed of the new guidelines on their yearly, or half-yearly follow up visits. During the period, the patients have had the possibility to contact the dietician for advice how to reduce the phenylalanine level. Among other age groups, children aged 4-12 are influenced by the changes. The previous Phenylalanine recommendations were as follows: 4-10 year = 120-400 µmol/L and 10-12 year = 120-600 µmol/L. The new Phenylalanine recommendations are: 4-12 year = 120-360 µmol/L. The purpose of this project was to investigate the impact on children aged 4-12 years, with classical PKU. We wanted to know whether compliance was influenced by the new recommendations. **Methods:** We collected phenylalanine results from 29 patients during the period from February to April 2016, and compared these results with same period in 2017, as the samples from the months in 2016 were not influenced by the newsletter to the patients. All test results from 2016 to 2017 influenced by intercurrent illness have been removed. **Results:** In February to April 2016, 77.6% of all the blood samples were within the recommended range. In February to April 2017, 70.3% of all the blood samples were within the recommended area; thus, after reducing recommendations for phenylalanine level, 7.3% more blood samples were above the recommended area. **Conclusions:** Despite the fact that the patients were informed a year before, with newsletters, meeting, and normal follow-up consultations, it shows that it is very difficult to lower the phenylalanine range.

260 - PKU Genotype in Argentinean Patients

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Background: In phenylketonuria (PKU), a relatively frequent congenital metabolic disorder, over 900 mutations in the PAH gene (NM_000277.1) have been described (www.pahdb.mcgill.ca). The available data on PAH mutations are useful for the understanding of the clinical features although genotype/phenotype correlations are not always conclusive. In Argentina, 1:13 000 newborn are affected with either any clinical forms of the disease. **Objective:** To describe the genotype of a large group of Argentinean PKU patients. **Patients and methods:** 82 PKU patients of 72 families with hyperphenylalaninemia were genotyped. 10 pair of siblings were included (1 with discordant phenotype). According to their phenylalanine tolerance at age 4-7, patients were classified as classic (n:30) with Phe intake <350 mg, moderate (n:28) (350-800 mg/day), mild (n:9) PKU (800-1200 mg/day) or persistent Hyperphenylalaninemia (PHPA) (n:15) (>1200 mg/day). DNA from peripheral blood lymphocytes was extracted. Codifying and adjacent intronic regions of the 13 exons of the PAH gene were amplified by PCR and sequenced by Sanger. Allelic frequency was calculated for the whole cohort and for each phenotypic group. **Results:** 49 different mutations of the PAH gene were identified. 90% of patients were compound heterozygotes. The 9 pair of siblings with similar phenotype had an identical genotype while the discordant one had also a discordant genotype, presenting only one common allele. So, 145 PKU alleles from 73 patients were analyzed. Nine mutations accounted for 53.6% of mutated alleles p.R408 W (9.6%), p.R261Q (8.3%), p.A403 V (6.2%), p.V308 M (5.5%), p.Y414C (5.5%), p.I65 T (4.8%), p.L68 S (4.8%), p.R158Q (4.8%), and c.1066-11G>A (4.1%). With great heterogeneity the most prevalent mutations in the classical group were p.R408 W, p.R158Q, c782G>A and c.1066-11G>A that were found in 30% of alleles in this group. p.R261Q, p.V388 M, p.E390G, p.I65 T, p.Y414C, nd ap.L48 S affected 50% of alleles of the moderate group. Mild forms harbored p.L48 S, p.A403 V, p.IVS1216A, p.R408 W, and p.I306 V representing 61% of alleles while in PHPA predominant mutations (73%) were p.A403 V and p.R408 W. **Conclusion:** We describe the distribution and frequency of PKU mutations in the

Argentinean population confirming a high heterogeneity. These findings will surely be useful for the understanding of PKU in our country and will help in the individual follow up of affected patients

261 - Performance on a Neurogenesis Sensitive Pattern Separation Task Suggests Hippocampal Deficits in Adult Phenylketonuria (PKU) Patients

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Cognitive deficits in frontal lobe-related (executive) functions in Phenylketonuria (PKU) have been well documented. However, despite studies in PKU mice revealing reduced hippocampal synaptic connectivity, and volumetric MRI studies showing hippocampal volume loss in PKU patients, we are not aware of any studies assessing aspects of cognition specifically relevant to hippocampal functioning in the condition. fMRI studies have identified increased dentate gyrus activity when subjects are required to discriminate very similar objects in an object pattern separation task (e.g. deciding whether or not a closely similar image is original image they saw). Studies using object pattern separation paradigms have demonstrated selective age-related declines in the ability to discriminate closely similar images. Such deficits have also been shown using this type of task in people with amnesic Mild Cognitive Impairment and mild Alzheimer's Disease. Research suggests that there is a relationship between compromised neurogenesis in the hippocampus and decreased ability to make these difficult discriminations. This study aims to assess hippocampal function in adult PKU patients compared to healthy controls, using the CogTrack^{TM} pattern separation task. To date, 36 subjects (n = 19 PKU patients (13 Female [Mean Age 29.31 [SD:9.2], 6 Male [Mean Age 38.33 [SD:9.7] n = 17 healthy controls (11 Female [Mean Age 34.09 [SD:8.7], 6 Male [Mean Age 30.17 [SD:8.9]), have participated in an online study. Subjects were asked to complete a cognitive test battery on two occasions. The task reported here required subjects to distinguish closely similar images (neurogenesis sensitive stimuli) from previously presented images (original stimuli) following a 10-minute interval during which they engaged in other cognitive tasks. The pattern separation task accuracy scores were subject to a two factor Mixed Model Repeated Measures ANCOVA with age and gender as covariates. The analysis identified an interaction between the type of stimulus and the presence of PKU ($P = .0195$). The PKU patients were notably more impaired than controls in their ability to discriminate the closely similar pictures (the neurogenesis sensitive stimuli) than the original pictures. This suggests that cognitive functions which reflect hippocampal neurogenesis may be compromised in adult PKU patients. Data collection is ongoing.

262 - Estimation of Resource Use and Quality of Life in Phenylketonuria (PKU) Patients in Ireland

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Objectives: Objectives of this study were to estimate health-related quality of life (HRQoL), medical resource use and productivity loss and in patients and parents of children with phenylketonuria (PKU). **Methods:** A literature review was conducted to describe how HRQL is affected in PKU. Literature was identified that described the clinical manifestation of the disease and the range of symptoms and loss of functioning that patients can experience. Using this information case vignettes were developed representing 4 different health states (controlled, partially controlled, uncontrolled and asymptomatic). These states were defined based on phenylalanine (Phe) levels and symptoms. The health states were validated with clinical experts in Ireland. This was followed by an advisory board, where clinical experts provided a proxy based assessment to reflect how the quality of life of the person described in the vignettes would be affected. They completed the EuroQol EQ5D-5 L and the Short Form SF-6D tools. To assess resource use, the experts provided an assessment of direct medical resource use and productivity loss. To assess the impact of PKU on children, the experts were asked to assess the child health states using the EQ5D-Y. In addition, for the child states, the experts were asked to rate the extent to which the parents would not be able to work and also any medical resource use associated with PKU. **Results:** The health state utility values show significant differences between the four health states. EQ-5D-5 L and SF-6D showed a reasonable degree of consistency in terms of the overall scores for the four health states. EQ-5D-5 L showed a slightly larger range, 1.00 to 0.54 compared to 0.84 to 0.57 for SF-6D (1-full health, 0-death) in adults. Similarly, health state utility values for children show large differences between states (0.92 to 0.39). The data show a very substantial decline in HRQL associated with uncontrolled PKU compared with an adult or child with no symptoms with blood phenylalanine in the target range (controlled). Medical resource use and productivity losses associated with different health states showed similar patterns with higher resource burden associated with uncontrolled and partially controlled health

states. Conclusions: PKU is a severe disease with poorer health states associated with significant decline in quality of life and high resource burden.

263 - The perceived quality of life and the ability to cope with challenges in 73 adult phenylketonuric patients from newborn screening—The experience of a Portuguese center

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Introduction: Phenylketonuria is associated with neurocognitive deficits and behavior problems. A normal IQ seems to be preserved when good dietary compliance and metabolic control are achieved. However, the deficits observed in specific neurocognitive functions may interfere with the performance in different domains and therefore with patients' quality of life (QoL). A Portuguese study with PKU patients revealed that, despite higher scores in *General* QoL on the WHOQOL-Bref questionnaires, in more specific domains such as *Environment* and coping, differences were observed between patients with different levels of quality of dietetic control (QDC), giving us some clues about the complexity of QoL's concept. The aim of this study was to characterize adult PKU patients not only on their self-perceived QoL but also on their actual achievements or difficulties in real life, trying to understand possible factors influencing these outcomes. **Methods**: A sample of 73 adult PKU patients aged 18-36 y was studied. Disease severity was categorized according to NBS blood [Phe]. Good metabolic control was considered when median blood [Phe] was < 8 mg/dL. Patient's outcome was evaluated using last global IQ scores and the neurocognitive profile on Wechsler Intelligence Scale for Adults (WAIS), the educational level, the professional career and general autonomy. Presence of comorbidities was recorded from clinical files. **Results**: Mean $\pm$ SD global IQ scores (93.50 $\pm$ 17.31) were found to be below normal range. A significant negative correlation was found between last global IQ score and last blood [Phe] (r = -,635; $P < .001$). Patients with bad metabolic control showed lower global IQ scores (104.8 $\pm$ 11.41 vs. 85.10 $\pm$ 16.21, $P \leq .001$) and lower scores on WAIS Performance IQ (103.0 $\pm$ 17.07vs. 85.7 $\pm$ 13.90, $P < .001$) revealing deficits in specific neurocognitive functions. Comorbidities were more frequent in classical PKU patients and in mild PKU patients with a bad metabolic control (63%) and may interfere with their ability to cope with more stressful situations:18.5% gave up from studies or professional career. **Conclusions**: There are significant differences in neurocognitive functioning between adult PKU patients regarding their QDC. The presence of co-morbidities should be considered in the study of PKU patients as they interfere with the ability to cope with daily challenges and consequently in their QoL.

264 - Potentials Effects of Phe Levels on the Cognitive Development, School Performance, Professional Career, and Socio-Affective Behavior of 73 Adult PKU Followed-Up by a Multidisciplinary Team at CGM-CHP

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Introduction: Phenylketonuria (PKU) is an autosomal recessive disorder characterized by disrupted metabolism of phenylalanine (Phe). Caused by mutations in the gene that code for the liver-based enzyme phenylalanine hydroxylase (PAH), it results in a decreased conversion of Phe into Tyrosine and in elevated plasma Phe levels. Severe neurological disabilities caused by untreated PKU can largely be prevented with the implementation of an early and adequate dietary treatment. However, a slightly decreased intellectual quotient (IQ) and specific cognitive impairments have been reported even for those treated early and continuously. The degree of neuropsychological impairments seems to be associated with high levels of Phe and potentially interferes with school performance, professional career and socio-affective behavior. **Methods:** From the total PKU population followed up at our center, we selected 73 PKU patients for this study, based on the following inclusion criteria: early diagnosed at the neonatal screening, aged 18 or more and without additional disorders. Annual values of all Phe median concentrations available were recorded and considered as independent variables. Patient's outcome was evaluated according to the last global IQ value on Wechsler Intelligence Scale for Adults (WAIS), IQs subscales, educational level as well as their professional career and socio-affective behavior. Results: Compared to the reference norm population, last global IQ values observed in PKU patients were slightly below the mean value (mean = 95.36, SD = 17.71). A similar trend was observed while analyzing WAIS - III subscales IQs. A significant negative correlation ($P <$ .01) between the last median of Phe levels and last global IQ scores was observed. We have also found significant negative correlations between IQ values and the annual Phe median values in the majority of age groups till the age of 18 years. Moreover, it was noted that the difficulties observed on the neurocognitive evaluations had influence on their school progress, professional career, and socio-affective behavior. Cognitive impairments were noticed in ten patients (IQ values < 70), being associated with a poor dietary compliance since the first years of life. **Conclusion:** These results illustrate the need of a centralized multidisciplinary team in PKU treatment, throughout life, in order to improve physical and neurocognitive development as well as their quality of life.

265 - Can Other Genetic Diseases be Hidden Under Hyperphenylalaninemia? A Single Centre Experience

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We have evaluated among our patients affected by hyperphenylalaninemia the incidence of other genetic diseases. Out of a total of 630 patients affected by hyperphenylalaninemia, whose diagnosis was genetically confirmed, 250 are under diet therapy and 380 are not. Amongst the patients following dietetic therapy seven have been diagnosed with a second genetic disease: three are also affected by a different inherited metabolic disease (IMD) such as Short-Chain Acyl-CoA dehydrogenase (SCAD) deficiency, Medium-Chain Acyl-CoA dehydrogenase (MCAD) deficiency and cystinuria. One patient was found to have Moebius syndrome, another was diagnosed with pseudo-para-hypothyroidism. A genetic neuropathy Charcot-Marie-Tooth like was detected in an adult patient and a girl was diagnosed with chromosome 17 deletion (17.p.13.2). The second genetic disease was suspected and therefore investigated considering the presence of signs and symptoms not completely justified by hyperphenylalaninemia (neurological symptoms, mental delay, facial dysmorphisms, hypocalcemia and growth delay). A second genetic disease was discovered also in three of the 380 patients not following diet therapy. One child has been diagnosed with Noonan syndrome and another with the X-fragile syndrome. These two patients were investigated due to mental retardation and facial dysmorphism. A young woman also affected by congenital deafness was diagnosed with familial adenomatous polyposis. Out of a total of 630 patients affected by hyperphenylalaninemia 10 patients (1.6% of our population), up to now, have received a second diagnosis of genetic disease. It is therefore important when signs and symptoms not completely justified by a diagnosis of hyperphenylalaninemia are detected to continue the diagnostic process also with other genetic analysis. Our experience suggests that a first diagnosis of hyperphenylalaninemia does not exclude the possibility of a second genetic disease. Considering the possible phenotypical overlapping of different genetic disease, we should not settle for a single definite diagnosis particularly when we find ourselves in front of mental retardation or neurological symptoms of any severness.

266 - Analysis of the West of Scotland Maternal Phenylketonuria Clinic

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Aim: To report the maternal and infant outcomes of pregnant women who attend the West of Scotland PKU clinic. **Method**: Retrospective case note analysis of maternal and birth outcomes in 17 mothers with PKU from 20 pregnancies (21 live births) between 2008 and 2016. Primary outcomes were maternal weight gain, complications during pregnancy, infant anomalies, weight z-score at birth, and weight gain at 6 months old. Predictors were: phenylalanine (Phe) control around conception, planned conception and socio-economic status using the Scottish Index of Multiple Deprivation. In addition, adherence to the amino acid supplement, protein exchanges and monitoring of blood spot Phe levels were examined. **Results**: 11 pregnancies were planned. Blood spot Phe were within target range in 8/19 (42%) pregnancies (data missing for 2) around conception increasing to 80% by 12 weeks gestation (16/20; missing data for 1). 9 (43%) were on PKU diet at conception. There was no significant difference in maternal weight gain between planned and unplanned pregnancy (planned: 12.7 vs. unplanned: 9.5 kg; $P = .184$). Adherence to diet preconception tended to be associated with higher maternal weight gain (adherent: 13 vs. non-adherent: 8.7 kg; $P = .090$) and higher infant birth weight z-score (adherent: 0.31 vs. non-adherent: -0.45 SD; $P = .008$). Infant birth weight z-score was also higher in planned pregnancies (planned: 0.20 vs. unplanned: -0.41 SD; $P = .087$). At six months post-partum, the deficits persisted but were not statistically significant. Socio-economic status did not predict infant birth weight z-score ($P = .224$). A positive association was observed between the degree of diet adherence during pregnancy and infant birth weight z-score. A tendency was also observed between the frequency of Phe blood monitoring and birth weight z-score (Spearman $\rho = .485$; $P = .093$). 4/17 (23.5%) infants presented with congenital anomalies associated with maternal PKU syndrome. The percentage of congenital anomalies was significantly lower in the mothers on preconception diet ($P = .005$) and planned their pregnancy ($P = .005$). **Conclusion**: Our experience indicates adherence to diet preconception improves: adherence to diet during pregnancy, maternal weight gain, infant birth weight and suggests better weight gain 6 months post-partum. The analysis emphasizes the need for continuous education to older girls and women of the crucial role a planned pregnancy and preconception diet has on infant outcome.

267 - Early Treated Phenylketonuria: DTI to Evaluate Microstructural Integrity of White Matter in a Spanish Pediatric Population

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Background: Despite early and continuous dietary intervention, individuals with early treated phenylketonuria (ETPKU) could have neurocognitive sequelae. **Objective**: To study the white matter (WM) abnormalities in 15 ETPKU with normal intellectual quotient and metabolic profile through magnetic resonance imaging (MRI). We used diffusion weighted MRI (DW-MRI) to obtain parameters related to WM microstructural integrity. We calculated Fractional Anisotropy (FA), Mean Diffusivity (MD) and Radial Diffusivity (RD) values of WM tracts across the whole brain. We compared the results with those from 11 healthy controls. **Methods:** From a sample of 33 early treated PKU patients studied during 18 months, we recruited 15 patients (median age: 12 years range: 8-18 years). WISC-IV and WAIS-III test was administered to estimate general intellectual ability. Concurrent phenylalanine(Phe) (day of the scanning session), last year Phe (median of all Phe values sampled during the last year), variability of Phe (standard deviation of all Phe values sampled during the last year), monoamines (homovanilic acid (HVA), vanil lactic acid (VLA), 5-hidroxiindolacetic acid (5HIAA)) concentrations were analyzed in plasma and urine, by chromatographic procedures. Within WM regions showing between-group differences, Pearson's correlations were computed between DW-MRI values (representing WM integrity) and age, and the biochemical variables. **Results:** ETPKU showed bilaterally decreased MD values as compared with controls in the body and splenium of the corpus callosum, superior longitudinal fasciculus, the corona radiata and in the posterior limb of the internal capsule. RD values followed a similar pattern, although decreased RD values in PKU patients were also found in the anterior limb of the internal capsule and in the cerebral peduncle. FA values showed no significant differences between groups. Decreased MD values within the aforementioned regions significantly correlated negatively with age ($r = -0.80$, $P < .001$), VLA concentration ($r = -0.66$, $P < .007$) and last year and concurrent phenylalanine values ($r = -0.65$, $P < .008$ and $r = -0.71$, $P < .004$). RD values displayed similar correlations. **Conclusions**: MD and RD were significant decreased compared with healthy controls, and they are promising neuroimaging markers to evaluate the microstructural integrity of WM. Age, concurrent Phe and last year Phe were the most powerful predictors of widespread microstructural WM integrity compromise in our group of ETPKU.

268 - New Tools to Investigate Phenylketonuria as a Perturbation of the Equilibrium Between Resting-State Phenylalanine Hydroxylase and an Architecturally Distinct Activated Phenylalanine Hydroxylase

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Phenylalanine hydroxylase (PAH) exists as a slowly equilibrating mixture of an autoinhibited Resting-State structure and an architecturally distinct Activated structure; the relative population of these structures is governed by Phe binding allosterically to a site present only on Activated PAH. Phenylketonuria (PKU) can occur when the amount of Phe needed to favor accumulation of Activated PAH rises to neurotoxic levels. Because this "alternate-structures" description departs from the common view of PKU as a disorder of "protein folding", it remains unknown how many disease-associated PAH variants adhere to this model. Nevertheless, our ultimate goal is to find pharmacological chaperones that can restore the intrinsic PAH tipping-point to safe Phe levels for common disease-associated variants. To this end, we have determined that a benign human PAH variant (C29 S) and the use of a cleaved His-SUMO tag facilitates investigation of human PAH structure and function. We report compounds that can potentiate the stabilization of Activated PAH by Phe. Excellent sensitivity was obtained screening potential candidate molecules using an enzyme kinetics approach in the normal physiological range of $\sim$100 μM Phe, rather than the less sensitive and saturating 1 mM Phe. PAH activation is complex and may reflect release of autoinhibition that is independent of the stabilization of the regulatory domain dimer now known to be specific to Activated PAH. Therefore, in addition to enzyme kinetic analysis, we monitored for the position of the PAH structural equilibrium using intrinsic protein fluorescence. The intrinsic fluorescence approach has been shown to provide a measure of the formation of the PAH regulatory domain dimer.

269 - 10 Year Retrospective Review (2003-2013) of 56 Inpatient Admissions to Stabilize Elevated Phenylalanine Levels

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Objective: The current practice within the National Centre for Inherited Metabolic Disorders is to admit PKU patients with phenylalanine (Phe) levels consistently out of range, despite intensive multidisciplinary input via outpatient and/or telephone review. The objective was to examine whether or not the admissions were having a sustained impact post discharge at 2 time points—six months and twelve months. Methods: The Metabolic ward admission books were used to identify patients admitted for stabilization of Phe levels only. Demographic data, the reason for admission (compliance with synthetic protein, over-exchanging or multiple issues) was supported by the patient's medical and dietetic notes. Patient's individual Phe level records were used to collect their Phe levels 6 months prior to admission, during admission and at respective time

points 1-6 and 7-12 months post discharge. Data were entered into excel and analyzed via SPSS by the Department of Research. Results: The number of admissions totaled at 56 admissions (under 12 years = 37; over 12 years = 18). The median length of admission was 5 days. The reasons for admission were 71% due to multiple issues, 16% due to compliance issues with synthetic protein, 11% due to over exchanging and 2% due to poor intake of low protein foods. There was a significant decrease in median Phe levels from prior to the admission to during the admission (754 to 505 μmol/L) ($P < .0001$). However, there was a significant increase in median Phe levels from during the admission (505 μmol/L) to both the 1-6 months and 7-12 months post discharge time points (618 and 654 μmol/L respectively ($P < .0001$)). Overall there was a 13.6% reduction in median Phe levels prior to admission to 12 months post discharge (754 to 651 μmol/L) ($P < .0001$). **Conclusion:** The results highlight that while inpatient admissions can stabilize levels within the acute setting, changes to current post admission practices are warranted to further support and sustain phenylalanine level reductions such as structured peer support learning programs.

270 - Hyperphenylalaninemia Impairs Brain Energy Metabolism in Rats

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Phenylketonuria (PKU) is a disorder of phenylalanine (Phe) metabolism caused by impaired phenylalanine hydroxylase activity, resulting in increased levels of Phe and its metabolites in fluids and tissues of patients. PKU patients present neurological signals and symptoms, such as hypomyelination and intellectual deficit. It has been shown that oxidative stress, calcium homeostasis and neurotransmitters metabolism are affected in PKU. The aim of this study was to analyze brain bioenergetics in the pathophysiology of hyperphenylalaninemia (HPA). For this study, 30-day-old Wistar rats were randomized into two groups. HPA group received a single subcutaneous administration of Phe (5.2 μmol/g) plus p-Cl-Phe (PAH inhibitor) (0.9 μmol/g). Control group received a single injection of NaCl 0.9%. One hour after injections animals were euthanatized and cerebral cortex, hippocampus and striatum were collected. The activities of lactate dehydrogenase (LDH), creatine kinase (CK), Krebs cycle enzymes and respiratory chain complexes were measured. It was also assayed concentrations of glycogen, free and total phosphate levels, as well as mRNA expression of PGC-1α, TFAM, NFR-1, SIRT3, SIRT5, MFN1, Polγ, and DRP-1. In cerebral cortex, HPA group showed decreased CK activity, glycogen levels, complex I-III and IV activities and CS activity, as well as increased LDH, α-KGDH and IDH activities, and the levels of free and total Pi. In striatum, HPA animals presented increased LDH and IDH activities, and decreased α-KGDH and complex IV activities. In hippocampus, HPA rats had increased α-KGDH and IDH activities, decreased complexes I and IV activities, and increased mRNA expression of MFN1. Our data demonstrated impaired bioenergetics in cerebral cortex, striatum and hippocampus of HPA rats. In conclusion, it may be speculated that disruption of brain bioenergetics is involved in neuropathology seen in PKU patients.

271 - Cutaneous Manifestations of Phenylketonuria in the 21st Century

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Introduction: Phenylketonuria (PKU) is an autosomal recessive disorder of the metabolism of aromatic amino acids (OMIM # 261600) in which phenylalanine can not be converted into tyrosine. PKU is caused by a deficiency of phenylalanine hydroxylase (PAH) or hepatic PAH cofactors. The distinctive clinical features of PKU are mental retardation, diffuse hypopigmentation, seizures, eczematous dermatitis and photosensitivity. One of the earliest signs of PKU may be early childhood eczema, indistinguishable from atopic dermatitis, which affects 20%-50% of children with the disease during the first year of life. Later, the incidence may be even higher. In some cases, substantial disappearance of eczema and seborrheic dermatitis have been observed with a diet low in phenylalanine content and supplemented with tyrosine. Another skin sign of PKU is pigment dilution, with striking pale pigmentation, blond hair and blue eyes in 90% of patients. Dilution of the pigment is reversible with diet (phenylalanine restriction) and tyrosine supplementation. **Objectives:** To define the cutaneous manifestations of PKU at the present time. **Material and method:** Prospective descriptive observational study of cutaneous manifestations in PKU currently in the field of query of innate Errors of Metabolism of Hospital Universitario Virgen del Rocío (Seville), where 201 patients with altered metabolism of the Phenylalanine. **Results:** The cutaneous manifestations of 86 PKU of the different phenotypes are described. The frequencies of atopic dermatitis, atopiform dermatitis, features of atopic dermatitis (follicular hyperkeratosis, pityriasis alba, . . .), vitiligo, Sutton's nevus and other dermatological alterations in these patients are collected. **Conclusions:** There

is a disagreement between the literature regarding the cutaneous manifestations in patients with phenylketonuria and the lesions that present at present with an adequate metabolic control.

272 - Brain-Derived Neurotrophic Factor and Platelet-Derived Growth Factors Levels Are Decreased in Plasma of Phenylketonuric Patients

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Accumulation of phenylalanine (Phe) in tissue and body fluids of patients is the hallmark of phenylketonuria (PKU), an autosomal recessive disease. Brain injury is a clinical characteristic of PKU patients, although the pathophysiology of this damage is poorly understood. Therefore, the aim of this study was to evaluate the levels of neurodegeneration markers in plasma of PKU patients. Peripheral blood of 12 healthy individuals (control group) and 24 phenylketonuric patients were collected in heparinized tubes. Plasma Phe level was measured by high-pressure liquid chromatography. Quantification of plasma levels of brain-derived neurotrophic factor (BDNF), cathepsin D, neural cell adhesion molecule (NCAM), plasminogen activator inhibitor-1 (PAI-1), platelet-derived growth factor types AA, and AB/BB (PDGF-AA and PDGF-AB/BB), regulated upon activation normal T cells expressed and secreted (RANTES), intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) was performed by ELISA method using commercial kits according to the manufacturers. It was observed that phenylketonuric patients presented a decrease in plasma levels of BDNF, involved in neuroplasticity and cell survival, PDGF-AA and PDGF-AB/BB, important molecules for oligodendrogenesis, when compared to control group. On the other hand, the levels of cathepsin D, NCAM, PAI-1, RANTES, ICAM-1, and VCAM-1 were not altered in plasma of phenylketonuric patients. Taken together, the present results suggest that the neurological damage and hypomyelination found in PKU patients might be related to impairment of neuron survival and plasticity and alterations in oligodendrogenesis, respectively.

273 - Epidemiological Clinical Profile of Patients With Phenylketonuria Treated at the Newborn Screening Service of Amazonas

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Objectives: To know the clinical and epidemiological profile of the patients with Phenylketonuria accompanied by the Newborn Screening Service of the Amazonas. **Methodology**: The Newborn Screening Service of Amazonas serves the entire state and performs screening for 6 diseases. The evaluation of the medical records of patients followed up with a diagnosis of Phenylketonuria was carried out in order to collect clinical and epidemiological data. **Results**: Currently, 27 patients with a diagnosis of phenylketonuria, 13 females and 14 males. The origin of the patients in 22 cases is from Manaus, capital of the state, and in this total, we have 3 families from the state of Pará. Only 5 patients from the interior of the state. Inbreeding was present in the parents of 5 patients. The mean onset of treatment was 2 months of age. The baseline PKU dosage was on average 18 to 20 mg / dL. The PKU dosage after treatment was on average below 10 mg / dL. Using the year 2014 as a reference, we have a prevalence of 1: 16 000, which indicates that Phenylketonuria in the state of Amazonas is a rare disease with a prevalence lower than estimated in other populations. **Conclusion**: The age of initiation of treatment and the maintenance of low levels of phenylalanine are below that recommended by the Brazilian Ministry of Health. However, taking into account the local difficulties, the follow-up of patients is done on a regular basis.

274 - Comparison of Amino acid Autoanalyzer and Tandem Mass Spectrometry With Fluorometric Delfia Method for Phenylalanine Monitoring in PKU Patients

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Background: Metabolic control of Phenylketonuria (PKU) and compliance with the low-phenylalanine (phe) diet are frequently monitored by measuring phe concentrations in blood. Differences in phe values depending on the method used for analysis can difficult optimal management of patients by clinicians. **Objectives**: Our objective was to investigate the difference in blood phe concentrations analyzed by three different methods used in our laboratory. **Methods:** We performed a retrospective analysis of Phe measurements in blood samples (n: 60) corresponding to PKU patients who came to our laboratory for control. The Phe levels obtained by Fluorometric DELFIA method (PerkinElmer, dried blood spot DBS collected on Whatman 903 paper), commonly used for newborn screening, were compared to those obtained by Amino Acid

Autoanalyzer (AAA; Biochrom 30) in plasma and Tandem Mass spectrometry (MS/MS; Acquity UPLC System, Waters) in DBS. DELFIA vs MS/MS: n = 22; DELFIA vs AAA: 48 Med Calc software V13.1 was used for statistical analysis. To evaluate agreement between the three methods, Passing Bablok regression was performed and Pearson's correlation coefficient (r) were calculated and Bland-Altman plots were evaluated. **Results:** DELFIA (Y) vs MS/MS (X): Y = 5.408526 + 0.873690 X (Passing Bablok regression). $r = 0.957$ (<0.0001; n: 22). DELFIA (Y) vs AAA (X): Y = 16.182018 + 1.467532 X. $r = 0{,}843$ (<0.0001; n: 48). Bland-Altman plots showed a positive difference (MS/MS-DELFIA): Mean: 46.8 µmol/L (95% CI: −130.6 to 224.2); %(MS/MS-DELFIA): Mean: 6.3% (95% CI: −45.1% to 57.8%) and (AAA-DELFIA): Mean: 141.4 µmol /L (95% CI: −303.4 to 586.3); %(AAA-DELFIA): Mean: 28.5% (95% CI: −30.1% to 87.1%) **Conclusions:** Although these results are preliminary since the number of samples is low, Phe levels measured by DELFIA Fluorometric method are lower to those obtained by MS/MS and AAA. These discrepancies could be the consequence of unrecognized preanalytical factors, different blood hematocrit levels, storage and handling prior to analysis, and type of blood sample (serum or DBS). The clinical relevance of this difference depends on the level of Phe and should be taken into account when Phe levels in PKU patients are monitored. Clinicians need to be informed about the method used and monitoring must be, if possible, done with the same method to ensure the optimal management of the patient with PKU.

275 - Relationship Between Body Mass Index and Plasmatic Phenylalanine Concentration in Patients With Phenylketonuria

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Introduction: The Phenylketonuria (PKU) is an inborn metabolic disease associated in the metabolism of the phenylalanine (PHE). The principle of the treatment is a severe restriction of this essential amino acid associated to a supplemental semi synthetic diet PHE- free enriched with tyrosine, vitamins and minerals. **Objective:** Connect the variables, body mass index (BMI) and age with the plasmatic phenylalanine concentration of adult patients with the diagnostic of PKU secondary to enzyme phenylalanine hydroxylase (PAH) deficiency. **Methodology:** 21 patients records with age between 18 and 45 years were analyzed. The parameters utilized were: age, weight, height, BMI and the average of plasmatic phenylalanine concentration accomplished in 2016. The BMI was classified according to the World Health Organization's references of 1997. Plasmatic phenylalanine concentration up to 10 mg/dL were considered adequate. Statistical analysis was done by Spearman correlation and the partial adjusted correlation. It was used the Statistical Package for Social Science *software* for Windows (SPSS) and utilized the level of significance of $P \leq 0.05$. **Outcome:** Among the 21 patients evaluated, 13 were females (61.9%) and 8 were males (38.1%). In regard to the classification of BMI, 76.2% (n = 2) were eutrophic, 4.8% (n = 1) were overweight, and 19% (n = 4) were obese. The plasmatic phenylalanine concentration was above the reference value in 57.1% (n = 12) on the sample. In the statistical analysis, there was a moderate positive correlation between BMI and phenylalanine rates (r = 0.44; $P = .048$) and a weak inverse correlation among age and phenylalanine rates ($r = -0.169$; $P = .46$). The correlation amid BMI and blood phenylalanine concentration maintained significant after the age adjustment ($r = .645$; $P = .002$). **Conclusion:** The obesity reached only 19% of the patients, showing that the dietetic alterations required to the disease treatment did not represent a risk to the weight gain. It was noted that more than 50% of the patients had an annual average of phenylalanine above the reference value, testifying the difficulty of adhesion to the diet in the adulthood, with correlation between BMI and phenylalanine rates. The inverse correlation amid age and blood phenylalanine, despite being unobtrusive, probably is due to the fact that older patients have more neurologic impairment because of a lag diagnostic, what makes them care dependent, enabling a greater diet control.

276 - Behavioral and Emotional Problems in Brazilian Early-Treated Children and Adolescents With Phenylketonuria

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Introduction: Phenylketonuria— PKU—is an inborn error of phenylalanine - Phe metabolism, caused by the deficiency of the enzyme phenylalanine hydroxylase (PAH). When untreated, PKU leads to a significant intellectual deficiency. The early institution of dietary therapy allows a normal cognitive development. However, low adherence to treatment with consequent high or fluctuating levels of Phe may result in neuropsychological deficits, including attention problems. Objectives: To evaluate emotional and behavioral problems, including signs and symptoms of inattention and Attention Deficit Hyperactivity Disorder (ADHD) in early-treated children and adolescents with PKU using the Child Behavior

Checklist questionnaire CBCL/6-18. **Methods:** Emotional and behavioral problems of the patients were assessed from parents' ratings of CBCL/6-18. The mean scores of internalizing, externalizing and total problems, syndrome scales (anxious/depressed; withdrawn/depressed; somatic complaints; social, thought, and attention problems; rule-breaking and aggressive behaviors) and DSM-IV oriented scales (affective, anxiety, and somatic problems; ADHD; oppositional defiant disorder; conduct problems) of PKU patients were compared with those of the controls - a normative sample of Brazilian schoolchildren. **Results:** It was possible to apply CBCL/6-18 to 36 parents. There were no significant differences between PKU patients and the controls for almost all CBCL/6-18 scales, with the exception of the Attention Problem Scale - CBCL-APS. The mean ($\pm$ SD) of the CBCL-APS scores was 7.86 ($\pm$ 5.33), considerably higher than the mean of the controls (6.07 $\pm$ 4.37; $P = .016$). The difference between the mean of the scores of DSM-IV/ADHD scale of patients (6.72 $\pm$ 4.07) and controls (5.73 $\pm$ 3.56; $P = 0.102$) was not statistically significant. There were no considerable differences in the mean of the CBCL-APS scores between patients with adequate and inadequate Phe mean levels. Conclusion: Attention problems have been reported as an important neuropsychological disorder in children, adolescents and adults with PKU. Using the CBCL-APS scale, we found evidence for a significant prevalence of attention problems in patients with PKU. Although ADHD has also been observed in these patients, our data seem to confirm previous observations that point to a lesser importance of hyperactivity in PKU. A connection between CBCL-APS scores and adherence to treatment could not be demonstrated.

277 - The Effect of Intact Protein from foods and Phenylalanine Free Medical foods on Large Neutral Amino Acids in Patients With Phenylketonuria

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Objective: The primary aim of this retrospective cohort study was to determine the association between the source of dietary protein and the sum of plasma concentration of large neutral amino acids (LNAA) in patients with Phenylketonuria (PKU). A second aim was to examine the effect of dietary compliance on plasma LNAA. **Methods**: The analysis included combined participant data from two previous studies conducted at the Emory University School of Medicine. Subjects are males (n = 34) and females (n = 43) with PKU ages 4-50 years. A Student t-test was used to compare total combined plasma LNAA (excluding tryptophan) by dietary compliance status (alpha = 0.05). Correlation statistics were used to determine the association between the ratio of reported intact food protein to medical food protein (IFP: MFP) on plasma LNAA. Multiple regression was used to examine the contribution of IFP: MFP ratio and other variables to plasma LNAA. **Results**: The median ratio of IFP: MFP reported was 0.354 (IQR: 0.188, 0.914). Median percent of PHE intake over the PHE intake recommendation was 31.64 (Interquartile range [IQR]; 7.44, 104.98). Plasma concentration of LNAA did not differ significantly between those with plasma PHE levels within vs above the therapeutic range <360 μmol/L (compliant; 611.7 μmol/L [n = 19]) (noncompliant; 595.3 μmol/L [n = 47]); $P = .613$). There was a marginal negative correlation between the ratio of IFP: MFP and plasma concentration of LNAA for those who were compliant ($r = -0.436$, $r = 0.1$) although not statistically significant ($P = .08$). No correlation was found for patients who were non-compliant. Regression analysis revealed that plasma concentration of LNAA was not significantly affected by the IFP: MFP ratio, age, or gender. **Conclusions**: Although not statistically significant, a negative trend was observed between plasma LNAA concentration and the IFP: MFP ratio in patients compliant with their recommended PKU diet. This suggests that the ratio, as reported by patient diet records, may influence the potential benefits of increased plasma LNAA levels in the treatment of PKU. The majority of the population (74%) were non-compliant with diet based on plasma PHE levels. Future studies are needed to determine the consequences of non-compliance by decreased intake of medical food protein or increased intake of intact protein on plasma LNAA.

278 - Blood Phenylalanine Reduction Corrects Monoamine Neurotransmitter Deficiencies and Improves Behavioral Performance but has no Effect Upon Cognitive Disability in Adult Mice With Phenylketonuria

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Central nervous system (CNS) deficiencies of the monoamine neurotransmitters dopamine and serotonin have been implicated in the pathophysiology of neuropsychiatric dysfunction in human phenylketonuria (PKU). In this study, we confirmed the occurrence of brain dopamine and serotonin deficiencies in association with severe behavioral alterations and cognitive impairments in hyperphenylalaninemic C57BL/6-*Pah*$^{enu2/enu2}$ mice, a model of human PKU. Phenylalanine-reducing treatments, including either dietary phenylalanine restriction or liver-directed gene therapy, initiated during adulthood were associated with increased brain monoamine content along with improvements in nesting behavior but without a change in the

severe cognitive impairments exhibited by these mice in the water maze. Following the behavioral and cognitive testing, the mice were euthanized for biochemical analyses. Brains of $Pah^{enu2/enu2}$ brain showed significant reductions in the protein abundance and maximally stimulated activities of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), the rate limiting enzymes catalyzing dopamine and serotonin synthesis respectively, in comparison to those seen in wild type animals. Phenylalanine-reducing treatments initiated during adulthood did not affect brain TH or TPH content or maximal activity. Despite this apparent fixed deficit in striatal TH and TPH activities, initiation of phenylalanine-reducing treatments yielded substantial correction of brain monoamine neurotransmitter content suggesting that phenylalanine-mediated competitive inhibition of already constitutively reduced TH and TPH activities is the primary cause of brain monoamine deficiency in Pah^{enu2} mouse brain. We propose that CNS monoamine deficiency may be the cause of the reversible adverse behavioral effects associated with chronic hyperphenylalaninemia in Pah^{enu2} mice, although phenylalanine-reducing treatments initiated during adulthood are unable to correct the neuropathology and attendant cognitive deficits that develop during juvenile life in late-treated Pah^{enu2} mice.

279 - Treatment Decreases Homocysteine Levels in PKU Patients

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Introduction: Treatment of patients with Phenylketonuria (PKU) is based on dietary restriction of phenylalanine (Phe) with a low protein diet and ingestion of a mixture with amino acids free of Phe, supplemented with vitamins and minerals. The "PKU-diet" may be insufficient but also patients who do not follow properly the treatment are at risk of developing deficiency of methionine and vitamins like B12 or folic acid. Plasma total homocysteine (tHcy) is a marker of diet insufficient of B-vitamins, in particular vitamin B12 and folate. In literature, it is debated if tHcy is increased in PKU patients. The study's objective was to determine in PKU patients tHcy before and during Phe restricted diet. **Patients and Methods:** We included in this study plasma samples from 3 PKU patients and compare: before treatment (age: 18 days, 1 and 3 months), and plasma samples from the same patients on treatment (median age: 31 months). Patients were being treated with a Phe-free infant formula with an average intake of 0.8 g/day of methionine, and a diet poor in Phe with an average intake of 340 mg/day. The Phe-free formula contains vitamin B12 ($\sim$3.3 μg/day), B6 ($\sim$1.5 mg/day) and folic acid ($\sim$180 μg/day). We measured Phe by HPLC and tHcy by LC-MS/MS. **Results:** As expected Phe levels (μmol /L) decreased from 1113.9 $\pm$ 532.7 before to 284.5 $\pm$ 175.6 ($P = .007$) after treatment. tHcy (μmol /L) decreased from 11 $\pm$ 1.4 before treatment to 6.2 $\pm$ 0.9 on treatment ($P < .001$). **Discussion and Conclusion:** We found a relative high tHcy in PKU patients before dietary intervention. We like to hypothesize that high Phe levels may interfere with homocysteine homeostasis. The decrease of tHcy due to Phe restricted diet may be due to restoration of homocysteine balance and the supplementation of vitamin B12 and folate due to the PKU diet.

280 - Phenylketonuria: Nutritional survey with quantification of Phenylalanine intake in childhood: Assessing the need of nutritional guidance in patients with Hyperphenylalaninemia. Preliminary Report

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Food habits differ worldwide from one culture to another. This is important when recommending a normal diet to patients with mild forms of metabolic disorders. **Objective:** Estimate the average intake of nutrients, especially Phenylalanine (Phe), in children without metabolic or nutritional pathology of our country, to determine the need of nutritional guidance in patients with hyperphenylalaninemia (HPA) and to indicate the protein and Phe intake during pregnancy in the HPA mother. **Methods:** A specially designed survey was administered to 61 children attending the Endocrinology Division of the R. Gutierrez Children's Hospital, Buenos Aires, Argentina. Those who had previous nutritional intervention or health condition that could have modified their diet, were excluded. They were divided in three age groups: 6-23 months (n = 12), 2-5 years old (n = 21), 6-10 years old (n = 28). A 24-hour food record of the consumption of everything ingested the day prior to the survey was taken. Visual models (Nasco Food) were used to specify portions, in raw and cooked weight. Preparation's composition was standardized prior to the survey. Nutritional information was analyzed with SARA system (Food composition analyzer). Own constructed tables with Phe values, and information provided by food processing companies in cases in which the other sources lacked information were used. Protein intake (PI), exceeding percentage from RDAs (E), %High biological value proteins (HBVP), Phe intake (PHI), % Phe covered from food >0.75mg% or beverages containing aspartame (HPF). **Results:** expressed as Median (M) and Range (R) 6-23 months: PI: M: 30.9 g/day (12.4-49.6); E: M: 159.6% (13.2-281.5); HBVP: 87% (19-98); PHI: M: 1348.7 mg/day (469-2424); HPF: M: 95.4% (83-100). 2-5 years: PI: M: 46.1 g (17-73); E: M: 202.7 (30.8-461.5); HBVP: M: 65% (20-84); PHI: M: 2264 mg/day (913 mg-3308 mg); HPF: M: 98% (61-100) 6-11 years: PI: M: 49.2 g (18.9-102); HBVP: M: 66% (16-86); E: M: 112.8 (0-436.8); PHI: M: 2350.5 mg/day

(900- 4228); HPF: M: 99% (28-100). **Conclusion:** Our preliminary results show that the protein and Phe intake in Argentina exceeds the protein recommendations issued by the RDAs in the groups assessed by the survey. If confirmed with a larger number of observations, our data support the need for nutrition education in patients with HPA to ensure an adequate clinical course of the pathology.

G) Phenylketonuria: Treatment, BH4 (281 to 301)

281 - Increasing the Dose of Tetrahydrobiopterin (BH4) Improves QOL of Patients With BH4-Responsive PKU

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Objectives: To evaluate the efficacy and dose of BH_4 therapy among BH_4-responsive phenylketonuria (BPKU) patients with or without diet therapy. **Patients and Methods:** At the end of 2015, 61 BPKU patients were receiving BH_4 therapy, of whom 27 were treated with BH_4 for more than 7 years. We conducted a longitudinal retrospective study which examined plasma phenylalanine (Phe) levels and BH_4 doses of BPKU while on both normal diets (31 patients) and on protein-restricted diets (30 patients), and compared them based on plasma phenylalanine levels in relation to guideline recommendations from the American College of Medical Genetics and Genomics; 360 μmol/L or higher is poor control (PC), less than 360 μmol/L is good control (GC). **Results:** The mean values of plasma phenylalanine and BH_4 doses were 319 μmol/L and 11.9 mg/kg on protein-restricted diets, and 415 μmol/L and 8.91 mg/kg on normal diets, respectively. The mean value of BH_4 doses on protein-restricted diets was much less than the maximum dose of 20 mg/kg. Among BPKU patients on normal diets, the mean value of plasma phenylalanine and BH_4 doses were 284 μmol/L and 8.37 mg/kg in GC, and 538 μmol/L and 9.41 mg/kg in PC, respectively. The mean value of BH_4 doses for PC on normal diets also was much less than the maximum dose of 20 mg/kg. **Conclusions:** The mean value of the BH_4 dose on protein-restricted diets and moreover on normal diets with PC were much less than the maximum dose of 20 mg/kg. Patients with BPKU would benefit by increasing the dose of BH4, allowing them to eliminate diet therapy and thereby gain both better control of their condition and improved quality of life.

282 - Outcomes of Pediatric Patients With Mild Hyperphenylalaninemia Who Were Diagnosed With Neonatal Screening Program

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Objective: The aim of this retrospective study is to evaluate the course of plasma phenylalanine (Phe) levels of children with mild hyperphenylalaninemia (mild HPA, plasma Phe level between 2.1-6.0 mg/dL) up to 6-year-old. **Methods**: 66 patients (30 boys, 45.5%, actual age (median [25-75 percentiles]) 49.5 [31.7-83.2] months) with mild HPA were included. The diagnosis was made by neonatal screening program in all children and all of them were regularly followed at our department. Patients were divided into two groups according to their first (neonatal) plasma Phe level: Group 1 (Phe levels between 2.1 and 4.0 mg/dL, 45 patients, 68.2%) and group 2 (Phe levels between 4.1 and 6.0 mg/dL, 21 patients, 31.8%). **Results**: Median follow-up duration was 47 [29.0-82.5] months. Sapropterin dihydrochloride was initiated to five patients in total (7.6%) during the follow-up period. In group 1, sapropterin was initiated to two patients (2/45) with the Phe levels of 10.0 and 10.8 mg/dL at 15th and 18th months of age, respectively. In group 2, sapropterin was initiated to two patients (2/21) with the Phe levels of 8.2 and 7.8 mg/dL at 12th and 36th months of age, respectively. One patient in group 2 was given Phe restricted diet at the plasma Phe level of 13.2 mg/dL at 12th months of age. The family did not follow the diet, therefore sapropterin was initiated to this patient at 6 years-old and the diet was ceased. Median plasma Phe level of these patients was 2.7 [1.8-3.7] mg/dL after initiation of sapropterin treatment. All of the children in this cohort have normal anthropometric, neurologic and developmental findings. **Conclusion**: Almost 8% of the patients whose baseline plasma Phe levels were between 2 and 6 mg/dL had elevated plasma Phe levels after 1-year-old, therefore sapropterin therapy had to be initiated. Possible explanations regarding high Phe levels are; relatively lower weight gain and higher protein intake of children after infantile period compared with the first year of life. Children with mild HPA should be follow regularly during childhood period.

283 - Improving PKU Management: Effect of Medical Education on Clinical Knowledge and Competence

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Objective: To determine if online continuing medical education (CME) could improve clinical knowledge and competence of endocrinologists and primary care physicians (PCP) regarding phenylketonuria (PKU) management. **Methods:** The CME activity was a 30-minute online video discussion between 3 experts with accompanying slides. Effect of CME was assessed using a repeated pairs pre-/post-assessment study design. The survey consisted of 3 knowledge questions and 1 self-reported confidence question. Differences between pre-to-post

responses were determined using McNemar's chi-squared test. P values <.05 were considered statistically significant. The activity launched March 17, 2017 and data were collected through April 17, 2017. **Results:** Data set included 49 endocrinologists and 89 PCPs. At baseline on preassessment, 14% of endocrinologists and 9% of PCPs answered all 3 knowledge questions correctly; on postassessment these more than doubled to 29% and 19%, respectively. Statistically significant improvements were seen regarding knowledge of data for a new therapeutic option in development [59% ($P = .026$) and 45% ($P = .033$) relative improvement for endocrinologists and PCPs, respectively]. Improvement in competence was seen related to next steps for treatment of a PKU patient [23% ($P = .218$) and 10% ($P = .453$) relative improvement for endocrinologists and PCPs, respectively. Self-reported confidence increased in 49% of endocrinologists and 48% of PCPs related to managing PKU with current treatments. Additional educational gaps were identified in one third to half of the participants and were related to managing different cohorts of patients with PKU and knowledge of data on emerging therapeutic options. **Conclusion:** This study demonstrates that a CME intervention in the form of a video-based panel discussion can improve clinically-relevant knowledge and competence related to PKU management.

284 - DNA Damage Induced by Phenylalanine and its Metabolites: Effect of L-Carnitine

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Background: Phenylketonuria (PKU) has been associated with oxidative DNA damage. L-Carnitine (LC) has an antioxidant property. **Objectives:** Considering that previously we demonstrated evidence of DNA damage in PKU (*in vitro* and *in vivo*), possibly due to high levels of Phenylalanine (Phe), and we also demonstrated that LC reduced the *in vitro* DNA injury induced by high concentrations of Phe, in the present work, we extended these investigations, analyzing the influence of Phe and its metabolites (phenyllactic acid - PLA, phenylacetic acid - PA and phenylpyruvic acid - PP) on chromosome damage and the effect of LC on this injury, evaluated by micronucleus frequency assay. **Methods:** The *in vitro* effect of LC (30 and 100 μM) on chromosome damage-induced by Phe and its metabolites ([Phe600μM+PA5μM+PP10μM+PLA10 μM], [Phe1200μM+PA10μM+PP15μM+PLA20 μM]) was examined in whole blood cells from normal individuals using micronucleus assay. **Results and Discussion:** We verified an increase in micronucleus frequency in healthy red blood cells incubated with Phe and its metabolites ([Phe600μM+PA5μM+PP10μM+PLA10 μM], [Phe1200μM+PA10μM+PP15μM+PLA20 μM]) when compared to control group ($P < .05$). We observed that the *in vitro* cotreatment with Phe and its metabolites and LC (30 and 100 μM) reduced significantly chromosome damage index when compared to group with Phe and its metabolites ($P < .05$). **Conclusions:** The present study yields experimental evidence that LC can reduce the in vitro chromosome injury induced by Phe and its metabolites, as well as, allows to hypothesize that LC protects against DNA damage in patients with PKU Financial support: CAPES, CNPq, FIPE/HCPA.

285 - Phase 3 PRISM-2 Long-term Extension Study Evaluating Efficacy and Safety of Pegvaliase for Treatment of Adults with Phenylketonuria

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Background: Pegvaliase, PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase, is a potential enzyme substitution therapy to lower blood phenylalanine (Phe) in individuals with phenylketonuria (PKU). **Methods**: The PRISM-2 open-label, long-term extension (OLE) objectives were to evaluate efficacy, safety, and tolerability of pegvaliase treatment. This study enrolled subjects from pegvaliase phase 2 and phase 3 studies and allowed subcutaneous pegvaliase dosing of 5-60 mg daily. Blood Phe, inattention (inattention domain of the Attention Deficit Hyperactivity Disorder Rating Scale), and safety were assessed at pre-treatment (pegvaliase naïve) baseline and throughout the OLE. **Results**: At data cut (Sept 23, 2016), 202 subjects in the OLE had a mean (SD) duration of pegvaliase treatment of 465 (236) days in the OLE and the majority (52%) were receiving pegvaliase at a daily dose of

40 mg (5 mg: <1%; 10 mg: 10%; 20 mg: 19%; 60 mg: 18%). Mean (SD) blood Phe decreased from pre-treatment baseline of 1234 (381) μmol/L to 808 (573), 519 (501), and 451 (519) at OLE week 1 (n = 181), 17 (n = 189), and 41 (n = 170), respectively. Blood Phe <360 μmol/L was achieved by 52/181 (29%), 91/189 (48%), and 97/170 (57%) subjects at OLE weeks 1, 17, and 41, respectively. The 35 subjects in the quartile with greatest blood Phe reduction (2209 to 1184 μmol/L) at OLE week 41 from pre-treatment baseline had the greatest change (~8-point decrease) in inattention scores. The most common adverse events (AEs) in OLE were arthralgia (36%), headache (33%), nasopharyngitis (29%), and upper respiratory tract infection (29%). In the OLE, 98% of subjects had AEs, 9% had serious AEs, and 74% had hypersensitivity AEs. Nine (5%) subjects had anaphylaxis (per National Institute of Allergy and Infectious Disease and the Food Allergy and Anaphylaxis Network criteria) that resolved (7 subjects continued treatment and 2 subjects discontinued) in the OLE. PAL IgG, PAL IgM, TAb, and NAb titers remained stable with long-term treatment and did not change after pegvaliase dose increases. **Conclusion**: Treatment with pegvaliase was associated with sustained blood Phe reduction and improved inattention scores compared to pre-treatment (pegvaliase naïve) baseline for subjects who continued with long term treatment, including those subjects unable to reach a blood Phe response in earlier studies. Pegvaliase has a generally acceptable safety profile with arthralgia and headache as the most common AEs.

286 - Biochemical and Genetic Investigation of Phenylketonuria Patients Treated With Sapropterin

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Background: Sapropterin dihydrochloride is a synthetic formulation of BH4 that has been shown to be effective in lowering serum phenylalanine (Phe) concentrations and/or improving dietary Phe tolerance in a subset of patients with phenylketonuria (PKU) or mild hyperphenylalaninemia (HPA). In this study, we aimed to investigate the biochemical and genetic findings of PKU patients who have been treated with sapropterin in our clinic. **Methods:** Demographic, biochemical, and genetic findings of PKU patients treated with sapropterin were analyzed retrospectively. The diagnosis was made by neonatal screening program in all patients. **Results:** Six-teen HPA and 11 PKU patients treated with sapropterin were enrolled in the study. Among these, 10 (37%) were females. Actual age was (median [25-75 percentiles]) 76 months [41-115]. Frequency of *parental* consanguinity was 37% (10 patients). Median initiation age of sapropterin was 53 months [20-94]. Before the sapropterin treatment, median maximum plasma Phe level of patients was 792 μmol/L [658-1749]. With the BH4 responsiveness test, median lowing in plasma Phe level was 60% [51-75]. Phenylalanine tolerance of patients raised from 630 mg/day [480-1200] to 1395 mg/day [895-2290] with the sapropterin treatment. In 15 (55.6%) patients, Phe-restricted diet was stopped with the initiation of sapropterin treatment. Cessation of Phe-restricted diet of the patients whose Phe levels between 360-900 μmol/L before the sapropterin treatment (12 patients, %75) were significantly higher the patients whose Phe levels higher than 900 μmol/L (3 patients, %27.3) ($P = .019$). In genetic analysis of patients, homozygous mutation (*p.Glu178Gly, p.Arg261Gln, c.IVS10-11G>A, p.Arg243Gln, p.Pro281Leu, p.Leu48Ser and p.Glu390Gly*) was revealed in 10 (37%) patients. Seventeen patients had compound heterozygous mutation. The most common mutations were *c.IVS10-11G>A* (18.5%), *p.Glu390Gly* (16.7%), and *p.Arg261Gln* (11.1%). **Conclusion:** In our study, *responsiveness* to *sapropterin* was related with several mutations and *c.IVS10-11G>A* was determined as the most common mutation in PKU patients treated with sapropterin. Furthermore, our study suggested that plasma Phe level of patients before the sapropterin treatment gives an idea about the degree of response to sapropterin treatment.

287 - Bone Mineral Density and BH4 Treatment

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Background: Bone Mineral Density (BMD) is compromised in PKU patients. New therapeutic approaches would benefit bone acquisition. **Objective:** To describe the changes in BMD in PKU patients under treatment with BH_4. **Patients and methods:** 14 well controlled PKU patients were studied. Six (Group 1 (G1) (5 boys) median (M) age 18.7 years (y) (range: 12.6-19.7) with a M phenylalanine (Phe) tolerance of 500 (450-1000) mg/day had been treated with BH_4 for a M period of 2.3y (1.3-3.8). The other 8 (Group 2 (G2) (5 boys)) M age 18.1y (R: 14.5-19.8), M Phe tolerance of 400 (300-1000) mg/day were always under conventional treatment (diet + protein substitute). In them, a period of 4.2 y (2.1-5.5) was evaluated. Initial and final data on Phe intake and protein and calcium intake and source were recorded. Initial and final data of Total and Lumbar spine (L2-4) BMD assessed by Lunar DXA and adjusted by bone age were retrieved, expressed as SDS according to local normal references and analyzed. Also, BMD changes between initial and final data were calculated for each group. Mann Whitney test was used for statistical analysis. **Results:** Initial Total and Lumbar spine L2-4 BMD were similar in both groups although with a trend of lesser Total and Lumbar spine BMD in G2. Total: G1: 0.5 (−0.4 to 2.3) vs. G2: 0.39 (−1.6 to 0.6) Lumbar: G1: 0.03 (−1.7 to 2.2) vs. G2: −1.17 (−2.5 to 0.07) (both $P = .08$). During the studied

period G1 increased Phe tolerance 3.5 ±1.6 times and protein intake 3.2 ±1.4 times (4 patients only natural protein, 2 patients 80% natural protein/20% protein substitute) and calcium intake from foods reached 53±32% of RDI while G2 continued with the same intake pattern. Final Total and L2-4 BMD were not different between groups: Total G1: 0.26 (−0.14 to 2.9) vs G2: −0.18 (−1.69 to 0.68) *P*: Nonsignificant (NS). Lumbar G1: 0.45 (−1.6 to 2.65) vs G2: -0.13 (−3.4 to 1.18) *P* : NS. Differences achieved in total and L2-4 BMD by both groups during the studied period did not differ. δTotal G1:0.19 (−0.61 to 0.61) vs. G2:0.06 (−1.4 to 1.1) *P* : NS. δLumbar G1:0.41 (−1.13 to 0.47) vs. G2:0.57 (−1.9 to 2.3) *P* : NS. **Conclusions:** We weren't able to evidence improvement in BMD or significant changes in its acquisition in a homogeneous group of well controlled moderate PKU patients, although those treated with BH_4 changed the intake of protein, calcium and Phe during the studied period. Further observations are needed with more prolonged follow-up to draw out definitive conclusions.

288 - Phase 2 Long-term Pegvaliase Treatment for Adults With Phenylketonuria: Updated Year 5 Safety and Efficacy Data from the PAL-003 Extension

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Background: Phenylketonuria (PKU) is caused by phenylalanine hydroxylase deficiency resulting in phenylalanine (Phe) accumulation. Pegvaliase, PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase, converts phenylalanine (Phe) to trans-cinnamic acid and ammonia, and is a potential enzyme substitution therapy to lower blood Phe in adults with PKU. **Methods**: The objectives of the ongoing phase 2 PAL-003 extension study are to evaluate long-term efficacy, safety, and tolerability of subcutaneous pegvaliase injections in adults with PKU. Subjects entered the PAL-003 extension study after completing a parent study (PAL-002, PAL-004 or 165–205), with dose adjustment as needed for safety or to maintain blood Phe of 60-600 µmol/L. **Results**: 68 subjects entered PAL-003 with a mean of 17 (range 11-24) weeks of prior pegvaliase exposure and PAL-003 mean (SD) baseline blood Phe of 1022 (530) µmol/L. The PAL-003 mean pegvaliase dose was 26 mg per day (range 4-107 mg per day) administered in 1-7 doses per week and mean treatment duration was 176 (range 4-354) weeks. At PAL-003 Year 1, mean (SD) blood Phe decreased 66% (34%) from pre-treatment (pegvaliase naive) baseline of 1332 (327) to 435 (446) µmol/L. This decrease persisted through PAL-003 Year 5. A total of 58 (85%) subjects had blood Phe ≤360 µmol/L and 59 (87%) subjects had blood Phe ≤600 µmol/L during PAL-003. Long-term pegvaliase treatment was tolerated. All patients reported an adverse event (Grade 1, 22%; Grade 2, 68%; Grade 3, 10%) and 4 subjects discontinued the study drug due to an adverse event. The most common adverse events were headache (63%), nasopharyngitis (59%), rash (52%), injection site reaction (52%), injection site erythema (50%), and arthralgia (50%). Six subjects had anaphylaxis events (per National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network criteria) that resolved (4 continued treatment and 2 withdrew from the extension study). All subjects developed a sustained antibody response against phenylalanine ammonia lyase and most developed a transient antibody response against polyethylene glycol. **Conclusion**: Subjects treated with pegvaliase had a substantial and persistent decrease in mean blood Phe. Long-term treatment was tolerated with most subjects experiencing Grade 1 or 2 adverse events. **Funding Disclosure:** BioMarin Pharmaceutical Inc. provided funding for the study.

289 - Dietary Treatment in Phenylketonuria (PKU): Only Benefits? Main Issues to Be Accounted

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Patients with phenylketonuria (PKU) encompass an 'at risk' group for a wide range of imbalances, affecting not only nutritional issues but also other aspects, such as quality of life (QoL). We reviewed recent literature aiming to focus topics to always consider when dealing with PKU population. Micronutrient status: based on PKU dietary limitations, though supplements are commonly added to phenylalanine(Phe)-free mixtures, there's still a risk of vitamin and mineral imbalances. This refers mainly to B12 vitamin, Se, Zn, Fe, folate, and ω3, with reference to age of treatment commencement, type of treatment, and dietary compliance. The need for supplementation in addition to what assured by mixtures is well established. Bone health: bone mineral density (BMD) has been widely used in scientific reports as main marker of bone status, being significant for Z-scores < or = −2 SD compared to general population (gp). Recent literature highlighted ↓ BMD in 90% PKUs but, deepening data, Z-scores were < −2.5 SD only in 5-14% PKU vs. 0.5% in gp. Further studies are needed to better establish fractures' risk (lack of data in PKUs). Overweight and obesity: available data show PKUs BMI comparable to gp, though ↑ carbohydrates (CHO) dietary intakes are confirmed with consequences on daily glycemic index (GI) known to be

correlated to non-communicable diseases (NCDs) in healthy adults. This highlights the need for GI to be primarily considered framing dietary intakes. Psychological issues: despite well-known sense of anxiety and guilt related to poor adherence to dietary treatment and pledge to maintain Phe levels within safe ranges, PKU QoL scores seems to be comparable to gp. Still, PKU patients tend to show ↑ vulnerability to psychiatric disorders than gp, with those showing better metabolic control being more diagnosed with such disorders, confirming the need of a psychologist/psychiatrist support. Our research confirms PKUs need to be strictly monitored throughout life achieving the best well-weighed nutritional intervention. A simple relationship between dietary intakes and nutritional status is not sufficient: many independent and interrelated complex factors should be evaluated other than quantitative issues. Much more is still needed to be done in order to guarantee the optimal equilibrium for each PKU patient considered in his entirety.

290 - Spanish Hyperphenylalaninemia Cases Bearing a Highly Frequent Mutation in the DNAJC12 gene

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Most cases with hyperphenylalaninemia (HPA) are caused by loss-of-function mutations in the PAH gene or by genetic defects in enzymes involved in the synthesis and regeneration of the PAH active cofactor tetrahydrobiopterin (BH4). In our lab, we have sequenced the PAH gene in more than 1036 cases after differential routine biochemical diagnosis to rule out a BH4 metabolism disorder. We sequenced the PAH gene by conventional Sanger sequencing combined with MLPA or in the last three years by massive parallel sequencing of the entire coding sequence in order to detect deep intronic mutations or genomic rearrangements. Biallelic or monoallelic pathogenic mutations were detected in 1021 cases. In this work, we report the genetic analysis of the recently described DNAJC12 gene in order to elucidate the molecular basis of 14 HPA cases from Spain and one from Chile with no mutations in the PAH gene. We have identified three novel nucleotide variations (c.524G>A, c.502+1G>C and c.298-2A>C) in 11 cases. All cases presented Phe levels at diagnosis >120 μM (120-337 μM), normal DHPR activity and normal urinary pterins levels. All cases are currently clinically asymptomatic but one presented psychomotor delay and seizures. Some cases presented Phe increase (400-500 μM) under febrile process. All are under normal diet and some of them are treated with BH4 with favorable response. The nucleotide change c.524G>A (p.Trp175Ter) was detected in 18 mutant alleles, eight homozygous and two heterozygous cases in combination with the splicing variations. All three variations were predicted to be pathogenic using the functional platform Alamut. The splicing variations were not detected in the worldwide population database ExAC while c.524G>A variation is present in EXAC and in a Spanish consortium database with a frequency less than 0.013% and 0.1%, respectively. These results suggest a likely frequent and founder mutation in our population that can be used as specific genetic marker. In summary, our DNAJC12 cases are in general clinically asymptomatic under normal diet although they should be included in follow-up in order to avoid in the future neurological symptoms related to neurotransmitter defects.

291 - Efficiency of Sapropterin Dihydrochloride in 112 Turkish Phenylketonuria Patients

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Background: Phenylketonuria (PKU) is the most frequent inherited metabolic disease caused by the deficiency of phenylalanine hydroxylase (PAH). It's devastating-irreversible effect on development can be avoid by a strict diet. Tetrahydrobiopterin (BH4) is the cofactor of PAH and used in the treatment of BH4-responsive PKU patients. It provides a better metabolic control and an increase in Phe-tolerance and the quality of life. Some genotypes are associated more with BH4 responsivity. **Objectives:** The increase in Phe-tolerance and the correlation between the genotype and BH4-responsiveness were evaluated in 112 patients. **Methods**: After a loading test, BH4 was initiated to 112 responsive mild-moderate PKU patients (≥30% decrease in phe level) with a dose of 10-20 mg/kg/d in either 2 divided doses or a single dose. Then, Phe-tolerance and blood Phe levels were followed. **Results**: The mean age of the initiation of BH4 was 40 months (1–149). A significant increase in Phe-tolerance with the reduction in blood Phe levels were the indicators of BH4 efficiency seen in our study. BH4 was discontinued in 27 patients because of secondary unresponsiveness. The median duration of BH4 treatment was 21,9 months.

Genotype determined in 103 patients were compound heterozygous: 49 (43,8%) and homozygous: 54 (48,2%). The most commonly found BH4-responsive *PAH* alleles were: [IVS10−11G>A];[IVS10-11G>A] (14,2%), [p.R261Q];[R261Q](14,2%), [IVS4+5G>T];[IVS4+5G>T] (3,5%), [p.Y417C];[Y417C] (1,7%), [p.E178G];[IVS10-11G>A] (1,7%), [p.A300 S];[IVS10-11G>A](1,7%), [IVS10-11G>A];[IVS4+5G>T] (1,7%), [IVS10-11G>A];[p.A403 V] (1,7%), [p.L293 S];[E178G] (1,7%), [IVS1011G>A];[p.F331C] (1,7%), [IVS10-11G>A];[p.R261Q] (1,7%); and [p.Y417C];[R261Q] (1,7%). The most common BH4-responsive mutations p.R261Q, IVS10-11G>A and p.A300 S were found in 54 patients. From the remaining compound heterozygous patients 17 had new, 13 had unknown-response and 5 had unresponsive mutations in one allele. **Conclusion:** This study is one of the biggest single center experience about the efficiency of BH4 and the genotype-phenotype correlation in PKU patients from Turkey. BH4 is found to be effective in increasing Phe-tolerance and maintaining an ideal blood Phe level. Parallel with the new reports, both homozygous and compound heterozygous mutations are found equally BH4-responsive. Although the mutations of our patients are nearly the same with the most commonly seen responsive mutations.

292 - Deficiencies of BH4 in Minas Gerais, Brazil

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Introduction: BH4 deficiencies are genetic diseases caused by mutations in the synthesis or regeneration of the tetrahydrobiopterin cofactor (BH4). Although rare, they are serious and, if left untreated, lead to developmental delays, movement disorders, seizures, and premature death. Of the 6 types of BH_4 deficiencies, 4 present with hyperphenylalaninemia: GTPCH I deficiency in homozygosity, PTPS deficiency, DHPR deficiency and PCD deficiency, thus being possible to be identified by the Neonatal Screening Program for hyperphenylalaninemias. Treatment consists of the control of hyperphenylalaninemia with diet or use of Sapropterin and the replacement of neurotransmitters through the precursors Levodopa and 5-Hydroxytryptophan. In DHPR deficiency folinic acid is also used. **Objective:** To describe the cases of BH4 deficiencies identified in the Neonatal Screening Program of Minas Gerais. **Results:** Since the implantation of the Neonatal Screening Service in Minas Gerais, 14 cases of BH4 deficiencies have been identified. Currently, 10 patients are followed up and 4 have died. The prevalence of the disease is 2.1 to 1 million live births, with the frequency between hyperphenylalaninemias being 1.71% of cases. Six patients were identified with PTPS deficiency (42.8%), 4 patients (28.5%) with GTPCH I deficiency and 4 patients (28.5%) with DHPR deficiency. Of the 10 patients in follow-up, the family of 2 patients (siblings) decided not to use Sapropterin in the control of hyperphenylalaninemia, preferring, for this purpose, the performance of restricted diet in proteins. Five were diagnosed from clinical suspicion and five from neonatal screening. Those diagnosed by neonatal screening have satisfactory neuropsychomotor development. **Conclusion:** Early treatment shows good results in the prevention of intellectual disability and it is justified the investigation of these deficiencies in children with hyperphenylalaninemia by neonatal screening programs for Phenylketonuria.

293 - Deleterious Effects of Sepiapterin on Developing Reaggregated Rat Brain Cell Cultures

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Background: Phenylketonuria (PKU) is an inborn error of phenylalanine metabolism caused by phenylalanine hydroxylase (PAH) deficiency. Patients with mild or moderate PKU are often successfully treated with Sapropterin dihydrochloride (SD) with a starting of treatment authorized now below 4 years of age. SD is a pharmaceutical version of tetrahydrobiopterin (BH4), a cofactor of the deficient enzyme PAH. Similar BH4 concentrations to those observed in plasma have been found in CSF of patients after a single oral BH4 administration. As BH4 is not stable, we used sepiapterin, the precursor of BH4. Concentrations corresponding to plasma concentrations in patients have been applied to study the effects of BH4 on developing brain cells in a rat 3D organotypic brain cell culture in vitro model. **Methods:** 3D organotypic rat brain cell cultures aggregates derived from E15 Sprague Dawley rat embryos were exposed to 60 or 120 ng/mL sepiapterin from DIV11 to DIV14 corresponding to early childhood. BH4/BH2 measurements were performed to evaluate the conversion of sepiapterin to BH4. Changes in morphology and viability of different brain

cell types were studied by immunohistochemistry and confirmed by western-blot. We investigated potential alterations of the GABA-ergic system by analyzing the expression of glutamic acid decarboxylase (GAD; the enzyme synthesizing GABA) as well as GABA-A receptor ($GABA_AR$). **Results:** BH4/BH2 measurements showed successful conversion of sepiapterin to BH4. Brain cell-type specific markers revealed swollen astrocytes and a decreased number of astrocytic fibers (120 ng/mL) and oligodendrocytes (120 ng/mL). Western blot analyses confirmed a significantly decreased expression of oligodendrocytes. Immunofluorescence for activated caspase-3 revealed an increased apoptosis rate for both concentrations. We found decreased levels of GAD and $GABA_AR$ expression (120 ng/mL). **Discussion:** Exposure of rat developing brain cells to sepiapterin leads to significant toxicity on different brain cell types in our in vitro model. The altered expression levels of proteins of the GABA-ergic system are similar to what is seen in attention deficit hyperactivity disorders (ADHD). The long-term neuropsychological follow-up of patients treated with SD since early age should be performed to evaluate the potential correlation with ADHD. Metabolomics studies and RNA sequencing are ongoing to decipher the molecular mechanisms underlying these observed effects.

294 - Challenges and Advances in Neurological Intervention for Control and Treatment of Phenylketonuria

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This work has the purpose to analyze the importance of the neurological intervention for control and treatment of phenylketonuria as a way to minimize this genetic disease of autosomal recessive inheritance, whose basic biochemical defect is the enzyme phenylalanine hydroxylase (PAH) deficiency, responsible for liver conversion of phenylalanine to tyrosine, and Intellectual disability is the most important sequel. **Methods:** an integrative literature review on Lilacs, Bireme, Medline and Scielo, whose inclusion criteria for the sample selection were: articles published in Portuguese and English that portray the theme under study, published and indexed in these databases over the last 15 years. **Results:** Through the selected articles, it was possible to investigate part of the Brazilian reality on the progress and challenges of control and treatment of phenylketonuria, reviewing not only the diagnostic methods of this genetic disease, but also the direct effects that diet therapy has on children's growth and development, the use of alternative diets of synthetic amino acids based on protein hydrolysates with low phenylalanine content, controversies about discontinuity and emerging problems of prolonged treatment of some age groups. **Conclusion:** It's important that managers of targeted programs to treatment and control of phenylketonuria can develop more efficient and broad health policies, inserting more resources to professional training and better working conditions to care and attend properly patients affected by this disease, and thus advance towards better quality of life for them, especially among the most disadvantaged communities.

295 - Seventh Interim Analysis of the Kuvan® Adult Maternal Pediatric European Registry (KAMPER): Interim Results in BH4 Deficiency Patients

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Objective: To assess long-term safety and efficacy of patients with hyperphenylalaninemia due to a defect in tetrahydrobiopterin metabolism treated with sapropterin dihydrochloride (Kuvan®, BioMarin Pharmaceutical Inc.) **Methods:** Data on growth, neurocognitive outcomes, blood phenylalanine (Phe) levels, and medication management including BH_4 were studied in patients with primary BH_4 deficiency within the KAMPER observational, multi-center drug registry. **Results:** The seventh-year interim analysis from 68 clinical sites in 9 countries included data on 49 BH_4 deficiency patients, with a mean (SD) age of 11.4 (10.8) years, including guanosine triphosphate cyclohydrolase deficiency, pyruvoyl-tetrahydropterin synthetase deficiency, and dihydropterin reductase deficiency, of whom 10 (20%) were detected through newborn screening. Mean and median blood Phe levels for patients of all age groups were maintained below 360 μM (<4 years, 25- 270 μM; 4 - <18 years, 47- 397 μM; ≥18 years, 36- 351 μM). The majority of subjects <10 years old maintained their mean z-score for height and body mass index (BMI) within 1 SD. BMI scores increased above 1 SD for subjects >10 years old. Two female subjects >10 years old had a mean z-score for height between −2 and −1 SD. The average sapropterin dose ranged from 1.5 to 19 mg/kg/day. Concomitant medications included dopa or dopa derivatives (81% of subjects), folic acid (19%), and monoamine oxidase inhibitors (17%). Sixteen patients experienced 66 adverse events (AEs); 4 experienced

6 serious AEs, including generalized tonic–clonic seizure, laryngitis (moderate), dystonia (moderate), epistaxis (mild), and a laceration; the rest were mild to moderate and none considered related to sapropterin. In the 4 -<18 years age group over 50% of patients were in the appropriate school level with a third at average levels for reading, writing, and math. **Conclusions:** Results show that BH_4 deficient patients demonstrated appropriate growth rates, had acceptable tolerance to sapropterin and maintained somewhat acceptable educational status.

296 - Seventh Interim Analysis of the Kuvan® Adult Maternal Pediatric European Registry (KAMPER): Interim Results in Phenylketonuria Patients

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Objective: To assess long-term safety and efficacy of patients with hyperphenylalaninemia due to phenylketonuria (PKU) treated with sapropterin dihydrochloride (sapropterin, Kuvan®, BioMarin Pharmaceutical Inc.). **Methods:** Growth, neurocognitive outcomes, dietary adherence, and long-term sapropterin sensitivity were studied by use of the KAMPER observational, multi-center drug registry on PKU patients. **Results:** The seventh-year interim analysis from 68 clinical sites in 9 countries included data from 575 PKU patients. In total, 436 (76%) of patients were genotyped. The most common genotypes were p.R261Q/ p.R261Q (n = 14), c.1315+1G>A/p.Y414C (n = 12), p.R408 W and p.L48 S (n = 12), and p.L48S/L48 S (n = 8). Mean and median blood Phe levels for patients 0-<12 years were maintained near or just above 360 μmol/L; older patients had Phe levels increasing with age, but generally <600 μmol/L. Dietary Phe and natural protein intakes increased in all age groups by 1.5 to 2 times their intakes prior to sapropterin treatment. Mean (SD) sapropterin dose was 14.4 (4.5) mg/kg/day, ranging from 5.0 to 22 mg/kg/day. Adverse events considered related to sapropterin occurred in 37 (6.4%) of subjects; the most frequently reported were nervous system disorders (n = 19) and gastrointestinal disorders (n = 12). None were considered serious. Mean anthropometric z-score measures were maintained between −1 and + 1 SD for both males and females over the 6-year time period. Around 90% of patients 4 to ≤18 years of age were in school with ≥70% of the group at average reading, writing and math levels, ≤10% below average levels and ≥20% above average. The most common psychiatric /behavioral symptoms reported included Attention Deficit Disorder (ADD/ADHD) and anxiety, with four new cases of ADD/ADHD and two new cases of anxiety reported since baseline. **Conclusions:** Results of the KAMPER registry show that PKU patients were able to maintain blood Phe levels near the recommended ranges according to the European PKU guidelines, while maintaining an increased dietary Phe intake; sapropterin was noted with a favorable safety profile. KAMPER provides a unique opportunity to gather a large collection of long-term follow-up data related to sapropterin treatment in PKU in 9 European countries.

297 - Interim Analysis of the Phenylketonuria (PKU) Patients Enrolled in the PKUDOS Registry

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Objective and Methods: The PKUDOS registry was designed to collect 15 years of safety and efficacy data on PKU patients of all ages who are currently or were previously treated with sapropterin dihydrochloride (sapropterin, Kuvan®), or who plan to initiate treatment. **Results:** As of February 2017, 1639 patients were enrolled in the PKUDOS registry; 908 have been continuously treated with sapropterin, 262 intermittently treated, 381 previously treated, and 88 could not be classified. Data on the previously and continuously treated patient groups are presented. Approximately 8% of these patients are <4 years, 49% 4-<18 years, 43% 18-<65 years, and <1% ≥65 years of age. Genotype data are available for 729 patients. The most common mutations (n = number of patients with mutation on either allele) are p.R408 W (n = 134), c.1315+1G>A (n = 72), p.165 T (n = 50), and p.Y414C (n = 42). Median blood phenylalanine (Phe) levels were 614 μM at baseline (n = 379) and 723 μM at 6 years (n = 55) for those previously treated with sapropterin, and 357 μM at baseline (n = 893) and 420 μM at 6 years (n = 215) for continuously treated patients. Baseline values were recorded upon study entrance, and are not

necessarily sapropterin treatment-naïve values. Median prescribed dietary Phe intake of the continuously treated group was ~1.7 times that of the previously treated group (500-550 mg Phe/day vs 295-333 mg Phe /day), which remained consistent over 6 years. Actual Phe intake in the continuously treated group was likewise higher, though this data was more variable. The overall mean (SD) sapropterin dose reported was 18.7 (3.2) mg/kg/day, with a median dose of 20 mg/kg/day. Adverse events (AEs) considered related to sapropterin in the continuously treated group occurred in 116 (12.8%) patients; the most frequently reported were gastrointestinal disorders (n = 62; 6.8%) and nervous system disorders (n = 30, 3.3%). Of these AEs, 3 (0.3%) patients reported gastrointestinal disorders that were considered serious. **Conclusions:** The PKUDOS registry allows for the longitudinal follow up of patients with PKU. Patients taking sapropterin had higher prescribed and actual dietary Phe intakes while maintaining lower blood Phe levels as compared to those who discontinued drug therapy for any reason. The PKUDOS registry is an opportunity for investigators to engage in active research regarding management and long-term outcomes of PKU patients who have, have had, or will have exposure to sapropterin.

298 - An Interim Analysis of the Kuvan® Adult Maternal Pediatric European Registry (KAMPER) and Phenylketonuria Developmental Outcomes and Safety (PKUDOS) Registries: Pregnancies

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Objective: To describe the efficacy and safety of the use of sapropterin dihydrochloride (Kuvan®, BioMarin Pharmaceutical Inc.) in a group of 44 women with phenylketonuria (PKU) during 55 pregnancies. **Methods:** Data were collected from the maternal sub-registries of KAMPER and PKUDOS, on the management, efficacy, and safety of sapropterin use prior to and/or during pregnancy in women with PKU. **Results:** Data from 12 pregnancies in 10 women, mean maternal age (SD) 29.7 (3.9) years from KAMPER, and 43 pregnancies in 34 women, mean maternal age 30.4 (4.8) years, from PKUDOS registries were provided. Of those, 7 (58%) KAMPER and 14 (32%) PKUDOS women were reported to be on Phe-restricted diets prior to becoming pregnant. Mean (SD) dose of sapropterin was 11.4 (5.8) mg/kg/day and 18.6 (3.3) mg/kg/day, with mean (SD) duration of sapropterin use 268.9 (14.2) and 265.7 (60.8) days during pregnancy from KAMPER and PKUDOS respectively. Mean blood Phe levels per trimester were maintained within the range of 120 to 360 μmol/L, except for 3 pregnancies during the first trimester in KAMPER and 2 in PKUDOS, and 4 in the second and 2 in the third trimester in PKUDOS. Two women withdrew from the KAMPER registry; one was lost to follow-up, and the other discontinued due to investigator decision. Six women in KAMPER experienced adverse events (AE) during pregnancies, all were mild with the exception of 1 serious AE, an arrhythmia. One (8%) woman in KAMPER and 5 (11%) women in PKUDOS had spontaneous or elective terminations of their pregnancies. In KAMPER, 10 pregnancies were carried to term, with 9 infants reported to be normal at birth, and 1 with abnormal appearance associated with head, ears, nose, and throat as reported by the investigator. In the PKUDOS registry per investigator report, 38 pregnancies were carried to term; 34 (90%) normal, and 4 (10%) infants with abnormal outcomes: 2 major (1 microcephaly and 1 cleft palate) and 2 minor (1 born at 35 weeks gestation and 1 ankyloglossia). The KAMPER abnormal offspring and 2 PKUDOS major abnormal outcomes were associated with poor metabolic control during the pregnancy. Limited breastfeeding data were reported; 4 (33%) KAMPER and 12 (27%) PKUDOS women breastfed. **Conclusions:** In a small population of PKU pregnant women, additional evidence on sapropterin therapy during and/or prior to gestation with a phe-restricted diet is presented, demonstrating efficacy with maintaining blood Phe within the targeted range.

299 - Discovery of Novel Compounds With Pharmacological Chaperone Potential for Therapeutic Correction of PKU

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Phenylketonuria (PKU) is the most common inborn error of metabolism, caused by mutations in the gene encoding phenylalanine hydroxylase (PAH), resulting in increased phenylalanine levels in blood and toxic levels in brain. The current treatment consists of a strict phenylalanine-free diet from birth

and supplementation with Kuvan®—a synthetic form of the cofactor tetrahydrobiopterin (BH4)—is also effective for some patients with milder phenotypes. But both treatments present pitfalls, including neurodevelopmental or psychosocial problems and low response-frequency among patients, respectively, and new therapeutic strategies are accordingly needed. One of the mechanisms of action of the cofactor is as a pharmacological chaperone, increasing the intracellular half-live of PKU-associated mutant PAH. We have performed a high-throughput screening protocol leading to the selection of 109 primary stabilizing compounds, which through validation of binding, activity assays and cellular studies were reduced to one final hit compound. This compound displayed good affinity ($K_D \approx 10$ μM), enthalpically driven binding and excellent scores in cultured cells, with an increase of 50% activity for wild-type (WT) PAH, and up to 250% increase of activity and protein levels for recurrent PKU mutations such as p.I65 T and p. 261Q. Extensive derivatization of the compound has provided two series of compound analogues with increased affinity and improved potency in cells expressing PAH mutants, which are very effectively stabilized, reaching protein amounts and activity levels characteristic of the WT enzyme. These compounds exhibit a great pharmacological chaperone potential for the development of a novel treatment option for PKU.

300 - Experience With the Use of Tetrahydrobiopterin in the Medical Genetics Service of the Hospital de Clínicas de Porto Alegre

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Objective: To report the experience of using the tetrahydrobiopterin cofactor (BH_4) in patients with Phenylketonuria (PKU) and BH_4 Deficiency followed in the Medical Genetics Service of the Hospital de Clínicas de Porto Alegre. **Methodology:** The data comes from the Metabolic Disorders Outpatient Clinic, which currently follows 85 patients with PKU, due to phenylalanine hydroxylase deficiency, who are undergoing dietary treatment. Four patients with BH_4 Deficiency are also followed in this service and the treatment consists of administration of BH_4 along with adjuvant drugs and none of these patients follow diet therapy. For PKU patients, two different protocols were used to assess BH_4 responsiveness: one based on BH_4 loading test and the other based on the combined L-Phe + BH_4 loading test (Giugliani et al., 2011; Nalin et al., 2011). **Results:** Thirty-four PKU patients performed, at least, one of the BH_4 responsiveness protocols and twelve of them are classified as responsive to the medication, by reducing in, at least, 30% the phenylalanine plasma values. None of these patients are currently using BH_4, but one is awaiting judicial decision to start treatment. On the other hand, all patients with BH_4 Deficiency use the cofactor in their treatment, keeping Phe levels within normal range. **Conclusions:** Our findings are in agreement with the literature and indicate that a relevant number of Brazilian patients with PKU are responsive to BH_4. It was observed that, for patients with BH_4 deficiency, the treatment with BH_4 has been used, possibly because there is no other therapeutic option. Currently in Brazil, access to this medication is only through a judicial process. We reinforce the importance of conducting BH_4 responsiveness tests in the Brazilian patients in order to know the population that can benefit from it, as it can be an adjuvant in the treatment of the patients and improve their quality of life.

301 - One-year Follow-Up of B vitamin and Iron Status in Patients With Phenylketonuria Responsive and Nonresponsive to Sapropterin

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Background: People with Phenylketonuria (PKU) who respond to sapropterin (tetrahydrobiopterin-BH_4) are often able to increase phenylalanine (Phe) tolerance and/or decrease dependence on medical food while maintaining therapeutic blood Phe levels. Saproterin responders may experience a reduction in B12, B6, folate, and iron if nutrients are not compensated through intact foods. **Objective:** This study investigated B vitamin and iron blood levels and dietary intake among patients with PKU over one year based on sapropterin response status. **Methods**: 58 male and female PKU patients, age 4-50 years were recruited. 33 with clear response status and complete data were included in the analysis. Nutrient intake was determined by NDSR analysis of 3-day diet records. Blood biomarkers were analyzed by Quest Diagnostics. Statistical analyses were performed using SAS software 9.4. T-tests were used to assess one-year changes in B6, B12, folate, and iron. Differences between sapropterin response groups at baseline and final visit were evaluated via analysis of covariance (ANCOVA), run through a general linearized model with response status and continuous age as independent variables, stratified by age group. Proportion of nutrients obtained from medical food and intact food was evaluated by response group. Patients were matched to controls from the National Health and Examination Survey (NHANES) to examine adequacy of nutrients. **Results**: Both responders and non-responders maintained lab and dietary values within reference ranges, though responders experienced a significant decline in serum B12 during the

study ($P = .028$), whereas non-responders did not. The difference in one-year B12 change by response group was most significant among those less than 18 years ($P = .04$). Serum B12 in both response groups was lower than NHANES controls at both time points. At 1 year, responders obtained a greater percentage of B12 from natural foods (mean = 84%), compared with non-responders (mean = 36%) ($P = 0.005$). **Conclusion**: Although mean dietary and lab values for B12, B6, folate, and iron in sapropterin responders and non-responders with PKU were adequate, responders had a significant decline in serum B12 over one year. This may be explained by decreases in fortified medical food among responders. This cohort had lower serum B12 than NHANES controls at both time points, indicating a need to monitor B12 concentrations and consider supplementation if necessary.

H) Sulphur Amino Acid Disorders (302 to 318)

302 - Analysis of the Mutation Spectrum of the CBS Gene in Russian Patients With Classical Homocystinuria

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The purpose of the study is to detect and analyze mutations in the *CBS* gene of classical homocystinuria in Russian patients. **Materials and methods:** A sample of 13 patients from 11 unrelated families with clinical signs of homocystinuria was formed. The PCR method with subsequent restriction analysis was used for screening mutations Ile278Thr and IVS11-2A-> C. The direct non-radioactive sequencing method was used to analyze the open reading frame of the *CBS* gene for all patients in the sample. **Results:** Mutation Ile278Thr, which is considered the most frequent among various populations of the world and responsible for the formation of a lighter B_6-dependent phenotype, was detected only in one patient. Another common mutation Gly307Ser, which leads to the development of a more severe B_6-resistant form of pathology, was not detected at all. All mutations found (except for the four of them) have been described in scholarly works. Most of the mutations cause the development of B_6-dependent homocystinuria. Data on mutations c.216-217delAT (1 patient), c.1498_1499delT (1 patient) and p.1560-1569delCACCGGGAAG (2 siblings) are not available and their effect is unknown. Most likely, these mutations are responsible for the formation of the B_6-resistant form of the disease, since it has been proved that these mutations lead to a shift in the reading frame and formation of premature stop codons in positions 103 and 540 of the protein chain, respectively. Furthermore, missense mutation Asp444Tyr detected in one patient in the homozygous state and nonsense mutation Gln368Term found in another patient in the heterozygous state have not yet been described in research. The most common mutation was the known splice site mutation IVS11-2A-> C, which was registered in 6 out of 13 patients examined. In 6 out of 11 patients, the B_6-dependent form of homocystinuria was confirmed by DNA diagnostics and analysis of biochemical and X-ray functional data. In 2 siblings, the new mutation found was manifested by severe eye pathology (dislocation of the lenses with the formation of malignant secondary glaucoma) and cardiovascular disorders with the development of crises (confusion and delirium) lasting up to 4 days, which are poorly managed medically. Thus, the detection of mutations of the *CBS* gene allows us to verify diagnosis, predict the course of the disease, select optimal therapy and provide efficient medical and genetic counseling for families.

303 - Chronic Mild Hyperhomocysteinemia alters Inflammatory and Antioxidant Parameters: Is Acetylsalicylic Acid a Neuroprotector?

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Homocysteine is a sulfur-containing amino acid derived from the metabolism of methionine. When plasma homocysteine levels reach more than 10-15 μM, there is a condition known as hyperhomocysteinemia. It may occur as a result of an inborn error of methionine metabolism or by non-genetic causes. Hyperhomocysteinemia also is considered a risk factor for development of neurological and cardiovascular diseases. Our research group has developed a chronic chemically-induced model of mild hyperhomocysteinemia (up to 30 μM) in young adult rats and has shown its association with cerebral oxidative stress and inflammation. The objective of present study was to evaluate whether acetylsalicylic acid has neuroprotective effect on inflammatory parameters (acetylcholinesterase and interterleukin-1β) and antioxidant enzymes (catalase e superoxide dismutase) in rats subjected to mild hyperhomocysteinemia. Wistar male rats received homocysteine (0.03 μmol/g of body weight) by subcutaneous injections twice a day and acetylsalicylic acid (25 mg/kg of body weight) by intraperitoneal injections once a day from the 30th to the 60th postpartum day. Control rats received saline. Cerebral cortex was dissected for posterior biochemical analysis. Results showed that rats subjected to mild hyperhomocysteinemia increased acetylcholinesterase activity ($P < .001$) and interterleukin-1β levels ($P < .05$). Regarding antioxidant defenses, homocysteine increased catalase activity ($P < .05$) and decreased superoxide dismutase activity ($P < .05$). Acetylsalicylic acid per se did not alter these parameters, but prevented the increase of

acetylcholinesterase and catalase activities. In summary, our findings showed that chronic chemically-induced model of mild hyperhomocysteinemia alters some inflammatory and antioxidant parameters and acetylsalicylic acid seems to have neuroprotective effect on some parameters that are associated to neurotoxicity of homocysteine. Supported by CNPq.

304 - Elevated Superoxide Levels, Mitochondrial Dysfunction, and Endoplasmic Reticulum-Mitochondria Crosstalk Disruption in ETHE1 and Sulfite Oxidase-Deficient Fibroblasts

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ETHE1 and sulfite oxidase (SO) deficiencies are autosomal recessive disorders that affect the catabolic pathway of sulfur-containing amino acids. Both disorders are characterized by neurological symptoms that include seizures, hypotonia, and mental retardation, whereas ETHE1-deficient patients also present with chronic diarrhea, petechiae and orthostatic acrocyanosis. To better understand the pathophysiology underlying these symptoms, we investigated superoxide production, mitochondrial bioenergetics, and levels of endoplasmic reticulum (ER)-mitochondria crosstalk components in fibroblasts from patients. Primary human dermal fibroblasts obtained from 4 patients with ETHE1 deficiency and 1 patient with SO deficiency were cultured in media containing high glucose levels. We measured the levels of ETHE1 and SO proteins as well as of ER-mitochondria crosstalk components (IP3 R, Grp75 and VDAC1). Superoxide generation, mitochondrial membrane potential and mass, and ATP production were also determined. Our findings show absence or marked decrease of ETHE1 in ETHE1-deficient cell lines. Decreased SO content was observed in SO-deficient cells. We also verified decreased IP3 R and VDAC1 levels, and increased superoxide production in all ETHE1- and SO-deficient fibroblasts. While increased mitochondrial membrane potential and decreased ATP levels were observed in the SO-deficient cell line, no alterations of these parameters were found in ETHE1-deficient cells. Finally, mitochondrial mass was increased in two ETHE1-deficient cell lines. These data provide evidence that mitochondrial dysfunction generating high reactive oxygen species levels and ER-mitochondria crosstalk disruption are mechanisms involved in the pathophysiology of symptoms observed in ETHE1 and SO deficiencies.

305 - Natural History, Characterization, and Outcome of Classical Homocystinuria in the Qatari Population

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Background: Classical Homocystinuria (HCU) is the most common inborn errors of metabolism in Qatar with an estimated incidence of 1 in 1800 newborns due to consanguinity and founder mutation R336C in the cystathionine β-synthase (CBS) gene. The objective of this study is to describe the phenotype of this severe devastating B6 nonresponsive HCU. **Method:** A single center study was carried out between 2016 and 2017 with a total of 85 Qatari patients (37 female, 48 male from 57 families) with HCU. Detailed clinical and biochemical data were collected. Stanford-Binet Intelligence Scales, Quality of life (PedsQL) and adherence to treatment (Morisky scale) assessments were carried out prospectively. Patients were stratified into three groups according to mode of diagnosis: 1) Late Diagnosis Group (LDG), 2) Family Screening Group (FSG), and 3) Newborn Screening Group (NSG). **Results:** The main manifestations that led to the diagnosis in LDG were ocular manifestations (47.5%), intellectual disability (ID) (37.8%), thromboembolic event (5.8%), seizure (5%), and hyperactivity and behavioral changes (3.9%). Interestingly, fair hair and brittles nails were observed in (6%) of LDG at diagnosis. During the disease course in LDG, 100% developed marfanoid habitus and osteoporosis, 92.1% developed ocular complications, 82.3% developed ID, 23.5% had fractures, 23.53% had psychiatric problems, 17.6% had cardiac complications, 17% developed hypertension, 11% had thromboembolic events and 5.8% developed severe neurological impairment and died at age of 18-30 years. Other rare complications in the LDG included diabetes mellitus (4%), bronchiectasis (4%) and gastrointestinal bleeding (2%). In the FSG, 28.5% developed bilateral lens dislocation, 28.5% had cardiac abnormalities and 14% fractures. On the other hand, 48.9% of NSG patients were classified as adherent to treatment and diet while 3% and 10.2% in FSG and LDG were adherent, respectively ($p<0.05$). The range and median of IQ were 39-113 and 79 in LDG, 84-110 and 97.5 in FSG, and 84-116 and 97 in NSG ($P < .01$) and the median of QoL in LDG, FSG and NSG were

86.9%, 92.7% and 96.6%, respectively ($P = .001$). **Conclusion:** Our data show a direct association between time of diagnosis and the outcome of HCU with emphasis that early detection by newborn screening and early treatment significantly improves the outcomes of HCU. This study further contributes to a better understanding of the natural history of classical HCU.

306 - Method Validation of Plasma Homocysteine by Cat-Ion Exchange Liquid Chromatography and its Comparison With a Chemiluminescence Immunoassay

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Objective: To optimize total homocysteine (tHcy) method using cat ion exchange high-pressure liquid chromatography (HPLC) and compare it with a Chemiluminescence immunoassay. **Methods:** Thirty-two (EDTA) samples from patients, controls and proficiency test material of tHcy were analyzed for imprecision, linearity and method comparison on Immulite 2000, from Siemens and Biochrom 30+ Cat ion exchange HPLC with photometric detection at Biochemical Genetics Laboratory of Aga Khan University. A shortened detection method was developed on biochrome for separation and quantification of tHcy and methionine using norvaline as the internal standard using 200 × 4.6 mm physiological high-performance column, standard lithium citrate buffers and standard physiological separation program. All statistical analysis was done using EP Evaluator. **Results:** Precision study on HPLC showed CV of 2.1% using pooled patient samples with mean tHcy 30.04 μmol/L. Four standards were run in triplicate to verify analytical measuring range/linearity from 0.5-500 μmol/L with slope1.01, intercept 0.0. On method comparison, tHcy showed mean positive bias of +13.25(43.25%) μmol/L on HPLC in comparison to Immulite 2000 for a tHcy range of 3-69 μmol/L. Sample carryover protocol was followed by analyzing samples with high tHcy followed by low tHcy samples on HPLC. Carryover study revealed 10-15 μmol/L (peak on chromatogram) of tHcy from positive to blank sample. Further re-optimization of the separation program was done and running time of lithium citrate buffer 3 was increased from 5 minutes to 5 minutes 30 seconds and flow rate of buffer 3 was lowered from 35 mL/h to 30 mL/h in standard program. Repeat carryover study showed no carry-over (no peak) between injections and therefore enables accurate quantification of homocysteine. Homocysteine was well resolved from methionine. Reanalysis of 20 samples was done on both HPLC and Immulite 2000 simultaneously with average error index of −0.1 μmol/L (−0.68 to 0.45); $r = 0.96$, slope = 0.83 (CI = 0.73-0.93), and intercept 1.5 (CI = −1.1 to 4.1). **Conclusion:** We optimized a short program for tHcy on HPLC for monitoring of patients with Cystathionine beta synthase deficiency. The tHcy results on HPLC correlated well with the immunoassay.

307 - Biomarkers of Oxidative Stress, Inflammation, and Vascular Dysfunction in Inherited Cystathionine β-synthase Deficient Homocystinuria and the Impact of Taurine Treatment

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Background: Cystathionine β-synthase deficient homocystinuria (CBSDH) has excessive clotting, dislocated lenses, osteoporosis, and neurologic problems. Current treatment suffers from partial biochemical correction and poor dietary compliance creating a need for a new approach. Mouse model data indicate a role of oxidative stress and inflammation that is mitigated by taurine. **Study objective:** A phase 1/2 clinical trial was done with aims to: 1) assess pharmacokinetics and safety of taurine therapy 2) evaluate oxidative stress, inflammation and vascular function, 3) evaluate the impact of 4 days taurine treatment. Methods: We enrolled 14 patients (8-35 year; 8 male, 6 female) with confirmed B_6-unresponsive CBSDH, homocysteine levels >50 μM. Biomarkers of oxidative stress and inflammation, disease metabolites, platelet aggregation and brachial artery flow-mediated dilation (FMD) studies were obtained at baseline and compared to 22 matched controls. Patients were treated with taurine twice daily and response assessed after 4 days. After two subjects were treated on low dose, the remaining subjects received the intended taurine dose 150 mg/kg/day. **Results**: Taurine treatment had minor, transient adverse effects of gastrointestinal discomfort 43% and headache 7%. Triglyceride levels increased (77 ± 19 mg/dL to 117 ± 33, $P = .01$). Baseline taurine was 40 ± 12 μM in patients (32 ± 6 in controls), and increased rapidly to a maximum at 1.2 h (396-1192 μM) returning to baseline at 12 h, but with slow accumulation and elevated predosing after 4 days (82 ± 36 μM). Only 2 of 5 oxidative stress markers (2,3-dinor-8-isoprostaglandin-F2α-III and superoxide dismutase) were increased, with no impact of taurine. There was only marginal

evidence for inflammation (borderline elevated TNFα, MCP1). Taurine decreased hsCRP marginally; betaine had a larger impact. Thromboxane B_2 metabolites were normal. FMD improved significantly with taurine for patients with homocysteine >125 μM, and particularly if pretreatment FMD was depressed (<10 mm, $P = .01$). 2 of 4 patients with elevated Lp[a] levels (>30 mg/dL) had a vascular clot, compared to 0 of 8 patients with low levels. **Conclusion:** Taurine has rapid kinetics and is safe when excluding preexisting hypertriglyceridemia. The study confirms mild oxidative stress unresponsive to taurine and a positive taurine treatment effect in patients with depressed endothelial function. Lp[a] levels should be investigated as a potential modifying factor for clotting events.

308 - Outcome of Late Detected Homocystinuria Due to CBS Deficiency in India

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Introduction: Homocystinuria due to CBS deficiency has an autosomal recessive inheritance with severe abnormalities of the eye, skeletal system, vascular system and central nervous system. The worldwide incidence of Homocystinuria has been reported to be 1 in 344,000, while that in Ireland is 1 in 65,000. No data about incidence in India is available. **Material and Method:** This is a retrospective study of cases from 2000 to April 2017. We suspected total 6 cases biochemically of which molecular confirmation is available for 4 and molecular testing is awaited for the remaining 2. Children who presented with marfanoid features, lens dislocation and neuropsychiatric symptoms were included in this study. Serum Homocysteine, Plasma Methionine, and cysteine were measured quantitatively. Diagnosis of CBS deficiency was confirmed by molecular testing performed at Centogene, Germany. **Result:** 4 patients confirmed both biochemically and molecularly were females and 2 patients confirmed only biochemically were males. All 6 patients had ectopia lentis and were operated. All of them had Marfanoid features. 5 of them had behavioral problems and learning difficulty. No seizures or cardiovascular events occurred till today. They were detected late and were neurologically affected. The mutations detected were c.346G>A (p.Gly116Arg), c.19del(p.Gln7Argfs*75), c.700G>A(p.Asp234Asn), and c.209+1G>C. Patients were initially treated with low methionine diet along with Pyridoxine. All 6 patients had a poor response to initial treatment; hence Betaine was added in the dose of 3-6 g/day. One of the patients required 9 g/day. The patients showed a good response to betaine. Initial Hcy level before treatment and last level after treatment in μmol/L are #1 (109-22), #2 (289-102), #3 (172-63), #4 (161-88), #5 (50-108), and #6 (201-167). Dietary management is difficult in India as special diets are prohibitively costly. Betaine is a cost-effective treatment for these patients. **Conclusion:** All 6 patients had a satisfactory response to betaine despite poor dietary control and absence of special diets. Indian diet being rich in cereals posed a challenge in controlling the methionine levels. Post betaine therapy methionine levels increased to more than 1000 μmol/L in 2 patients. We had to reduce the dose in one patient and start strict methionine restricted diet in another. Early detection by NBS would help in preventing neurological damage and in early initiation of therapy.

309 - In Vitro Evidence that Hydrogen Sulfide Disturbs Bioenergetics and Induces Mitochondrial Permeability Transition in Rat Brain

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Ethylmalonic encephalopathy (EE) is a mitochondrial disorder caused by ETHE1 deficiency and biochemically characterized by hydrogen sulfide accumulation. Affected individuals have neurological dysfunction with cerebellum and basal ganglia abnormalities. We evaluated the *in vitro* effects of hydrogen sulfide (10-500 μM) on creatine kinase (CK) activity, mitochondrial respiration, membrane potential and swelling in brain of young rats. The effects of the free radical scavengers glutathione (GSH), melatonin (MEL), trolox (hydrosoluble analogue of vitamin E; TRO), and lipoic acid (LA) and of the xanthine oxidase inhibitor allopurinol (ALP) on CK activity were also examined. CK activity was measured in cerebellum and striatum supernatants. Mitochondrial respiration was assessed in cerebellum homogenates, whereas mitochondrial membrane potential ($\Delta\Psi m$) and swelling were determined in cerebellum mitochondrial preparations. Our data demonstrated that hydrogen sulfide decreased CK activity in rat cerebellum and striatum, indicating that this metabolite disturbs energy transfer. Hydrogen sulfide-induced decrease of CK activity was prevented by GSH, MEL and TRO in cerebellum and

striatum, while LA and ALP did not alter it. Furthermore, hydrogen sulfide decreased ADP- and CCCP-stimulated respiration, that represent oxidative phosphorylation and electron transfer system capacity, respectively, supported by complex I- and complex II-linked substrates in cerebellum. Hydrogen sulfide also decreased $\Delta\Psi m$ and induced swelling in the presence of calcium in cerebellum mitochondria. Decrease of $\Delta\Psi m$ was prevented by ruthenium red, while swelling was prevented by cyclosporine A and ADP, suggesting that hydrogen sulfide induces mitochondrial permeability transition (mPT) with the involvement of calcium. Taken together, our data indicate that impairment of energy transfer and production, and mPT induction may play an important role in the pathophysiology of cerebellum and basal ganglia damage observed in patients affected by EE. **Financial support:** CNPq, Propesq-UFRGS, FAPERGS, PRONEX, FINEP, INCT-EN.

310 - Disturbance of Redox Homeostasis by S-adenosylmethionine in Cerebral Cortex of Young Rats: Implications for the Pathophysiology of inherited Methylation Disorders

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Tissue accumulation of S-adenosylmethionine (AdoMet) occurs in various inherited methylation disorders, such as S-adenosylhomocysteine hydrolase (SAHH), glycine N-methyltransferase, and adenosine kinase deficiencies. Patients affected by these disorders usually have severe neurological features whose pathogenesis is practically unknown. We therefore investigated the effects of this metabolite on important parameters of cell redox status in cerebral cortex of young rats. Malondialdehyde (MDA) levels, carbonyl formation, 2′,7′- dichlorofluorescein (DCFH) oxidation, nitrate and nitrite levels, aconitase activity, glutathione (GSH) concentrations, and sulfhydryl content were determined. AdoMet markedly increased MDA levels and carbonyl formation, implying induction of lipid and protein oxidation, respectively. We also observed that AdoMet significantly increased DCFH oxidation but did not change nitrate and nitrite levels, indicating stimulation of reactive oxygen species generation. In contrast, this compound decreased GSH concentrations and sulfhydryl content, suggesting a decrease of antioxidant defenses. The activity of aconitase, which is highly vulnerable to oxidative attack, was also reduced by this metabolite. Furthermore, AdoMet-induced lipid peroxidation and GSH decrease were prevented by the antioxidants melatonin, alpha-tocopherol and resveratrol, indicating a role for reactive species in these effects. It is therefore presumed that disturbance of redox homeostasis by a major metabolite accumulating in SAHH and other methylation disorders may contribute to the pathogenesis of these diseases.

311 - Bezafibrate Pretreatment Prevents Glial Reactivity, Neuronal Damage, and Myelin Injury Induced by Sulfite Administration in Rat Striatum

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Sulfite oxidase (SO) deficiency is an autosomal recessive disorder which can arise either from the isolated deficiency of the enzyme SO or from defects in the biosynthetic pathway of its molybdenum cofactor. Patients present with severe seizures in infancy and progressive neurological damage, often resulting in early death, as well as tissue accumulation of sulfite. Since the pathomechanisms involved in the neuropathophysiology of SO deficiency are not fully understood, we determined the effects of sulfite administration on glial reactivity, neuronal damage, and myelin compaction and structure. We also evaluated the influence of a pretreatment with the pan-PPAR agonist bezafibrate on sulfite-induced toxicity. Thirty-day-old rats were intrastriatally administered with sulfite (2 μmol) or NaCl (2 μmol; control) and euthanized 7 days after injection. Pre-treatment with bezafibrate (30 mg/kg/day) was performed by gavage during 7 days before sulfite administration. After euthanasia, striatum sections were used for immunohistochemical analysis. Sulfite administration increased S100B and GFAP staining in rat striatum, indicating that this metabolite induces glial reactivity. Furthermore, sulfite decreased NeuN and increased Fluro-Jade® C staining, suggesting neuronal damage. Bezafibrate pre-treatment prevented sulfite-induced increase of GFAP and decrease of NeuN. In addition, sulfite caused myelin injury, as reflected by decreased myelin basic protein (MBP) and FluoroMyelin staining, while bezafibrate prevented MBP decrease. Our data provide evidence that glial reactivity, neuronal damage, and myelin injury provoked by sulfite may underlie neurological symptoms and abnormalities found in SO deficiency. The fact that bezafibrate prevented sulfite-induced alterations also suggests that this protective compound may be a promising clinical candidate for SO-deficient patients. **Financial support:** CNPq,

PROPESq/UFRGS, FAPERGS, PRONEX, FINEP IBN-Net, INCT-EN.

312 - Rescue of CBS Mutants by Pyridoxine Administration: Demonstration of In Vivo Effect by Measuring Plasma CBS Activity

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Background: Molecular mechanism of pyridoxine responsiveness in a subgroup of patients with homocystinuria is elusive with a possible chaperoning activity of pyridoxal 5'-phosphate. However, direct demonstration of *in vivo* effect on the enzyme is lacking. We described previously that CBS is released into circulation from several organs, mostly liver. Here we used this method to assess the *in vivo* effect of pyridoxine administration on the rescue of mutant CBS in 15 patients with homocystinuria. **Methods:** We studied 8 pyridoxine non-responders (homozygotes or compound heterozygotes for mutations p.A69fs*94; p.C165Y; p.A71Pfs*24; p.V10Wfs*71; p.K211*; p.G246Dfs*52; p.W409_G453del; p.A155 T; p.E144 K) and 7 partial or full responders (homozygotes or compound heterozygotes carrying mutation p.P145 L; p.I278 T; p.P49 L; p.R336 H; p.R336C on at least one allele). We determined CBS activity in plasma by LC-MS/MS using deuterium labeled serine, and assessed the metabolite fluxes by measuring the methionine-to-cystathionine (Met/Cystat) and total homocysteine-to-cystathionine (tHcy/Cystat) ratios in plasma. **Results:** Plasma CBS activity ranged 0% to 0.7% of the median of controls in all eight non-responders even on combined therapy with methionine restriction, pyridoxine and/or betaine. In contrast, residual plasma CBS activity in the group of 7 responders not taking pyridoxine was 5.1% (range 0-20.3%) of the median of controls and significantly increased on pyridoxine to 26.2% of median of controls (range 7.3-72.1%, Wilcoxon pair test $P < .043$) indicating the in vivo rescue of the activity of mutant CBS in the liver. This correction of enzymatic activity in responders resulted also in an improved flux of sulfur amino acids as shown by a significant drop in Met/Cystat and tHcy/Cystat (6.7 times and 10.7 times, respectively, Wilcoxon pair test $P < .018$ and $<.043$, respectively). **Conclusion:** This study demonstrates that pyridoxine administration partially rescues the activity of selected mutant CBS enzymes in vivo. In contrasts, even large doses of pyridoxine do not rescue the activity of CBS in non-responsive patients supporting the recent recommendation (Morris et al, *J Inherit Metab Dis.* 2017; 40:49-74) that therapy with vitamin B_6 is not necessary in this subgroup of patients with homocystinuria.

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313 - Sulfite Intrastriatal Administration Alters Signaling Pathways and Neuronal Injury Markers: Possible Mechanisms Involved in Sulfite Oxidase Deficiency Neuropathology

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Sulfite oxidase (SO) deficiency is a recessive inborn error of metabolism biochemically characterized by high urinary excretion and tissue accumulation of sulfite. The main clinical and neuropathological findings include progressive encephalopathy, severe neonatal seizures, and basal ganglia abnormalities. Although the pathophysiology of neurological dysfunction in SO deficiency is not totally elucidated, sulfite accumulation has been suggested to play a major role. Therefore, we investigated the effect of sulfite intrastriatal administration on the content of proteins involved in key signaling pathways for cell survival and death (MAPK and Akt) and on neuronal injury (Tau protein, synapthophysin, and α-synuclein) and autophagy (LC3B) markers. Acridine orange staining was also used to evaluate autophagy. Thirty-day-old rats were instrastriatally injected with sulfite (2 μmol) or NaCl (2 μmol; control) and euthanized 30 min after injection. Striata were dissected and dissociated for acridine orange staining, or homogenized in RIPA buffer containing protease and phosphatase inhibitors to measure the immunocontent of proteins by western blotting. Our results showed that sulfite altered the content and phosphorylation of the MAPK ERK1/2 and p38 but not of JNK. Sulfite decreased ERK1/2 content, but increased its phosphorylation. On the other hand, sulfite decreased p38 phosphorylation without altering its protein content. Furthermore, sulfite increased Akt, synaptophysin and Tau protein content, whereas Tau phosphorylation was decreased. However, α-synuclein and LC3B content, and acridine orange staining were not altered by sulfite. Our findings suggest that alterations on MAPK and Akt signaling pathways caused by sulfite are possibly involved in neuronal injury observed in patients affected by SO deficiency. **Financial support:** CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP IBN-Net, INCT-EN.

314 - Erymethionase, Methioninase Entrapped in Red Blood Cells: An Innovative Treatment Approach for Classical Homocystinuria

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Classical Homocystinuria, resulting from cystathionine beta-synthase (CBS) deficiency, is recognized as the most common inborn error of sulfur amino acid metabolism. CBS metabolizes homocysteine into cystathionine, leading then to the synthesis of cysteine. Biochemical hallmarks of this disorder are elevated levels of homocysteine (Hcy) and methionine (Met). Patients symptoms include intellectual disabilities, thromboembolism, osteoporosis and ocular lens dislocation. Current treatments, for non-responsive Vitamin B6 patients, are methionine/protein restricted diets supplemented with betaine. However, adherence to this strict diet is not easy. As an alternative treatment approach to reduce the toxic accumulation of Hcy and Met we envisioned an innovative enzyme therapy. Erymethionase is a Methionine gamma-lyase (MGL from *P. putida*) entrapped in red blood cells using ERYTECH's proprietary ERYCAPS technology platform to provide effective, long-acting therapeutic activity with reduced toxicity. MGL displays enzymatic activity with both Met and Hcy substrates with similar kms and a higher Vmax for Hcy (Ito et al, *J Biochem* vol 79, p 1263 and personal data). Reducing the level of both Hcy and Met is critical in restoring the metabolic balance in individuals with classical homocystinuria. Moreover, the red blood cell is the perfect transporter for PLP-dependent MGL enzyme as it provides the enzymatic cascade reaction to produce PLP from Pyridoxine (PN), molecule crossing easily the red cell membrane. PK, PD and safety parameters of Erymethionase were first evaluated in healthy mice. Once entrapped into erythrocytes, MGL maintained more than 30% of its specific activity after 5 days in circulation. In comparison, administration of the free form of MGL at similar doses led to a rapid loss of the enzymatic activity in few hours. The role of RBC in PLP biosynthesis from exogenous uptake of PN was also investigated *in vivo*. We are currently conducting experiments to demonstrate the potential of Erymethionase to lower plasma Hcy and Met in homocystinuria mouse model (Gupta et al, *FASEB J*, vol 28, p781). Preliminary results show an abrupt decrease of plasma Hcy following Erymethionase administration (131 μM vs the pathophysiological values 326-371 μM), when PN administration alone did not cause any decrease of Hcy or Met plasma levels. Further investigations are undergoing to determine the potential of Erymethionase as a therapeutic option for homocystinuria patients.

315 - S-Adenosylhomocysteine Hydrolase Deficiency—Intrafamilial Variability of Intellectual Disability Among Four Siblings

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S-adenosylhomocysteine hydrolase (SAHH) deficiency is an inherited methionine cycle disorder with multisystem involvement. Disturbed methylation is presumed, but the pathogenesis is not completely understood. Until now, ten patients have been described. Most of them had psychomotor delay, hypotonia, muscle weakness and liver involvement, but clinical presentation is variable. Two siblings had very severe course and died in early infancy and one patient was asymptomatic at seven years. We report follow-up of a family with four siblings suffering from SAHH deficiency due to mutations c.336G>A; p.W112X and c.428A>G; and p.Y143C of *AHCY* gene. Three boys have been reported previously. This is the first report on the fourth sibling, a female. Proband was started on low methionine diet, phosphatidylcholine, creatine and N-acetylcysteine at the age of 13 months, second child at the age of 3.5 months and two youngest siblings have been treated since birth. All patients had psychomotor delay, myopathy and mild hepatopathy. The myopathy is most severe in the first child who was started on diet later than other siblings. All patients had permanently elevated CK (10-100xN), permanently elevated aminotransferases and coagulopathy. Three boys had behavioral problems, mainly due to ADHD. At the age of 16, 13.5, and 10.5 years they had mild intellectual disability and attended school with individualized education plan. Psychological testing showed better verbal comprehension and perceptive reasoning, while they achieved lower scores in working memory and processing speed tests. The girl had severe developmental and speech delay, autistic behavior with stereotyped movements and temper tantrums. She started to walk unassisted at the age of 4 years. At the age of 7.5 she had moderate to severe intellectual disability and completely undeveloped speech. Her brain MR was normal and there were no signs of other neurological disease. Although all four siblings with SAHH deficiency had otherwise similar clinical presentation, there is quite variability in cognitive outcome. It is unclear why female sibling in whom the treatment was started and maintained since birth has the worst outcome. No other contributive factors could be identified. There is a report on patient who underwent liver transplantation and short follow up showed better cognitive functioning. Future studies on brain involvement in SAHH deficiency are needed to yield optimal treatment strategy.

316 - Intraleukocitary Cystine Quantification: Reference Values for Colombian Population

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Introduction: Cystinosis is a lysosomal storage disease characterized clinically by renal and ocular compromise. The disease is caused by a defect in the protein called cystinosine which is in charge of transporting cysteine from the lysosome to the cytosol. Thus, the main biochemical feature of this disease is the intralysosomal accumulation of cysteine in form of cystine crystals that can be measured in leukocytes to stablish the biochemical diagnosis. Up to now, in Colombia the cystine quantification was not available. **Aim:** To implement the protocol for cystine quantification in blood polymorphonuclear cells and to stablish reference values for our population. Methods: The method for cystine quantification in blood polymorphonuclear cells using HPLC was adapted to our laboratory conditions. Reference values were established using 50 samples from healthy volunteer adults. The method was tested against five samples from patients and results were compared with the results obtained by the current reference laboratory. **Results:** Results from control subjects showed that 90% of the population has values below 0.34 nmol1/2cystine/mg. The method implemented was able to appropriately distinguish between unaffected individuals (0-0.64 nmol 1/2cystine/mg) and cystinosis patients, even after treatment (1-12 nmol1/2cystine/mg) with good correlation in the interlaboratory tests. **Conclusions:** This work allowed the implementation in Colombia of the intraleukocitary cystine quantification. In addition, this study considers a higher number of controls compared to previous reports allowing detecting a wider normal values distribution. This implementation improves, in terms of time and costs, the diagnosis of cystinosis in our country allowing a faster instauration of the treatment for these patients.

317 - Inflammation in Classical Homocystinuria: Analysis of 20 Cytokines in Brazilian Patients

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Classical homocystinuria (HCU; CβS deficiency) is characterized by high levels of homocysteine and methionine and cysteine deficiency. Its main clinical signs include lens dislocation, thromboembolism, intellectual disability, psychiatric disorders and osteoporosis. Increased pro-inflammatory cytokines are believed to play a role in the etiology of many chronic diseases, but the role of inflammation in HCU is unclear. In a previous study of 16 cytokines in HCU patients from USA (Keating et al., 2011), increased pro-inflammatory cytokines were found in non-treated HCU patients, while treated patients had normal levels of most cytokines studied. **Objective**: To determine 20 inflammatory cytokines in plasma of poorly controlled HCU patients and healthy controls. **Methods**: in this cross-sectional study, 9 HCU patients and 10 healthy controls were included. Plasma total homocysteine, cysteine, and methionine were measured in plasma by LC-MS/MS. The cytokines quantification assay was performed through EMD Millipore's MILLIPEX® MAP Human Cytokine kit, accordingly manufacturer's instruction. All samples were measured in duplicates for 20 cytokines (G-CSF, GM-CSF, GRO, IFN-γ, IL-1a, IL-1b, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17a, MDC; IP-10; MCP-1; MIP-1α, MIP-1β; TNF-α, TNF-β, and VEGF). Measurements with divergence $\geq$30% between duplicates were excluded from analysis. **Results**: Patients and controls had similar age (median: 24 vs 27 years, respectively) and sex (masculine: 66 vs 70%). All patients received homocysteine-lowering treatment (pyridoxine 7/9, folic acid 8/9, betaine 7/9, methionine-restricted diet 3/9); but only 3/9 had total homocysteine <100 μmol/L (median = 130 μmol/L, reference values: 5-15 μmol/L). Most patients (7/9) were pyridoxine nonresponsive. Cytokines plasma levels were similar in patients and controls, with the exception of IFN-γ, which was significantly reduced in patients (median patients: 3 pg/mL, controls: 10 pg/mL; $P = .008$). An inverse association between total homocysteine and IFN-γ was found ($r -0.487$; $P = .03$). **Conclusions**: No evidence of increased inflammation was found in our sample of HCU patients. In fact, reduced IFN-γ levels have anti-inflammatory properties, as demonstrated in previous studies. To our knowledge, this is the first study to evaluate IFN-γ in HCU patients, and its implications in HCU need further investigation. Despite poor metabolic control, treatment might have contributed to these results.

318 - Sulfite Oxidase Deficiency: New Mutation and Phenotypic Variability (Case Report)

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Sulfite oxidase (SO) deficiency is a hereditary autosomal recessive metabolic disorder due to mutations in the SUOX gene, which encodes the sulfite oxidase enzyme responsible for catalyzing sulfite oxidation to sulfate, causing a plasma and

urinary accumulation of S-sulfocysteine. Clinical manifestations begin early after birth and are characterized by refractory seizures, delayed neuropsychomotor development, facial dysmorphisms, and dislocation of the lens. The diagnosis is based on clinical suspicion, magnetic resonance imaging of the brain, urine sulfite test, enzymatic activity in cultured fibroblasts and mutation research on the SUOX gene. **Objective:** To report the case of a patient with clinical and molecular diagnosis of SO deficiency. **Methodology:** Revision of medical records of a patient diagnosed with SO deficiency accompanied at a reference center for diseases of inborn errors of metabolism in Bahia. **Case report:** Male patient, 2 years and 8 months, child of non-consanguineous parents, with history of neuropsychomotor development since 6 months and facial dysmorphism, evolves with acute extrapyramidal syndrome after the episode of pharyngitis at 13 months, associated with regression Neuropsychomotor development, extreme irritability, axial hypotonia, double hemiparesis, oculomotor dyspraxia, dysarthria, dysphagia and ophthalmoplegia. Magnetic Resonance of encephalon shows hypersignal in T2 and Flair, bilateral and symmetric of the lentiform nuclei, central area of malacia in pale globes, reduction of cephalic parenchyma, accentuation of cortical sulcus with diffuse widening of arachnoid space, alteration of signal in periventricular white substance with Hypersignal in T2 and Flair with predominance in adjacent body of lateral ventricles and discrete ectasia of ventricular and supratentorial systems. Homocysteine: 3.72 µmoL / L (5.46-16.20); Taurine: 69.00 µmoL / L (38.00-153.00). Exome sequencing revealed a homozygous mutation (p.Arg376Ser) in the SUOX gene, of uncertain pathogenicity. **Conclusion:** The uncertain pathogenicity of the mutation identified in the SUOX gene has not been previously reported in the literature and appears to be associated with a milder phenotype of sulfite oxidase deficiency. However, to strengthen this association, biochemical confirmation is necessary.

I) Other Amino Acid Disorders (319 to 358)

319 - Successful Pregnancy in a Woman With Alcaptonuria Treated by Nitisinone

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Alkaptonuria (AKU) is caused by homogentisate 1,2-dioxygenase deficiency, an enzyme converting homogentisic acid (HGA) to maleylacetoacetic acid in the tyrosine degradation pathway inducing joints and cardiac progressive disease. Nitisinone (NTBC) is a reversible inhibitor of 4-hydroxyphenylpyruvate dioxygenase indicated in the treatment of hereditary tyrosinemia type 1. This drug has been recently used off-license for patients with AKU, resulting in a striking decrease in the urinary and serum HGA concentrations, while increasing serum tyrosine (TYR) concentration. We describe here the first off-licensed use of NTBC during a pregnancy in a patient with AKU who had no history of joint or cardiac complication. A 33 years old woman was diagnosed with AKU in infancy and exhibited only a moderate ochronosis. No significant joint space narrowing was observed at the coxo-femoral joint. The echocardiography was normal. She has been treated by low-dose of NTBC (0.2 mg/day) since February 2013 with a mildly protein restricted diet without amino acids substitutes or low protein foods. After 9 months, serum HGA concentration was decreased by 94% while serum TYR concentration increased form normal to 406 $\pm$ 83 µmol/L. Our patient became pregnant in April 2016 and to avoid any secondary increase of HGA excretion, she gives her consent to continue NTBC treatment. Considering the toxicity of high phenylalanine level in maternal phenylketonuria, and in order to reduce any potential risk for the fetus, we aimed at keeping a target concentration of TYR <400 µM/L during all pregnancy (364 $\pm$ 104 µmol/L). As urinary HGA excretion increased during pregnancy, NTBC the dosage was increased at 0.5 mg/day from November 2016 until delivery without increased of blood TYR levels. During pregnancy, a metabolic and nutritional monthly follow up was performed and zinc and cobalamin supplementation were prescribed. She delivered in January 2017, and her son was fully healthy. In conclusion, we successfully used NTBC treatment during the pregnancy of a woman with AKU, with continuous metabolic efficacy and without any side effect for the mother and her son.

320 - Clinical, Biochemical, Outcome, and Molecular Features of 8 Patients Affected of Non Ketotic Hyperglycinemia

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Objective: Nonketotic hyperglycinemia (NKH) or glycine encephalopathy is an autosomal recessive metabolic disorder of glycine due to cleavage enzyme defect, results in glycine accumulation in brain and disturbs brain functions. The enzyme consists of 4 subunits: the P protein (pyridoxal containing), the H protein (hydrogen carrier) T protein (tetrahydrofolate requiring), and the L protein (lipoamide dehydrogenase) encoded by GLDC, GCSH, AMT and L protein gene respectively. NKH has three main forms. The most common is most severe neonatal(classic);85% presenting most often in the first week. Attenuated neonatal forms with residual activity;15%. Those presenting in infancy 50% have severe and 50% have attenuated, forms, overall 20% of either neonatal or infantile have a less severe form with developmental delay but may learn to sit and walk. Mild NKH forms present after one year, have mild developmental delay to normal intelligence, but with movement disorders. **Objective** This is a retrospective study of

8 cases with NKH referred to us within 9 years. **Results**: 8 cases (5♀,3♂), consanguinity 3/5, Classic neonatal:5/8; onset at 2- 7 days of life presented lethargy, poor feeding, frequent hicupping, seizure, coma and death in the first week of life:3/5 and 3 weeks to 3 months;2/5 . Attenuated forms:3/8, one female: onset at 45 days who developed intractable convulsion and spasticity, mental & developmental delay, despite treatment with sodium benzoate& dextromethorphan treatment, diet and seizure control, died at 2 years, a male with three encephalipathic attack at 2 days,2.5and 7 months with severe hypotonia now alive with severe mental retardation, spontaneous smiling, neck control and a female with convulsion at 20 and 48 days, microcephaly, referral at 3 years and now 4 years old, only can walk & know her parents. Diagnosis confirmation: Normal acyl carnitine(MS/MS) & organic acid in urine (GC/MS), no acidosis, very high plasma glycine: mean; 1210.6 μmol/L (N; up to 380) and mean CSF glycine131.7 μmol/L;(n;0-12). Mutation detection revealed 2 novel heterozygous mutation: c.1282 C > T & c.1279 del C on GLDC in attenuated neonatal confirmed by their parental gene sequencing. No mutation was detected on 3 genes of NKH in our 3 classic neonatal phenotypes mutations on GLDC, GCSH and AMT). **Conclusion**: We reported 5 fatal neonatal and 3 attenuated NKH forms.

321 - Abnormal Social Behavior in Mice With Tyrosinemia Type I is Associated With an Increase of Myelin in the Cerebral Cortex

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Tyrosinemia type I is caused by mutations in the fumarylacetoacetate hydrolase (FAH) gene, the template for the final enzyme in the tyrosine catabolism pathway. If left untreated this deficiency of functional FAH leads to a buildup of toxic metabolites that can cause liver disease, kidney dysfunction, and high mortality. The current treatment with the drug NTBC prevents the production of these metabolites and has consequently increased the survival rate in tyrosinemia type I children. As a result of this increased survival, long-term complications of tyrosinemia type I are now being observed, including slower learning, impaired cognition, and altered social behavior. **Objective:** We studied a mouse model of tyrosinemia type I to gain insight into the effects of tyrosinemia type I and treatment with NTBC on social behavior in mice. **Methods:** We used the Crawley three chambered sociability test to determine social preference and social novelty preference in mice with tyrosinemia type I (FAH^{mut}). Brains were removed and brain slices were stained with Luxol Fast Blue to determine myelin expression. The cerebral cortex was also examined for levels of myelin related gene expression (MBP and PLP1). **Results:** We showed that mice with tyrosinemia type I display abnormal social behavior in that they spend more time in the absence of another mouse. The FAH^{mut}-NTBC mice spent close to twice as much time in the empty chamber with the dummy mouse 283.1 $\pm$ 24.75 s (n = 10) compared to the side containing the real mouse 148.1 $\pm$ 23.25 s (n = 10, $P = .0009$). The WT-NTBC mouse did not discriminate between the two choices ($P = .1468$). This altered behavior was due to tyrosinemia type I and not treatment with NTBC. Quantification of cerebral cortex myelin in mice with tyrosinemia type I showed an increased myelin expression. Luxol Fast Blue pixel intensity was measured as 28.46 $\pm$ 5.93 (n = 3) for WT-NTBC and was about four times more intense in FAH^{mut}-NTBC mice (117.90 $\pm$ 11.11, n = 4, F (2, 7) = 26.31, $P = .0006$). **Conclusions:** Our findings suggest that absence of FAH expression in the brain produces an altered brain biochemistry resulting in increased expression of myelin. This increase in myelination could lead to abnormal action potential velocity and altered neuronal connections that provide a mechanism for the altered learning, social behavior, and cognitive issues recently seen in tyrosinemia type I.

322 - Asparagine Synthetase Deficiency Detected by Whole Exome Sequencing: The First Description in Two Brazilians Siblings

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Deficiency of asparagine synthetase is a very rare AR neurometabolic disorder caused by homozygous or compound heterozygous mutation in the *ASNS* gene (7q21.3) that encodes an asparagine synthetase enzyme. Congenital microcephaly; progressive encephalopathy, resulting in severe intellectual disability; brain abnormalities, axial hypotonia; appendicular spasticity; intractable seizures and hyperekplexic activity characterize this newly inborn error of non-essential amino acid synthesis. Additional variable dysmorphic features include micrognathia, receding forehead and large ears. The aim of this report is a clinical and molecular description of two Brazilian children with ASNSD analyzed by whole exome sequencing. We describe two siblings of a healthy and unrelated couple. A 11-year-old-boy with spastic quadriplegia, congenital microcephaly, seizures, and profound developmental delay was referred to a Clinical Genetics Division of HCFMRP due to a history of sister who died at 7 years of age with the same clinical picture and after extensive investigation in their state of origin. He was born by cesarean section due to fetal distress. His birth weight was 3.2 kg, length 48 cm and OFC: 30 cm. Seizures, global delay, axial hypotonia, and hypertonic extremities marked his clinical evolution. Laboratory examinations were normal including metabolic screening (plasma amino acid and very long-chain fatty acids analysis, urine organic acids, and isoelectric focusing of transferrin), chromosomal analysis, and array-based comparative genomic hybridization. Magnetic Resonance Imaging (MRI) showed microcephaly, Dandy Walker malformation, and cerebral and cerebellar global volumetric reduction. Exome sequencing (Illumina HiSeq®)

showed a compound heterozygous mutation in *ASNS* gene (c.893 T>G, p.Asp295Asn and c.1213 T>C, p. Ala405Thr) and both were predicted to be pathogenic by bioinformatics analyses. This is the first description of ASNSD in Latin America and the second in Western countries. Despite the impossibility of carrying out confirmatory molecular examination in the proband's sister, there are sufficient clinical data that support these siblings represents about twenty patients around world. Whole exome sequencing has allowed the diagnosis of extremely rare diseases that are heterogeneous and/or overlap with other conditions. This report expanded the mutational spectrum of ASNSD and allowed the end of a painful diagnostic odyssey.

323 - Melatonin Supplementation as Preventive Therapy to Neurological Damage Caused by the Induction of Hypermethioninemia in Wistar Rats Offspring

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Hypermethioninemia is an inborn error of methionine metabolism caused by deficiency of the S-adenosylmethionine enzyme, leading to an accumulation of this essential amino acid and its metabolites. Recent studies showed that pups of mothers submitted to gestational hypermethioninemia presented oxidative stress and inhibition of Na^+K^+-ATPase activity in encephalon. During fetal development, these changes could lead to memory deficit and/or development of neurodegenerative diseases in postnatal life. Melatonin is a hormone released by the pineal gland and is involved, among other functions, to circadian rhythm control, removal of reactive species, antiapoptotic effect and reduction of lipid peroxidation. The aim of this study was to verify the possible neuroprotective effect of melatonin on biochemical (Na^+K^+-ATPase) and behavior parameters (memory by water maze task) in hypermethioninemic rats offspring at 30-day-old. Methionine (2.68 μmol/g body weight) was administered subcutaneously in Wistar female rats, twice a day during entire pregnancy. Melatonin (10 mg/kg body weight) was administered at the same way, but once a day. Control rats were treated with saline or melatonin. Pups were submitted to Morris water maze task in order to verify spatial memory and 12 hours after the animals were killed by decapitation without anesthesia. Results showed that hypermethioninemia reduced Na^+K^+-ATPase ($P < .05$) activity and melatonin was able to significantly reverse such effect. Results also demonstrate that the pups treated with methionine presented memory deficit since the average learning time of these pups was longer when compared to the control group ($P < .05$). On the other hand, pups treated with melatonin obtained values closer to the control, but not significantly. In accordance with our studies, methionine inhibits Na^+K^+-ATPase activity, which is essential to brain function and changes in its activity could be, at least in part, one of the reasons memory deficit caused by gestational hypermethioninemia. Mechanisms of melatonin action must be further investigated to elucidate how it can work as a possible preventive therapy to reduce neurological disorders found in patients with hypermethioninemia. Supported by CNPq.

324 - Tyrosinemia type 1: A Treatable Cause of Hypertrophic Cardiomyopathy in Infancy

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Introduction: Tyrosinemia type 1 (TT1) is an autosomal recessive disorder caused by a deficiency of the enzyme fumarylacetoacetate hydrolase (FAH). TT1 usually presents in early infancy with failure to thrive, vomiting, jaundice, hepatomegaly, elevated liver enzymes, and bleeding tendency. **Method**: A retrospective review of two patients with TT1 was conducted. **Results**: A 2-month-old boy presented with fever, vomiting, and refusal of feeding. Examination revealed a sick looking infant with signs of severe dehydration and hypovolemic shock. He was jaundiced, had hepatomegaly and elevated liver enzymes. An echocardiography was done in view of the lack of response to inotropes, which showed biventricular and interventricular septal hypertrophy. The ventricular ejection fraction was 65%. Urine for organic acids showed elevated succinylacetone, consistent with the diagnosis of TT1. *FAH* gene study identified c.1 A>G homozygous mutation. The patient responded to intensive cardiorespiratory therapy, tyrosine free formula, and oral NTBC. Echocardiographic findings revert to normal in four weeks. Another 2-month-old baby boy presented with hematuria coagulopathy and low factor 7. Ammonia and liver enzymes were high. He developed septic shock. ECG and Echocardiography revealed hypertrophic cardiomyopathy. Amino acid showed high tyrosine, methionine, and phenylalanine. Urine showed no succinylacetone. A homozygous mutation in FAH detected (c.601G>T). The patient responded to NTBC and special formula and Echocardiographic findings revert to normal in 3 months. The pathogenesis of cardiomyopathy associated with TT1 is not well understood. It may be related to the elevated tyrosine or accumulation of the toxic succinylacetone in the cardiac tissues. This view is supported by the fact that cardiomyopathy resolved in almost all patients treated with NTBC or liver transplant. **Conclusion**: This report highlights tyrosinemia type 1 as a treatable cause of cardiomyopathy in children.

325 - Clinical Spectrum of Alkaptonuria from a Country with High Consanguinity Rate: A Systematic Review

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Objective: To determine the patient characteristics and clinical presentation of Alkaptonuria (AKU) cases reported from Biochemical Genetics Lab (BGL) of Aga Khan University Hospital between 2013 and 2017 and its comparison with local data from Pakistan. **Methods:** Reported cases of AKU diagnosed on basis of homogentisic acid peak in urine by GC-MS were retrieved. Their demographics and clinical data was collected from questionnaire for patients tested at BGL. Systematic review was done from the databases independently searched by two reviewers for studies published in English from 1996 to 2017 on AKU and Pakistan. Eight studies were identified; Cochrane n = 0, Medline n = 0, PubMed n = 2 and HEC Digital Library n = 1, Google Scholar n = 7. Common studies extracted by data bases were excluded. Data for study design, year of study, demographic details, symptomatology and treatment prescribed was extracted. **Results:** Nine cases of AKU were diagnosed in 3 years at BGL while systematic review showed 1 literature review and 7 case reports where one study reported two cases from Pakistan. Male to female ratio in lab data was (2:1) while the ratio was 3:1 in published literature. Median age of patients diagnosed with AKU in BGL was 31.2 years (32 days-55.5); however, 3 cases presented in infancy. Presenting age in systemic review was after fourth decade of life except two cases presenting in second decade and infancy and almost all patients were harboring symptoms for 10-15 years. Only 3/9 of our patients had dark urine on standing; on comparison with published data urine darkening was observed in all. Homogentisic acid in urine was performed by colorimetry in all published cases except one where paper chromatography was done. Musculoskeletal involvement was seen in 3/9 patients from BGL while it was reported in all case reports. Brownish-black osler spots on sclerae (n = 2), bluish discoloration of pinnae (n = 2) were noted in BGL patient data also reported in 7/8 case reports from Pakistan. **Conclusion:** Few cases have been reported till to date from Pakistan. However, diagnosis of 9 cases in a short time span of 3 years suggest significant disease burden. Lack of diagnostic facilities, non-consideration of AKU by physician due to lack of awareness are probable factors.

326 - Characterization of a Series of Patients With Homocystinuria in the Western Center Region of Colombia

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Objective: Characterize a series of patients with Homocystinuria in the Western Center Region of Colombia. Methodology: Information was collected between 2013 to 2017 in the Clinica Comfamiliar Risaralda with the measurement of blood and urinary levels of homocysteine and methionine, and in some patients with the identification of the molecular defect. For each patient, the following variables were reviewed: age at diagnosis; predominant symptom at the time of diagnosis; antecedent of consanguinity; family history of stroke in possible disease carriers; presence on physical examination of dislocation of the optic lenses, arachnodactyly and/or scoliosis; associated heart disease confirmed by echocardiography; and current medical treatment. **Results:** 10 patients with homocystinuria were identified on a total population of one million inhabitants. Two patients belonged to the same family, and 3 had antecedent of consanguinity in their parents. Of the total, 9 were diagnosed before the age of 18 years and 7 were women. To date, the molecular defect has been identified in 3 individuals: (*CBS*): c572C>T (p.Thr191Met), homocystinuria, pyridoxine- non-responsive. The preponderant symptom at the time of diagnosis was dislocation of the lens in 3 patients; neurological complications including anxiety, hyperactivity, learning difficulties, and/or epilepsy in 5 patients, and in 2, venous thrombosis. Six patients have developed "marfanoid" habitus with presence of arachnodactyly, 3 scoliosis, and by echocardiogram 3 patients had abnormal findings but without clinical repercussion. After diagnosis all patients received betaine, metabolic formula restricted in methionine and nutritional support resulting in medical improvement. In four cases, we found a familial history of vascular events; two of those relatives are being followed and will be screened for homocystinuria. **Conclusions:** Homocystinuria has a wide variety of clinical presentations with symptoms that may be specific but also nonspecific at any age. Measuring homocysteine in a high number of patients with various clinical symptoms is recommended for a timely diagnosis and treatment, which resulted in clinical improvement of our patients. The high frequency of cases found in this region of our country, emphasize the importance of implement a newborn screening as a public health policy and specific molecular defect detection.

327 - Inborn errors of metabolism: Anthropometric evaluation and body composition of a group of patients

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Inborn errors of metabolism constitute a group of diseases in which the genetic alteration affects different metabolic pathways within the organism. They can be caused by deficiency of the enzymatic activity or by the failure in the mechanisms of transport of different compounds. In recent years, these diseases have been identified more frequently. Their knowledge allows to realize the diagnosis early and to establish the specific treatment to prevent the mental disabilities that they produce. Periodic anthropometric evaluation guarantees post-statural follow-up and adequate channeling during childhood and adolescence. **Objectives:** To perform the anthropometric and body composition evaluation in a group of patients with inborn errors of metabolism, to identify patients with poor nutrition. **Methodology:** An observational, descriptive cross-sectional study was performed. **Population under study:** Patients with inborn errors of metabolism treated at the National Reference Center of the Centro Habana Pediatric Hospital. For the anthropometric evaluation, the following measurements were taken: weight, height, brachial circumference, triceps, Subscapular; Suprailiac and brachial folds. The Body Mass Index, the Muscle Area, the Fat Area and the Summation of Folds were calculated. Reference standards and cut-off points were used for the Cuban population up to 19 years. Data were processed in the SPSS program. Results: We evaluated 25 patients between 3 and 18 years old, of both genders with Hyperphenylalaninemias, Histidinemia, Homocystinuria. According to MC: Normal 76.0%, Overweight 20%, Obese 4%; Weight / Size: Normal 80%, Overweight 16%, Obese 4%; Weight / Age: Normal 76%, High 24%; Size / Age: Normal 80%, High 20%. Body composition. AM: Normal 88%, High 12%; AG: Normal 76%, High 24%; SP: Normal 76%, High 24%. Nutritional treatment was indicated and includes the special formula to patients with Hyperphenylalaninemias and Homocystinuria. **Conclusions:** Most had an adequate nutritional status. Patients with hyperphenylalaninemias who had increased body fat, secondary to excess consumption of simple sugars and fats, were identified. Feeding alternatives were indicated for the control of obesity and / or overweight of the patients.

328 - Tyrosinemia Type I and Todan's VI Cystic Duct: An Unexpected Association

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Background: The cystic duct cysts are rare lesions, which are classified according to the modified classification of Todani, subtype VI of this classification is the rarest subtype of which few cases have been reported in the literature. **Case presentation:** We report the case of a masculine 13 days old newborn, admitted in our unit for cholestatic jaundice beginning 3 days after birth. Investigations showed no infection, liver function tests were abnormal with cytolysis, cholestasis and liver failure. Alpha-fetoprotein was elevated and abdominal ultrasound suspected a malformation of the cystic duct, the bili-MRI performed confirmed cystic duct cyst type IV according to Todani's classification. Given the significant elevation of alpha-fetoprotein, a chromatography of amino acids in the blood and urine was carried out for the diagnosis of type 1 tyrosinemia. **Conclusion:** No cases of association between these two entities have been previously described in the literature according to our research. Cystic duct abnormalities are among the etiological diagnosis of neonatal cholestasis, but the clinician should be alert to signs that can guide the associated metabolic disorders.

329 - Growth Patterns in the Irish Pyridoxine Non-responsive Homocystinuria Population and the Influence of Metabolic Control and Protein Intake

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Background: A low methionine diet is the mainstay of treatment for pyridoxine nonresponsive homocystinuria (HCU). Adequate protein intake is necessary for normal growth and development. There are various guidelines for recommended protein intakes for HCU patients and clinical practice varies from center to center. Inferior growth has been associated with poor metabolic control, particularly low cystine levels. **Aim**: The objectives were to investigate growth patterns in the Irish HCU population including the relationship between protein intake and biochemical control. **Methods:** A chart review of 48 Irish pyridoxine non-responsive HCU patients focused on weight, height, body mass index (BMI), protein intake, and metabolic control up to 18 years at nine set time points. Patients diagnosed via newborn screening (NBS) were compared to late diagnosed (LD) patients. Data was collected and analyzed using SPSS. A linear mixed effects model was the primary analysis comparing the groups. **Results:** By age 18 **t**he LD group (n = 12, mean age at diagnosis = 5.09 years) were heavier - estimated effect 4.97 kg ($P = 0.0058$) and taller - estimated effect 7.97 cm ($P = 0.0204$) than the NBS group (n = 36). There was no difference in growth rate between the groups after 10 years of age. The HCU population was also heavier and taller than the general population by one standard deviation. There was no difference in BMI. There was no association between metabolic control, including intermittently low

cystine levels, and height. Three protein guidelines were compared - Great Ormond Street, Genetic Metabolic Dietitians International (GMDI), and the Ross guidelines. There was no difference in adult height between those who met the lowest of the protein guidelines (GMDI) and those who had a higher protein intake. **Conclusion:** This is the largest study to date examining the growth patterns of HCU patients with their metabolic control. It highlights a significant difference in the way LD patients grow in comparison to those diagnosed through NBS. There was no association between intermittently low cystine levels and poor growth. Achieving a higher total protein intake offers no added benefit in terms of adult height reached and patients may benefit from a lower dose of synthetic protein in terms of compliance and calorie intake.

330 - Recommendations for Diagnosis and Treatment of Tyrosinemia Type I

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Tyrosinemia type I (Hepatorenal Tyrosinemia, HT-1) is an autosomal recessive condition resulting in hepatic failure with comorbidities involving the renal and neurologic systems and long-term risks for hepatocellular carcinoma. An effective medical treatment with 2-[2-nitro-4-trifluoromethylbenzoyl]-1,3-cyclohexanedione (NTBC, nitisinone) exists but requires early identification of affected children for optimal long-term results. Initial efforts at newborn screening (NBS) to detect presymptomatic infants with HT-1 by measuring tyrosine levels identified some but not all HT-1 children. Recent methods using blood succinylacetone as the newborn screening marker is predicted to identify all infants affected with HT-1. If correctly identified and medically managed, the majority, if not all, of these infants can anticipate a life free of hepatic or renal disease. A satisfactory clinical management scheme is clearly needed for infants with HT-1 identified by newborn screening (NBS) or following presentation with clinical symptoms. To this end, a group of eleven clinical practitioners, including eight biochemical genetics physicians, two metabolic nutritionists, and clinical research psychologist, from the United States and Canada with experience in providing care for patients with HT-1 initiated a consensus-based process to establish uniform recommendations for identification and treatment of affected children. Using results from a systematic literature review, practitioner management survey, and a nominal group process involving two face-to-face meetings, recommendations were developed and are summarized in this presentation. Final recommendations were proposed if there was at least 80% agreement (strongly agree or agree). There was strong consensus in favor of newborn screening for HT-1 using blood succinylacetone as the primary marker, followed by diagnostic confirmation and early treatment with NTBC and diet. Consensus recommendations for both immediate and long term clinical follow-up of both positive newborn screened individuals and those identified through clinical symptomatic presentation are provided. In addition, organ specific complications and recommendations for follow-up and treatment will be discussed. Given the availability of sensitive newborn screening techniques and effective therapy, the outcomes for this multisystemic disorder should be dramatically improved with the use of more consistent approaches advocated in this presentation.

331 - Intermittent Maple Syrup Urine Disease (MSUD)—A Difficult Diagnosis

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Intermittent MSUD may be difficult to diagnose, due to absence of biochemical abnormalities between symptomatic episodes. Patients with intermittent MSUD have normal growth and development, but when ill, may present with severe neurological impairment. If not recognized and treated, the outcome may be fatal. We report here two Danish patients with intermittent MSUD, one with a fatal outcome. **Case report: Patient** 1: A 5-year old boy was admitted to the local pediatric department due to neurological impairment (ataxia and slurred speech), somnolence, dehydration and evolving encephalopathy. Metabolic acidosis was detected. Treatment with 10% glucose iv was initiated and the patient recovered completely. A urine metabolic screen obtained during the symptomatic episode, showed massive excretion of branched-chain keto acids suggestive of MSUD, but normal excretion of branched-chain amino acids (including allo-isoleucin). All samples (urine and plasma), taken subsequently when the child was clinically well, were normal. Molecular genetic analyses of genes involved in the BCKDH complex revealed two pathogenic variants in *DBT* (c.175+1A>G; c.901C>T), the latter described previously in seven Norwegian patients with intermittent MSUD[1]. Patient 2: A 15-year old boy hospitalized with similar symptoms as patient 1. Guillain-Barré syndrome and Addison crisis was suspected and hydrocortisone administered. Shortly after, the

patient developed severe encephalopathy and multi-organ failure, and respiratory assistance was required. Amino acid analysis in CSF revealed grossly elevated concentrations of branched-chain amino acids, including allo-isoleucine, diagnostic of MSUD. Hemodialysis was initiated immediately but unfortunately the patient died shortly after subsequent to brain incarceration. Sequencing of *DBT* revealed two pathogenic variants (c.75_76delAT; c.827T>G). Both patients had normal NBS results. **Conclusion:** Intermittent MSUD should be suspected in patients that present with somnolence and atypical neurological signs in connection with an infection. [1]Brodtkorb et al. *Mol Gen Met*. 100 (2010) 324-332

332 - Biochemical Diagnosis of Nonketotic Hyperglycinemia in Cuba

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Nonketotic hyperglycinemia is an inborn error of glycine metabolism, due to a deficit in the glycine cleavage system. The accumulation of glycine in tissues, blood, cerebrospinal fluid, and urine is a distinctive feature of this disorder. The seizures and central nervous system damage are the most important clinical symptoms. There are three variants: the classic form, the atypical variants and the transitory form. Biochemical diagnosis includes urine organic acid analysis and glycine quantification in plasma and CSF. In Cuba, there is not a neonatal screening program for identifying this disease, so the diagnosis we performed by clinical features. The aim of this work is to implement a protocol for the biochemical diagnosis of Nonketotic hyperglycinemia. A cross-sectional study was performed in 507 patients under clinical suspicion of aminoaciduria or organic aciduria in a period of three years (January,2014 -April,2017). In the patients with hyperglycinuria and a normal organic acids profile (GC-MS) we quantified glycine in blood and CSF by HPLC. Five patients with treatment for refractory seizures showed high glycine levels in urine and serum, and normal organic acid profiles. In all of them, the glycine CSF/plasma ratios were over than 0.08. Considering the clinical symptoms and the age of symptoms onset, the most common variants were neonatal classic and the infantile. A protocol for the biochemical diagnosis of Nonketotic hyperglycinemia in Cuba was implemented.

333 - Case Report of Treatment of Non-Ketotic Hyperglycinemia (NKH) With Amino Acid Based Glycine Free Powdered Infant Formula

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Objective: To describe three cases who presented with neonatal seizures and hiccup who were diagnosed with nonketotic hyperglycinemia (NKH). Non-ketotic hyperglycinemia (NKH) is a rare, genetic, metabolic disorder caused by a defect in the enzyme system that breaks down the amino acid glycine, resulting in an accumulation of glycine in the body's tissues and fluids. There is a classical form of NKH and a variant form of NKH. The classical form is then further divided into severe disorder or an attenuated form (mild form). The incidence of NKH is predicted to be approximately 1:76 000. NKH can occur in individuals of any ancestry. **Methods:** Case series. It is described three newborns who presented with hiccups and seizures who were diagnosed with NKH. All patients presented on the first week of life with seizures, hypotonia, poor feeding, and abnormal movements including jitteriness, hiccups, and myoclonias. One of the babies had transient seizures, for a couple of days in the first week of life with later presentation of poor feeding, severe hypotonia, and severe respiratory distress. All three cases needed extensive investigations before reaching the diagnosis including metabolic screen, lumbar puncture, electroencephalography or PSG, and magnetic resonance imaging. Two newborns needed intubation on their first day of life because of distress and apneas in whom later continue ventilation for 3 to 4 weeks. Another newborn was discharged home on oral sodium benzoate, levetiracetam, topiramate, dextromethorphan, and amino acid based glycine free powdered infant formula. **Results:** Case 1- No history of importance, and normal delivery a 4-day-old baby boy presented with lethargy, poor feeding, seizures (myoclonias) and occasional hiccups. Physical examination revealed hypotonia with poor suction and no dysmorphic features. **Conclusions:** Early treatment with dextromethorphan and sodium benzoate sufficient to normalize plasma glycine levels is effective at improving outcome if used in children with attenuated disease and when started from the neonatal period. These 3 patients had NKH diagnosis, and treated under guideline of metabolic disease of our center, the metabolic team initiate the dietary therapy with amino acid based glycine free powdered infant formula (NKH anamix) to improve the support. The early diagnosis and definitions of prognosis, also helps in genetic counseling and prenatal diagnosis can be offered at the subsequent pregnancy.

334 - Acute and Long-Term Effects of Intracerebroventricular Administration of α-Ketoisocaproic Acid on Oxidative Stress Parameters

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Background: Patients affected by Maple Syrup Urine Disease (MSUD) are biochemically characterized by elevated levels of leucine, isoleucine and valine, as well as their corresponding transaminated branched-chain α-keto acids in tissue and biological fluids. Neurological symptoms and cerebral abnormalities, whose pathophysiology is still unknown, are typical of this neurometabolic disorder. In the present study, we evaluated the early effects (1 hour after injection) and long-term effects (15 days after injection) of a single intracerebroventricular administration of α-ketoisocaproic acid (KIC) on oxidative stress parameters. **Methods:** Wistar rats at 30 days old were divided into two groups: KIC and control (which received vehicle: *artificial cerebrospinal fluid*; ACSF). KIC was administered by stereotaxic into the lateral ventricle of the animal at a concentration of 0.8 μmol of KIC dissolved in 2μL ACSF; in the control group, ACSF was injected in the same way. One hour or 15 days after the administration the animals were killed by decapitation and cortex, hippocampus and striatum were isolated for analysis. **Results:** Our results showed that KIC induced early and long-term effects; we found an increase in TBARS levels, protein carbonyl content in the hippocampus, striatum and cerebral cortex both one hour and 15 days after KIC administration. Moreover, a remarkable increase in SOD activity was found in the hippocampus and striatum one hour after injection, whereas after 15 days SOD activity was increased only in the striatum. On the other hand, KIC significantly decreased CAT activity in the striatum one hour after injection, but 15 days after KIC administration, we found a decrease in CAT activity in the hippocampus and striatum. **Conclusions:** From these results biochemical, we speculate that KIC provokes short- and long-term oxidative stress in brain; this fact may play an important role in the pathophysiology of the neurological damages present in patients with MSUD.

335 - Intracerebral Administration of Glycine Increases Blood-Brain Barrier Permeability and Induces Myelin Injury in Rat Brain

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Non ketotic hyperglycinemia (NKH) is caused by defects in the glycine (GLY) cleavage system, leading to the accumulation of GLY in urine, plasma and cerebrospinal fluid (CSF) of patients. The most common presentation of this disorder is the neonatal form, that is characterized by lethargy, hypotonia, seizures, hiccups, and apnea appearing within the first week after birth. Common neuropathological findings include cortical atrophy, corpus callosum agenesis or dysgenesis and leukodystrophy. Although GLY accumulation has been implicated in the onset of the disease, its pathophysiology is not totally established. The objective of this study was to evaluate the effects of a single intracerebral injection of GLY (0.2 μmol/g) on blood-brain barrier (BBB) integrity and myelination in rat pups. On post-natal day (PN) 1, animals received either GLY or PBS (vehicle) into the ventricle. After reassuring the wellbeing of the pups, they were returned to their mother and kept under ideal conditions of temperature and light for two weeks. On PN 15, the rats were euthanized and samples were prepared to evaluate Evans Blue (EB) extravasation, and immunohistochemistry for myelin basic protein (MBP) and myelin-associated glycoprotein (MAG). Our results evidenced that GLY increased EB staining in brain, suggesting that GLY impairs BBB integrity. Regarding myelination, GLY decreased MBP and MAG staining in corpus callosum, and MBP in striatum. In contrast, no alterations on MBP and MAG staining were seen in cerebral cortex. Our data show that GLY increases blood-brain barrier permeability and induces myelin injury. It may be presumed that these pathomechanisms are involved, at least in part, in the neuropathology of NKH. **Financial support:** CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP IBN-Net, INCT-EN.

336 - Lack of Correlation Between Serum Ornithine Concentration and Chorioretinal Changes in Gyrate Atrophy Patients—One Center Experience

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Background: Gyrate atrophy is a rare metabolic disease caused by mutations in *OAT* gene, resulting in the ornithine aminotransferase deficiency. It leads to high serum ornithine concentrations that are toxic for chorioretina and leads to its atrophy circumferentially in the periphery, myopia and early cataract. Age at diagnosis and disease progression varies respectively of the severity of phenotype and patients' compliance to diet. Patients remaining on an arginine-restricted diet (precursor of ornithine) may reduce serum ornithine and the progression of chorioretinal atrophy and visual loss. The aim of this study was to assess if there is a correlation between ornithine and chorioretinal sequential images in patients respective of diet. **Methods**: Patients attending our Adult Metabolic Medicine Clinics had investigations performed as part of their routine care. We usually aim for ornithine <400 μmol/L. Ornithine concentration, as part of plasma amino acids, was

measured within weeks of retinal imaging. Chorioretinal images were performed by optical coherence tomography (OCT) in a local Ophthalmology Department. **Results**: Seven adult patients (1 male, 6 females) were affected by gyrate atrophy. Median age was 24 years (19-30). 4/7 patients remained on low protein diet. In all cases, ornithine was above the target of 400umol/L irrespective of treatment. All 7 patients had at least one measurement of ornithine and one retinal imaging performed. In 2 cases, the ornithine concentration and retinal imaging was repeated sequentially. In one case, within 4-year-follow- up, there were no marked changes in their central retinal thickness (CRT) that was 225 μm. Ornithine concentration varied between 619-717 μmol/L despite no strict diet. In another case ornithine decreased within 3 years (fell from 1339 to 808 μmol/L) with pyridoxine, biotin and low protein treatment. However, cystoid macula edema with intra retina fluid persisted and CRT varied 363-448 μm and was above the average 260 μm. Other retinal changes included thickened myopic retina, peripapillary atrophy, scalloped changed progressing to surround arcades. **Conclusions**: There was no clear correlation between ornithine and severity of retinal changes. In some cases, changes were longstanding and irreversible with low protein diet, lysine, pyridoxine and dialamine treatment. Patients find it difficult to comply with strict low-arginine diet and dialamine supplements that are not palatable.

337 - Chronic Administration of L-Tyrosine Alters Oxidative Stress Parameters in Brain of Rats Supplemented With Omega-3 Fatty Acids

Milena Carvalho-Silva[1], Lara Gomes[1], Letícia Teixeira[2], Joyce Rebelo[1], Fernanda Malgarin[1], Maria Gomes[1], Bruna Ferreira[1], Patricia Schuck[1], Gustavo Ferreira[3], and Emilio Streck[1]

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Background: Tyrosine aminotransferase deficiency characterizes the inborn error of metabolism tyrosinemia type II, leading to increased levels of tyrosine and its by-products in plasma, resulting in eye, skin and neurological injuries. The mechanisms of brain injury are still not well known, but several studies suggest that oxidative stress in brain is involved in the pathophysiology of tyrosinemia. Docosahexaenoic acid (DHA; C22:6) and eicosapentaenoic acid (EPA; 20:5) are omega-3 fatty acids which play important roles in the development and maintenance of the central nervous system. Thus, the present study aimed to assess DHA and EPA administration effects on oxidative stress parameters, such as 2′,7′-dichlorofluorescein (DCFH) oxidation, nitrate and nitrite levels, sulfhydryl (thiol) group content and thiobarbituric acid-reactive species (TBA-RS) levels, in brain of young rats subjected to an animal model of hypertyrosinemia. **Methods:** Wistar rats were divided into 4 groups: control, L-tyrosine, DHA + EPA, and L-tyrosine + DHA + EPA. The animals received L-tyrosine (500 mg/kg of body weight, i.p., 12/12 hours), and DHA + EPA (0.1 g/kg body weight by gavage, once a day) from the 7th to the 28th postnatal days (control group received vehicle). Twelve hours after the last administration, the animals were killed by decapitation; hippocampus, striatum, and cortex were isolated for analysis. **Results:** Our results showed that L-tyrosine increased the oxidation of DCFH and TBARS levels in the cortex, and administration of DHA and EPA prevented these effects. The levels of nitrates and nitrites were increased in hippocampus and striatum of rats, but the administration of DHA and EPA did not prevent this effect. Sulfhydryl group content was decreased in striatum in the tyrosine group, and the administration of **DHA and EPA** partially prevented this diminution. **Conclusions:** From these results, we speculate that chronic administration of L-tyrosine may induce oxidative stress in the hippocampus, striatum and cortex and the supplementation omega-3 fatty acids can be a potent adjuvant treatment for patients with tyrosinemia type II.

338 - Effect of Acute and Chronic Administration of L-Tyrosine on Working Memory in Young Rats

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Background: Tyrosinemia type II is an inherited autosomal recessive disorder of L-tyrosine metabolism caused by a deficiency in the hepatic enzyme tyrosine aminotransferase (TAT); due to this deficiency, tyrosine accumulates in blood and other fluids. Previous studies reported that acute and chronic administration of L-tyrosine affect both some neurotrophins as well as the cholinergic system. Thus, the objective of this study was to evaluate the effects of acute and chronic administration of L-tyrosine on working memory in young rats. **Methods:** In the acute protocol, L-tyrosine (500 mg/kg body weight) was administered once and 1 hour after administration the animals were subjected to the object recognition task; control group received vehicle. Chronic administration of L-tyrosine (500 mg/kg body weight) was performed 12/12 hours from the 7th to the 28th day of life and 12 hours after the last administration the animals were submitted to the object recognition task; control group received vehicle. **Results:** We verified that short- and long-term memory was impaired by acute administration of L-tyrosine. On the other hand, chronic administration of L-tyrosine did not affect this behavioral task. **Conclusions:** Based on the present findings, we speculate that the administration of high doses of L-tyrosine may impair cognition in

animals, corroborating with other studies that show neurological alterations in patients with tyrosinemia.

339 - Acute and Chronic L-Tyrosine Administration Increase Dopamine Levels in Rat Brain

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Background: Tyrosinemia Type II is an inborn error of metabolism caused by liver enzyme tyrosine aminotransferase deficiency. Several studies report cognitive deficits, motor and speech problems in patients with tyrosinemia type II; these symptoms may occur due to a change in the dopaminergic system, since tyrosine is precursor of dopamine. Taking into account that tyrosine is a precursor of dopamine, the present work evaluated the levels of L-tyrosine and dopamine in rat brain in a chemically induced animal model of tyrosinemia type II. **Methods:** In the acute and chronic experiments, the animals were divided into two groups: vehicle (tween) and L-tyrosine (500 mg/kg). Chronic administration was performed from the 7th to the 27th day of life; L-tyrosine or tween was administered twice a day, every 12 hours. Twelve hours after the last administration, the animals were submitted to euthanasia and cerebral cortex, hippocampus and striatum were separated for analysis of L-tyrosine and dopamine levels. For acute administration protocol, animals received one single injection of L-tyrosine; one hour after the animals were submitted to euthanasia and the same brain areas were separated. **Results:** Our results showed that L-tyrosine and dopamine levels were increased in cerebral cortex, hippocampus and striatum in both protocols, acute and chronic. **Conclusions:** Our hypothesis is that high levels of tyrosine and dopamine may lead to cognitive deficits, taking into account that increased dopamine may increase reactive species by its auto-oxidation and that this may alter neurotrophins, resulting in cognitive problems.

340 - Tyrosinemia Type I. How much NTBC is enough?

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Tyrosinemia type 1 (Tyr 1) is a rare autosomal recessive disorder of tyrosine metabolism with an incidence of 1:125,000 in Europe. The deficiency of the enzyme fumarylacetoacetase causes an accumulation of tyrosine and toxic metabolites. Mainly affects liver and kidney function. Diagnosis is based on the elevated levels of succinylacetone (SUAC) in urine and/or blood, which is pathognomonic. NTBC treatment is costly but lifesaving. It has to be combined with natural protein restriction supplemented with essential amino acids. NTBC dosage should be reduced to the minimal dose allowing metabolic control. Standard treatment is 1mg/k/d. Metabolic control is judged by SUAC normalization in blood or urine, plasma tyrosine <500 μM and NTBC levels in the therapeutic range 20–40 μM. Is our aim, to present a Tyr I case that archives metabolic control receiving half of standard doses of NTBC. Clinical case: 13 months of age male patient. Nonconsanguineous healthy parents. Normal growth and development. At 8 months of age pediatrician detected hepatosplenomegaly with hard liver on examination. High alpha-fetoprotein and coagulopathy. Ultrasound identified multiples nodules in the liver and normal kidneys. MRI: several cirrhotic nodules. Elevated blood and urinary succinylacetone confirmed diagnosis. NTBC was started on standard doses associated to diet at 11 months of age. Because economic issue during 16 days NTBC was changed to half of the doses and during 10 days, it was suspended. Urine and blood SUAC was not detected during this last 26 days. Blood NTBC was in therapeutic range during the period of half of doses and there was traces when he was 10 days without drug. Conclusions: Although the patient received half of standard doses of NTBC during a short period of time, the SUAC was not detected in blood neither in urine and blood drug remained in therapeutic range. We think our patient could be treated with this low doses and strict clinical and biochemical control is necessary for a long time. The achievement of therapeutic objectives with low doses is important in our developing country where it is difficult to access to high cost medication.

341 - The R588Q Mutation in SLC25A13 is a Common Cause of Citrin Deficiency in Pakistani Patients With a Highly Variable Phenotype in Children

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Objective: Citrin deficiency (CD), caused by mutations in *SLC25A13* has been classically described in association with 2 major phenotypes: Neonatal Intrahepatic Cholestasis caused by Citrin Deficiency (NICCD) & Adult Citrullinaemia Type 2 (CTLN2) though there is increasing evidence that clinical problems may manifest in patients in later childhood including

failure to thrive & dyslipidemia. A homozygous c.1763G→A (p.Arg588Gln) mutation in exon 17 was first described in a 38-year old Pakistani adult with CTLN2. We describe a cohort of pediatric patients diagnosed with CD due to homozygosity for the same mutation with a wide variety of clinical features. **Methods:** Retrospective chart review of patients at a single pediatric metabolic center diagnosed with CD due to the c.1763G→A mutation. Results: 9 patients with CD have been managed at our center over a 10-year period. 8 were from consanguineous Pakistani families and 1 from a non-consanguineous Caucasian family. Of the Pakistani cases, 7 children from 4 unrelated families were found to be homozygous for the c.1763G→A mutation. Symptoms suggestive of NICCD were recorded in only 2 of these children—and resolved spontaneously. 5 children with CD due to the c.1763G→A mutation were investigated for recurrent hypoglycemia and impaired fasting tolerance between 2-4 hours was confirmed in 3 patients. Controlled fasting even in the 2 patients without proven fasting intolerance, demonstrated an initial delay in ketone production though when hypoglycemic the patients were mostly ketotic. A further cousin of 2 affected patients, shows the same delay in ketone production on controlled fasting & is likely to have CD. Molecular investigations are pending. 2 patients have been asymptomatic from CD and were diagnosed only following investigation of a symptomatic sibling. 2 children had comorbid conditions: coeliac disease and Megalencephalic Leukoencephalopathy with Subcortical Cysts. Carbohydrate aversion was noted in 3 patients. Conclusions: The c.1763G→A mutation appears to be relatively common in CD patients from the Pakistani community in the UK. Not all patients show typical symptoms of NICCD. An absence of this history should not preclude testing for CD in patients with suspicious symptoms. CD should be considered in the differential diagnosis of children with recurrent ketotic hypoglycemia, especially with evidence of delayed ketone production on controlled fasting.

342 - Non-Ketotic Hyperglycinemia in Twin Premature Infants: Novel Mutation in GLDC With two Phase Evolution

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Objective: Non-ketotic hyperglycinemia is a genetic disorder characterized by deficient activity of the glycine cleavage enzyme, resulting in high glycine concentrations in the brain. We report two related patients with novel mutation in *GLDC*. **Results:** At age 2 days, twin neonate girls with prematurity of $32^{6/7}$weeks presented central hypotonia, lethargy and apnea requiring ventilatory support. Deep tendon reflexes were obtained. EEG showed a constantly discontinuous pattern. Metabolic work-up was irrelevant except for both increased cerebrospinal fluid (CSF) glycine levels and CSF to plasma glycine ratio. In both infants, oral benzoate dose (250-500 mg/kg) produced a decreased of both plasma and CSF glycine levels associated with neurological improvement. No seizures were observed, but myoclonic jerks were present. In both infants, DNA analysis showed new homozygous mutations in *GLDC* (c478 C>T) resulting in truncated protein (p.Gln160*). Both parents were heterozygous for this mutation. Twin zygosity DNA testing identified dizygotic twins. In both infants, on brain MRI performed at corrected age of 40 weeks of gestational age, diffusion restriction was seen in the posterior limb of the internal capsule and in the corticospinal and central tegmental tract. Short (TE 35 ms) and long (TE 144 ms) proton (1 H) MR spectra disclosed a singlet at 3.56 ppm, which was assigned to glycine. At corrected age of 3 weeks, repeated EEG showed a discontinuous pattern including burst suppression pattern. Subsequently, despite of higher oral benzoate dose (750 mg/kg), apnea and lethargy reappeared. Hiccups were noticed. No further treatment was attempted in accordance with the family's wish. **Conclusion**: In spite of an initial improvement, both infants exhibit later on, the severe classic non-ketotic hyperglycinemia even on higher dose of oral benzoate.

343 - Late Diagnosis of Rare Type of Homocysteinuria and Treatment With Betaine (Cystadane): The First Experience in Russian Federation

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Patient A, 2 3/12 year-old male, was diagnosed with homocysteinuria (defect of remethylation) at the age of 1 4/12 year, when complications of the disease occurred as partial atrophy of the optical nerves and blindness, breath difficulties, replacement hydrocephalus of atrophic type, severe delay of mental development. The patient was presented to a doctor at the age of 2 months with delay of growth of head circumflex noticed by the mother, microcephaly (circumflex of the head 38.5 sm), and diffuse muscle hypotonia. Ultrasonography revealed signs of severe diffuse abnormalities of brain structure in the form of mixed connected hydrocephalus of atrophic type, and

retrocerebellum cyst. Antibodies to cytomegalovirus infection (CMV) class M were detected. Laryngomalation and micro aspiration syndrome were diagnosed. At the age of 4 months, ENMes showed signs of prior muscle leisure of upper and lower limb muscles. Metabolic myopathy was suggested. At the age of 7 months, MRI of the head showed picture of abnormal development of Denni-Yoker type, replacement hydrocephalus and hypoplasia of corpus callosum. Amino acid blood analysis revealed a decrease in the level of methionine, 5.52 mkM/L (N 6.00-110.00). Therefore, homocysteinuria was suspected. Severe hyperhomocysteinemia was found with total homocysteine level of 198.47 μmol/L (5.46-16.20 μmol/L). Vitamin B12 (419.85 nmol/L) and folic acid (24.35 nmol/L) indexes were normal. Methylmalonic aciduria was not detected. Cystadane 100 mg was prescribed of. Pathologic reactions were not noticed and the patient's condition stabilized. Respiratory support lasted continuously for 7 months, was dismissed. The level total homocysteine decreased up to 77.1 μmol/L. Subsequent sequencing of the MTHFR gene by Sanger method showed mutations Ile256Phe and Pro348Ser. **Conclusion**: The earliest clinical symptoms of infant homocysteinuria caused by MHFTR gene defect are as follow: delay of the head growth circumflex, microcephaly, muscle hypotonia, and breath disorders. Determination the total homocysteine level is mandatory for patients presented with such clinical manifestations, to avoid late diagnosis and provide timely treatment.

344 - Patients Diagnosed With Hepatocellular Carcinoma With Tyrosinemia Type I

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The clinic symptoms of tyrosinemia type1 are numerous and can appear in any term extending from neonatal period to adulthood. Liver is the primary organ that is affected; kidney can also be influenced in the follow-up, there is a hepatocellular carcinoma (HCC) development risk. Four patients diagnosed with tyrosinemia type1 with HCC development were presented to draw attention to the association of these clinics. **Case 1:** A 3-year-old patient diagnosed with Fanconi syndrome and admitted to our hospital at 9 with abdominal distention complaint. On the physical examination, severe growth failure, hepatosplenomegaly and acid were noticed. In the laboratory analyses; elevated liver function tests, increase in alpha-feto protein (AFP) and tyrosine level in blood; succinylacetone in urinary organic acid analysis were determined. In the abdominal imaging; multiple nodules compatible with HCC and metastatic nodules were noticed in thorax analysis. The patient was diagnosed with advanced stage HCC developed at the base of tyrosinemia type I. **Case 2:** A 14-month old patient was found to have an increase in blood tyrosine level in workups performed due to hepatomegaly, succinyl acetone excretion in the urine and was diagnosed with tyrosinemia type I. In the follow-up period, the patient—incompatible with the treatment—was diagnosed with advanced stage HCC with increase in AFP value and imaging findings. **Case 3:** An 11-month-old patient with Fanconi syndrome was found to have tyrosinemia type I at the age of 2 years as a result of mutation identified in FAH gene analysis, succinyl acetone excretion in urine, increase in blood tyrosine level. At the age of 11 years, the patient was diagnosed with HCC with increased AFP values and imaging findings. **Case 4:** The patient who had been diagnosed with hypophosphatemic rickets at the age of 6, Fanconi syndrome at the age of 9 was discovered to have tyrosinemia type 1 in the evaluation conducted due to hepatomegaly when 10 years old as a consequence of succinyl acetone excretion in urine, rise in blood tyrosine level. The patient was diagnosed with advanced HCC with increase in AFP and imaging and pathology findings when 16 years old. In tyrosinemia, it is important to commence nitisinone at an early period and comply with the follow-up and treatment. HCC development risk has reduced since nitisinone came into use. HCC development risk increases with age.

345 - Nonketotic Hyperglycemia: Case Report

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Introduction: Glycine encephalopathy, also known as nonketotic hyperglycinemia (NKH), is an inborn error of glycine metabolism caused by deficiency in the glycine cleavage system and characterized by large quantities of glycine accumulated in all body tissues. **Case Report:** A 2-day-old baby boy presented with lethargy and poor feeding. He was a full-term baby, her parents not consanguineous, pregnancy uneventful, and labor and vaginal delivery uncomplicated. Apgar scores at birth were 9 at 1 minute and 10 at 5 minutes, birth body weight 3410 g (50th-75th percentile), birth height 52 cm (75th-90th percentile), and head circumference 33 cm (50th-75th percentile). Examination revealed gross hypotonia with poor suck and weak cry, shallow breathing and no dysmorphic features. Laboratory examination showed no evidence of infection,

acidosis, or ketosis, with blood cell count, electrolyte, glucose, and lactate all within the normal range. Brain echography showed negative findings. Thyroid function tests were normal. Ammonia levels were elevated 1.2 μg/mL (normal range: 0.2-0.8 μg/dL). Extensive metabolic investigations were carried out. After being NPO for few hours there was improvement in muscle tone. After oral feedings was restarted he was noted to have apneic episodes requiring ventilatory support. No spontaneous breathing was observed while on ventilator despite not being on sedation. He was noted to have some abnormal movements including jitteriness, for which he was treated with Levetiracetam. His electroencephalography showed normal pattern. Magnetic resonance imaging showed mild hypoplasia of corpus callosum. New measurement of ammonia was 5.4 μg/dL (normal range: 0.2-0.8). He was kept NPO and sodium benzoate was stared. Oral feedings were restarted with an Amino Acid-Modified Infant Formula (Nonessential amino acid-free). His serum glycine levels were high 1331.1 μmol/L (normal range:145.6-518.9) which is diagnostic of NKH. Regimen of frequent feedings, together with antiepileptic drugs, including levetiracetam, sodium benzoate and dextromethorphan and L-carnitine were prescribed. **Conclusion:** Despite the progress in the management of patients with NKH, the long-term morbidity remains poor. It is important to recognize the condition early as genetic counseling, and prenatal diagnosis can be offered at the subsequent pregnancy.

346 - L-carnitine Prevents Oxidative Stress in the Brains of Rats Subjected to a Chemically Induced Chronic Model of Maple Syrup Urine Disease

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Maple syrup urine disease (MSUD) is an inborn error of metabolism associated with acute and chronic brain dysfunction that is caused by severe deficiency in the activity of the branched-chain α-keto acid dehydrogenase complex, enzyme involved in the branched-chain amino acid (BCAA) degradation pathway. Due to this blockage, high concentrations of the BCAAs as well as the respective keto acids accumulate in patients when on an unrestricted diet and during episodes of metabolic decompensation during intercurrent illness. The main symptomatology presented by MSUD patients includes ketoacidosis, failure to thrive, poor feeding, apnea, ataxia, seizures, coma, psychomotor delay and mental retardation, although, the neurological pathophysiologic mechanisms are poorly understood. It was recently reported that treated MSUD patients have L-carnitine deficiency (L-car), a compound with antioxidant properties that is used as adjuvant therapy in some inborn errors of metabolism. In the present study, it was investigated BCAAs effect on several oxidative stress parameters and evaluated the L-car efficacy against these possible pro-oxidant outcomes of a chronic MSUD model in the cerebral cortex and cerebellum of rats. The chemically chronic model of MSUD used in this study demonstrated that BCAAs induce lipid and protein oxidation (measured by thiobarbituric acid-reactive substances and protein carbonyl content, respectively), impaired brain antioxidant defenses (analyzed by reduced glutathione content and catalase, superoxide dismutase, glutathione peroxidase and glucose 6-phosphate dehydrogenase activities), and increase reactive species production (verified by 2′7′-dichlorofluorescein oxidation assay), particularly in the cerebral cortex. L-car treatment was able to prevent these effects, improving the activity of antioxidant enzymes, increasing the non-enzymatic antioxidant defenses, and diminishing the lipid and protein oxidative damage and reactive species production. Taken together, the present data indicate that chronic BCAA administration significantly increased oxidative stress in the brains of rats subjected to a chronic model of MSUD, and considering that the current treatment of MSUD involves a protein restricted diet, which is low in antioxidants and L-car, supplementation with this compound may be considered a suitable adjuvant therapy for MSUD patients because it can improve redox homeostasis in this disorder. Financial Support: CNPq, CAPES, FAPERGS, and FIPE-HCPA

347 - Nonketotic Hyperglycinemia: First Case of Patient With Biallelic Mutations in GCSH gene

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The rapid analysis of genomic data is providing an effective tool in the diagnosis of Nonketotic Hyperglycinemia, a monogenic cause of neonatal epilepsy caused by a deficient activity of the glycine cleavage system (GCS), however the growing challenge is the correct interpretation of the clinical significance of identified variants. GCS consist of four different proteins, P-protein (*GLDC*), T-protein (*AMT*), L-protein and a small lipoylated H-protein (*GCSH*). Up to now, biallelic mutations in *GCSH* have not been identified. Here we present the

genetic test and functional analysis of two novel variants identified in the *GCSH* gene of a patient who died at 18 days of age with a clinical and biochemical diagnosis of classic NKH. Massive-parallel exome sequencing using a customized gene panel designed to capture exome sequence of *GLDC, AMT, DLD* and *GCSH* genes identified the c.170A>G (p.His57Arg) and c.148-?_228+? del variants in the *GCSH* gene. Sanger sequencing of nucleotide variants in parent's samples confirmed the Mendelian segregation. The scoring of pathogenicity of the missense change using several computational predictive programs returned a concordant result of pathogenic variants. To evaluate the functional effect of missense mutant protein we performed the ectopic expression of the mutant cDNA *GCSH* construct in a COS7 cell system, followed by enzymatic assay, western blot analysis to evaluate protein stability and immunofluorescence microscopy to evaluate subcellular location. The results rendered a mutant protein normal in quantity and size, properly located in the mitochondria and able to participate in the removal of CO_2 from glycine. The nature of this functional test doesn't discard an effect on total GCS activity, but our data point to p.His57Arg as a probably a hypomorphic variant which combined with the loss-of-function variant c.148-?_228+? del, could be responsible of the patient's NKH phenotype. PI12/02078; PI16/00573; MINECO-FEDER. Fundación Isabel Gemio

348 - Ex Vivo Gene Therapy in a Pig Model of Hereditary Tyrosinemia Type 1: Spheroid Suspension Hepatocytes are a Valid Alternative to Single Cells for Intraportal Infusion

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We aimed to treat and cure a porcine model of hereditary tyrosinemia type 1(HT1) through ex vivo gene delivery via intraportal transplantation of single cell or spheroid suspension hepatocytes. We performed laparoscopic partial hepatectomies on six-week-old (15-20 kg) fumarylacetoacetate hydrolase (FAH)-deficient pigs (n = 6) and isolated hepatocytes *ex vivo*. Hepatocytes were transduced in suspension with a lentiviral vector expressing the *FAH* gene. Animals received autologous hepatocyte transplantation by percutaneous portal vein infusion of single cell (n = 3) or spheroid (n = 3) suspension. Portal pressures were measured during transplantation and ultrasound used to evaluate presence of thrombotic events. Engraftment and expansion of *ex vivo* corrected autologous hepatocytes were followed through biochemical/histological analysis and animals' ability to thrive off protective drug NTBC. This experiment was repeated in two wild-type pigs and a cohort of wild-type mice: here, hepatocytes were labeled with Zirconium-89 to evaluate biodistribution through PET-CT. Animals receiving single cell suspension hepatocytes, 4.8-15.0 g, experienced a mean change in portal pressure of 0.8-6.0 mm Hg during injection. No thrombus was noted. Animals receiving spheroid suspension hepatocytes, 9.1-10.8 g, experienced a mean portal pressure change of 10.9-12.5 mm Hg. Portal vein thrombi were noted in two animals; portal infusions were stopped and enoxaparin administered for 7 days, at which time ultrasounds showed no thrombus. On PET-CT imaging post-operatively, no significant difference in biodistribution was found between single cell and spheroid hepatocytes in neither pigs nor mice. Liver biopsies at 6 months post-transplantation show presence of multiple FAH-positive nodules; biochemical analysis shows a trend towards normalization of tyrosine levels. All spheroid animals are clinically stable, with one animal currently gaining weight off NTBC treatment for over 6 months, suggesting effective treatment of the metabolic disorder. In this preclinical study, we show that ex vivo gene correction of autologous hepatocytes in FAH-deficient pigs can be performed using spheroid as well as single cell suspension hepatocytes, with single cell suspension allowing for infusion of larger numbers of hepatocytes but no significant difference in biodistribution. In addition, transplantation of larger spheroids presents a higher short-term risk for portal vein thrombosis and increased portal pressures.

349 - Ex Vivo Gene Therapy and Ectopic Hepatocyte Transplantation into Lymph Nodes for the Treatment of Hereditary Tyrosinemia Type 1 in a Large Animal Model

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Objective: To treat and cure a porcine model of hereditary tyrosinemia type 1 (HT1) through ex vivo gene delivery via ectopic transplantation of hepatocytes into mesenteric lymph nodes. **Methods:** We performed laparoscopic partial hepatectomies on five fumarylacetoacetate hydrolase (FAH)-deficient pigs at five weeks of age and isolated primary hepatocytes ex vivo. Hepatocytes were transduced in suspension with lentiviral vectors expressing the human *FAH* and the sodium-iodide symporter (*NIS*) genes. The NIS reporter is a non-invasive method to monitor hepatocyte expansion using non-invasive nuclear imaging. All animals received autologous hepatocyte transplantation by direct injection of single cell

hepatocytes into mesenteric lymph nodes. Engraftment and expansion of ex vivo corrected autologous hepatocytes are being followed through biochemical and histological analysis, SPECT-CT or PET-CT imaging, and through the animal's ability to thrive off the protective drug 2-(2-nitro-4-trifluoromethylbenzyol)-1,3 cyclohexanedione (NTBC). **Results:** All animals were injected with six hundred million cells. No complications were noted during resection or transplantation. Two months post-transplantation, liver function tests and tyrosine levels are trending towards normalization with no significant increase in bile acid levels. SPECT-CT scanning two months post-transplantation showed multiple NIS-positive lymph nodes at the root of the mesentery, demonstrating successful engraftment of ex vivo transduced hepatocytes. All animals are currently clinically stable, and are being cycled on and off NTBC to stimulate expansion of FAH-positive hepatocytes. **Conclusion:** In this study, we show that lymph nodes can be used as a safe, ectopic site for hepatocyte transplantation after ex vivo gene therapy. Engraftment and expansion of these cells in the lymph nodes may be sufficient for improvement and possibly cure of metabolic disease.

350 - Treatment of Hereditary Tyrosinemia Type 1 in a Large Animal Model Through In Vivo Lentiviral Gene Therapy

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Objective: To treat and cure a porcine model of hereditary tyrosinemia type 1 (HT1) through in vivo liver-directed lentiviral vector gene therapy. **Methods:** We performed direct percutaneous portal vein infusions of a lentiviral vector carrying the human *FAH* gene under control of the liver-specific alpha1-antitrypsin promoter in four fumarylacetoacetate hydrolase (FAH)-deficient pigs at six weeks of age. Pigs were pretreated with corticosteroids and antihistamines, and received a dose of 2×10^{10} TU/kg. Correction of the metabolic disorder was followed through biochemical analysis of liver function and tyrosine metabolite levels, histological analysis of liver fibrosis and presence of FAH in hepatocytes, as well as clinically through the animal's ability to thrive off the protective drug 2-(2-nitro-4-trifluoromethylbenzyol)-1,3 cyclohexanedione (NTBC). Integration profile of the lentiviral vector will be studied through next generation sequencing and bioinformatics analysis of over ten different tissue types. **Results:** One animal developed an acute inflammatory reaction and died in the 48 hours following treatment. The other three animals were cycled on and off NTBC to stimulate expansion of FAH-positive hepatocytes. At two months post-treatment, liver harvest in one pig showed presence of multiple FAH-positive hepatocyte nodules with no evidence of fibrosis. Preliminary PCR analysis of this animal's tissues demonstrated preferential lentiviral vector integration in the liver. The two remaining animals are clinically stable, and are gaining weight off NTBC for progressively longer periods of time, suggesting effective treatment of the metabolic disorder. **Conclusion:** In this study, we show that in vivo gene transfers can correct the metabolic deficiency in FAH-deficient pigs with no evidence of substantial lentiviral integration in non-hepatic tissues. Further studies are required to evaluate optimal viral vector dosing.

351 - Blood levels of Glutamine and Alanine are Signature Amino Acids for determining Aminoaciduria by Reverse Phase LCMS

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Blood levels of Glutamine and Alanine are Signature Amino Acids for determining Aminoaciduria. We analyzed 76 pediatric cases with different grades of encephalopathy without metabolic acidosis (Plasma Lactic acid was performed to rule out mitochondriopathies). Blood ammonia and lactic acid were analyzed in fresh plasma samples and the same was run on Reverse Phase HPLC (Agilent). High blood ammonia correlated well with high plasma levels of glutamine and alanine. Retention time(RT), the main determinant in amino acid peak identification for a particular amino acid can shift causing confusion as to what it signifies, RT is highly depended on temperature, pressure and humidity of the place of analysis. Retention time of that specific Amino acid can also overlap with artefacts or background noise giving false-positive results and hence misinterpretation. The high peak of glutamine and alanine can easily be identified and then could be further processed for specific amino acid in question. We conclude that LC-MS is the most sophisticated & robust investigative tool of non-volatile metabolites in diagnosing IEM viz alpha aminoacidopathies and aid in early diagnosis and the appropriate intervention.

352 - An Unusual Family Illustrating the Complexities of Enzyme and Molecular Analysis in Nonketotic Hyperglycinemia

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Nonketotic hyperglycinemia (NKH) is a rare metabolic disorder with an estimated disease incidence of approximately 1/76 000 (Coughlin et al., 2017). NKH is attributable to defects of

the glycine cleavage system (GCS). Approximately 80% of patients have biallelic mutations of the *GLDC* gene, encoding the GCS P protein, and ~20% of patients have mutations of the *AMT* gene (T protein). A pair of siblings and their cousin presented as infants with increased glycine in blood (324 μmol/L, 340 μmol/L and 475 μmol/L respectively), CSF (55 μmol/L, 58 μmol/L and 72 μmol/L) and CSF: plasma glycine ratios (sibling 0.17, cousin 0.15) consistent with a diagnosis of NKH. GCS enzyme analysis of a liver biopsy from the proband showed normal activity excluding a P protein defect. Mutation analysis of the *AMT* gene identified two variants in the proband and her sibling, the previously reported c.664C>T p.(Arg222Cys) and a rare variant, c.-58C>T, of uncertain significance within the 5'UTR. Other rare variants in this region have been reported and suggest an important regulatory region of the *AMT* gene although this is not functionally proven (Coughlin et al., 2017). The cousin has the familial p.(Arg222Cys) variant and a different variant c.230C>T p.(Ser77Leu) in *AMT*, considered likely to be pathogenic. All variants were classified according to the American College of Medical Genetics guidelines for the interpretation of sequence variants (Richards et al., 2015). This rare family with three segregating *AMT* variants illustrates the complexities of enzyme and molecular analysis in NKH. Coughlin et al., Genet Med. 2017 19(1):104-111. Richards et al., Genet Med. 2015 17(5):405-24.

353 - Tyrosinemia Type III: Outcome in Two Siblings

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Background: Tyrosinemia type III is a rare autosomal recessive disorder caused by a deficiency 4-hydroxyphenylpyruvate dehydrogenase. Only a few cases have been described in literature, with the majority presenting neurological symptoms as intellectual impairment, dyslexia, hyperactivity and speech delay. **Case report:** Case 1 is a male patient, the third child of a consanguineous couple, with an unremarkable family history, born at term following an uneventful pregnancy. He presented an elevated serum tyrosine level (526 μmol/L) on newborn screening, with a high excretion of tyrosine and phenolic metabolites. He was started on a low-protein diet, with a consistent decreased in tyrosine levels. The diagnosis of tyrosinemia type III was confirmed by genetic study, with identification of the homozygous mutation p.A33 T (c.97G>A). He had a normal psychomotor development during his first years, but around the age of three began to reveal a speech disorder and a hyperactive and impulsive behavior, with the inability to follow orders. He was diagnosed with Attention-Deficit/Hyperactivity Disorder (ADHD) at 5-year-old, beginning treatment with methylphenidate. During this time, he maintained tyrosine levels consistently bellow 300 μmol/L. Currently, at 7-year-old, he shows a slightly improvement in terms of behavior and attention time and maintains speech therapy sessions, with no learning difficulties. Case 2 is a 15-year-old girl, sibling of case 1, that was diagnosed with tyrosinemia type III at 8- year-old, in the context of a family screening following her brother's diagnosis. By that age she presented ADHD, medicated with methylphenidate, and learning difficulties benefiting of special education. She started a low protein diet, but with no benefits in terms of behavior, beginning diet liberalization two years later. Nevertheless, she has maintained tyrosine levels below 300 μmol/L without dietary restriction or supplementation. **Discussion:** The clinical spectrum of tyrosinemia type III is still not well defined and it is unclear whether lowering plasma tyrosine levels will alter its natural history. Nonetheless, it has been considered reasonable to treat patients with a low protein diet, at least in early childhood. Our patients have maintained tyrosine levels below 300 μmol/L. Clinically, they both present ADHD, although the one that begun treatment in neonatal period has a better cognitive performance.

354 - BCAT2 Mutations cause a Novel Disorder of Hypervalinemia/Hyperleucine-Isoleucinemia, Autistic Features, and Developmental Delay Detectable by Expanded Newborn Screening

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Objective: The first step in the enzymatic breakdown of branched-chain amino acids (BCAA) leucine, isoleucine and valine is catalyzed by the two BCAA transferases (BCAT),

cytoplasmic BCAT1 and mitochondrial BCAT2. The pathophysiological and clinical consequences of hypervalinemia/hyperleucine-isoleucinemia are still something of an enigma. **Methods:** We present clinical, biochemical and molecular data in three patients with BCAT2 deficiency. One patient was diagnosed following metabolic work-up for developmental delay, the second patient was diagnosed on Whole Exome Sequencing (WES) for learning disability and autistic traits and the third patient was identified on expanded Newborn Screening (NBS). **Results:** Patient 1 and 2 presented with developmental delay and autistic features. Metabolic and genetic work-up revealed profoundly elevated plasma BCAA concentrations (Case 1: leucine 3446 μM/L, valine 3935, isoleucine 2774, alloisoleucine not detected; Case 2: leucine 874, valine 1963, isoleucine 518, Allo-isoleucine n.d.). Case 3 was identified on expanded NBS (leucine 325, valine 606, and isoleucine 248 μM/L). Maple Syrup Urine Disease could be ruled out. Fibroblast studies were available in Case 1 and revealed considerably reduced valine and moderately impaired leucine oxidation. Targeted Sanger sequencing revealed a novel homozygous in-frame deletion in the *BCAT2* gene in Case 1. WES performed in Case 2 revealed two missense variants in the *BCAT2* gene. Molecular genetic testing in Case 3 showed a homozygous frame-shift *BCAT2* mutation. **Conclusion:** Comparing these three cases to the first published case who had presented as an adult with headache and memory loss (Wang et al., JIMD 2015), our findings suggest that BCAT2 deficiency also causes a novel developmental disorder with autism. Furthermore, it is detectable on NBS as demonstrated in Case 3, raising the possibility that timely diagnosis and early treatment could alter clinical course and improve outcome.

355 - Syndromic Progressive Infantile Neurodegeneration Caused by Novel Homozygous Variant in 3-hydroxyisobutyrl-CoA Hydrolase -First Case in Colombia

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Introduction: 3-hydroxyisobutyrl-CoA hydrolase deficiency (HIBCHD, MIM: 250620) is a rare disorder of infancy associated with hypotonia, developmental delay, and cerebral atrophy on magnetic resonance imaging (MRI). **Case Presentation:** A Colombian female infant born to consanguineous parents with persistent vomiting, anorexia, irritability, swallowing difficulties, poor feeding and psychomotor developmental delays, no language, and development regression. At 9 months old, she developed multiple episodes of seizures and myoclonus. Physical examination showed a short forehead, wide palpebral fissures, epicanthal fold, synophrys, nasal bones hypoplasia, low nasal bridge, prominent philtrum groove, small mouth with cupid's bow, microcephaly, anterior fontanelle punctate, axial hypotonia, decreased tendon reflex and hepatomegaly. We reported a case of female Colombian infant with HIBCH deficiency carrying a new homozygous missense mutation (c.808A>G,(NM_014362.3) at the cytoband 2q32.2, including a newly identified mutation (p.Ser270Gly) revealing a clinical correlation. **Conclusion:** This knowledge is essential for the elaboration of correct diagnosis, clinical management, and drug considerations as part of integral treatments for patients with HIBCH deficiency.

356 - Later Presentation and Management of Maple Syrup Urine Disease in an Infant With Developmental Delay and Failure to Thrive

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Maple syrup urine disease is a rare autosomal recessive disorder caused by a deficiency in the activity of the branched-chain a-ketoacid dehydrogenase complex, being an inborn error of amino acid metabolism first described by Menkes in 1954. This rare disorder represents one of the causes of acute neonatal illness which results in disturbances of neurological development, such as seizures, lethargy and refusal of feeds, besides clinical significant fasting hypoglycemia, metabolic acidosis, and ketoaciduria, soon followed by hypertonicity, opisthotonus, and death if untreated. The abnormal odor of maple syrup is found in the urine, sweat and cerumen of most patients. The aim of the study was to evaluate the clinical course and alterations of marker metabolites since early diagnosis. Laboratory data as well as information on clinical course and the management were obtained retrospectively in medical record of the patient. The patient in this case report had a global development delay diagnosed since 2-years-old followed by hypotony and poor weight gain. Gas-chromatographic-mass spectroscopy of urine showed low rates of 2-hydroxisovaleric acid, 12-hydroxisocaproic and 2-hydroxi-3-metilvaluric. Plasma organic acids assay showed high titles of leucine, isoleucine and valine. Since that, the child was treated with thiamine, iron supplementation and dietary protein restriction and the intake of an iron fortified synthetic formula devoid of leucine, isoleucine and valine, with a remarkably improve on his development. A treatment protocol on maple syrup disease was designed to inhibit endogenous protein catabolism, sustain protein synthesis, prevent deficiencies of essential amino acids and maintain normal serum osmolarity, once persistent increases in the branched-chain amino acids, leucine, isoleucine and valine is observed, and frequently greater than 10-fold over normal.

Treatment often requires a low branched-chain amino acid diet. The patients must be carefully followed by monitoring plasma amino acid concentrations repeatedly. Some patients respond to treatment with large doses of thiamine. Specific diagnosis by tandem mass spectrometry of this inherited disorder of amino acid metabolism mostly in newborns with seizures and other signs of acute severe metabolic decompensation aims to improve brain development and minimize severity.

357 - Alkaptonuria: Not and "Adult Disease" Anymore—Early Diagnosis for Treatment is Essential

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Aims: To describe two patients with alkaptonuria diagnosed in different ages and with different clinical involvement. **Methods:** Clinical history assessment associated to charts reviews. **Results:** Patient 1, LAS, nine years old. The diagnosis was suspected at 1 year and 6 months due to altered urine staining observed in diaper. At 3 years of age, the diagnostic was reached by the result of an increase of homogentisic acid (HGA) in the urine. Molecular examination was performed and the parents were heterozygous carriers. After the confirmation of the diagnosis, examinations for investigation of the pathology were also performed, and at echocardiography exam it was visualized mitral valve thickening without hemodynamic repercussion, and it was not found compromise of other systems. Patient 2, VCM, 71 years old. Symptomatology was reported at 66 years. The initial symptoms were pain and stiffening of the fingers with slow progression, associated with paresthesia (in median nerve territory), Raynauld phenomena. In this period, were noted hyperchromic punctate lesions on the first and second fingers. At the physical examination, blackened hyperchromic spots were observed on the palms of the hands and face, ears, and sclera of both eyes. Results of laboratory tests showed elevation in HGA urine. Electroneuromyography evidenced carpal tunnel syndrome, magnetic resonance of wrists showed tendon ruptures. At echocardiography, aortic insufficiency of mild to moderate degree and moderate mitral regurgitation were observed. **Conclusion:** Alkaptonuria, also called endogenous ochronosis, is a rare metabolic autosomal recessive disease, which affects 1:1 000 000 people. Management of joint pain tailored to the individual; physical and occupational therapy to help maintain muscle strength and flexibility; knee, hip, and shoulder replacements when needed; aortic stenosis may necessitate valve replacement. Therapy with NTBC could be an important advance in the treatment of such rare and disabling disease.

358 - Methylamine Dynamics: Carnitine, Trimethylamine, and Trimethylamine Oxide

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Trimethylamine-N-oxide (TMAO), a carnitine metabolite produced from microbiome-derived trimethylamine (TMA), has been associated with cardiovascular risk, raising long-term safety concerns for carnitine supplementation in IEMs. We are studying dynamics of carnitine and its derivatives in patients with various IEMs receiving oral or intravenous carnitine. Methods: We measured plasma and urine carnitine, TMA, TMAO, betaine, dimethylglycine and N6-trimethyl-lysine. Fractional excretion (FE) was calculated from results in urine and plasma. We included 2 patients with medium-chain acyl-CoA dehydrogenase deficiency (MCADD), 3 with 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency, 3 with propionic acidemia (PA), and 1 respectively with ethylmalonic encephalopathy, isovaleric acidemia, glutaric acidemia type II (MADD), methylmalonic acidemia, primary carnitine deficiency (SLC22A5 mutation), beta ketothiolase deficiency, and trimethylhydroxy-lysine epsilon (TMHLE) dioxygenase deficiency. Results: Plasma or urine levels of carnitine or TMAO did not correlated simply with oral carnitine dose, implying multiple factors influencing their levels. Plasma TMAO correlated strongly ($r = 0.92$) with urine TMAO, but that wasn't the case for carnitine ($r = 0.27$). Plasma and urine TMAO levels were very closely correlated; the FE was close to 1 for most patients. FE of creatinine was much lower for all patients, speaking for very efficient renal tubular carnitine reabsorption. Two patients had significantly higher carnitine FE: the patient with MADD and the patient with SLC22A5 deficiency. The explanation is obvious in the latter case of transporter deficiency, and the first case displayed renal tubular acidosis, likely due to mitochondrial dysfunction. The TMAO FE and carnitine FE were not correlated. Patients with PA had remarkably high levels of plasma TMAO. The ratio TMAO/(TMAO+TMA) was close to 1 for most patients except for the two patients receiving oral antibiotics. Plasma TMAO was significantly reduced (20-fold) in one patient with MCADD during IV carnitine infusion. Conclusions: We hypothesize that the TMAO renal reabsorption transporter system is not as efficient as the carnitine reabsorption system, even though it has been described recently that TMAO is reabsorbed by the same transporter (organic cation transporter 2). Antibiotic therapy affects the TMAO/TMA ratio likely due to microbiome disturbances. Patients with PA are at risk for high plasma TMAO levels.

J) Urea Cycle Disorders (359 to 383)

359 - The Diagnosis of Argininosuccinate Lyase Deficiency in a Screened Population

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Argininosuccinate lyase deficiency is a urea cycle disorder which can present in the neonatal period with hyperammonemic encephalopathy, or later in childhood with episodic vomiting, growth and developmental delay. Diagnosis is made by detecting argininosuccinate in the plasma, CSF or urine samples of affected individuals. With the advent of expanded newborn screening, diagnosing presymptomatic infants has been possible through the measurement of citrulline in the dried blood spot sample. In New South Wales since the introduction of expanded newborn screening in 1998, 10 children have been diagnosed with this disorder. Seven, of these 10, had an increased citrulline on their newborn screening sample and three children, from two separate families, were diagnosed when investigated for other reasons. The oldest of these patients is now 17 years of age and this cohort of patients shows the spectrum of this disorder.

360 - First Report of Carglumic Acid in a Patient With Citrullinemia Type I (Argininosuccinate Synthetase Deficiency)

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Carglumic acid is a structural analogue of human N-acetylglutamate, which can enter mitochondria to activate carbamoyl phosphate synthetase 1, hereby enhancing the activity of the urea cycle and promoting ureagenesis. In this report, we present a 6.5-year-old male patient with argininosuccinate synthetase deficiency who was successfully treated for 3.5 years with carglumic acid. **Case:** A male newborn admitted to hospital at the age of 9 days with complaints of restlessness, feeding problems, and vomiting. The patient was pale, lethargic, and dehydrated at the time of admission. He was diagnosed with citrullinemia after serum amino acid analyses revealed markedly elevated citrulline concentration at together with elevated glutamine, urine orotic acid concentrations and homozygous c.1168G>A (p.Gly390Arg) mutation in *ASS1* gene. The ammonia concentration decreased and blood gas analysis normalized after peritoneal dialysis was performed for three days. Also, sodium benzoate, L- arginine, and parenteral nutrition with glucose and lipid therapy were initiated. He remained under follow-up with sodium benzoate, L-arginine, and protein restricted diet. Until 1 year of age, low adherence to sodium benzoate therapy due to unpleasant taste caused hyperammonemic episodes at 3, 5, 7, and 11 months and obligated us to initiate carglumic acid (100 mg/kg/day) therapy. During treatment with carglumic acid, the median ammonia level was 45.6 μmol/L. Owing to the high cost of carglumic acid and tolerable taste of sodium phenylbutyrate, the patient's treatment was switched from carglumic acid to sodium phenylbutyrate (250 mg/kg/day) when he was 4.5 years old. Currently, the patient is 6.5 years old and remains under follow-up with sodium phenylbutyrate, L-arginine, and protein restricted diet. **Laboratory Findings**: There were no differences in median ammonia, citrulline and glycine levels between the sodium benzoate, carglumic acid and sodium phenylbutyrate treatments. Ornithine level was found to be significantly lower during the carglumic acid treatment compared to other treatments ($P = .039$). Also, glutamic acid was found to be higher during the sodium benzoate treatment period compared to other treatment periods ($P = .024$). In conclusion, this is the first case of citrullinemia type 1 for whom follow-up was performed with carglumic acid treatment. Our case provides insight on the efficacy of carglumic acid in the long-term treatment of citrullinemia type 1.

361 - Correction of Ureagenesis in OTC-Deficient spf-ash Mice Using Naked-DNA Minicircle-Vector Gene Transfer to Periportal Hepatocytes

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Background: The aim of this project is to exploit the potential of non-viral naked-DNA, so called minicircle (MC) vectors for liver-directed gene therapy as viral-based vectors, including adeno-associated virus (AAV), although at the forefront of clinical use, are increasingly raising safety concerns for human application to treat, e.g., liver defects. Ornithine transcarbamylase (OTC) is a key enzyme of the urea cycle that is fully expressed exclusively in periportal hepatocytes. OTC deficiency is the most common and a severe urea cycle disorder for which no curative therapy exists except liver transplantation. Physical targeting of MC vectors to periportal hepatocytes is challenging as it requires hydrodynamic administration through vessels connecting directly to the portal triad composed of branches of portal vein, hepatic artery and biliary duct. As a model for treatment, we used the *spf-ash* mouse that carries a genetic variant that is also found in patients and exhibits residual OTC enzyme activity between 3-6% of normal,

resulting in mice in a mild phenotype with elevated urinary orotic acid but no clinical significant hyperammonemia. **Methods:** To treat male *spf-ash* mice, we generated various MC vectors. Our superior vector contained a codon-optimized murine *Otc*-cDNA plus a truncated intron, and was under transcriptional control of a synthetic liver-specific promoter-enhancer. **Results:** MC vectors were delivered to mouse livers via hydrodynamic portal vein injections; however, mortality rate in *spf-ash* (but not in wild-type) mice was high and the transfection rate was not sufficient for treatment (below 1% of hepatocytes). We next established hydrodynamic retrograde intrabiliary injection (HRII) as an alternative. Injection of MC vectors using HRII was feasible and safe with 100% survival (more than 50 mice). More importantly, *in vivo* ureagenesis (using stable isotopes) in at least two treated *spf-ash* mice was corrected to wild-type levels accompanied with the improvement of enzyme activity (over 70% of wild-type). Survival after induction of severe hyperammonemia by a short hairpin (shRNA)-mediated knock-down of residual endogenous OTC expression in the treated mice confirmed long-term correction of OTC deficiency (more than 150 days). **Conclusion:** Using nonviral, naked-DNA MC-based vectors, we show for the first time the ability of sustained gene therapeutic correction of male *spf-ash* mice, a model for human X-linked OTC deficiency.

362 - Management of an Adult Patient With Late-Onset Ornithine Transcarbamylase Deficiency After Severe Adverse Reaction to Glycerol Phenylbutyrate

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Patient JS was seen initially at 43 years of age after he presented with episodes of altered behavior, agitation and combativeness to a local emergency room. No significant childhood illnesses or protein aversion was reported. Initial ammonia was reported as 207 μM with a CPK of 409 UI/L, negative urine toxicology, normal liver function tests and normal imaging with no evidence of cirrhosis or portal hypertension. He had reported a prior history of anxiety and obsessive–compulsive disorder treated with desvenlafaxine and citalopram. There was no history of alcoholism. He was involved in power lifting for ten years prior to his admission and was intermittently using dietary supplements and testosterone boosters over the counter to become more competitive. On initial assessment at the clinic while asymptomatic, his plasma ammonia was 46 μM with a plasma citrulline of 16 μM (17-46 μM); arginine of 33 μM (32-120) and urine orotic acid of 0.6 mmol/mol/cr (0.4-1.2). Acylcarnitine profile and lactate were within normal limits. Sequencing for *NAGS*, *CPS1* and *OTC* was completed. *OTC* sequencing showed a c.562G>C mutation at exon 6 (p.G188A). No mutation was detected in the other genes. He was started initially on sodium benzoate at 250 mg/kg/day and was readmitted due to lack of compliance, dietary indiscretion and continued involvement in intense physical activity at work and at the gymnasium. With addition of supplemental citrulline and increased compliance with protein restriction, he gradually decreased his levels of daily activity resulting in adequate control of his ammonia levels and decreased frequency of hospitalizations over time. After stable management for 6 months he was switched to glycerol phenylbutyrate but developed three weeks later a severe reaction consisting on angioneurotic edema requiring management for anaphylaxis including epinephrine and corticosteroids. No other potential allergens were detected. No allergy testing assay was available for glycerol or phenylbutyrate to directly confirm allergenicity. The adverse event was reported. Patient was switched back to sodium benzoate and has been kept on 20 grams a day of citrulline and sodium benzoate with no major adverse medical problems for the next 3 years. Levels of citrulline and arginine have been kept above control values and Benzoate levels have never been in the toxic range.

363 - Gene Therapy Corrects Hyperammonemia and Highlights Nitrosative Stress-Related Cerebral Disease in Argininosuccinate Lyase-Deficient Mice

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Background: The conversion of argininosuccinic acid into arginine is essential to two metabolic pathways: i) the urea cycle for clearance of ammonia in the liver and ii) the citrulline-nitric oxide cycle for synthesis of nitric oxide in most organs. This reaction is catalysed by argininosuccinate lyase (ASL). Patients deficient in ASL present argininosuccinic aciduria, the second most common urea cycle defect. The phenotype is characterised by hyperammonaemic crises and a multi-organ disease with a debilitating neurological phenotype.

Best-accepted therapeutic guidelines aim to correct hyperammonemia but not the systemic nitric oxide imbalance. **Methods:** We investigated the pathophysiology of the neurological disease in argininosuccinic aciduria using the hypomorph $Asl^{Neo/Neo}$ mouse model. An adeno-associated viral vector serotype 8 (AAV8) vector containing the murine *Asl* gene under the transcriptional control of the EFS promoter was administered systemically in neonatal or adult mice and monitored over 9 and 12 months, respectively. **Results:** In mutant mice, we observed neuronal pathology associated with oxidative/nitrosative stress. AAV-treated mice showed correction of both pathways: i) the urea cycle with long-term correction of hyperammonemia although the macroscopic phenotype and other biomarkers were only normalized in the adult-treated $Asl^{Neo/Neo}$ mice; ii) the citrulline-nitric oxide cycle in the brain of neonatally-treated but not in adult-treated $Asl^{Neo/Neo}$ mice. Neuronal disease persisted after normalization of hyperammonemia only in adult-treated animals but was dramatically reduced after correction of the neuronal ASL activity in neonatally-treated mice. Improvement of behavioral studies supported these results. **Discussion:** This highlights i) a new pathophysiological feature of the neurological disease with neuronal oxidative/nitrosative stress not associated with hyperammonemia and ii) the proof of concept of AAV-mediated gene therapy in targeting the central nervous system and the liver, acting on two different metabolic pathways *via* sequential systemic injections of a single vector. This endorses new avenues for treating hepatocerebral inherited metabolic diseases.

364 - Gyrate Atrophy With Hyperornithinemia: Report the First Case in Cuba

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Gyrate atrophy of the choroid and retina (GACR) is an extremely rare inherited retinal dystrophy. The disease is an autosomic recessive disorder, caused by mutations in the ornithine aminotransferase gene (OAT), which is localized on chromosome 10q26. OAT gene mutations result in hyperornithinemia, typically with a 10- to 20-fold elevation of plasma ornithine levels. OAT deficiency causes hyperornithinemia, which results in progressive chorioretinal atrophy. Based on these observations, the aim of this work is to describe the diagnosis of a patient with gyrate atrophy of the choroid and retina through ophthalmological tests and quantification of ornithine in plasma by HPLC. This is the first report of GA in Cuba, diagnosed by ornithine levels in blood and the ophthalmologist assessment.

365 - Hyperphenylalaninemia in Argininosuccinic Aciduria: A Case Report

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Argininosuccinic aciduria (ASA) is an autosomal recessive disorder of the urea cycle, caused by argininosuccinate lyase (ASL) deficiency. ASA is characterized by life-threatening acute attacks with hyperammonemia. Therefore, hyperammonemia should be treated immediately. Herein, we present an 18-month-old male diagnosed with ASA, which was confirmed by biochemical and genetic analyses. This patient initially had normal blood phenylalanine, but due to resistant hyperammonemia, he had to receive long-term and high-dose intravenous infusion of sodium benzoate and sodium phenylbutyrate combination, which led to markedly increased blood phenylalanine levels (1440 μmol/L). The 18-month-old male was from consanguineous parents and diagnosed with ASL deficiency during the newborn period with p.R113 W (c.337C>T) homozygous mutation on ASL gene. He had several metabolic decompensation attacks despite protein restricted diet, oral sodium-benzoate and arginine treatment. In the last attack, he had persistent hyperammonemia, for which we had to administered intravenous sodium-benzoate and sodium phenylbutyrate combination drug infusion and oral phenylbutyrate. We detected high blood phenylalanine level with these drug therapies. There was also a septic infection caused by *Escherichia coli* and vegetation in right atrium which was diagnosed as endocarditis on echocardiography. After the infection was treated, drug therapies were gradually discontinued and a new diet with 0.35 g/kg natural protein was initiated, blood phenylalanine level returned to normal. It is known that phenylbutyrate is converted to phenylacetate in vivo. High phenylethylamine was detected in this patient's urine, which suggested the presence of alkaline pH. We hypothesized that, in the presence of infection and alkaline medium in the blood, high blood phenylacetate may be decarboxylated to phenylalanine, causing an increase in blood phenylalanine levels.

366 - A Mild Intermittent Hyperammonemia Caused by a Novel Mutation in Ornithine Transcarbamylase Gene

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Introduction: Hyperammonemia is caused by inherited and acquired diseases. Urea cycle disorders (UCD) are the main inborn errors that present with hyperammonemia.1 All the urea

cycle enzyme defects are autosomal recessive except ornithine transcarbamylase (OTC) deficiency, which is an X-linked disease. **Clinical Report**: We report a 3-year-old Saudi boy who is a product of a non-consanguineous marriage. He was well until the age of one year when he was admitted to the hospital with pneumonia. He presented again at the age of 2 years with 3 days history of vomiting, poor feeding, and altered mental status. His physical examination showed a lethargic, confused child with signs of moderate dehydration. His liver and spleen were not palpable. His initial laboratory investigations showed serum ammonia of 135 μmol/L (normal <50 μmol/L) with a normal serum lactate and negative urine ketones. Septic workup was normal. He improved on intravenous fluid. He was discharged and booked for follow-up in the outpatient clinic, but unfortunately, missed his appointment. One year later, he presented with vomiting, decreased activity, and feeding associated with sleepiness. Clinical assessment revealed signs of moderate dehydration. Apart from lethargy, central nervous system examination was normal. His initial ammonia level was 178 μmol/L and increased to 269 μmol/L in 6 hours. Intravenous fluids and arginine, Sodium benzoate, and Sodium phenylbutyrate infusion were given. His urine organic acids showed a large elevation of orotate, uracil, 3-hydroxybutyrate, and acetoacetate. Serum amino acids showed glutamine of 630 μmol/l (normal <700), arginine of 105 μmol/l (normal 100-150), and citrulline of 80 μmol/l (normal <200). Our patient responded nicely to ammonia scavenger drugs. His ammonia levels returned to normal within 48 hours, and his clinical examination was normal. He was maintained on oral Arginine, Sodium benzoate, Sodium phenylbutyrate, and low protein formula. Follow-up for one year showed normal development without metabolic decompensation or hyperammonemia. Sanger sequencing of the ornithine transcarbamylase (OTC) gene revealed a novel hemizygous deletion at the fourth nucleotide of intron 4 (c.386+4delT) in the proband and his asymptomatic mother. In conclusion, we have identified a novel splice site mutation in the OTC gene in a patient with mild intermittent hyperammonemia and his asymptomatic mother.

367 - Hepatic Organoids in a Urea Cycle Disorder, Citrullinemia

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In vitro three-dimensional (3D) organoid cultures hold great promise for modeling and cell-based therapies for a variety of complex, genetic, rare and degenerative diseases, some of which have no current treatments available. For liver diseases, such cell-based therapies are lacking and whole organ transplantation and surgical resection are still the major treatment options. This is partly because hepatocytes cannot be grown efficiently in cell culture. Here, we utilize human iPSCs-derived liver organoids for modeling Citrullinemia type I, an inherited urea cycle disorders of the liver which results from deficiency of the enzyme argininosuccinate synthase (ASS1), Dermal fibroblast was used to generate patient-specific human iPSCs via episomal reprogramming and differentiated toward mature hepatocytes under chemically defined conditions. Upon differentiation FACS-enriched progenitor cells were used to develop functional liver organoid cultures. The iPSCs-derived organoids from healthy donors and citrullinemia patients exhibited properties of mature hepatocyte functions such as LDL uptake, albumin secretion, cytochrome P450 metabolism, glycogen storage as well as marker gene expression. Organoids from citrullinemia-derived iPSCs exhibited phenotypes such as increased ammonia and citrulline accumulation, similar to the disease pathology observed in patients. These phenotypes could be rescued by ectopic expression of the wild-type ASS1 gene in patient derived organoids. Furthermore, organoids could be transplanted intrahepatically into immunocompromised mice and were able to integrate into the liver tissues. Thus, in this study, we report for the first time the generation of liver organoids from patient-specific human iPSCs for citrullinemia disease. These personalized iPSC derived-organoid cultures have the potential to be utilized in disease modeling, drug screening, personalized and regenerative medicine. This study was supported by TUBITAK projects with no: 115S465 and 213S182.

368 - Mutation Spectrum and Outcomes of 18 Patients With Citrullinemia Type I: A Single Center Experience From Argentina

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Background: Classical citrullinemia type I (CTLN1) is an autosomal recessive disorder caused by defects in the *ASS1* gene encoding for argininosuccinate synthetase (ASS), the rate-limiting enzyme in the urea cycle. The exact incidence of UCDs in Argentina is not known due to absence of newborn screening or a registry. Most of the patients were detected symptomatically, adding to high morbidity and mortality. Our aim was to evaluate the spectrum and outcome of Argentine patients with CTLN1. **Methods:** Measurement of plasma amino acids and urine orotic acid by HPLC, and ammonia by spectrophotometry; molecular diagnosis by PCR and restriction assays and/or sequencing; *in silico* validation was

performed for new mutations. Inbreeding in couples at risk was achieved by three polymorphic autosomic markers of high frequency in our population, comparison was performed with χ^2 test and GenAlEx. **Results:** We diagnosed 18 CTLN1 patients presenting with hyperammonemia, vomiting, convulsions, failure to thrive and/or lethargy. We confirmed the diagnosis by genetic analysis in all patients. We identified three different genotypes: one patient with c.79T>C/c.847G>A showing a severe early onset phenotype; one patient with c.79T>C/c.970G>A with early onset but mild phenotype; and 16 patients homozygous for the common mutation c.1168G>A from 13 unrelated families from San Luis province and neighboring areas, who all died during the neonatal period. Mutation c.79T>C (p.Gln27*) is novel and causing premature termination of translation in codon 27 instead of 413. The incidence of CTLN1 was previously reported by our group to be 1/2427, suggesting a populational cluster. After this, inbreeding in this population was approached through polymorphic markers indicating no endogamy and a high degree of kinship between the pairs of risk (Fst = 0.25). Additionally, another effect that increases the frequency of carriers could be the transmission ratio distortion of the mutated allele. Out of 18 patients identified 16 died in neonatal period, 1 died at one year of age and the late form continue stable under treatment. **Discussion:** CTLN1 it is the most frequent UCD in Argentina. There should be an increased awareness of preconceptional screening of CTLN1 among individuals/couples who are at risk as the most effective prevention for CTLN1 in the absence of satisfying treatment is good information about reproductive options.

369 - Ornithine Carbamyl Transferase Deficiency: Report of Two Cases

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Background: The urea cycle is the metabolic pathway that leads to the excretion of excessive nitrogen in the form of urea. This cycle involves six enzymes. When one of these enzymes is missing or partially functional, we call it "disorder of the urea cycle." These enzyme deficiencies induce accumulation of ammonia, which is toxic to the central nervous system and liver. We report the cases of two children admitted to our pediatric neurology unit in Rabat Children's Hospital of Morocco for ornithine carbamyl transferase OCT deficiency. **Case report 1:** This is a third female child in a family of four with a history of consanguinity. The child never had vomiting episodes until three months before her admission when she presented behavioral problems, night terrors and vomiting, complicated by a deep coma. The examination was normal except for a hepatomegaly. The biological screening revealed hyperammonemia, high rate of glutamate and a high orotic acid. The diagnosis of ornithine carbamyl transferase deficiency was confirm genetically by the presence in the heterozygous state of the p.Arg92Gln mutation at exon 3. The child was put under strict low protein diet with sodium benzoate, sodium phenyl butyrate, citrulline and arginine supplementations. **Case report 2:** A 2 years old female infant, the product of a nonconsanguineous marriage. The child was hospitalized three times for acute dehydration from vomiting since 8 months old. Admitted at age 17 months with apathy, lethargy and right hemiparesis. A CT cerebral scan made showed an ischemic cerebrovascular accident of the left middle cerebral artery. Clotting and thrombophilia tests are normal. The biological screening revealed hyperammonemia, high levels of glutamate and orotic acid confirmed the diagnosis of OCT deficiency. The genetic study is underway. The child was put under strict low protein diet with sodium benzoate and arginine supplementation. **Conclusion:** We conclude that one should think of hyperammoniemia diagnosis even if symptoms are atypical such as psychologic disorders.

370 - The States of Hyperammoniemia in Pediatrics: Lessons in 10 Years

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In Algeria, screening for hereditary diseases of metabolism is not yet mandatory. Among these conditions, the states of hyperammonemia are at the origin of a high mortality because they are underdiagnosed because little known and therefore not sought after and therefore not treated. **Goals:** Our goal is to collect the hyperammonemias found in children admitted to pediatric intensive care over a period of 10 years (2000-2010) and to investigate their cause. **Material and Methods**: It is a retrospective, monocentric study. The inclusion criteria are: arterial ammonia >150 µL / L on 2 occasions whatever the reason for admission and whatever the age. This assay is supplemented by gasometry, lactatemia, liver function tests (TP), procalcitonin assay and, as far as possible, an assay of orotic acid and citrulline. **Results:** During this period 20 children were recorded with a high inbreeding rate of 70%. Newborns account for 60% (12/20). Among them the majority (11/12) had no infectious risk factor and receive all the antibiotics. The pattern of admission to intensive care was neurological in 14 children (70%) and digestive in 9 children (45%). Biologically, we found an acidosis (70%), hyperlactatemia (70%), hyperammonemia (50%) between 170-250 µL / L and (40%) between 480 and 950 µL / L. The CAA and CAO could only be performed (in France) in only 3 patients to confirm the diagnosis of Triple H, Methylmalonic Acid and Citrullinemia. In the absence of a specific treatment, there is only one survivor (Triple HHH). **Conclusions:** Determination of NH3 in front of any acute vital distress (neurological and respiratory); -NH3 is an emergency parameter such as lactate and

gasometry; - Ammonia should always be integrated into the clinical context -Availability of specific treatment without delay.

371 - Clinical features, Biochemical profile, Neuroradiological Findings, and Treatment in Arginase Deficiency: A Single Center Experience

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Arginase deficiency (AD) is a rare autosomal recessive urea cycle disorder (UCD), caused by mutations in the ARG1 gene encoding arginase which catalyzes the hydrolysis of arginine to ornithine and urea. Patients have hyperargininemia and progressive neurological impairment, but generally suffer fewer episodes of metabolic decompensation compared to other UCDs. **Objective** To describe the clinical features, biochemical profile, neuroradiological findings and treatment of children with AD. **Methods:** We retrospectively examined the medical records of patients with AD treated in our center over the last 20 years. **Results:** 6 patients from 3 unrelated families were identified. All patients presented with gait abnormalities due to lower limb spasticity rather than metabolic decompensation. Mean age at first symptom was 3.8 (1.5-6) years, while mean age at diagnosis was 8.4 (4.1-11.5) years. Neurologically, 4/6 developed diplegia and 2/6 developed quadriplegia with classical features including spasticity, hyperreflexia, clonus, and toe walking. This resulted in gait abnormalities which have been assessed with physiotherapy repetitively, requiring tendon Achilles release surgery in 5 children. Generalized tonic–clonic seizures and/or absences were present in 3/6 children and were well controlled with anticonvulsants. All patients had moderate learning difficulties requiring special educational input. MRI showed cerebral/cerebellar hypoplasia in 4 patients and basal ganglia abnormalities in 2 of them. In terms of the biochemical profile, all patients had hyperargininemia at presentation. Mean arginine level at diagnosis was 642 (427-955), while over the follow-up period was 412 (336-594) μmol/L (normal 42-120). The mean arginine/ornithine ratio was 13.6 (11.7-18.2). Patients had relatively few lifetime hyperammonemic decompensations (ranging 0-6). Mean peak ammonia level during decompensations was 211 (117-426) μmol/L. Neither arginine levels nor arginine/ornithine ratio correlated with the number or severity of metabolic decompensations. Treatment included protein restriction, essential amino acid supplements and ammonia scavengers. Two patients died following severe metabolic decompensation in late adolescence. **Conclusions** Children with arginase deficiency continue to present a challenge to management of what appears to be an inexorable course of neurocognitive impairment. Further insight into disease mechanisms may provide insight into novel treatment strategies.

372 - Pathogenesis, Pathophysiology, and Treatment of Citrin Deficiency

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Citrin deficiency is now well known as a pan-ethnic disease, causing neonatal intrahepatic cholestasis (NICCD) in neonate, failure to thrive and dyslipidemia (FTTDCD) in infant, and adult-onset type II citrullinemia (CTLN2) in adult. Saheki et al described the enzymological properties of argininosuccinate synthetase (ASS) in the liver of the patients who suffered from citrullinemia and hyperammonemia, characterized by liver-specific decreases in ASS protein with no abnormality in ASS gene and named the disease adult-onset type II citrullinemia (CTLN2). Kobayashi et al [1] discovered the causative gene, *SLC25A13*, by homozygosity mapping and named the product of the gene citrin. We identified a number of mutations in *SLC25A13* and showed a wide distribution and a high frequency of the mutations in East Asia including Japan, China, and Vietnam. We also showed that citrin is a liver-type mitochondrial aspartate glutamate carrier (AGC) [2]. Citrin deficiency patients suffer from not only CTLN2 but also many other diseases such as NICCD [3-5] and FTTDCD [6], and the other diseases such as hepatoma, pancreatitis and NASH. The diversity of the clinical states of citrin deficiency is derived from the function of citrin: Citrin as an AGC not only functions in supply of aspartate from mitochondria to cytosol, but also plays a role in transport of cytosolic NADH reducing equivalent from cytosol to mitochondria as a member of malate aspartate shuttle. Related to the functions of citrin, one of the most important characteristics of citrin deficiency is nutritional aspects showing carbohydrate toxicity; intake of carbohydrates deteriorates the patients by causing hyperammonemia. The carbohydrate toxicity was reproduced by an animal model, citrin/mitochondrial glycerol 3-phosphate dehydrogenase double-KO mice [7]. Using the citrin deficiency model mice, we showed the therapeutic effects of sodium pyruvate, protein, amino acid such and medium-chain triglyceride [8]. In the present talk, I will discuss therapeutic effects of amino acids in more detail analyzed with the model mice. [1] Kobayashi K et al. *Nat Genet*.1999;22;159, [2] Palmieri L et al. *EMBO J*. 2001;20:5060, [3] Tomomasa T et al. *J Pediatr*. 2001;138:741, [4] Tazawa Y et al. J 2001;138:735, [5] Ohura T. et al. *Hum Genet*. 2001;108:87, [6] Song YZ et al. I*nt J Mol Med*. 2011;28:33, [7] Saheki T et al. *J Biol Chem*. 2007;282:25041, [8] Saheki T et al. *Mol Genet Metab*. 2012;107:322.

373 - Mitochondrial Carbonic Anhydrase VA Deficiency as the Cause of Neonatal Hyperammonemic Encephalopathy

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Introduction: Mitochondrial carbonic anhydrase VA (CAVA) deficiency is a recently recognized metabolic disorder, resulting from abnormalities in the *CA5A* gene, a gene that plays an important role in ureagenesis and gluconeogenesis. This disorder results in a secondary deficiency of several carboxylases and usually presents in the newborn period with hyperammonemic encephalopathy, hyperlactacidemia, mild hypoglycemia and ketonuria. Patients may later on present encephalopathic episodes during illness but neurodevelopment is mainly unaffected. Until today, less than 20 patients are described in the literature. **Case Report:** We describe an 18-month-old boy born at term to unrelated Caucasian parents who presented at the third day of life with tachypnea, hypotonia followed by encephalopathy associated with hyperammonemia (310 μmol/L). Unlike what is described in urea cycle disorders he presented with metabolic acidosis, hyperlactacidemia, transient hypoglycemia, and ketonuria. Expanded neonatal screening was considered unremarkable. Evaluation of plasma amino acids revealed high levels of glutamine and alanine and organic acids in urine showed high excretion of β-hydroxybutyric, and acetoacetic acids and normal orotic acid. Treatment with intravenous dextrose, a protein free formula, sodium benzoate and phenylbutyrate and L-arginine showed a transient improvement but symptoms resolved and ammonia normalized once carglumic acid was introduced. Sanger sequencing showed a c.774G>C (Gln258His) heterozygous mutation in the *CA5A* gene and MPLA analysis a large heterozygous deletion. He was on a low protein diet and medication for only a few months. Follow-up at 18 months shows normal growth and psychomotor development, normal ammonia levels, and no decompensations, despite several intercurrent illnesses although the patient is not on medication or diet. **Conclusion:** Hyperammonemia can present as a severe life-threatening disorder, secondary to several different etiologies. The correct diagnosis can be a challenge but may be essential for a correct treatment. Carglumic acid seems to be an effective therapeutic option in this disorder. As only few cases of a defect *CA5A* gene have been described, we underline that CAVA deficiency, a potentially treatable condition, should be excluded in neonatal hyperammonemia especially if it is associated with lactic acidosis, hypoglycemia and ketonuria.

374 - Clinical-Molecular Features and Outcome of 20 Turkish Patients With Urea Cycle Disorders

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Objective: The urea cycle is a pathway by which urea is produced from ammonia primarily in the liver. Urea cycle disorders (UCD) are inborn errors of this metabolic pathway. Decreased excretion of nitrogen in the urea cycle due to deficiencies of carbamylphosphate synthase (CPSI), ornithine transcarbamylase (OTC), argininosuccinate synthase (ASS), argininosuccinate lyase (ASL), arginase (ARG) and N-acetylglutamate synthase (NAGS) causes hyperammonemia. OTC deficiency is the most common form and accounts approximately two third of the total cases. Frequently seen sign and symptoms are nausea-vomiting, loss of appetite, failure to thrive, hyperammonemia, liver failure, fatty liver, hepatomegaly, abnormal brain magnetic resonance imaging findings, convulsions, and mental-motor retardation. **Methods:** In this analysis, the clinical data, mutations, and prognosis of 20 patients with UCD from the south part of Turkey were evaluated retrospectively. **Results:** 20 patients (12F/8 M) from 17 families were enrolled in the study. Symptoms were poor feeding (8/20), neurological problems (7/20), and nausea-vomiting (2/20). Two patients were diagnosed with neonatal screening program and one was diagnosed with family history. The median age of symptoms was 3 days (0 day-3 years). 12 patients whom presented in the neonatal period had hyperammonemia. The median level of plasma ammonia was 85 μmol/L (170-1783). Liver dysfunction and metabolic acidosis were detected in 7 and 4 patients, in respectively. Nine patients had citrullinemia, six arginase deficiency, two CPSI deficiency, one OTC deficiency, one ASL deficiency, and one NAGS deficiency. Genetic analyses of the patients revealed 8 novel mutations out of 14 different mutations. Five patients were died. Among the long-term complications growth retardation and mild to severe mental-motor impairment were observed in 18 and 19 patients, in respectively. **Conclusion:** We observed a higher percentage of citrullinemia than reported in the literature among urea cycle disorders. Also with this analysis, 8 new mutations were added to the genotypic spectrum of the disorder. The embarrassing part of our study was the impaired neurological outcome seen in 19 patients, due to late diagnosis and unawareness of physicians about inherited metabolic diseases.

375 - Automated urinary analysis by Nuclear Magnetic Resonance (NMR): Application of the Method to a Newborn With Hyperammonemia

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Introduction: Early-onset metabolic diseases leading to hyperammonemia are challenging emergency situations both for the therapy and the diagnostic workup. Because of the broad differential diagnosis, various analytical methods are necessary. To evaluate NMR technology as a single platform method, we later on also analyzed the original urine sample. **Patient and methods**: A 3 days old, severely ill newborn was transferred from an outside hospital with blood ammonia of >1400 μmol/L (normal <60 μmol/L) under arginine substitution. Common established diagnostic methods as GC/MS, HPLC/MS, Tandem MS, and Ion exchange chromatography (IEC) of blood and urine samples were applied. Later on, an aliquot of this urine sample was investigated by NMR: to 900 μL urine sample 100 μL buffer was added and analyzed with NMR (Bruker IVDr System at 600 MHz) providing automatically concentration of 150 analytes. Sensitivity and specificity of the metabolic panel were validated using concentration curves of spiked test substances. **Results**: Tandem MS in dried blood gave no evidence for organic acidemia or fatty acid oxidation disorder. Urine analysis using IEC showed high excretion of lysine, arginine (under therapy), glutamine and ornithine providing no conclusive diagnosis. In plasma, typical amino acid changes as high glutamine and alanine reflected high ammonia and secondary lactic acidemia. GC/MS in urine confirmed normal organic acids but elevated orotic acid. In addition, orotic acid and its metabolites using HPLC/MS were analyzed. Based on these results the most reliable diagnosis was OTC-deficiency. NMR analysis in urine also provided all diagnostic metabolites to confirm this diagnosis (mmol/mol creatine) (upper normal): glutamine 1522 (205), alanine 1505 (244), lysine 1925 (174), ornithine 2239 (19), arginine 2464 (14), orotic acid 213 (3), uracil 74 (24), lactate 2111 (431) with otherwise normal organic acids. Quantitative congruency with the established methods was excellent. **Conclusion:** NMR urinary analysis approach has the advantage of coverage a broad spectrum of different substance classes, exact quantification, short analysis time (20 minutes) and minimal sample preparation. Future developments have to prove the diagnostic specificity and sensitivity of this new method. Positive diagnostic metabolites are validated rapidly by comparison of the NMR spectra of the pure compounds.

376 - In Vitro Studies in a Rat 3D Brain Cell Model Suggest Creatine as Treatment for Neurotoxicity in Argininosuccinate Lyase Deficiency

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The urea cycle disorder (UCD) argininosuccinate lyase (ASL) deficiency (ASLD) is caused by a defective ASL enzyme (expressed in liver and brain). Patients exhibit a wide spectrum of phenotypes ranging from life-threatening neonatal hyperammonemia to no clinical symptoms but only elevated argininosuccinic acid (ASA) in body fluids. In contrast to other UCDs, even without hyperammonemia, ASLD patients often develop severe mental retardation and seizures. Our aim is to understand the impact on the developing brain of ASA and guanidinosuccinic acid (GSA), specific metabolites that accumulate in ASLD, and to test if creatine (Cr), decreased in ASLD, has beneficial effects. If confirmed, Cr could provide a novel cotherapy for ASLD, which would help prevent neurological damage. Primary rat brain cell 3D cultures were used to mimic ASLD by repeated addition of diverse combinations of ASA (0, 1, 10, 100 μM), ammonia (5 mM), Cr (1 mM) and GSA (0, 1, 10, 100 μM). Two time points were tested representing distinct developmental stages: from days in vitro (DIV) 5 to 13 (neonatal brain) and from DIV 14 to 22 (brain in maturation). After sample harvest and cryopreservation, metabolite measurements, immunohistochemistry, western-blotting and metabolomics were performed. A dose-dependent toxic effect for both ASA and GSA was observed; the astrocytes were the most affected cell type. Increasing ASA concentrations led to decreased expression of the astrocytic marker glial fibrillary acidic protein (GFAP) and to shorter astrocytic processes. At 100 μM ASA, swollen astrocytic cell bodies were observed. Ammonium exposure also led to shorter astrocytic processes and this effect could be reverted by addition of Cr. 100 μM GSA appeared toxic to developing brain cells, leading to a 150-fold increase in lactate dehydrogenase, which points out a drastic increase of cell death. Metabolomics showed separation between treatment groups and harvest dates, especially in presence of ammonia. Beside the well-known neurotoxic effect caused by ammonia, ASA and GSA also showed adverse effects in developing and mature brain cells. Co-treatment with Cr prevented some of the effects caused by ASA and ammonia in the 3D model used here. We therefore suggest further

evaluation of Cr as co-therapy for ASLD to prevent neurological damage. Important for later use in humans, Cr is commercially available, and it is used as treatment for disorders in Cr synthesis with well-established doses in all age groups.

377 - A Genetically Engineered E. coli Nissle to Prevent Hyperammonemia in Urea Cycle Disorder (UCD)

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Urea cycle disorders (UCDs) are a group of inherited diseases where the inability to efficiently convert waste nitrogen into urea leads to the toxic accumulation of systemic ammonia (NH_3). The gut is known to be a major source of systemic NH_3, thus capturing part of intestinal NH_3 may mitigate disease symptoms. To carry out this therapeutic strategy, *E. coli* Nissle (EcN), a well-characterized probiotic, was engineered to convert NH_3 to arginine (Arg) in the intestine by deleting a negative regulator of Arg biosynthesis and expressing a feedback-resistant Arg biosynthetic enzyme. In vitro data show that engineered strains, named SYNB1010 and SYNB1020 (SYNB), are able to consume NH_3 and produce Arg at a rate of 2.4 and 0.65 μmol/10^9 cells/hour respectively. Pharmacokinetic studies done in mice dosed orally with 10^{10} SYNB cells, showed that ~2.5% of bacteria survived transit through the GI tract, maintained their activity within the cecum and colon for 8 to 12 hours and gradually shed from the GI tract over 30 hours. qPCR studies of fecal shedding of SYNB strains in mice and the cynomolgus monkey showed no detectable sequences by 7 days following the last dose. Tracer studies using ^{15}N-labeled Arg were conducted to determine the fate of the Arg produced by SYNB in vivo. $^{15}N_4$-Arg spiked in mouse cecal content ex vivo was rapidly converted to ^{15}N-labeled citrulline, ornithine and NH_3, most likely by the combined activity of the microbiome arginases, arginine deaminases and ureases. In addition, $^{15}N_4$-Arg introduced into the mouse colon by enema was almost entirely excreted in urine in the form of $^{15}N_1$ and $^{15}N_2$-labeled urea, in a 3:2 ratio. Together, those results suggest that the Arg produced by SYNB in the mouse GI tract may be metabolized in the intestine, absorbed and incorporated in the urea cycle in the form of intact Arg and its degradation products. The efficacy of SYNB in lowering NH_3 was tested in *spf-ash* mice, a genetic model of UCD. In this model, SYNB was able to prevent systemic hyperammonemia caused by a high-protein diet. The safety of SYNB was tested in a 28-day non-GLP toxicology study in non-human primates and in a 28-day GLP toxicology study in mice. In both cases, no adverse test article-related effects were observed on animal health. The design of a phase 1 clinical trial evaluating the safety and tolerability of SYNB1020 following single and multiple doses in healthy volunteers, will be presented.

378 - OCT Deficiency in Females: 3 New Moroccan patients

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OTCD is the most frequent urea cycle disorder. It results from mutations in the OTC gene encoding a 354-residue polypeptide. The clinical picture in females is highly variable even within a family, depending on the X-inactivation pattern in liver. We present 3 girls, one aged of 2 years and two of 2 years and half who manifested recurrent vomiting, growth retardation, encephalopathic and psychotic episodes, and behavioral troubles. Investigations revealed hyperammonemia episodes, high plasma glutamine with decreased or normal citrulline and arginine. Elevated urinary orotic acid was the key parameter suggestive of ornithine trancarbamylase deficiency. DNA analysis (sequencing of all exons and adjacent intronic regions) in two patients revealed a heterozygous mutation respectively in the exon 3 (c.275G>A) and in the exon 4 (c.416T>G). The administration of a low protein diet, Sodium phenylacetate, sodium benzoate, and L. arginine led to rapid improvement of biological and clinical states. The mutations were explored by sequencing of the exon 3 and 4 of OTC gene in the parents and family members. No carrier was identified suggesting that the detected mutations could be neo-mutations.

379 - Clinical and Laboratory Characteristics of the Patients With Urea Cycle Disorders: 40 Years' Experience of Hacettepe University

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This study aimed to evaluate the characteristics of urea cycle disorders in Turkey. We retrospectively investigated the patients with urea cycle disorders diagnosed and followed in our clinic from 1979 to 2016. Hyperammonemia due to other metabolic disorders and unconfirmed cases were excluded. Patients' demographic characteristics, clinical and laboratory data at diagnosis, genetic investigation results, and current status of the patients were noted. Variance analysis, Mann-Whitney tests, and Kruskal-Wallis tests were used to compare between group variables. Pearson chi-square test was also used for correlation analysis. 85 patients and 129 hyperammonemic episodes were included. The most common type was citrullinemia type 1(51.8%) followed by ornithine transcarbamylase deficiency (24.7%). 42 of patients were confirmed by genetic

analysis and 4 of mutations had not been reported so far. Of the 85 patients, 26 were lost to follow-up, 3 were followed by another center and 46,4% of the follow-ups were dead. Mean follow-up duration were 4.17 years (0 days-26.83 years). 30% of surviving patients had normal intelligence, while the rest were found to have developmental and mental retardation at various levels. There were no obvious triggers in most of the hyperammonemic crisis. 84.8% of the hyperammonemic episodes were recovered and the rest resulted with death. The initial and peak ammonia levels were associated the most with mortality and neurological outcome. We also found that the high ammonia levels were associated with hypocalcemia, hypernatremia and polycythemia. We also identified 4 new genetic changes that needs to be confirmed by functional analysis.

380 - Citrin Deficiency: Assessment of the Carrier Frequency and Identification a Novel Ashkenazi Jewish Founder Variant with Unique Clinical Phenotype for an Underdiagnosed Urea Cycle Disorder in the US

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Introduction: Citrin deficiency (CD) is an autosomal recessive urea cycle disorder caused by mutations in the *SLC25A13* gene that encodes citrin, an aspartate-glutamate carrier on the mitochondrial inner membrane. CD can manifest as self-limited neonatal intrahepatic cholestasis (NICCD) during infancy, failure to thrive with aversion to carbohydrate-rich foods during childhood, or citrullinemia type II (CTLN2) characterized by acute onset of neuropsychiatric symptoms and life-threatening hyperammonemia during adulthood. While it was previously thought that this condition was confined to East Asian populations, an increasing number of non-Asian CD cases have been identified. Available natural history data suggest that individuals who manifest CTLN2 in adulthood do not necessarily manifest NICCD in infancy. Thus, it is challenging to make early diagnosis of asymptomatic individuals with CD who are at-risk for life-threatening CTLN2 due to limited information of CD disease frequency and natural history in non-Asian populations. **Objective:** To assess carrier frequency of CD in the US by NGS carrier screening and to characterize the clinical findings of CD in individuals in the testing cohort. **Methods:** We used the database of an NGS carrier screening test performed at the Mount Sinai Genetic Testing Laboratory to evaluate the frequency of pathogenic *SLC25A13* alleles. Retrospective chart review of patients with molecularly confirmed CD was performed. **Results:** Among the 50 054 alleles screened, we detected 30 different pathogenic/likely-pathogenic variants in *SLC25A13* in 122 heterozygous and, surprisingly, two homozygous individuals. Based on these findings, we estimated a carrier frequency of 1/199 in an unselected US population. Eleven variants were novel. Additionally, we identified three Ashkenazi Jewish individuals homozygous for c.1336A>C, p.T446P, including two asymptomatic adults-one identified by the screening. These adults were in good health without detectable biochemical abnormalities or growth deficiency but they were identified to have self-reported aversion to high-carbohydrate diet and to alcohol. Abdominal ultrasounds showed incidental hepatic steatosis. **Conclusions:** We have identified a CD founder mutation in the Ashkenazi Jewish population in the US. Aversion to alcohol and asymptomatic hepatic steatosis may be important clinical phenotypes for CD. CD may be more prevalent than previously thought and underdiagnosed in non-Asian populations.

381 - Comprehensive Biochemical and In Vivo Flux Studies in the Spf-Ash Mouse, A Model for Human X-Linked OTC Deficiency

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Background: The most common primary defect of ureagenesis is the X-linked ornithine transcarbamylase deficiency (OTCD), a main target for novel therapeutic interventions in urea cycle defects. To study human OTCD and to test potential new therapies, the sparse-fur and abnormal skin and hair (spf-ash) mouse is a valid and widely used animal model. These animals carry a point mutation in exon 4, c.386G>A (p.R129 H), which is also found in human patients and affects splicing with partial intron 4 retention. Furthermore, male spf-ash mice have a mild biochemical phenotype with low levels of OTC enzyme activity around 5%-10% of wild-type, resulting in elevated urinary orotic acid but no hyperammonemia. The aim of this work was to better characterize these mice and to test novel low- or non-invasive methods. **Methods:** For a full biochemical characterization of mice, we determined amino acids in dried blood spots, urinary orotic acid, blood ammonia levels, and liver OTC activity. In addition, we measured in vivo ureagenesis from dried blood spots using [^{15} N]ammonium chloride as tracer. **Results:** We observed in spf-ash mice, despite some overlap with wild-type animals, that arginine and citrulline were significantly decreased ($P < .0001$) while urinary orotic acid was normal to elevated, but still significantly different from wild-type ($P < .0001$). Some animals presented normal values of amino acids and also orotic acid, despite low enzyme activity. Likewise, ammonia levels fluctuated from normal to elevated, possibly dependent on the animal stress during blood collection. Follow-up of ureagenesis in spf-ash mice over time showed a relative small intra-individual variation, but some animals had a ureagenesis capacity up to wild-type levels.

Conclusion: The spf-ash mouse model, despite being used for many years for studies of new therapies, shows a highly variable biochemical phenotype; therefore, to follow individual mice is important to confirm the outcome of novel treatment applications. As in humans, amino acids, ammonia and urinary orotic acid are not reliable outcome parameters. Our studies provide a caveat for research using the spf-ash mouse and underline the need to carefully characterize animals at baseline and to consider especially the mouse background strain.

382 - Follow-up of the Two Cases With the Diagnosis of Neonatal Citrin Deficiency

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Citrin deficiency is due to lack of function of the hepatic mitochondrial aspartate/glutamate transporter/carrier citrin which is caused by the mutation in SLC25A13 gene. There are two main age-dependent clinical presentation. We presented two cases with Neonatal Intrahepatic Cholestasis Caused by Citrin Deficiency (NICDD). A 5-month-old girl referred to our clinic due to cholestasis, urinary reductant positivity, elevated blood total galactose and free galactose. On her physical examination hepatosplenomegaly and icterus was detected. In laboratory investigations, galactose1- phosphate uridyl transferase enzyme activity was normal, elevated direct bilirubin levels and transaminase levels were detected. Also, alpha fetoprotein level was very high at 767969 ng / mL (N: 46 $\pm$ 19). Blood amino acid analysis revealed high citrulline, methionine, tyrosine, threonine levels. SLC25A13 gene analysis revealed homozygous p. R319X (c.958C>T) mutation. Lactose (galactose) free diet was started, she was died at the age of 7 months. A two-year-old girl was admitted to our clinic with prolonged jaundice and increased in urine galactose when she was 4 months of age. Total galactose level was high. Galactose 1 phosphate and galactose-1- phosphate uridyl transferase enzyme activity were normal. Blood amino acid analysis revealed elevated citrulline, threonine, methionine, tyrosine levels. In SLC25A13 gene analysis, p.L598 R (c.1793T> G) mutation was detected. Lactose (galactose) free diet was started. Regular follow up demonstrated normal growth and development. NICCD deficiency should be considered as a differential diagnosis in any infant with cholestasis and persistently positive urinary reducing substances. Lactose (galactose) free diet shows improvement in clinical and laboratory findings in these patients. The cases were presented in order to emphasize the importance of early diagnosis of the patients with NICDD.

383 - Argininosuccinate Synthetase Deficiency: Sixteen Patients From a Single Center

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Background: Argininosuccinate synthetase deficiency (ASSD) (OMIM215700) is an autosomal recessive disorder of the urea cycle in which the cytosolic enzyme is lacking. In the classical form of this disease, symptoms manifest during the neonatal period as progressive lethargy, poor feeding, and central nervous system depression secondary to hyperammonemia. **Objectives:** The aim was to evaluate symptoms at presentation of patients with argininosuccinate synthetase deficiency (ASSD)(OMIM215700). **Methods:** Demographic data and clinical findings of 16 patients with ASSD at referral were reviewed. **Results:** Sixteen patients (9 females, 7 males) diagnosed with ASSD were studied. The present age of the patients ranged from 1,5 to 31 years with a mean of 13 years. Most of the patients (n:12) presented during newborn period and infancy (median 4 months). Mean age at referral was 35.8 $\pm$ 21.7months (range:1 day- 264 months). Consanguinity was reported in 10 patients. Clinical and biochemical evaluation of patients with ASSD showed that most cases presented with vomiting (n: 9), acute encephalopathy (n: 7), psychomotor retardation (n: 7), respiratory failure (n: 5) and hypotonia (n: 5). Two patients were diagnosed during pregnancy one with psychotic behavior due to acute hyperammonemia, and the other with acute hepatic failure. The diagnosis was confirmed by enzyme assay or molecular diagnosis following the determination of elevated citrulline levels in plasma. **Conclusion:** Evaluation of presenting symptoms of patients help to increase knowledge about the natural history of rare disorders. ASSD is an inborn error of metabolism that can present with a wide variety of clinical symptoms, during any period of life.

K) Organic Acidurias: Branched-Chain (384 to 415)

384 - Redox Homeostasis Disruption, Src Kinase Phosphorylation Increase, and Neuronal Damage Induced by the Major Accumulating Metabolites in 3-Hydroxy-3-Methylglutaric Aciduria in Rat Cerebral Cortex

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3-Hydroxy-3-methylglutaric aciduria (HMGA) is a neurometabolic disorder characterized by predominant tissue accumulation and high urinary excretion of 3-hydroxy-3-methylglutaric (HMG) and 3-methylglutaric (MGA) acids. Affected patients predominantly present neurological symptoms, whose pathophysiology remains to be established. In the present work, we evaluated the effects of an acute intracerebroventricular (icv) administration of HMG and MGA on redox homeostasis, reactive oxygen species (ROS)-sensitive signaling pathways and neuronal damage in cerebral cortex of Wistar rats. One-day-old Wistar rats received icv injection of HMG (0.5 μmol/g), MGA (0.5 μmol/g) or PBS (control group; vehicle), and were euthanized 24 h after the administration. Cerebral cortex supernatants were then prepared to evaluate malondialdehyde (MDA) and reduced glutathione (GSH) levels, antioxidant enzyme activities and content of proteins involved in ROS-sensitive kinase phosphorylation pathways, as well as of Tau protein and synaptophysin, indicators of neuronal injury. HMG and MGA icv administration increased MDA levels and superoxide dismutase activity. We also verified that both organic acids decreased the activity of glutathione peroxidase, whereas only MGA diminished glutathione reductase activity and GSH concentrations, the most important cerebral antioxidant. Furthermore, HMG increased Src kinase phosphorylation, while decreased Tau protein phosphorylation and synaptophysin content. In contrast, HMG did not alter p38, ERK1/2 and JNK phosphorylation, and heme oxygenase-1 content. Our findings suggest that disruption of redox homeostasis caused by the metabolites accumulating in HMGA leading to Src kinase phosphorylation increase and neuronal damage are relevant pathomechanisms involved in the neurological dysfunction found in patients affected by this disorder. **Supported by:** CNPq, CAPES, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP IBN-Net and INCT-EN.

385 - Autism Spectrum Disorder as a First Presentation of Propionic Acidemia

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Autism spectrum disorder (ASD) is a neurodevelopmental syndrome, diagnosed solely on the basis of the triad of persistent social and language deficits and stereotypic behaviors. Currently a genetic cause can be identified in 5% to 20% of children with ASD. Autism have been reported in association with inborn errors of metabolism (IEM) with a rate higher than that found in the general population. IEM can be found in less than 5% of autistic individuals. In these cases, autistic behavior is usually accompanied by clinical signs characteristic for IEM such as epilepsy, ataxia, lethargy, cyclic vomiting, and intellectual disability. In rare cases, however ASD may be considered as isolated at the onset of a metabolic disease. We describe a 3-year-old girl who was referred to the child psychiatrist because of behavioral problems such as repetitive and stereotyped movements, poor eye contact, isolation from other children. A diagnosis of ASD was made. Several months later she presented with severe metabolic acidosis, hyperammonemia, and hypoglycemia during a gastrointestinal infection. The patient was diagnosed with propionic academia (PA) after metabolic evaluation with tandem mass spectrometry and urine gas chromatography–mass spectrometry and a proper treatment was started. Early diagnosis of metabolic disorders in patients with autism is important because a timely treatment can improve behavioral disturbances.

386 - Identification of Six New Mutations on Chilean Population With Maple Syrup Urine Disease

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Introduction: Maple Syrup Urine Disease (MSUD) is an autosomal recessive hereditary metabolic disease caused by the deficiency of the branched-chain α-keto-acid dehydrogenase (BCKD) enzyme complex. BCKD is a mitochondrial complex encoded by four genes (BCKDHA, BCKDHB, DBT and DLD) and is involved in branched chain amino acid metabolism (BCAA). **Objective:** To identify the point mutations in BCKDHA, BCKDHB and DBT genes in a cohort of Chilean patients diagnosed clinically and biochemically with MSUD. **Methods:** A cross-sectional descriptive study with 18 patients diagnosed clinically and biochemically with MSUD. The BCKDHA, BCKDHB and DBT genes were analyzed by polymerase chain reaction (PCR) and sequencing. Sequencing reactions were done using the BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied®) kit. The sequences of the coding regions of the three genes were studied using DNA genomic from peripheral blood leukocytes. All analyzes were carried out at the Laboratory of Molecular Genetics and Bioinformatics of the Regional Hemotherapy Center of Ribeirão Preto, with support from the Research Support Center - Integrated Systemic Biology Center (NAP - CISBi) of the University of São Paulo. In silico analysis of nucleotide substitutions was performed to evaluate the pathogenicity of the new mutations

through the software MutPred® v1.2, Polyphen-2® - Polymorphism Phenotyping v2 and SIFT®. **Results:** Of the 18 patients 100% presented mutation in the BCKDHB gene and only 1 patient (5% of the total) presented mutation in the BCKDHA gene. A total of 11 mutations were found in the sample and 6 new mutations (54.4%) were found. Of the mutations already described in the literature, the I214 L mutation of Spanish origin was the one with the highest incidence, totaling 44.4% of the patients. The new mutations found were c [375T> A], c [1006G> A], c [1083T> G], c [1041_1042insGGTGGA], and c [718C> A], located in the BCKDHB gene and c. [1025G> A] localized in the BCKDHA gene. Exons 4 and 10 of the BCDKHB gene were the ones that presented the highest number of mutations, being considered hot spots, followed by exons 6, with 2 mutations and exons 5 and 10 with one mutation each. **Conclusion:** This study reported 6 new mutations in patients with MSUD in the Chilean population, which may help in future genetic diagnosis of the disease.

387 - Experimental Evidence That the Metabolites Accumulating in Propionic Acidemia Impair NADH-Linked Oxidative Metabolism

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Propionic acidemia is an inherited metabolic disorder biochemically characterized by predominant accumulation of propionic (PA) and 3-hydroxypropionic (3OHPA) acids. Maleic acid (MA), a product of the intermediary metabolism structurally similar to PA, is also found at high concentrations in urine of these patients and was shown to provoke proximal tubule cell damage resembling Fanconi syndrome. Considering that patients affected by propionic acidemia usually develop chronic renal failure along development whose pathogenesis is yet unknown, we investigated the effects of MA, PA and 3OHPA on mitochondrial oxidative metabolism in mitochondrial preparations from kidney of young rats. Resting (state 4), ADP-stimulated (state 3) and CCCP-stimulated (uncoupled) respiration measured by oxygen consumption were evaluated using NADH- and $FADH_2$-linked substrates, as well as the activities of α-ketoglutarate dehydrogenase (α-KGDH) and glutamate dehydrogenase (GDH). MA strongly decreased oxygen consumption in state 4, state 3 and uncoupled respiration using glutamate plus malate and especially α-ketoglutarate (up to 90%) as substrates, with less intense effects observed when using pyruvate plus malate and particularly succinate respiring mitochondria. In addition, PA moderately decreased state 3 and uncoupled respiration using pyruvate plus malate or glutamate plus malate-supported mitochondria (up to 40%), whereas 3OHPA provoked the less evident effects demonstrated by a mild decrease in states 4 and 3 respiration when mitochondria was supported by succinate (up to 20%). It was also observed that MA strongly decreased the activities of α-KGDH and GDH that may possibly be associated with the observed effects provoked by this organic acid on glutamate and α-ketoglutarate-supported mitochondria. It is therefore presumed that MA and PA but to a lesser extent behave as metabolic inhibitor of oxidative metabolism in kidney mitochondria and might be involved in the chronic renal failure occurring in propionic acidemic patients. **Financial support:** We thank PROPESQ/UFRGS, FAPERGS, and CNPq.

388 - Methylmalonic Aciduria in Saudi Patients: Report of Two Novel Homozygous Mutations in the Methylmalonyl-CoA Mutase Gene

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Introduction: Methylmalonic aciduria (MMA) is a genetically heterogeneous disorder caused by an inborn error of methylmalonate and cobalamin metabolism. Mutations in the methylmalonyl CoA mutase (*MUT*) gene are the main cause of isolated MMA. **Clinical report**: Patient 1 is 6-day-old baby boy presented with decreased activity and poor feeding His parents are first cousins. His initial evaluation showed a lethargic, sick looking baby with severe dehydration. The rest of the systemic examination was unremarkable. He received fluid resuscitation, covered with broad-spectrum antibiotics, and electively intubated and ventilated. His baseline investigations showed hypoglycemia, metabolic acidosis, ketonuria, and serum ammonia of 633 μmol/L (normal value [NV] <80 μmol/L). The working diagnosis was organic acidemia and accordingly, our patient was commenced on ammonia scavenger medications, oral carnitine, and hydroxocobalamin injections. Four hours later, ammonia was increased to 917 μmol/L and hence, continuous renal replacement therapy was started, and the ammonia gradually decreased to reach 298 in less than 24 hours. However, on the second day of admission he deteriorated, became hemodynamically unstable despite being on maximum inotropic support. He developed severe coagulopathy and died of presumed sepsis while the ammonia level was below 300 μmol/L. The result of urine organic acids was consistent with MMA. Urine organic acid showed highly elevated methylmalonic acids, 3-hydroxypropionic acids, and methylcitrate. Acylcarnitine profile showed high propionylcarnitine. Sanger sequencing detected a homozygous novel mutation (c.329A>G; p.Y110C) in the *MUT* gene in the patient and a heterozygous in parents. Patient 2 is a 3-month-old boy who presented with a sepsis-like picture, metabolic acidosis, and hyperammonemia. Investigations revealed high propionylcarnitine (C3), elevated urinary methylmalonic acids,

3-hydroxypropionic acids, and methylcitrate, consistent with MMA. Sanger sequencing detected a homozygous novel mutation (c.2200C>T; p.Q734X) and a heterozygous in parents. **Conclusion**: In conclusion, we identified 2 novel mutations in the MUT gene causing isolated MMA.

389 - 3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency: A Case Report and Literature Review

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Background: 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG-CoA-LD) usually presents in the neonatal period with vomiting, metabolic acidosis, hypoglycemia and absent ketonuria. The primary aim of management is a low protein, high carbohydrate diet, avoidance of extended fasts and with rigorous emergency management during infection. Fat restriction has also been advocated. However only few case reports are described and optimal dietary management is not established. **Case Report:** A 5-month-old girl with non-ketotic hypoglycemia, very low blood ketones and metabolic acidosis, elevated 3-hydroxyisovalerate, 3-methylglutaconate, 3-hydroxy-3-methylglutarate and 3-methlycrotonylglycine was diagnosed with HMG-CoA-LD. She commenced carnitine, low protein diet (1.5g/kg/day natural protein) without leucine-free L-amino acids or fat restriction. Her actual nocturnal fasting time was $\leq$ 8 hours but when fasting tolerance was formally assessed at 10 months, results indicated a maximum safe fasting time of only 7 hours with free fatty acids elevated to 1327 μmol/L. By the age of 11 months, she had 11 hospitalizations, mainly with recurrent vomiting, metabolic acidosis and hyperammonemia without obvious contributing factors. As a consequence, overnight continuous tube feeding was commenced and at her current age of 20 months, there has been no further recurrence of unexplained hypoglycemia. Her developmental progress is within normal limits. Her weight is between the 25 and 50th percentile and her length is on the 25th percentile. **Conclusion:** In HMG-CoA-LD, dietary recommendations have varied for protein, leucine and fat restrictions and the use of leucine-free L-amino acids and fasting tolerance is not well reported. This case study exhibited limited nocturnal fasting tolerance and poor metabolic control when treated with a low protein diet only. Establishing disorder severity at an early stage of management is essential to optimize clinical outcome. Evaluating fasting tolerance immediately post diagnosis will help establish the requisite for night feeding in individual cases. It is important to monitor clinical progress, document dietary intake and biochemical markers carefully to systematically evaluate the role of nutritional intervention. Higher numbers of case studies should be collected with detailed dietary management, fasting times and outcome to improve future treatment.

390 - Elucidating the Mitochondrial Architecture of Branched-Chain Amino Acid Metabolism Enzymes

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Introduction. Branched chain amino acid metabolism (BCAA) is based in the mitochondrial matrix and comprised of 17 enzymes, some shared, organized into three pathways for the catabolism of leucine, isoleucine, and valine (LEU, ILE, and VAL respectively). However, the physical relationships of the various enzymes in the pathways are unknown. Diseases such as isovaleric academia (IVA) and maple syrup urine disease (MSUD) are among the disorders caused by genetic deficiencies of BCAA metabolism. **Methods**. In this project, we examined the BCAA pathways and their physical interactions through in vitro proteomics analysis and in vivo using stimulated emission depletion (STED) super-resolution microscopy. The functional interactions of the pathways were measured by flux analysis with stably labeled LEU, ILE, and VAL in patient and wild type cell lines, with quantification of the labeled metabolic end-products of each pathway. **Results**. Proteomic and imaging studies are consistent with the existence of an energetically favorable, metabolite-channeling BCAA supercomplex. In the presence of a chemical cross-linking agent, liver mitochondrial proteins migrate on a denaturing gel to form single bands containing multiple BCAA proteins. Flux studies show that the end product of LEU, ILE and VAL metabolism are generated in both wild type and patient derived cells lines, implying cross-talk of BCAA pathways and closeness of shared enzymes. Additionally, we have found the end-product of ILE and VAL, propionyl-CoA, does not readily enter the TCA cycle, as previously thought. **Discussion**. These results provide novel insight into the BCAA metabolism and offer new opportunities for the development of therapeutic agents for their defects. Furthermore, because propionyl-CoA derived from odd-chain fatty acids does, in fact, readily enter the TCA cycle, results from the metabolic flux studies will impact potential anaplerotic therapies for disorders of fatty acid oxidation and BCAA metabolism.

391 - Oxysterol Levels in Organic Acidemia Patients: Preliminary Results

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Aim: Organic acidemias are a group of inborn errors of metabolism (IEM) caused mostly by a deficient enzyme in the catabolic pathways of amino acids and characterized by abnormal urinary excretion of organic acids. The pathophysiology of these diseases is poorly known and oxidative stress has been proposed as an important pathophysiologic feature of various IEM. Considering that there are few studies relating oxidative stress directly in organic acidemias, we aimed to evaluate markers of oxidative damage in organic acidemia patients. **Methods**: The blood samples of 71 organic acidemia patients were collected during their routine clinic visits or when they refer to emergency department with a complaint. The control group consisted of 60 healthy children. The plasma levels of oxidation biomarkers, 7-keto cholesterol (7-KC) and cholestane-3β,5α,6β-triol (C-triol) that result from non-enzymatic oxidation of cholesterol, were determined using the LC-LC/MS method. **Results:** Seventy-one organic acidemia patients (35 males and 36 females) were aged between 9 months and 25 years. The patient group consisted of 29 maple syrup urine disease, 16 methylmalonic acidemia, 11 propionic acidemia, 7 isovaleric acidemia, and 8 glutaric acidemia type 1. There were 75 episodes: 63 of them were during routine clinic visits and 12 of them were when they referred to emergency department with a complaint. Significantly higher plasma 7-KC levels were found in the patient group [median 32.59 ng/mL, min-max 26.94- 40.11 ng/mL] compared to the controls [median 29.73 ng/mL, min-max 10.36-22.69 ng/mL] ($P < .001$). We also observed a statistically significant increase in plasma C-triol levels in organic acidemia patients [median 21.34 ng/mL, min-max 18.10-25.70 ng/mL] compared to the control group [median 12.59 ng/mL, min-max 3.61-18.07 ng/mL] ($P < .001$). Plasma 7-KC and C-triol levels didn't significantly differ between organic acidemia patient subgroups ($P = .893$ and $P = .470$, respectively) and between routine clinic visit group and emergency department group ($P = .750$ and $P = .483$, respectively). There was no significant correlation between plasma 7-KC and C-triol levels with serum total cholesterol and LDL levels. **Conclusion:** These findings may suggest that pro-oxidant state occur in organic acidemia patients. In this context, it is possible to suggest that, in the future, antioxidants could come to be used as adjuvant therapy for patients affected by these diseases.

392 - Exposure of Normal Human Primary Hepatocytes to Propionate Replicates Aspects of Biochemical Features in Propionic Acidemia

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Propionic acidemia (PA) is an autosomal recessive disorder of propionate catabolism caused by a deficiency in the enzyme propionyl CoA carboxylase (PCC). The functional consequence of PCC deficiency is the inability to catabolize propionyl-CoA (P-CoA) to methylmalonyl-CoA, resulting in the accumulation of P-CoA and other metabolic intermediates, such as propionylcarnitine, 3-hydroxypropionic acid, and methylcitrate. P-CoA, at the high concentrations found in PA, causes the inhibition of liver N-acetylglutamate synthetase (NAGS), impacting ureagenesis and inducing hyperammonemia. In addition, P-CoA and its metabolic intermediates reduce mitochondrial energy production by inhibition of enzymes in the tricarboxylic acid (TCA) cycle. We previously showed that metabolic defects of PA could be recapitulated using PA patient-derived primary hepatocytes in a novel organotypic system (Chapman et al, MGM 2016). Here, we sought to investigate whether propionate treatment of normal human primary hepatocytes would recapitulate some of the biochemical features of PA in the same platform. We found that high levels of propionate resulted in high levels of intracellular P-CoA in normal hepatocytes similar to the levels found in PA hepatocytes. Propionate treatment also induced increases in ammonia and decreases in urea. Analysis of TCA cycle intermediates indicated that propionate is incorporated in the TCA cycle. However, the pattern of regulation of TCA intermediates, suggested that propionate treatment may also inhibit enzymes of the TCA cycle as shown in PA. In summary, propionate treatment on normal primary hepatocytes recapitulates some key biochemical features of PA.

393 - To Understand Genetic and Biochemical profiles in Indian Patients With Glutaric Aciduria Type I

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Objective: Glutaric aciduria type I (GA-I) is a metabolic disorder of amino acid metabolism caused due to deficiency of glutaryl-CoA dehydrogenase (GCDH). In India, there are few studies and controversial reports on its genetic etiology. There are no studies from this region, showing correlation between

genetic and biochemical phenotypes. Hence, the objective of this study is to understand genetic and biochemical profiles in GA-I patients from India and to recognize the existence of correlation between them. **Methods:** The study was approved by Institutional ethical committee. Blood and urine samples were collected from 33 confirmed GA-I patients from unrelated families. The urine samples were used for measuring glutaric acid, 3-hydroxyglutaric acid and glutaconic acid levels by GC-MS. The blood spots were used for measuring the glutaryl carnitine levels by tandem mass spectrometry. All the 11 exons of GCDH gene were amplified using exon specific primers by PCR and sequenced to screen mutations. Those patients carrying mutation/s in GCHD gene were included in establishing the correlation between genetic and biochemical phenotypes. **Results:** In this study, 30 different mutations were identified in GCDH gene. Thirteen (43.3%) were novel and 17(56.7%) were known or reported mutations. Among novel mutations, 6 (46.2%) were missense (I152 M, E99G, R234P, Q144P, R372G, and T344I), 1 (7.4%) nonsense (E414X), 1 (7.2%) deletion (g.11629delG), 2 (14.4%) insertion (g.10205_10206 Ins CTATGATCATC and g.11628_11629insT), and 3 (23.1%) were silent (Y295Y, Q29Q, and Q333Q). Three (10%) low excretor mutations were found in this study. Exons 7, 8 and 10 of GCDH gene are hotspots for mutations. There was no strong correlation exist between genotype and biochemical phenotype in our study subjects, but it was observed that combined homozygous condition showed high levels excretion of urine organic acids. **Conclusion:** We therefore conclude that despite ethnic diversity in India R402 W, is commonly occurring mutation in Indian patients with GA-I. The W225X mutation was found exclusively among GA-I patients from southern origin. In our population, low excretor phenotype exists and P286 S, is the most common low excretor mutation found among them. Clear correlation did not exist between genotype and biochemical phenotypes in GA-I. The finding from this study can be extended in DNA based non-invasive prenatal screening in high-risk pregnancies.

394 - Clinical, Molecular Data and Outcome of 25 Iranian Patients Affected of Isolated Methylmalonic Aciduria: A Case Series

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Objective: Isolated Methylmalonic aciduria (MMA) is a rare autosomal recessive disorder caused by a defect in enzyme methylmalonyl coA mutase (mut^0or mut^-) or its cofactor adenosylcobalamin (cblA, cblB, or cblD variant2). Identification of prognostic factors would be useful to prevent disease complications and good long-term follow-up. In this study, we aim to report a case series of isolated methyl malonic aciduria to explore clinical, molecular characteristics and their outcome. **Methods:** Isolated methyl malonic aciduria patients treated in Research Unit of Iranian National Society for Study on Inborn Errors of Metabolism from 2006 to 2016 entered the study. Diagnosis was made by died blood spot acyl carnitine profile studied with MS/MS and urine organic acid profile studied by GC/MS. Genotyping was done if the family could afford. After standard MMA therapy, a retrospective descriptive analysis was done on 25 patients' data. **Results:** 25 patients:13 ♀,12♂; 23/25 consanguineous (92%); Mean Birth Body Weight: 3074 (2450-3700 gr); Mean Birth Head Circumference: 34.4 (32-36 cm); Mean age of onset:10.77 (0.1-72 mo); Mean age at diagnosis: 17.63 (0.6-80 mo); Mean follow-up duration: 5.44 (0.2-23yrs); First presentations: vomiting 13/25 (52%), coma 4/25 (16%), developmental delay 2/25 (8%), respiratory distress 2/25 (8%), seizure 1/25 (4%), school age low IQ1/25 (4%), adolescent major depression 1/25 (4%); mean hyperammonemic episodes after treatment: 3.24 (range:0-12); Complications: permanent metabolic acidosis 16/25 (64%), developmental delay 15/25 (60%), Lactic Acidosis10/25 (40%), Nephropathy4/25 (16%), Seizure 5/25 (20%), Optic Neuropathy 2/25 (8%); Mean complication age: 3.56 (0.5-20 yrs); Mean plasma C3 Level: 14.12 (4-30, cut off:6 µmol/L); Mean Urine MMA:9799.6 (552-32640 mmol/molcr, NL<25); Treatment response: good (rapid improvement) 9/25 (36%), fair (slow improvement) 10/25 (40%), poor (worsening) 6/25 (24%); Genetic: 4/9 (44%) MMAB: 1/4poor response, 4/9 (44%) MUT: 1/4poor response, 1/9 (11%) MMAA: 1/1 poor response. Three new mutations: 2 in MUT (intron 12 c.2125-3C>G&c.284C>A, p.Pro95Gln) and 1 in MMAB (p.Gln231Ter, c.691G>T). Previously reported mutations:2 in MUT gene (121 Ins T AAT>t AAG and 231 Del C ATA>c ATT), 1 in MMAA gene (p.Arg359 Ter, c.1075C>G), and 3 in MMAB gene (p.Arg191Trp, c.571C>T). **Conclusion:** Isolated MMA in our Iranian studied group has heterogeneous presentation which needs further study for genotype-phenotype correlation, also it is a fairly good responding disease if treated early in life.

395 - Together: Methylmalonyl CoA Mutase and Oxoglutarate Dehydrogenase Complex Associate With the Inner Mitochondrial Membrane

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Background: Components of the tricarboxylic acid (TCA) cycle play a major role in mitochondrial energy production through their role in producing reducing substances. Several individuals have demonstrated associations of TCA cycle

enzymes including citrate synthase, malate dehydrogenase and fumarate hydratase (Srere 1987; Beecksmans et al 1989). However, a similar association has been more difficult to demonstrate for oxoglutarate dehydrogenase and succinate synthase. **Methods:** Mitochondria from livers of wild-type mice were isolated. They underwent serial ultra-centrifugations in a sucrose solution and sub-fractions were isolated. Resulting sub-fractions underwent immunoblotting using antibodies for succinate synthase component (SUCLG1), oxoglutarate dehydrogenase complex component (OGDH) and methylmalonyl mutase (MUT). Immunoprecipitation of these sub-fractions was also done. **Results:** These studied demonstrate that SUCLG1, OGDH and MUT can all be identified in the same sub-fractions which were consistent with inner mitochondrial membrane. **Discussion:** If these enzymes are in fact associated, then they could impact anaplerotic intermediate entry into the TCA cycle and impact potential therapies for disorders which affect the levels of these intermediates.

396 - Potential Role of miRNAs in the Development of Cardiomyopathies in Propionic Acidemia

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Propionic acidemia (PA) is a potentially lethal autosomal–recessive organic aciduria caused by defects in either the *PCCA* or the *PCCB* genes, encoding both subunits of the mitochondrial enzyme propionyl-CoA carboxylase (PCC). Considering the natural history of the disease, cardiac alterations (hypertrophy, dilated cardiomyopathy, long QT) are one of the major causes of mortality in patients surviving the neonatal period. We have previously identified a set of dysregulated miRNAs involved in apoptosis and mitochondrial function in tissue samples of the hypomorphic $Pcca^{-/-}$(A138 T) mouse model of the disease and in PA patients' plasma samples. In this work, we set out to investigate miRNAs highly expressed in cardiac tissue and reported to be involved in cardiomyopathies (cardiomiRs). A group of 11 cardiomiRs were selected (miR-1, miR-23a, miR-29a, miR-30c, miR-34a, miR-133a, miR-199a, miR-208a, miR-328, miR-350, and miR-378), with a documented relevant role in cardiac hypertrophy, apoptosis or fibrosis. Our results showed that all miRNAs analyzed except miR-328 were upregulated in heart samples from hypomorphic PA mice, as compared to wild type mouse samples. For the upregulated miRNAs, we have investigated their role in signaling pathways involved in the development of cardiac disease, through the analysis of reported direct or indirect downstream targets. For anti-apoptotic miR-133a, that protects cardiomyocytes from oxidative stress induced apoptosis, we detected a decrease in the levels of its target caspase 3. The increase in pro-hypertrophic miR-199a correlated with the decrease in its target PPARδ, a critical regulator of energy metabolism. Finally, pro-hypertrophic miR-208a, one of the most important heart-enriched miRNAs playing a crucial role in heart disease, was found 16-fold increased correlating with the increase of the pro-hypertrophic marker β-MHC, regulated by the transcription factor MED13, which is directly repressed by miR-208a. Recently, the identification of miR-208a and other cardiomiRs in plasma has fostered their clinical interest as potential biomarkers of cardiac injuries. We could detect increased levels of miR-208a in pooled plasma samples from PA patients, paving the way for more in-depth studies of cardiomiRs as biomarkers and therapeutic targets for heart disease in PA patients.

397 - Methylmalonic Aciduria cblB Type: Hepatocyte Differentiation From iPS

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The understanding of the cellular and molecular mechanisms underlying inherited metabolic disorders (IMDs) is essential for devising new strategies for their prevention and treatment. The difficulty to obtain a unique model for all the genotypes observed in IMDs and the upcoming of personalized medicine has prompted the emergence of new models. The aim of this work was the hepatocyte differentiation of induced pluripotent stem cells (iPSCs) generated by reprogramming of methylmalonic aciduria cblB type patient-derived fibroblasts. This organic aciduria is caused by the deficiency of ATP: cob(I)alamin adenosyltransferase (ATR) encoded by the *MMAB* gene. Fibroblasts from a patient bearing a hypomorphic destabilizing mutation in the *MMAB* gene (p.Ile96Thr) were reprogrammed using the commercial CytoTune Sendai vectors. After the molecular and functional characterization of the iPS cell line, iPSCs were differentiated in vitro into definitive endoderm and then incubated with specific factors, aimed at hepatocyte differentiation. IPSC-derived hepatocytes expressed relevant hepatic markers (HNF4a, AFP and albumin) analyzed by immunofluorescence. HepaRG, a hepatoma cell line highlighted for its high and inducible drug-metabolizing enzyme activities, was also used in the study to compare the expression of hepatic markers. Finally, the hepatocytes generated were used for evaluation of a potential pharmacological chaperone previously described (N-{[(4-chlorophenyl)carbamothioyl] amino}-2-phenylacetamide) in combination with hydroxocobalamin. The results provide evidences of its positive effect on the activity of the mutant ATR hepatocytes. Hence, our findings provide an experimental suitable model for the

investigation of the pathogenesis of this severe disease serving also as ex vivo platform for therapeutic applications.

398 - Successful Living Donor Liver Transplantation for Classical Maple Syrup Urine Disease

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MSUD is an autosomal recessive condition characterized by a deficiency in the enzyme, BCKDH, which catalyzes the breakdown of BCAAs. If left untreated, MSUD can result in mental retardation, central nervous system disorders, and even death. Most patients with MSUD are treated with a restricted protein diet and milk from which BCAAs have been removed. During times of illness, patients with MSUD can suffer from severe metabolic derangement, acute cerebral edema, and untimely death. Liver transplantation has been shown effective in patients with MSUD. Donor liver transplant restores the ability to metabolize branched-chain amino acids, even on an unrestricted diet, and prevents metabolic derangements during times of illness. This report describes the case of a 38-month-old boy with classical MSUD who received a liver graft from his mother at the age of 10-month-old. Transplantation was successful, and the patient was then able to ingest a normal diet. Post-operative BCAA levels normalized in our patient and remained so on an unrestricted protein diet and during times of physiological stress. Following transplantation, he did not require hospitalization in the last 28 months nor did any have new acute metabolic attack following a normal diet. He has excellent neurologic examination. These findings indicate that patients with MSUD can be successfully treated by LDLT, even when the donor is a heterozygous carrier of a mutated BCKDH gene.

399 - Alternating Hemiplegia as a Major Symptom of Maple Syrup Urine Disease: Case Report

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The aim of the present study is to draw attention to the rare association of maple syrup urine disease and alternating hemiplegia. We describe a case report from medical record review. RFD, 3 years and 6 months, male, white, adopted 3 months before, had abrupt onset of paroxysmal motor events characterized by alternating hemiplegia, with a duration of 30 minutes, evolving with total and spontaneous recovery in up to 24 hours, totaling 28 in 6 months. There was no information on prior development and symptoms of the child currently presenting language and fine motor delay. The physical exam showed dimorphisms: absence of lip groove, thin upper lip, large eyes, thick eyebrows, blond and fine hair, brachydactyly, hypotrophy, hypotonia, hyperreflexia, and low weight. Magnetic resonance imaging of the brain was performed with spectroscopy revealing hypersignal of globus pallidus, subthalamic nuclei, thalamus, mesencephalon and dentate nuclei; restriction areas in the cerebellar hemisferes and hypersignal in subcortical white matter; branched chain amino acid spike. Analysis of urinary organic acids showed elevated levels of 3-hydroxybutyric acid, 2-hidroxisovaluric,2-hidroxi-3-metil-valuric,2-hidroxisocaproic and 2- ketoisocapric acids, leucine, and isoleucine. The patient was diagnosed with MSUD and experienced resolution of neurological symptoms and milestones improvement after treatment with restriction of protein intake. MSUD has a worldwide incidence of 1: 185 000 live births and is not present in neonatal screening in our country, despite having treatment that alters the course of the disease. They can be divided into classical form and variants, that can vary according to the residual enzymatic activity. In the presenting case the lack of information of years previous to the adoption, associated with the rare atypical manifestation, lead to a difficulty in clinical suspicion and diagnosis. Despite the compatible laboratory and radiological diagnosis and good response to therapy for MSUD, gene sequencing was already requested for diagnostic confirmation of the subtype of the disease. There are only a few reports of the association between MSUD and alternating hemiplegia, making effective recognition and treatment a challenge. Therefore, it is necessary to approach the subject in order to improve these patient's morbidity and mortality, preventing the progress of neurological deficits.

400 - Improved Clinical Outcome Following Use of Citrate for Anaplerosis in Propionic Acidemia

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Objective: Propionic acidemia (PA) is an inherited disorder of branched chain amino acid metabolism treatable with a protein-restricted diet, carnitine supplementation, GI decontamination with metronidazole and acute management of illnesses. There is considerable variation in the severity of PA in children and some patients have considerably more frequent hospitalizations for metabolic instability than others despite

optimal medical and dietary management. The reduced production of succinyl-CoA in PA could theoretically lead to impaired function of the TCA cycle and may be ameliorated by anaplerotic therapy—however thus far chronic use of anaplerotic substrates in PA has not been recommended. We describe a child with prospectively treated severe PA whose clinical course appears to have stabilized since the introduction of citrate therapy. **Patient Details:** The patient is a 6-year-old child, born to first cousin consanguineous Pakistani parents, who was diagnosed at birth with propionic acidemia due to a family history of the same disorder in a cousin. She was treated from birth with a protein restricted diet and carnitine and metronidazole supplementation. She suffered one major neurological decompensation at the age of 3 years with acute basal ganglia infarction, from which she made a good recovery. Despite optimal medical and dietary management, and adequate treatment of constipation, at the age of six years the patient was still having frequent hospital admissions with vomiting and mild hyperammonemia. **Results:** In the three months prior to citrate initiation, the child had 5 hospital admissions spending 20 days in hospital. Citrate therapy was commenced as Albright Solution 10 ml three times daily via gastrostomy, delivering a dose of 19 mmol citrate (0.65 mmol/kg/day). In the 3 months immediately after citrate supplementation, the child has had 2 brief hospital admissions (including one unrelated to PA for a urinary tract infection) spending only 4 days in hospital. The parents give subjective reports of reduced fatigue and increased school attendance. No significant change was noted in biochemical measurements of metabolic stability. **Conclusions:** Citrate supplementation appears to have led to a significant and immediate improvement in metabolic stability in this patient with severe PA. Larger studies of this and other anaplerotic agents in PA are warranted.

401 - Cardiac Manifestations of Propionic Acidemia and Methylmalonic Aciduria: A Systematic Retrospective Review

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Introduction/ Methods: Cardiac involvement is a well-known complication of propionic acidemia (PA) but is less commonly seen in methylmalonic aciduria (MMA). The etiology of cardiomyopathy is not fully understood. We examined the medical records of 21 patients with PA (aged 5-27 years) and 52 patients with MMA (aged 6-22 years) attending our units. We compared cardiac disease in both disorders, as well as the clinical history and biochemical parameters of patients. **Results**: 6/21 (29%) of patients with PA and 6/52 (11%) of those with MMA had cardiomyopathy (CM). For PA, in patients for whom the CM subtype was known, 3 had DCM, 1 HCM and 1 mixed DCM/HCM. For MMA, 3 had DCM, 2 HCM and 1 restrictive CM. The mean age at diagnosis of CM was 11.3 years in PA and 9.3 years in MMA. The number of severe lifetime metabolic decompensations was similar for PA and MMA (4.3 and 4.2, respectively). Biochemically, patients with PA had a significantly higher methylcitrate/Cr ratio compared to those with MMA (393 vs 109 μM/mM, $P = .003$). Propionylcarnitine levels were similar between the two disorders (PA: 24.6 vs MMA: 30.7 μM, $P = .633$). The severity and management of CM varied. One patient with PA had a CM that was transient and reversible, two had mild-moderate CM needing long-term treatment with an ACE inhibitor, two had mild CM diagnosed shortly before death, and one had DCM and severe heart failure needing diuretics, ACE inhibitors, digoxin and ivabradine and fitting of a biventricular assistive device during a severe cardiac decompensation. He recovered well, but re-presented in cardiac failure 1.5 years later and died. Four patients with MMA had mild disease (continued surveillance), one had moderate HCM, diastolic dysfunction and renal impairment needing a brief period of treatment with antihypertensives and one patient had severe DCM needing diuretics, ACE inhibitors, and digoxin, and died of end-stage cardiac and renal failure. **Conclusions**: Cardiomyopathy is more than twice as common in PA as in MMA and variable as it can present as DCM, HCM or restricted CM. Patients have differing biochemical profiles and this may account for different pathogenetic mechanisms for CM. Regular echocardiography from an early age is essential to identify and monitor the development of cardiomyopathy which can be challenging to treat and carries a high mortality.

402 - Acute Metabolic Decompensations of Branched-Chain Organic Acidemias in the Pediatric Emergency Department: Clinical Presentation and Outcomes

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Branched-chain organic acidemias are a group of inborn errors of metabolism characterized by recurrent life-threatening acute metabolic decompensations (AMD). There is no single clinical

or biochemical marker established by consensus or strong evidence to diagnose AMD. However, presence of vomiting, food refusal or altered consciousness has been previously proposed to diagnose AMD in methylmalonic acidemia (MMA) and propionic acidemia (PA). In this study, we aimed to retrospectively analyze the clinical presentations and outcomes of patients with MMA, PA, isovaleric acidemia (IVA) and maple syrup urine disease (MSUD) that presented to the emergency department of a single metabolic center in Turkey. Children with MMA, PA, IVA, and MSUD that presented for any reason to the emergency department of Hacettepe University Children's Hospital during 2014-2016 were included. Clinical data were retrieved from hospital records. There were a total of 67 patients (20 MMA, 16 PA, 5 IVA, and 26 MSUD) and 278 presentations (90 MMA, 94 PA, 7 IVA, and 87 MSUD). 36.6% of all presentations (102 distributed as 17 MMA, 57 PA, 2 IVA, and 26 MSUD) were diagnosed as AMD. Presence of vomiting, food refusal, or altered consciousness did not significantly differ between MMA patients with or without AMD ($P > .05$), but all three of these clinical findings were significantly more common in PA patients with AMD than those without AMD ($P < .0001$, $P < .01$, and $P < .05$, respectively). In MSUD, altered consciousness was significantly more prevalent in those with AMD ($P < .001$), but vomiting and food refusal were not ($P > .05$). During the study period, 1 patient with PA died during an AMD. 2 MMA patients without an AMD died due to sepsis, which, in 1 patient, was precipitated by loss of protective skin and mucosa barrier due to severe isoleucine deficiency. Although MMA and PA are similar diseases in a common metabolic pathway, vomiting, food refusal, and altered consciousness were significantly more prevalent in AMD of patients with PA, but not with MMA. Vomiting and food refusal are common complaints that can be attributed to other causes in pediatric practice, such as acute infections or chronic diseases. Other clinical and laboratory parameters are necessary to differentiate these causes from AMD in patients with organic acidemias. It is important to note that deaths may not always be due to AMD. Infections and treatment complications should be prevented and treated to decrease mortality.

403 - Successful Management of Methylmalonic Aciduria During Pregnancy and Delivery—Case Report

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Background: There is an increasing number of reported pregnancies in inborn errors of metabolism, even in such serious condition as methylmalonic aciduria (MMA). But still detailed description of successful management is needed to ensure good maternal and newborn outcomes. We report on a case of pregnancy and delivery in the 31.5-year-old patient with isolated methylmalonic aciduria cobalamin non-responsive. Case Report: The patient comes from related parents. She was symptomatic since neonatal period (vomiting and diarrhea with metabolic acidosis). Diagnosis of MMA was established at age of 6 months and since then the patient has been treated and monitored in regular way. Intermittent metabolic decompensations, usually associated with intercurrent infections and stress, were observed. The most life-threatening episode occurred 3 years ago when the patient was exposed to steroid therapy. Chronic management included diet low in natural protein (0.9-1.5g/kg/d) and carnitine supplementation. The patient presented with poor appetite, so dietary compliance was unsuccessful. Since early pregnancy increase of calorie intake up to 2300kcal/d and natural protein up to 1.0g/kg/d were recommended. But until the sixth month of pregnancy, episodic vomiting occurred, what made this diet difficult to maintain. Formula and yoghurts with higher protein content were added. Throughout three trimesters mean daily energy, natural protein and total protein were: 1840-1863-2200 kcal, 0.78-0.9 -1.25 g/kg and 1.0 -1.17 -1.43 g/kg, respectively. But still low amino acids (mainly isoleucine and valine) levels were noted, so they both (at doses 100-150mg/d each) were supplemented during the whole pregnancy. No metabolic crisis was observed during pregnancy and delivery, although marked MMA elevation was noted just before delivery. Healthy baby was born through a C-section. Main concern was directed to postpartum period, also because of breastfeeding. In order to avoid catabolism, before, during and two days after delivery iv dextrose and iv carnitine were given with addition of a special formula without protein. Plasma amino acids, ammonia, urinary GC/MS profile, C3-carnitine, and free carnitine were evaluated every month, including perinatal period, and the management was adjusted based upon those data. Conclusion: Careful clinical, biochemical and dietetic monitoring during pregnancy and delivery is crucial to ensure favorable outcome for the mother with MMA and her newborn.

404 - Cognitive Development and Emotional Features in Chilean Patients With MSUD

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Maple syrup urine disease (MSUD) is an inborn error of amino acids catabolism characterized by the accumulation of branched-chain amino acids leucine, isoleucine and valine and their corresponding neurotoxic ketoacids. Little information regarding behavioral development of these patients is

available. The aim of this study was then to analyze long term cognitive development and observed emotional and behavioral features in MSUD patients. This retrospective study included 41 Chilean patients, 35 with classic form MSUD. The mean age of diagnose was 38.9 ± 96 days (3-600 d) with a mean leucine concentration of 1456 ± 854 μmol/L. Mean age last psychomotor development assessment with Bayley Scale of Infant Development of 31.7 ± 12.5 months, mean mental index score (MDI) was 55.1 ± 12.5. Only 24% patients showed normal development or mild delay (MDI > 70). The mean age last IQ assessment was 12.6 ± 3.6 years of age (5-20 years), all assessed with age-appropriate Wechsler Intelligence Scale, showing a mean verbal IQ (VIQ) of 65.2 ± 22.5, performance IQ (PIQ) of 60.6 ± 20.5 and full-scale IQ (FSIQ) of 61.1 ± 20.8 (39-103). 18% of patients showed a normal cognitive functioning (FSIQ 80-109), 34% borderline or mild disability and 46.9% moderate to severe cognitive disability. Of the 6 children that presented normal cognitive functioning, two had normal psychomotor development during infancy, two mild and two severe developmental delays. Of the patients that scored FSIQ >55 (n = 17), 9 presented lower scores in the PIQ when compared with the VIQ, with an average of 15.2 points of difference. Regarding observed behaviors, in patients with FSIQ>55 score, 14/17 patients were described as smiling, having good social response and 12 showed attention difficulties and/or mild restlessness/impulsive behaviors. In the group FSIQ<55, 18/21 showed good social response, but 15 of them had serious attentional difficulties and/or restlessness/impulsive behavior. Children diagnosed before the 14th day of life (9 ± 3.3 d, n = 18) presented a significantly better FSIQ (74 ± 19.7 vs 53 ± 18 $\rho = 0.001$) that the group of children diagnosed after (61 ± 124 d, n = 17). **Conclusion:** We found a high prevalence of cognitive impairment and difficulties in visuospatial abilities in MSUD patients. Early diagnose (even before day 14th) has a positive impact in cognitive prognosis. Good social response, but with noticeable attentional difficulties and restlessness/impulsive behavior is frequently observed in clinical setting.

405 - Unusual Triple Troubles of Genetic Disorders Lead to Clinical Presentations

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Background: Inborn errors of metabolism (IEMs) are rarely caused by copy number variations. We identified triple troubles in a two and half year-old girl born through in vitro fertilization. Initial visit revealed that she had history of failure to thrive, hypotonia, and development delay. Other clinical features included microcephaly and dysmorphic face. Brain MRI at age of 1-year-old showed that her frontal lobes size was slightly atrophy. Mass spectrometry results from her blood and urine suggested 3-methylcrotonyl-CoA carboxylase deficiency (3-MCCD). Close examination revealed that she had 20 teeth with small size and yellow discoloration. **Methods:** Medical Exome Sequencing covered with 4000 known diseasing genes that could cause clinically genetic diseases was used to screen suspected genetic causes on DNA extracted from her peripheral blood samples and parental samples for Trio analysis. Sanger sequencing and PCR were used to confirm the results. **Results:** Three genetic abnormalities were identified in the case. A homozygous c.1630delA (p.R544Dfs*2) of *MCCC1* gene was found. Her mother was confirmed to be heterozygous for this mutation. No point mutation of *MCCC1* gene was detected on the parental chromosome. A *de novo* 1.36 Mb deletion of 3q27.1 encompassing *MCCC1* gene was identified. This de novo deletion presumably occurs on the paternal chromosome. Compound heterozygous mutations of c.536C>G (p.S179*) and c.2686C>T (p.R896*) on *WDR72* gene were identified. Her father is a carrier of c.536C>G (p.S179*) and her mother is a carrier of c.2686C>T (p.R896*). **Conclusion**: A 1.36 Mb deletion on 3q27.1 has never been reported before. However, 3 cases on the similar region had been reported to have development delay, microcephaly and dysmorphic features. The identified de novo deleted region harboring *MCCC1* gene and one mutation of *MCCC1* inherited from her mother together with consistent biochemical findings lead to the confirmed diagnosis of 3-MCCD. The diagnosis of amelogenesis imperfecta IIA3 caused by *WDR72* gene was also established after closely clinical examination. The delineation of these mutational mechanisms provides additional insight for the diagnosis of IEMs. The triple troubles identified here present the advantage of NGS that could provide both CNV and SNV in single assay. The extra layer complexity has improved our understanding of the pathogenesis of complex diseases with unexplained clinical symptoms.

406 - MSUD: Levels of Leucine, Isoleucine, Valine, and Alloisoleucine During the First Seven Days of Life in Maple Syrup Urine Disease

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Maple Syrup Urine Disease (MSUD) results from a primary defect in oxidative decarboxylation of the ketoacids derived from the branched chain amino acids: leucine (leu), isoleucine(ile) valine(val) and alloisoleucine (alloile). Neonates with classic MSUD appear normal at birth but usually become symptomatic by 4 or 5 days of age. There are very few serial data available on the levels of branched chain amino acids at birth and through the first few days in neonates with this disease. **Objective:** Our objective was to determine the levels of leu, ile, val and alloile, in the first week. **Methods and materials:** We

studied two families who had previous known children with classic MSUD. Newborns of these families (without prenatal diagnosis) were studied. Amino acids to the newborn sisters of the affected children were quantified from the first day of birth to the seventh. Amino acids levels in plasma were determined by Amino Acid Analyzer (HPLC) and in dried blood spots by UPLC -MS/MS. **Results:** Patient 1: Day 1: val: 222, leu:387. Day 2: val:398, leu: 614. Day 5: leu: 353, val:268 (Reference values MS/MS μM: val:40-200, leu:70- 250) Day 0: val:20, leu:11.5, alloile:0.0, ile:3,9. Day 2: alloile 1.2, leu: 44.7, val:30.7, ile:12.5 (Reference values HPLC μmol/dl leu: 5.0-17.5, ile:0.6-11.0, leu:5.0-17.5, val: 6.4-29.4). Patient 2: Day 0 (8 h) val: 190, leu: 228. Day 1: val: 252, leu: 310. Day 7: leu: 963 val:191 (MS/MS). Day 3: alloile: 5.5, leu: 36.5, val: 48.6, ile: 19.9 (HPLC). At 24 hours, there was a slight increase in levels of valine and leucine in both girls. The alloisoleucine increased at 48 and 72 hours, respectively. **Conclusion:** There is a slight increase in levels of branched chain amino acids from the first 24 hours of life, and alloisoleucine rises between 48 and 72 hours, at least in these two cases studied. Much caution should be taken in measuring these metabolites, whenever the newborn screening samples are obtained earlier than 24hs of life.

407 - Molecular Characterization of Maple Syrup Urine Disease in Tunisia

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Introduction: Maple syrup urine disease (MSUD) is a rare autosomal recessive disorder of metabolism caused by the defective function of the branched-chain α-ketoacid dehydrogenase complex (BCKD). BCKD catalyzes the oxidative decarboxylation of branched-chain α-ketoacids (BCKAs) derived from the transamination of branched chain amino acids (BCAAs) leucine, isoleucine and valine. The disease-causing mutations can occur either in *BCKDHA*, *BCKDHB*, or *DBT* genes encoding respectively the E1α, E1β, and E2 subunits of the complex. MSUD might represent a major cause of mortality, disability, and chronic disease in Tunisia. **Aim:** The aim of this study was to perform the molecular characterization of 4 Tunisian patients affected by the classic form of MSUD. **Patients and Methods:** Molecular analysis was carried out using direct sequencing of the entire coding and flanking intronic regions of *BCKDHA*, *BCKDHB* and *DBT* genes. **Results:** The analysis of the entire coding region of *BCKDHA*, *BCKDHB* and *DBT* identified two novel putative mutations. Patients 1 and 2 were from Sidi Bouzid and share the same homozygous molecular alteration in *BCKDHB* gene. The nucleotide change is c.716A>G, which causes the replacement of a glutamic acid by a glycine (p.Glu239Gly). The functional impact analysis of the nonsynonymous substitution in silico predicted that this mutation was probably damaging. Patient 4, originate from central Tunisia, has a homozygous molecular alteration of the same gene: c.502C>T (p.Arg168Cys). Patient 3 was homozygous for a four base-pair deletion in *DBT* gene (c.1333_1336delAATG). This deletion creates a premature stop codon at residue 445 of E2 protein (p.Asn445X). **Conclusion:** MSUD shows a mutational heterogeneity in central Tunisia. The identification of these two novel mutations will facilitate prenatal diagnosis and genetic counselling in the families at Risk.

408 - Early Diagnosis of Neonatal Classical Maple Syrup Urine Disease By 1H-MRS

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Background: Classical neonatal Leucinose or classical maple syrup urine disease (MSUD; OMIM 248600) is a rare autosomal recessive inborn error of branched-chain amino acid (BCAA) metabolism all around the world. In Tunisia, it seems to be relatively frequent disease with prevalence of 1/13 716. Early diagnosis in neonate is challenging. Even in case of a neonate with neurologic symptoms and fenugreek urine odors, the diagnosis needs several days to be done. This can delay specific treatment and worsen the prognosis. We report two neonatal cases with rapid diagnosis by ^{1}H-MRS. **Cases report**. **Case N1**: A full-term female infant born from an uneventful pregnancy and delivery, with a birth weight of 3600 g and Apgar score 9/10, was hospitalized at 8 days old because of sucking difficulties and neurologic distress with abnormal movements, she was hypoglycemic with metabolic acidosis and her temperature was 37.8°C. Usual laboratory finding (BUN, electrolytes, CRP, LCR, .) was normal. She had a urines' odor of fenugreek. **Case N2**: A full-term male infant born from second cousins parents, birth weight of 3850 g. At 6 days old he developed sucking difficulties with neurological distress and abnormal movements with metabolic acidosis with hyperammoniemia and urines 'odor of fenugreek. **Case N3:** A baby boy from second cousins' parents developed neurologic distress by 10 days old with fenugreek odor of urines. **Brain MRI with spectroscopy:** It was performed for all our patients, by two weeks of life, showed bilateral and symmetrical hypersignal lesions on periventricular white matter along corticospinal faisceaux, cerebral peduncles. Hyperdiffusion with low ADC. Proton magnetic resonance spectroscopy (^{1}H-MRS) showed a large methyl resonance peak at 0.9 p.m. corresponding to accumulation of amino acids and Alpha-ketoacids. For all our patients, chromatography showed, three weeks later, an increase of the branched-chain amino acids in the plasma confirming the diagnosis of neonatal MSUD. **Conclusion:** ^{1}H-MRS imaging

would be one of the earliest clues to the diagnosis of Classical MSUD in the neonatal period, especially when specific analysis takes several days to be performed. Then, the diagnosis and the specific treatment could be started earlier improving neurologic outcome.

409 - Organic Acidurias and Other Inborn Errors of Metabolism Detected by Urinary Organic Acid Analysis: A 10-Year Period Experience of a Private Laboratory in Brazil

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Objectives: Organic acidurias/ acidemias form a heterogeneous group of inborn errors of metabolism - IEM that involve metabolic pathways related to the degradation of amino acids, carbohydrates and fatty acids. Gas chromatography/ mass spectrometry (GC/MS) of urine samples is the method of choice in the diagnosis and follow-up of these disorders. However, due to the high costs of equipment purchase, operation and maintenance, there are few reports on the detection of these IEM in Brazil. The objective of this study was to describe the organic acidurias/ acidemias, aminoacidopathies, urea cycle disorders, and fatty acid beta-oxidation defects detected by the analysis of urinary organic acids in a private IEM reference laboratory in Brazil. **Methods:** A total of 6024 occasional urine samples were obtained over a 10-year period from symptomatic and asymptomatic patients with a clinical suspicion of IEM. These samples were maintained at −20°C until analysis. The analytes of interest were extracted with ethyl acetate / ethyl ether following acidification of the urine and addition of internal standard. Sample extracts were thoroughly dried before derivatization with a 100: 1 MSTFA (*N*-methyl-trimethylsilyl-trifluoroacetamide)/ trimethylchlorosilane (TMCS) reagent mixture and identified as trimethylsilyl compounds on a gas chromatograph/ mass spectrometer. **Results:** A total of 471 urine samples (7.8%) were identified with a specific profile of one of the IEM detectable by the methodology. Methylmalonic acidemia (132 samples from 34 patients), glutaric aciduria type I (78/24), urea cycle disorders (27/24), maple syrup urine disease (24/20), and propionic acidemia (49/14) were the most frequent IEM. Other 706 samples (11.7%) presented nonspecific profiles, common to several conditions, such as lactic aciduria, dicarboxylic aciduria, and ketonuria. **Conclusions:** The analysis of urinary organic acids was useful in the diagnostic elucidation of patients with a clinical suspicion of IEM. This analysis may be included in the diagnostic workup of neonates with severe symptoms due to acute and early forms of IEM, often confounded with sepsis and other common conditions, as well as of patients with insidious or late-onset forms, frequently with a predominance of neurological symptoms.

410 - Effect of Liver Transplantation on Hyperammonemia and Metabolic Control in Propionic Acidemia

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Propionic acidemia is caused by a deficiency of propionyl-CoA carboxylase, the enzyme that converts propionyl-CoA to methylmalonyl-CoA. In its most severe form, it is characterized by recurrent episodes of hyperammonemia and metabolic acidosis resulting in progressive encephalopathy and organ damage. The objective of this study was to evaluate the effects of heterologous orthotopic liver transplantation on biochemical control and clinical outcome in 3 patients with propionic acidemia. Biochemical and clinical data were collected by retrospective review of medical records. All patients were followed at our center since birth, and all data were used for analysis. Values of continuous variables before and after transplantation were compared using analysis of variance. Three of our patients with neonatal presentation of propionic acidemia underwent orthotopic unrelated liver transplantation at 0.75-13 years of age. All patients tolerated the procedure well and were discharged home 16-26 days after the procedure. There was a decrease in plasma C3 (propionyl)-carnitine (from 81.4 $\pm$ 16.4 to 47.3 $\pm$ 14.3 μmol/L) and glycine (from 921 $\pm$ 397 to 359 $\pm$ 126 μmol/L) after the liver transplant with an increase in glutamine (a marker of anaplerosis, from 373 $\pm$ 127 to 556 $\pm$ 98 μmol/L). Ammonia levels were significantly reduced (from 77.6 $\pm$ 42.7 to 43 $\pm$ 22.9 μmol/L) after the transplant with no new hyperammonemic events (follow-up time 1.6-3.9 years). Number and length of hospital admissions (normalized per year) decreased significantly after transplant. Patients had improvements of developmental milestones, decreased the use of medical foods (although supplements of carnitine and citrate were continued) and increased the intake of natural proteins. These data indicate that liver transplant can improve metabolic control and quality of life in patient with severe forms of propionic acidemia, while reducing hospital admissions. Liver transplant remains a procedure with considerable risk and longer follow-up is necessary to determine the overall risk–benefit ratio in propionic acidemia.

411 - Methylmalonic Aciduria as a Differential Diagnosis of Primary Immune Deficiencies: A Case Report

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Background: Hematological manifestations have already been described in organic aciduria especially during acute decompensations, but they are a rather rare mode of presentation. **Aim:** We report a case of methylmalonic aciduria (MMA) presenting with a severe infection and hematological abnormalities mimicking primary immune deficiencies. **Case report**: Our patient was the fifth child of consanguineous parents. His brother was dead in early infancy of a severe sepsis. The pregnancy and the birth were uneventful. From the age of two months, he was investigated for a severe failure to thrive with neurological abnormalities. On examination, he had fever, truncal hypotonia and hepatomegaly. The biological tests showed a high C-reactive protein level, a pancytopenia, a hypogammaglobulinemia, a slight elevation of liver enzymes, and a hypokaliemia with altered tubular function tests and persistent acidosis with a high anion gap. These findings associated to evidence of Klebsiella pneumoniae sepsis and Candida albicans urinary tract infection suggested the diagnosis of a primary immune deficiency. However, the diagnosis of an inherited metabolic disease was considered because of the persistent unexplained acidosis. The patient died at the age of three months because of uncontrolled infection. The urinary organic acid profile showed characteristic features of MMA. Genetic counselling was done and prenatal diagnosis can be proposed in further pregnancies. **Conclusion:** In our patient, the clinical presentation of MMA was misleading. We emphasize the importance of metabolic workup in severely ill infants with unexplained clinical or biological features such as metabolic acidosis. The confirmation of the diagnosis will allow the family to have a prenatal diagnosis in further pregnancies and avoid the recurrence of the disease.

412 - Recurrent Hypoglycaemia Revealing a Propionic Aciduria: A Case Report

Rim Ben Abdelaziz[1], Arij Ayadi[2], Hela Hajji[2], Hela Boudabous[1], Amel Ben Chehida[1], Awatef Jlassi[3], Mohamed Slim Abdelmoula[1], Moncef Fekih[3], Hatem Azzouz[1], and Neji Tebib[1]

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Background: Hypo- and hyperglycemia may be found in acute episodes of classical organic acidurias but they are not usual as presenting features. **Aim:** We report a case of infantile propionic aciduria revealed by recurrent episodes of hypoglycemia in an otherwise healthy baby. **Case report**: Our patient was the first child of non-consanguineous parents. He had no familial history of neurological illness or early deaths. The pregnancy and the birth were uneventful. he was admitted and treated during the first weeks of life for a neonatal meningitis with negative bacteriological investigations. At age 5 months, he was admitted for dyspnea and wheezing. An asymptomatic hypoglycemia was found but no further investigation was made at this stage and the baby was discharged within few days. Thenafter, his mother has noticed some episodes of drowsiness and the capillary blood glucose was as low as 38 mg/dL. At age 8 months, he had normal developmental milestones (he was able to sit by himself and babble), his weigh was slightly below the third percentile with normal height and head circumference. His fasting blood sugar was low but all the other standard biological tests were normal (no acidosis, no ketonuria, no hyperammoniemia). Urinary Organic acids profile was performed as part of metabolic work-up of hypoglycemia and showed characteristic features of propionic aciduria on two different samples. The patient was treated with Carnitine, metronidazole and branched chain amino acid-controlled diet with no acute or chronic complications six months after treatment initiation. **Conclusion:** Propionic aciduria is not a common cause of apparently isolated recurrent hypoglycemia in infants. Urinary organic acids should be systematic in any patient with recurrent unexplained hypoglycemia. Discovering organic aciduria in an early stage allows the initiation of the treatment before the occurrence of severe complications.

413 - Glutaric Aciduria III in Female Patient Detected Through Chromosomal Microarray Analysis (CMA 750 k CGH+SNP)

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We present an 8-year-old female patient with development delay and intellectual disability. Parents weren't consanguineous. Family history was positive for schizophrenia on a paternal uncle. Medical history: vaginal delivery, weight birth 3,500 g, height birth and cephalic perimeter are unknown. On physical examination, short stature, microcephaly, underdeveloped supraorbital ridges, short, woolly, sparse hair; trichorrhexis nodosa (optical microscopy) and bilateral clinodactyly was found. Developmental milestones: walking at 3 years 6 months old, first words at 4 years old, currently on elementary school (1st grade). Chromosomal microarray analysis showed homozygous deletion of 125 kb on chromosome 7 [7p14.1(40,140,770-40,265,451)x0], compromised two genes: *MPLKIP, C7orf10,* producing two entities: Trichothiodystrophy 4, non-photosensitive (TTD4) and glutaric aciduria III (GA III). Although patients with GA III may have no disease phenotype, some patients showed failure to thrive, hypertension,

diarrhea, vomiting, goiter and hyperthyroidism. All patient's clinical characteristics are due to TTD4, and *C7orf10* deletion is probably asymptomatic.

414 - Experimental Evidence That α-Ketoadipic and α-Aminoadipic Acids Provoke Marked Oxidative Damage and Reduces the Antioxidant Defenses in Rat Brain

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α-Ketoadipic aciduria is biochemically characterized by tissue accumulation and high urinary excretion of α-ketoadipic (KAA) and α-aminoadipic (AAA) acids. Although many patients are asymptomatic, especially those diagnosed by NBS at birth, many others present neurological symptoms of unknown pathogenesis. We investigated the *in vitro* effects of KAA and AAA on a large spectrum of redox homeostasis parameters in brain of young rats. 2′,7′-Dichlorofluorescein (DCFH) oxidation, nitrate and nitrite levels, malondialdehyde (MDA) concentrations, carbonyl formation, sulfhydryl content, glutathione (GSH) concentrations and aconitase activity were determined in rat cerebral cortex. AAA and more markedly KAA increased reactive oxygen and nitrogen species (increase of DCFH oxidation and nitrite/nitrate levels) generation as well as induced lipid (increase of MDA concentrations) and protein oxidative damage (increase of carbonyl formation and decrease of sulfhydryl content), besides decreasing the antioxidant defenses (reduced glutathione, GSH) and aconitase activity. We also observed that KAA-induced lipid peroxidation and GSH decrease were prevented by the antioxidants α-tocopherol, melatonin and resveratrol, suggesting the involvement of reactive species in these effects. Furthermore, the classical inhibitors of NMDA glutamate receptors MK-801 and memantine did not prevent KAA- and AAA-induced reactive species formation (increase of DCFH oxidation) and GSH levels decrease, making unlikely a secondary induction of oxidative stress through overstimulation of glutamate receptors. The present data indicate that disturbance of redox homeostasis by the major metabolites that accumulate in α-ketoadipic aciduria may possibly contribute to the pathophysiology of the neurologic symptoms occurring in some affected patients. **Financial support:** PROPESQ/UFRGS, FAPERGS and CNPq.

415 - Delayed Diagnosis of Glutaric Aciduria Type 1 (GA1): Clinical Case Report

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Introduction: Glutaric aciduria type 1 (GA1) is an organic acidemia caused by the deficiency of Glutaryl CoA dehydrogenase, which is involved in the catabolic pathway of L-lysine, L-hydroxylysine, and L-tryptophan. Typical biochemistry is characterized by an accumulation of glutarate, 3-hydroxy glutarate, and glutaconate in urine and glutarylcarnitine (C5 DC) in the blood. GA1 is classified into two groups: low and high excretors, based on their urinary metabolite excretion. **Clinical case report:** The patient is a 6-year-old female, born after a term pregnancy. Her parents were not consanguineous. She has an older sister who is in good health. Her newborn screening sample showed elevated C5 DC 0.4 μmol/L (reference value < 0.25 μmol/L). Follow up testing on plasma showed C5 DC at the upper level limit of the normal range 0.2 μmol/L (reference range < 28 days 0-0.2 μmol/L). Urine organic acids performed at the same time was reported as normal. The conclusion was that these results did not support the diagnosis of GA1. At the age of 3 years and 4 months she presented with dystonia. At the age of 5 years further investigations were performed including a movement disorder gene panel testing. The genetic testing showed two heterozygous variants in the gene *GCDH1,* one on each allele. Subsequent analysis of urine and plasma showed a normal glutarate but trace increase in 3-hydroxy glutarate and a slight increase C5 DC respectively. These results were consistent with a low excretor GA1. Review of the initial urine organic acids revealed a small but significant 3-hydroxyglutarate peak which was not identified by the software. **Conclusion:** Despite late detection of GA1, patient is doing well. Her gross motor performance is very good, although she still has the dyskinetic movements. She is now treated with low protein diet and taking L- carnitine. The case highlights the difficulties in detecting low excretors and the need for careful follow up of newborn screening positive findings.

L) Organic Acidurias: Others (416 to 440)

416 - Simultaneous Estimation of Elevated Biomarkers of Organic Acidemia in Neonatal Urine using LC-MS

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Inherited metabolic disorders include a variety of disorders which are individually rare but frequently observed, affecting the pediatric population causing mortality or morbidity. Despite the advancements in bioanalytical methods, it still remains a challenge to the analysts to develop a method for small molecules in complex matrices. The LC-MS technique clubbed with the separation power of HILIC column technology has given sufficient specificity and sensitivity avoiding derivatization. In this study, an LC-MS based analytical method was developed and validated for screening of biomarkers responsible for maple syrup urine disease and methyl malonic aciduria. This method has achieved recoveries of 80%-105% by a "dilute and shoot" method, thus avoiding time consuming extraction techniques. Complete separation of all biomarkers and the internal standard were achieved within a retention time of 3.6 minutes. The intra and inter-day precision were < 11% and 12%, respectively, with linearity r^2 of ≥ 0.990 for biomarkers in their respective calibration ranges. The stability studies for the biomarkers were promising at all stability study conditions. Analyses of clinical samples with the developed method were in confirmation with the gold standard techniques such as GC-MS and tandem mass spectrometry. The method was applied for analysis of samples for confirmation of maple syrup urine disease and methylmalonic acidemia.

417 - Oxidative Stress in Patients With L-2-Hydroxyglutaric Aciduria and In Vitro Protective Effect of L-carnitine Upon DNA Damage Caused by D-2-Hydroxyglutaric and L-2-Hydroxyglutaric Acids

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D-2-hydroxyglutaric (D-2-HGA) and L-2-hydroxyglutaric acidurias (L-2-HGA) are 2 distinct neurometabolic disorders biochemically characterized by increased levels of D-2-hydroxyglutaric (D-2-HG) and L-2-hydroxyglutaric acids (L-2-HG) in biological fluids and tissues, respectively. Patients affected by D-2-HGA are classified into 2 variants, D-2-HGA type I or D-2-HGA type II. D-2-HGA type I is caused by mutation of D-2-hydroxyglutarate dehydrogenase gene while D-2-HGA type II is caused by a gain of function mutation in isocitrate dehydrogenase 2 gene. L-2-HGA is caused by mutation in the L-2-hydroxyglutarate dehydrogenase gene. Considering that the pathophysiology of these diseases is not fully understood and that many studies have been shown the involvement of oxidative stress in inborn errors of metabolism, the main objective of this work was to investigate oxidative and nitrative stress parameters in the urine of L-2-HGA patients and to investigate the in vitro DNA damage caused by the accumulated acids of D-2-HGA and L-2-HGA as well as the protective effect of L-carnitine on this damage. It has been found that concentrations of 50 μM of D-2-HG and 30 μM of L-2-HG induce DNA damage and concentrations of 30 μM and 150 μM of L-carnitine significantly reduced the in vitro DNA damage, measured by comet assay, compared to controls. In addition, urine samples from L-2-HGA patients were analyzed. It was observed a significant increase of oxidized guanine species, an oxidative DNA damage biomarker as well as a significant increase of urinary di-tyrosine level, indicating protein oxidative damage in the patients. However, there was no significant difference in the levels of urinary isoprostanes and reactive nitrogen species. In conclusion, these results suggest, at least in part, proteins and DNA oxidative damage and highlight the L-carnitine antioxidant potential as a promising adjuvant in the treatment of patients affected by L-2-HGA or D-2-HGA. **Financial Support:** CNPq and Fipe-HCPA.

418 - Striatum Histopathological Alterations Provoked by Acute Lysine Administration in Glutaryl-CoA Dehydrogenase Deficient Mice

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Glutaric acidemia type I (GA I) is an inborn error of lysine (Lys) catabolism caused by glutaryl-CoA dehydrogenase (GCDH) deficiency. Patients affected by this disorder are highly susceptible to develop striatum acute degeneration during stress catabolic situations mainly from 3 to 36 months of age that significantly worse their prognosis. Although the underlying mechanisms of striatum damage are still on debate, they may be related to increased brain concentrations of glutaric (GA) and 3-hydroxyglutaric acids. In the present work, we investigated the effects of an acute intrastriatal administration of Lys (1.5 and 4 μmol) to 30-day-old wild type (WT) and

GCDH deficient (*Gcdh−/−*) mice fed a baseline chow (0.9% Lys) on brain histopathology. Mice were sacrificed 48 hours after Lys injection. Striatum and cerebral cortex morphology was evaluated by hematoxylin and eosin, whereas astrocyte activation and neuronal viability were determined by immunohistochemistry of GFAP and NeuN, respectively. Lys administration at the lowest dose (1.5 μmol) provoked marked vacuolation/edema in the striatum of *Gcdh−/−* but not in WT mice, implying a higher susceptibility of *Gcdh−/−* mice to acute intrastriatal Lys. Similarly, astrocytic GFAP protein staining was markedly increased only in *Gcdh−/−* Lys (1.5 μmol)-injected mice, suggesting astrocytic reactivity. Furthermore, NeuN staining was reduced in the striatum of *Gcdh−/−* but not in WT mice submitted to 1.5 μmol Lys treatment, indicating neuronal loss. It is stressed that no alterations were observed in the cerebral cortex of both WT and *Gcdh−/−* mice injected with 1.5 μmol Lys. However, Lys, at the highest dose (4 μmol), also provoked extensive vacuolation in cerebral cortex of *Gcdh−/−* mice, as compared to WT mice, indicating that cerebral cortex of GCDH deficient mice may be also affected in the knockout animals when exposed to high Lys concentrations. It is concluded that striatum of *Gcdh−/−* mice is severely damaged presenting astrogliosis and neuronal loss when acutely injected with Lys that is presumably converted to GA and possibly 3HGA in the brain of these animals. The present data therefore support the hypothesis that increased brain concentrations of the accumulating metabolites may be responsible for the striatal damage occurring in GA I patients during episodes of metabolic decompensation. **Financial support:** PROPESq/UFRGS, FAPERGS, CNPq, FINEP IBN-Net, and INCT-EN.

419 - Cobalamin C Deficiency Detected Through Family Screening and Treated Prenatally—Case Report

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Background: Currently cobalamin C (cblC) deficiency may be detected by newborn screening (NBS). But despite early identification and initiation of therapy, the patients are still at risk to develop some complications. **Case Report:** Older sister of our index patient was detected by NBS due to elevated C3-carnitine. Soon combined form of methylmalonic aciduria (MMA) and hyperhomocysteinemia was diagnosed. Unfortunately, the child suddenly died at age of 2 weeks. Finally, cblC deficiency was identified. The patient's mother became pregnant again and prenatal diagnostics showed affected fetus (the same genotype; c.565delC homozygote). The parents decided to continue this pregnancy. Due to previous single reports of prenatal treatment in cblC deficiency, intramuscular (IM) injection of hydroxycobalamin (OHCbl) 5 mg 3 times per week were started since 22 gestational week. The index child was born at term without any complication with no dysmorphy or congenital anomalies. Initial biochemical findings (taken after birth) were: no MMA, C3-carnitine—2.4 μmol/L, tHcy—137 μmol/L , Met—12 μmol/L , OHCbl > 4000 pg/mL. The boy was treated with: OHCbl 1 mg/d, betaine 230 mg/kg/d, folinic acid 10 mg/d, L-carnitine 100 mg/kg/d. Dosing of above drugs was adjusted according to biochemical results and body weight increase. No protein restriction was recommended. The patient is doing well, during infections constant IV glucose infusions are given to avoid catabolism. At age of 9 months, he was able to sit unsupported and at age of 15 months he started to walk by himself. At age of 18 months the child was sociable, expressed single words. No neurologic, cardiologic, or hematologic abnormalities were observed. Now 21-month-old boy presents with nystagmus, retinal degeneration and maculopathy on ophthalmologic examination, but psychomotor development is normal. Current treatment: OHCbl 4 mg/d IM, oral betaine 180 mg/kg/d, folate 5 mg/d, L-carnitine 60 mg/kg/d, and Met supplementation 200mg/d. Last laboratory results are: tHcy 34 μmol/L , Met 28 μmol/L , OHCbl 895 000 pg/mL. **Conclusion:** Previous single reports showed beneficial effect of prenatal OHCbl administration. In our patient with cblC deficiency, early prenatal treatment with OHCbl and subsequent complex pharmacotherapy has resulted (until now) in normal psychomotor development and favorable outcome, although retinopathy has occurred.

420 - D, L-3-Hydroxybutyrate Treatment of HMG-CoA Lyase Deficiency in a Patient

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3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) lyase deficiency is a rare inborn error of metabolism characterized by recurrent metabolic crises generally presenting in neonates

(30%) or infancy (60%) during catabolic states triggered by prolonged fasting or intercurrent illness. Acute decompensations with lethargy, vomiting, hypotonia, hypoketotic hypoglycemia, and metabolic acidosis may evolve into Reye-like syndrome without treatment, with acute liver failure, hyperammonemic encephalopathy, dilated cardiomyopathy and death in 20% of cases. Long-term health complications include psychomotor retardation, white matter abnormalities, epilepsy, hepatic steatosis, pancreatitis, cardiomyopathy, and arrhythmias. The enzyme catalyzes the cleavage of HMG-CoA to acetyl-CoA and acetoacetate, the common final step of ketogenesis and leucine degradation, resulting in a diagnostic urinary organic acid pattern with absence of ketonuria, elevated 3-hydroxy-3-methylglutaric, 3-methylgutaconic, 3- methylglutaric, and 3-hydroxyisovaleric acids. Definitive diagnosis requires enzyme activity assays and/or genetic testing. Standard treatment includes protein (leucine) and fat restriction, carnitine supplementation, avoidance of fasting, and use of high carbohydrate based caloric intake when unwell. Here, we describe biochemical findings, mutation studies and clinical course in a patient with severe neonatal onset disease presenting with metabolic acidosis, non-ketotic hypoglycemia, hyperammonemia, and white matter changes on brain MRI. In addition to the standard management, D, L-3-hydroxybutyrate was used as an adjunct therapy to prevent potential cerebral dysfunction and cardiomyopathy, with the rationale that decreased ketone bodies (major energy source for the brain and the heart during starvation), synthesis occurs in this disorder. D, L-3-hydroxybutyrate therapy appears safe and effective in minimizing frequency and severity of acute decompensations.

421 - Glutaric Aciduria Type I Misdiagnosed as Dystonic Cerebral Palsy

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Introduction: Glutaric aciduria type 1 (GA1) is an inherited autosomal recessive metabolic disorder caused by a deficiency of glutaryl Co-A dehydrogenase (GCDH). **Clinical Report**: Here, we report a 14-month-old Saudi boy who was born at term by spontaneous vaginal delivery following an uneventful pregnancy with no history of perinatal asphyxia. He is the first baby of first-cousin parents with no family history of metabolic disorders or early neonatal deaths. He was noted to be floppy since early infancy with delayed gross and fine motor development. He achieved head control after the age of 8 months and started to sit unsupported at 10 months of age. However, he lost some of the developmental skills that he had gained after an acute gastroenteritis at the age of 11 months. This acute illness was complicated by encephalopathy and seizures. An extensive workup was carried out including cerebrospinal fluid analysis that was negative for viral and bacterial infections. He remained with severe spasticity, not responding to extensive physiotherapy and Baclofen therapy. He was subsequently diagnosed with cerebral palsy. He was referred to our center for a second opinion. His initial evaluation showed a spastic child with severe dystonic posture and failure to thrive. His investigations revealed normal serum lactate and ammonia with no evidence of metabolic acidosis. His brain magnetic resonance imaging (MRI) showed abnormal high signal intensity at basal ganglia and widened Sylvian fissure. Based on clinical presentation along with the MRI findings, the diagnosis of GA1 was considered. urine organic acids result showed elevated 3-hydroxyglutaric acid, while serum amino acids were normal. Acylcarnitine profile revealed high glutarylcarnitine, consistent with the diagnosis of GA1. The DNA analysis confirmed homozygosity for a mutation in the GCDH-coding gene (c.482G>A; p.R161Q). **Conclusions**: This case alerts pediatricians to consider GA1 as a differential diagnosis of children presenting with dystonic CP. Also, all children with neurologic symptoms of unknown origin such as CP should work up for inborn errors of metabolism.

422 - Dietary fibers and propionate production in an in vitro gut fermentation system

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Introduction: Gut microbiota metabolites could be critically important in genetic metabolic disorders such as propionic acidemia (PA). In this disorder, the biochemical and clinical features are caused by the accumulation of propionyl-CoA and other metabolites. This accumulation occurs due to the deficiency of the propionyl-CoA carboxylase enzyme. Propionate is a precursor of propionyl-CoA, and, in addition to acetate and butyrate, it is one of the main short chain fatty acids (SCFA) produced by the gut microbiota. Dietary fibers are known to influence the composition and activity of gut microbiota and, consequently, the production of SCFA. Therefore, dietary fibers—recommended to the PA patients to manage constipation, frequent in this disease—could play a role in the clinical features of PA patients. The objective of the present study was to evaluate the effect of different commercially available fiber mixtures, including mixtures used in clinical practice for children with PA, on fecal microbiota composition and fecal SCFA production. **Methods:** The effect of 2 fiber mixtures with the 6 fibers: oligofructose, inulin, resistant starch, cellulose, arabic gum, and soy polysaccharides (with and without Guar Gum) and 2 partially hydrolyzed Guar Gum (with and without starch) mixtures as well as several control oligosaccharides (GOS, FOS, lactose), on fecal microbiota composition (16 S rRNA gene amplicon sequencing) and SCFA production were measured after 24-hour incubation in a high-throughput anaerobic

colon model (i-screen ™ platform, TNO, Zeist, the Netherlands). The i-screen ™ model was inoculated with standardized healthy human adult gut microbiota mixtures (from fecal donations of 6 Caucasian adult individuals). **Results:** The most striking difference between the various fiber mixtures was observed in the propionic acid production, which was lower in the incubations of both 6 fiber mixtures as compared to the incubations of both Guar Gum fiber mixtures. Microbiota composition was comparable between the different mixtures. **Conclusion:** Different fiber mixtures result in variant levels of propionic acid production, and, thereby, further research is needed to explore whether fiber supplementation could play a role in modulating the clinical features of patients with PA.

423 - Fumarase Deficiency: A High Fat/Low Carbohydrate Diet is Safe and Potentially Disease Modifying

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Fumarate hydratase deficiency (FHD), caused by biallelic alterations of the fumarate hydratase (*FH*) gene, is a rare disorder of the tricarboxylic acid (TCA) cycle. The clinical phenotype is varied, ranging from a fulminant course with progressive infantile encephalopathy, profound psychomotor retardation, refractory seizures, and fatal outcome in early childhood to a less common, milder phenotype with profound speech delay and longer term survival. Severely affected children generally have FH enzyme activity less than 10% of the control mean. There are currently no recognized therapies and only isolated reports of unsuccessful dietary modifications. Herein, we describe the safe and possibly disease modifying effect of a high fat, low carbohydrate diet in a 12-year-old female with FHD. A severe phenotype was predicted based on her presentation by 6 months of age, with failure to thrive, hypotonia, developmental delay, MRI brain abnormalities with cerebral atrophy and polymicrogyria, subsequent seizures and fibroblast enzyme activity 9% of normal controls. Molecular analysis revealed compound heterozygosity for two mutations previously reported in severe cases with early fatality. FH catalyzes the reversible interconversion of fumarate and malate, and its deficiency leads to impaired energy production due to interruption in the flow of the TCA cycle with subsequent accumulation of various TCA intermediates including fumarate, succinate, 2-ketoglutarate and citrate. Each complete beta-oxidation of palmitate generates 8 molecules of acetyl-CoA which subsequently releases 2 molecules of NADH per acetyl-co A molecule fed into the TCA cycle, preceding the fumarate block. As a long chain of reduced carbon atoms, fatty acids fuel more cycles of the TCA cycle than glycolysis, and additionally generate one molecule each of NADH and FADH2, for each cycle of beta-oxidation. Hence, we propose that a high fat diet may increase the amount of reduced high-energy molecules, allowing increased ATP generation through the mitochondrial electron transport chain. Interestingly, we have not observed the expected increase in urinary fumarate levels with this dietary management. Contrary to predictions, her clinical progression has followed a milder phenotype with well-controlled seizures and mild-moderate intellectual disability, possibly due to the novel dietary intervention commenced at 14 months of age.

424 - L-2-Hydroxyglutaric Aciduria Treated With Riboflavin: A Case Report

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L-2-Hydroxyglutaric aciduria (L2HGA) is a rare, autosomal recessive, cerebral organic acid disorder with a characteristic disease course. It is a disorder of metabolite repair and presents exclusively neurological symptoms. The deficiency of FAD-linked L-2-hydroxyglutarate dehydrogenase (L2HGDH) leads to accumulation of L-2-hydroxyglutarate in CSF, plasma and urine. Patients exhibit a slowly progressive disease course, with mental and psychomotor delay, accompanied by symptoms such as seizures, cerebellar ataxia, macrocephaly and extrapyramidal, pyramidal or pseudobulbar signs. Brain imaging shows a characteristic pattern of diffuse, mostly subcortical cerebral white matter abnormalities and basal ganglia (especially putamen and globus pallidus) and dentate nuclei abnormalities. The objective of this study is to report a case of L2HGA and review the literature available on the disease and differential diagnoses. The patient was the only son of a consanguineous Brazilian couple and presented at 6 months with generalized tonic-clonic seizures. He had mild developmental delay and macrocephaly. He evolved with poor seizure control and was admitted at 11 months for inpatient treatment, when medication dosage was adjusted and a computerized tomography (CT) scan was obtained, revealing diffuse white matter hypodensity. Magnetic resonance imaging (MRI) of the brain revealed bilateral and symmetrical abnormalities of the basal ganglia (putamen and globus pallidus), the dentate nuclei and the frontal-parietal-temporal-occipital brain white matter, with sparing of the corpus callosum and brain stem. Qualitative mass spectrometry in two urine samples showed elevated levels of 2-hydroxyglutaric acid without other Krebs cycle metabolites. Plasma amino acids analysis was normal. Based on clinical

signs, imaging study and laboratory results, he was diagnosed with L2HGA and started on riboflavin 100 mg/day at 18 months. At 3 years old, patient remains stable, with some developmental delay, gaining milestones, with no new symptoms. Fast diagnosis is the key for timely commencement of treatment. Although reports regarding the use of riboflavin on L2HGA are still anecdotal, they suggest that it might slow down disease progression. Although enantiomeric analysis and genetic tests weren't available in this case, the accumulation of 2-hydroxyglutaric acid in the context of typical clinical and imaging findings allowed for the start of treatment with minimal delay.

425 - A New Case of Molecularly Confirmed Combined Malonic and Methylmalonic Aciduria in Poland

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Background: Combined malonic and methylmalonic aciduria (CMAMMA) is a rare recessive inborn error of metabolism characterized by elevations of urine malonic acid (MA) and methylmalonic acid (MMA), with higher levels of MMA than MA. CMAMMA presents in childhood with symptoms suggestive of an intermediary metabolic disorder: metabolic acidosis, elevated transaminases, developmental delay, axial hypotonia, dystonia, seizures, microcephaly, cardiomyopathy and failure to thrive. CMAMMA is caused by mutation in the gene *ACSF3* on chromosome 16q24.3, encoding acyl-CoA synthetase family member 3, which form an activating thioester linkage between the fatty acid and CoA. **Case report:** The patient is a boy, who was born from the sixth uncomplicated pregnancy by cesarean section at 36/37 weeks of gestation, with Apgar score of 10 and body weight of 3 kg. Neonatal intestinal atresia was treated using laparotomy after birth on second day of life and temporary stoma was placed. Short bowel syndrome was recognized due to the surgical removal of a large portion of the small intestine. Total parenteral nutrition was administered. He suffered from severe sepsis two times, which was associated with infection of central venous catheter. The urine organic acid profile analysis was performed for the first time at 1 month of age and showed elevated MMA = 696 mmol/mol creat. (ref. <20) and MA = 145 mmol/mol creat. (ref. ND). Test was repeated twice with similar results. Acylcarnitine profile was conducted twice and revealed decreased free carnitine C0 = 6.29 μmol/L (ref. > 11.8). Currently child aged 15 months, reveals generalized hypotonia, psychomotor delay, failure to thrive. Dietary treatment is aimed, high in carbohydrates, low in protein (total daily protein intake 1.75g/kg/day), adequate lipids, based on infant formula and partially parenteral nutrition. **Results:** Molecular genetic analysis revealed compound heterozygosity for two novel mutations: c.348_351dup, p.ser118Aspfs*153 and c.1061g>A, p.Arg354Gln in the *ACSF3* gene. In silico predictions suggest a pathogenic effect for these mutations. **Conclusions:** The phenotypic spectrum of CMAMMA is broad (Sloan et al. 2011), ranging from asymptomatic individuals, to adults with neurological syndromes and psychiatric problems. Children presented with variety of clinical features. Until now 12 patients have been reported in the literature, the natural history of this disorder and clinical management remain to be elucidated.

426 - Severity and Variety of Management in Five Patients With Methylmalonyl-CoA Mutase Deficiency

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Objective: Newborn mass screening using MS/MS (NMS) enabled early diagnosis for fatty acids and organic acids metabolism disorders and early therapeutic intervention has shown successful results. On the other hand, an evaluation of severity became more important for selection of the therapeutic strategies. Here, we evaluated the clinical course in 5 patients with methylmalonyl-CoA mutase (MCM) deficiency to elucidate their severities even before their first metabolic attack. **Patients and method**: Pt 1: 15-year-old female. Onset: 2-month-old. Vomiting, convulsive seizure, severe mental retardation, frequent acidosis attacks (4-6 hospitalization per year) were observed and living donor liver transplantation was performed at 5 years old. Pt 2: 10-year-old male. Onset: 12-month-old. Developmental delay and acidosis attack were observed, but no metabolic crisis after the first attack was observed with early hospitalization and his development improved. Pt 3: 4-year-old female. Detected by NMS. No severe metabolic crisis was observed with early hospitalization

to prevent attack. Pt 4: 3-year-old female. Detected by NMS. Hypoglycemia with metabolic acidosis crisis with unconsciousness at infectious was observed. Pt 5: 1-year-old male. Onset: 2-day-old. Apnea with high NH3 level: 970 μg/dL and metabolic acidosis (pH:7.18, BE:-14.5 at day 3) were observed. NH3 level became normal with treatment at day 5 but encephalopathy was obvious after resuscitation due to MRSA septic shock. In these 5 patients, natural protein intake (g/kg/day), amount of L-carnitine supplementation and serum acylcarnitine levels in their stable condition were compared. **Result and discussion**: According to the amount of natural protein intake and clinical course, Pt 2 was considered to be a mild case. C3 and C3/C2 levels in stable condition might be useful biomarkers to determine their severities, because obviously low levels were observed in Pt 2 compared to the other 4 patients. More than 1.0 g/kg/day natural protein intake were hardly achieved in severe type. Pt 3 is considered as severe type; however, her parents have not decided to have liver transplantation (LT) since she has shown normal development with strict diet and early hospitalization to prevent metabolic attacks. Effectiveness of LT in MCM deficient patients was previously reported, however we should carefully think about the best strategy considering the risk and benefit of LT for each individual to improve their quality of life.

427 - Vitamin B12 Responsive Methylmalonic Acidemia (MMAr): Medical and Dietetic Treatment, Biochemical Profiles, and Outcomes of Patients From a Single Center Cohort

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Objective: There is limited published data on treatment and outcome of MMAr. Dietary treatment varies internationally. Our objective was to describe dietary and medical treatment, biochemical profiles, and outcome of our cohort. **Methods**: Longitudinal data was collected on current patients by retrospective review of medical notes and routine out-patient biochemical results including, urinary MMA excretion, MMA/creatinine ratio μmol/mmol (UMMA) measured by GC-MS and plasma MMA μmol/L (PMMA) by MS/MS. **Results**: Eleven MMAs were identified (8 Asian, 3 mixed race). Nine were cblA, one cblD-MMA, and one mut^-. Nine initially presented with metabolic decompensation (2 required hemofiltration), and two were screened due to family history (diagnosed at birth, 1.5y). Treatment commenced at birth to 4.5 y (median 13wks); current age range 2-17 y (median 10y). Diagnostic UMMA median concentration was 9251, range 2390-33900. Vitamin B12 responsiveness was tested on fibroblasts (propionate incorporation test) or clinically. All were treated with vitamin B12: 9 with 1 mg hydroxycobalamin i.m. every 1-14 days (median 2d), 2 with oral adenosylcobalamin. All were on L-carnitine, restricted protein diet, vitamin and mineral supplement and emergency regimen. Total daily protein intake was: median 112%, range 94%-149% of safe intake for age (WHO/FAO/UNU 2007). Initially protein intake was counted and foods weighed. All fed orally, 3 used energy supplements. Growth data: median weight z-score −0.2, height z-score 0.3. No further episodes of metabolic decompensation were observed on treatment. GFR (mL/min/1.73m^2) was measured 1-2 yearly from age 2 y, current range 85-124. Once stabilized on treatment serial results for all patients: UMMA concentrations median 426 (range 33-10 125), PMMA median 44 (range 4-482). Common reasons for higher UMMA levels were attributed to B12 non-compliance, need for B12 to be administered more frequently and intercurrent illness. All attend mainstream school, 5 reported behavioral problems. **Conclusion**: Acute presentation was associated with greatly elevated UMMA and metabolic decompensation. Despite B12 treatment, protein restricted diet and L-carnitine, UMMA and PMMA remained chronically elevated in all. No patient developed chronic kidney disease but this remains a risk. Normal growth was achieved on diet. To help prevent toxicity from increased methylmalonic acid and optimize outcomes we advise a protein restricted diet is combined with B12 therapy and L-carnitine.

428 - Mevalonate Kinase Deficiency Syndrome: A Case Report

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The hyperimmunoglobulinemia D / Mevalonate kinase deficiency (MKD) is a rare autosomal recessive inherited autoinflammatory disease characterized by recurrent fever associated to persistent episodes of hyperinflammation, abdominal pain, arthralgias, and mucocutaneous lesions. It is caused by loss of function mutations in *MVK*, which encodes for the protein mevalonate kinase. Mevalonate kinase is an essential enzyme

in the common pathway leading to both cholesterol and non-sterol isoprenoids, which are necessary for several essential biologic processes. Typically, patients start to show symptoms in the first years of life. As clinical manifestations are unspecific and often mimic a chronic inflammatory disease, the diagnosis is challenging and requires immunologic, genetic, and biochemical investigations. Here we present a 19-year-old male, child of consanguineous parents, with recurrent episodes of fever since he was 2 months old. At three months of age, he presented lymphadenopathy and three months later hepatosplenomegaly. At the age of four, a liver biopsy showed granuloma suggesting mycobacteriosis, however, no mycobacteria were found and tuberculin skin test was negative. He constantly suffered from painful cervical lymphadenopathy, tonsillitis and suppurative otitis, as well of skin lesions. At the age of seven, he was submitted to a diagnostic laparoscopy due to acute abdomen, which led to a final diagnosis of primary peritonitis. Laboratorial tests showed anemia, leukocytosis and elevation of inflammation markers, as well as increased levels of IgA and IgG. He presented normal lymphocyte immunophenotyping and normal myelogram. At the age of 11, after exclusion of several immunodeficiency syndromes, a diagnosis of MKD was suspected. Analysis of the *MVK* gene confirmed a mutation in homozygosis, and urinary organic acids analysis showed an increased mevalonic acid excretion, confirming the diagnosis of MKD. Patient was first managed with simvastatin, however with poor adherence. After several years of treatment with corticosteroids and antibiotic prophylaxis, he showed an improvement of the autoinflammatory symptomatology. This case illustrates an example of an inborn error of metabolism affecting the cholesterol biosynthesis with an autoinflammatory phenotype, characterized mainly as fever of unknown origin. Determination of mevalonic aciduria may be helpful to support the diagnosis if molecular tests are not available.

429 - L-2-Hydroxyglutaric Aciduria Case Series

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Objective: L-2-hydroxyglutaric aciduria (L2HGA), D-2-hydroxyglutaric aciduria (D2HGA) and combined D, L-2-hydroxyglutaric aciduria (D, L2HGA) are autosomal recessively inherited cerebral organic acidurias. They are usually diagnosed with urinary organic analysis of a patient with unexplained developmental delay and/or other neurological dysfunction. In this report, we aimed to define our patients' clinical findings with mutation analysis results in order to state the heterogeneity of L2HGA. **Methods:** Medical records of 13 L2HGA patients were evaluated retrospectively. Demographic features, clinical data and molecular analyses' results were summarized. **Results:**13 patients were included in this report. Male/female ratio was 1,6. The mean age of the first symptom was 3.65 $\pm$ 3.12 years (min: 0.25 and max: 10.5 years). The mean current age was 10.8 $\pm$ 6.48 years (min:3.0 and max: 26.8 years). One of the patients was diagnosed at 25 years of age, even though she had symptoms before one year of age. 3 patients were siblings, and 6 had positive family history. 12 patients' parents were consanguineous. The initial symptom was convulsions in 3, developmental delay in 8, and 2 patients were diagnosed with family screening without any complaints. Totally, 5 patients have abnormal cerebellar tests, 6 patients have epilepsy and 11 had psychomotor retardation. Interestingly, one female adult patient had Graves 'disease and pseudotumor cerebri in addition to L2HGA. None of the patients had intracerebral neoplasms. Of the pathogenic mutations on *L2GHDH* gene, 3 patients had 2 different unidentified mutations described as highly damaging with *in silico* programs. **Conclusion:** 2-hydroxyglutaric acidurias are first defined in 1980. Patients show neurological manifestations, including mild to moderate psychomotor retardation, cerebellar ataxia, tremor, dystonia, variable macrocephaly, and epilepsy. Our patients' striking sign was psychomotor retardation, and they have variable epilepsy and cerebellar signs. Also, the age of the first symptoms were very diverse in our patient series. In the literature, central nervous system neoplasms, bone tumors and nephroblastomas are also reported in L2HGA. In our case series, none of our patients had any type of malignancies despite a relatively long follow-up interval. Although there are no specific therapy for these disorders, clinicians should regularly follow-up these patients for not only neurologic manifestations but also for malignancies.

430 - Early Detection, Treatment and Prognosis of Combined Malonic and Methylmalonic Aciduria in a Mexican Newborn: Case Report

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Combined Malonic and Methylmalonic Aciduria (CMAMMA) (OMIM #614265) is an autosomal recessive inborn error of metabolism characterized by elevations of urine malonic acid (MA), and methylmalonic acid (MMA), secondary to malonyl-CoA decarboxylase (MCD) deficiency. CMAMMA is claimed to be the most common intracellular disorder of cobalamin metabolism with an incidence of 1/100 000 to 1/37 000 live births worldwide. Patients with CMAMMA have normal

development, although they may also have severe complications such as: metabolic acidosis, developmental delay, seizures, cardiomyopathy, gastrointestinal distress, and dysmorphic features. The possibility to improve the prognosis in patients with CMAMMA depends upon the early detection. We present an asymptomatic 1-month-old Mexican female with CMAMMA detected by newborn screening. The quantification of organic acids in urine showed significantly increased levels of methylmalonic acid (1369 mg/dL). The molecular study found a variant within the *ACSF3* gene supporting the diagnosis of CMAMMA. The treatment includes adjustments in her diet combining a formula MTVI free (methionine, threonine, valine, and isoleucine) and breast milk, whose content quantities varied upon the follow-up test. We aim to report the followed pathway through a correct diagnosis, the therapeutic strategy and to compare it with the ones reported in literature. Early detection and treatment with parenteral OHCbl have decreased the mortality and reversed the complications in newborns with CMAMMA. Therefore, it is important to make a complete prenatal care, newborn screening, and to raise awareness.

431 - The Clinical, Biochemical Features, and Mutational Analyses in Glutaric Acid Type 1 Patients

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Introduction: Glutaric acid type 1 (GA1) is a rare autosomal recessive neurometabolic disorder caused by glutaryl-CoA dehydrogenase deficiency due to GCDH gene mutations. Patients may present with brain atrophy, macrocephaly, and acute dystonia secondary to striatal degeneration typically triggered by an infection, fever, and/or dehydration. Motor–mental retardation (MMR), seizures, and extrapyramidal symptoms are subsequently presented. Neuroimaging studies commonly show brain atrophy and abnormal myelination. Herein we described the clinical and biochemical profiles together with the, mutation results on GCDH gene of 48 patients with GA1 in a single center. **Subjects and methods:** This retrospective study was conducted with hospital records of patient with 48 followed by Hacettepe University, which has one of the biggest metabolic center in Turkey. The clinical and biochemical features and mutational analyses were evaluated. **Results:** A total 48 patients (n:25, 52.1% girls, n: 23, 47.9% boys) were included. The mean age of the patients was 13.18 year (std. deviation: $\pm$7.34). The median age of onset of findings and age of diagnosis were 0.58 year (IQR: $\pm$0.7) and 1.0 year (IQR: $\pm$4), respectively. Initial findings of patients were dystonia (66.7%), seizure (41.6%), retardation (37.5%), hydrocephaly (16.6%), subdural effusion (4.1%). and discomfort (2.1%). Furthermore, five patients (10.4%) were asymptomatic and detected with the sibling history. There was macrocephaly in 30 (62.5%), speech impairment in 43 (89.5%), and neuromotor retardations in 47 (97.9%) patients. There were typical CT/MRI findings in 45 (93.8%) patients. Thirteen patients (27.1%) were given antiepileptic therapy. Clinical outcomes of patients were mild MMR (20.8%), moderate MMR (20.8%), severe MMR (37.5%), gross motor delay (4.2%), mild dystonia with normal mental activity (2.1%), severe MMR with autism (2.1%), and exitus (12.5%). Mutations in the GCDH gene of 39 patients were detected. 13 patients of them had novel mutations. **Conclusion**: GA1 is more often inherited metabolic disease in Turkey because of common consanguineous marriages. GA type 1 disease should be considered in patients presenting with dystonia, macrocephaly, seizure and MMR. But, when diagnosis is made after manifestation of neurological disease, outcome is poor, and therapeutic impact is limited. Therefore, early diagnosis and appropriate treatment are extremely important for achieving a better outcome.

432 - Analysis of Urinary Organic Acid Excretion Profile in Children

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Introduction: Inborn errors of metabolism (IEM) are rare disorders caused by mutations in a gene that codes for a protein involved in the metabolism disrupting its biological activity. Organic acidurias are a group of IEM characterized by the accumulation of organic acids (OA) in biological fluids, due to defects in the degradation of lipids, carbohydrates or amino acids. Organic Acidurias occurs more frequently in the pediatric population, with a broad spectrum of symptomatology that may cause patient's death if not treated. For the diagnosis of these diseases it is necessary to perform the analysis of urinary OA excretion profile by GC-MS. Previous studies have shown that factors such as diet, age, products of bacterial metabolism and medications may interfere with the excretion profile of these acids, making it difficult to interpret them correctly. **Objective:** To analyze the variations in short and medium chain OA urinary excretion profile in children during the first year of life. Methods: A semiquantitative analysis of the excretion profile of OA was carried out by GC-MS in 68 urine samples from children during the first year of life, divided in three groups according to their age and dietary habits: 0-4 months (breast feeding only); 5-8 months (onset of complementary diet); and 9-12 months (familiar diet). **Results:** Chromatographic profiles included 48 metabolites that included metabolic intermediaries already reported in children samples, and 2 metabolites not previously reported that were associated to diet. In addition, variations in the OA profile was mainly

explained by age, although among the three groups it was also observed variations in dietary derived OA. Additionally, the presence of basal levels of metabolites associated with organic acidemia were detected in this population. **Conclusions:** Although qualitative and semi quantitative OA profile analysis has a high sensitivity and specificity for organic acidurias diagnosis, it can be overshadowed by the presence of interferers derived from diet and medication, as well as age variations. For this reason, it is necessary to emphasize the importance of having a normal reference profile established in the same age and population of the patients being studied, in order to overcome the above-mentioned interferences in order to improve the diagnosis of organic acidurias.

433 - Analysis of Urinary Organic Acid Excretion Profile in Newborns Feed With Infantile Formulas

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Introduction: Organic Acidurias are inborn errors of metabolism that can present during the neonatal period. Their diagnosis is achieved through the analysis the urinary excretion profile of organic acids (OA) using GC-MS. This profile allows the observation of increased levels or the presence of metabolites characteristic of each aciduria. There is evidence, that specific diet habits may influence the OA excretion profile. Therefore, in newborns, such profile can be influenced by the use of infantile formulas, however, up to now there are no reports in the literature that address this issue. **Aim:** To describe the urinary excretion profile of short and medium chain organic acids in full-term newborns that were feed with breast milk and infantile formulas. Methods: The samples analyzed corresponded to urine samples from newborns between 0 and 4 months that were feed with breast milk($N = 30$), and infantile formulas ($N = 20$). The OA urinary excretion profile was obtained by GC-MS and analyzed semiquantitatively. **Results:** The analyzed profiles showed the presence of around 35 short and medium chain OA that include metabolites derived from TCA cycle, amino acids metabolism, and fatty acids oxidation, among other metabolic pathways. In both populations, the presence of metabolites that have not been previously reported in newborns was observed. In addition, metabolites associated to organic acidurias were also observed in the studied population. Some of these metabolites were present in low levels in both populations (v.g. Glutaric acid), whereas others were associated to the consumption of infantile formulas (v.g. 3-hydroxy-3-methyl-glutaric acid). **Conclusions:** The results obtained evidenced that the OA excretion profile reflects the metabolic changes in newborns caused by the diet, since the nutrients supply is different between breast milk and infantile formulas. Such changes may alter the qualitative interpretation of the excretion profile in the context of OA diagnosis.

434 - Glutaric Aciduria Type I: Twenty-Four Cases in Brazil Over a Ten-Year Period Experience

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Objectives: Glutaric aciduria type I (GA1) is an autosomal recessive inborn error of metabolism (IEM) caused by the deficiency of the enzyme glutaryl-CoA dehydrogenase. Untreated, most patients suffer an acute encephalopathic crisis, triggered by infection or immunization, characterized by hypotonia and seizures, which lead to the gradual development of a complex movement disorder. In some patients, the movement disorder develops insidiously, in the absence of an encephalopathic crisis. In symptomatic patients, quantitative urinary organic acids, and plasma or dried blood spot (DBS) and urinary acylcarnitine analyses are the preferred methods of diagnosis. We report the biochemical findings of a series of symptomatic patients that were diagnosed with GA1 in a Brazilian reference laboratory. **Methods:** Urine and DBS were obtained from 6024 patients with a clinical suspicion of an IEM. Urinary organic acids were identified by gas chromatography/mass spectrometry, and acylcarnitines in DBS and urine by electrospray ionization tandem mass spectrometry. **Results:** The diagnosis of GA1 was made in 24 patients, 11 females and 13 males, with ages varying from 7 months to 20 years. Most of the patients were diagnosed in the chronic neurodegenerative phase after an encephalopathic crisis. Three patients presented the insidious or the late forms of the disease. Suggestive clinical signs included epilepsy (9 in 24), extrapyramidal manifestations (9 in 24), macrocephaly (6 in 24), neuroradiologic abnormalities (4 in 24), and truncal hypotonia (4 in 24). Parental consanguinity was reported in four patients. A urinary peak of glutaric acid and a less conspicuous peak of 3-hydroxyglutaric acid (high excretor phenotype) were found in 22 patients; in eight of them, excretion of glutaconic acid was also observed. Two patients with a low excretor phenotype had undetectable or trace urinary levels of these acids. The diagnosis in these patients was accomplished by the finding of a urinary and/or DBS glutarylcarnitine peak (C5 DC). In all patients with a high excretor phenotype, in which acylcarnitines could be analyzed (12 in 22), it was possible to find a C5 DC peak. **Conclusions:** The profiles of

urinary organic acids and DBS acylcarnitines are indicated for all patients with macrocephaly, acute encephalopathic crisis, abnormal movements, or neuroradiologic studies revealing white matter abnormalities or frontotemporal hypoplasia, for which no other etiology has been established.

435 - Completing the Metabolic Network Around 2-Hydroxyglutarate

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2-Hydroxyglutarate (2HG) is an atypical metabolite that accumulates in neurometabolic diseases as well as in certain types of cancer. The mechanisms through which 2HG leads to cell transformation or neurodegeneration remain, however, poorly understood. Compared to the research on 2HG in mammalian systems, and despite certain advantages of yeast as a model organism for biomedical research, only a very limited number of studies reported on the occurrence and metabolism of 2HG in yeast. An extensive study performed over the last two years in our lab, revealed a panoply of new findings on 2HG metabolism of Saccharomyces cerevisiae. Among those the fact that the yeast phosphoglycerate dehydrogenases Ser3 and Ser33 convert α-ketoglutarate to D-2HG in addition to their primary metabolic role, which consists in catalyzing the first step of the serine synthesis way converting 3-phosphoglycerate to 3-phosphohydroxypyruvate. Our results also show, however, that the two identified D-2HG producing enzymes do not represent the only sources of this metabolite in yeast. Within our pilot study, the two dehydrogenases Dld2 and Dld3 were both shown to convert D-2HG to α-ketoglutarate in vitro and the purified recombinant enzymes were found to have a higher affinity for D-2HG than for D-lactate. Targeted metabolome analyses and biochemical characterization led additionally to the original finding that DLD3 is actually an FAD-dependent transhydrogenase that converts D-2HG to α-ketoglutarate, using pyruvate as a hydrogen acceptor. Based on our findings, we were for the first time able to propose a central carbon network of Saccharomyces cerevisiae integrating the metabolite D-2HG and connecting its metabolism to the mitochondrial respiratory chain (1). In the present research project, we aimed to further elucidate the metabolic network involved in 2HG formation and degradation in yeast as well as to gain a better understanding of the cell functions that are impacted by pathological intracellular 2HG concentrations. Using targeted metabolome analysis and high-throughput growth phenotyping, we analyzed the accumulation of D-2HG in natural yeast isolates. Applying the same methodologies on strains lacking genes known to be involved in the metabolism of 2HG in yeast, we compared the effect of 2HG accumulation under various metabolic states of the cell.

436 - Effect of EPI-743 on the Clinical Course of Visual Damage in Cobalamin C Patients: A Prospective Randomized Double-Blind Phase II Clinical Trial

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Background: Cobalamin C (CblC) defect is the most common inborn error of cobalamin metabolism causing methylmalonic aciduria and homocystinuria. Despite standard pharmacological treatment, long-term outcome in early-onset patients is characterized by progressive visual (maculopathy and optic atrophy) and neurological impairment. Pathophysiology of eye and brain damage remains unclear. Recently, the contribution of oxidative stress has been hypothesized. EPI-743 is a small molecule therapeutic that has shown beneficial effects in different diseases characterized by oxidative stress. Our aim was to test the potential benefits of add-on therapy with EPI-743 on visual and neurological damage in CblC patients. **Patients and methods.** A 1-year prospective randomized double-blind phase II clinical trial (ClinicalTrials.gov identifier: NCT01793090) was conducted in 30 genetically confirmed CblC patients, aged 2 to 22 years, with variable degrees of visual impairment. The patients were randomly assigned to receive a 6 months course of EPI-743 (100 mg TID) or placebo. After 6 months, all patients were switched to treatment for further 6 months. All patients were evaluated 6 months before, at 6 months intervals during the trial and 6 months after treatment, to compare our findings with individual natural history. Primary endpoint was a 20% improvement in visual function. Secondary endpoints were improvement in neurologic function and/or QOL and/or in electrophysiological tests (flicker VEP). **Results:** One patient dropped out. 5/30 patients were not assessable at least at one of the time-points of the study due to lack of collaboration. Despite the large heterogeneity of visual impairment, treatment with EPI-743 led to an improvement ($P < .05$) in visual acuity in 9/24 (37%) of the remaining patients. Younger age and absence of maculopathy were the main predictors of response. We did not observe significant changes in flicker VEP amplitude. However, subgroups analysis showed better results in patients without maculopathy ($P = .03$). Despite some reported progresses in adaptive behavior function, no significant changes were recorded in neurological function and QOL. There was no evidence of drug-related side effects. **Conclusions.** Our study shows that EPI-743 may

improve visual function in a selected subcategory of CblC patients (young patients without maculopathy), suggesting the need of larger multicenter clinical trials in more homogenous CblC patients cohorts.

437 - Glutaric Aciduria Type I Diagnosis Case With Normal Glutaryl Carnitine and Urine Organic Acid Analysis

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Glutaric aciduria type I (GAI) is a rare inherited metabolic disease, deficiency of glutaryl-CoA dehydrogenase results in accumulation of the putatively neurotoxic metabolites glutaric and 3-hydroxyglutaric acid (GA, 3-OH-GA) in body tissues, particularly within the brain. Here we presented a 3-year-old girl with hypotonia and dystonia diagnosed with GAI although the repeated analysis of the carnitine profile and organic acid analyzes were normal. The patient has motor, mental retardation, and hypotonia. Her weight was on 3-10 percentile, height on 25-50 percentile, and head circumference on 25 percentile at the age of 3 years. The physical examination was normal except severe hypotonia. Spot blood carnitine profile, blood amino acid, urine organic acid, lactic acid and pyruvic acid were normal in repeated analyzes. Dystonia and spastic tetraparesia developed on her follow-up. Cranial MR scan revealed bilateral striatal necrosis. In GCDH gene analysis, p. Y123C (c.368A> G) /p.L340F (c.368A> G) mutation was found. There was no history of encephalopathy. The patient treated with levodopa and trihexepenidil and lysine-restricted diet. In the presence of bilateral striatal necrosis and dystonia, glutaric aciduria type I should be kept in mind. Blood carnitine profile and urine organic acid analyzes may not be consistent. It is important to evaluate the cases for genetic investigation.

438 - Overview of Organic Acidemia Diagnosis and Other Inborn Errors of Metabolism from a Reference Center in Chile

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Introduction: Urinary organic acid (UOA) analysis by gas chromatography-mass spectrometry (GC-MS) is the essential analytical procedure for the laboratories focused in investigation and diagnosis of the inherited metabolic disorders. Our center has implemented in 2014 the analysis of UOA excretion profiles following a qualitative methodology and by quantitative isotopic dilution the determination of succinylacetone and orotic acid for tyrosinemias and orotic acidurias diagnosis confirmation, respectively. To date, our laboratory has received and analyzed 520 urine samples from patients suspecting for one of the 65 possible inherited metabolic abnormalities that could be confirmed by those approaches. In consideration with proper difficulties on the interpretative analysis of UOA, we actively participate in External UOA Qualitative Scheme from ERNDIM, (European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism) and in the Special Assay in Urine Program, reaching to the date positive and confident results. **Results:** From October 2014 to May 2017, we found a 7.75%of abnormal excretion pattern of organic acids from the total of processed samples that were characteristic for one of the classic profile of organic acidemias or EIM. The confirmatory diagnostic was based on the compatibility of chromatographic UOA findings profile and clinical signs of the patients documented in a special UOA form requested together with the sample. The age of patients at the time of diagnosis ranged from one month of age to 34 years, with an average time in the notification of the result of five days. **Conclusion:** After three years working as a specialist laboratory for analysis of organic acid analysis, we could evaluate our overall performance as positive. Among the achievements reached by the collaborative work of our laboratory, clinical and nutritional staff, we could mention the recognition by Health Ministry as the single providers for the confirmation of high cost pathologies (Tyrosinemia type I and other LD), included in national Subsidized programs (Ricarte Soto law). This scenario gives us new challenges and opportunities to improve access to the diagnosis of EIM and direct us to work on implementation of other diagnostic tools that allow the fast and reliable confirmation of EIM.

439 - Impaired Bone Health in Methylmalonic Acidemia and Propionic Acidemia: An Underrecognized Comorbidity

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Background: Decreased bone mineral density (BMD) is a rarely reported feature of methylmalonic acidemia (MMA) and propionic acidemia (PA). We report a systematic review of our cohort in a single center. **Objective:** Dual energy X-ray

absorptiometry (DEXA) were analyzed in Vitamin B12 responsive (MMAr) and non-responsive (MMAnr) MMA and PA patients. Second objectives were correlating bone markers, vitamin D levels and renal impairment. **Methods:** 42 active MMA and 17 active PA patients' medical records were reviewed, of which 15 MMA and 6 PA patients completed at least one DEXA scan. BMD age matched Z score of the lumbar spine was measured. Low BMD was defined as Z score < −2.5 standard deviation (SD). Renal impairment was defined as glomerular filtration rate (GFR) less than 60ml/min. Bone marker used in this study was alkaline phosphatase (ALP). **Results:** Of 15 MMA patients included; 8 were MMAr and 7 were MMAnr. Age range at the time of the DEXA was 6.2 to 17.1 years (median 12.8 years). Z score ranged from 0.2 to −3.9. Normal BMD were 3/15; Z score between −1.0 and −2.5 were 7/15 and Z scores < -2.5 were 5/15. BMD <-−1 was seen in MMAr (6/8) and MMAnr (6/7). All MMAnr cases had GFR <60. Vitamin levels were insufficient in 3/15 and ALP was increased in 4/13 with ALP results available. For PA patients, 4 were included, aged between 9 and 17 years (median 16 years). Z scores ranged -0.2 to -5.7. 2/4 had normal BMD whilst the other 2/4 had Z scores < -2.5; with the latter group that demonstrated raised ALP and low vitamin D. None of the PA patients had renal impairment. **Conclusion:** 7 of a total of 19 (37%) patients in the cohort study of MMA and PA had low BMD with Z scores less than -2.5. Therefore, poor bone health in MMA and PA is likely to be under-reported in literature and perhaps underestimated. Poor bone health in MMA and PA patients is likely to be multifactorial and not only secondary to renal impairment. Consequently, it should be closely monitored with caution to adequate treatment.

440 - Neuroradiological Findings in Glutaric Aciduria Type I a Key for Diagnosis

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Introduction: Glutaric aciduria type 1 is a rare metabolic disease caused by a deficiency of glutaryl-CoA dehydrogenase located in the catabolic pathway of lysine, hydroxylysine, and tryptophan, leaving to toxic metabolic accumulation of glutaric acid and 3-hydroxiglutaric acid. Most untreated patients present with an encephalitic crisis during the first years leaving to acute striatal necrosis, movement disorder and severe neurological sequelae. Diagnoses before the metabolic crisis without newborn screening is difficult because unspecific neurologic symptoms like macrocephaly and developmental delay, about a half of them remains asymptomatic until metabolic crises. Neuroradiology findings CT/ MRI in selective screening can lead to the metabolic workup and diagnosis. **Objective:** To describe neuroradiological findings in a series of cases with glutaric aciduria type 1 and to emphasize the role of neuroimaging in the diagnostic approach. **Methods:** Retrospective review of medical records in a single national reference center for metabolic diseases at INTA (Institute of Nutrition and food technology, University of Chile) from 1994 to 2017. **Results:** 28 patients were diagnosed with AG-1, 20/28 the diagnosis was made after the metabolic crises, 3/28 died post encephalitic crises because of medical complications. The medium age at diagnosis was x = 12.4 months, age at the metabolic crises was x = 8.9 months. Neuroimaging CT/MRI were available in 21 patients the principal findings were widening of sylvian fissure and/or bitemporal cystic lesions (15/21), symmetrical T2 hyperintensity at basal ganglia (12/21) affecting putaminal and globus pallidus region, subdural hematomas (9/21) and supratentorial diffuse white matter involvement (5/21). These findings suggested the diagnosis of AG-1 in all the patients even in the absence of clinical symptoms. Conclusion: Neuroimaging is an important tool for the diagnostic approach in AG-1 especially in countries that do not have expanded neonatal screening available for the entire population.

M) Carbohydrate Disorders (441 to 473)

441 - Normalization of Cardiomyopathy and Clinical Improvement on Ketogenic Diet in Patient With GSD Type IIIA

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Introduction: Glycogenosis type III (GSD III) is an autosomal recessive disorder caused by the deficiency of debranching enzyme, causing incomplete glycogen degradation and its accumulation. Besides hepatomegaly, which is present in GSD type IIIb, also variable skeletal myopathy and cardiomyopathy are characteristic for GSD type IIIA. Cardiomyopathy might be progressive and very severe; few previous reports show an improvement of cardiomyopathy following a ketogenic diet (KD). **Case report:** A 12-year-old girl with GSD IIIa (*AGL* gene mutation, p.W1327X) developed a severe and progressive left ventricular obstructive hypertrophy, while treated with frequent diurnal and nocturnal meals with cornstarch supplements. In addition, she also had hepatomegaly, skeletal myopathy, and recurrent hypoglycemias. She was introduced the KD (ketogenic ratios of meals were from 2.5:1 to 4:1; fats contributed 87% calories, proteins 11% and carbohydrates 2%); subsequently, continuous ketosis has been maintained for the last 18 months. No hypoglycemias were recorded in the period. Within few months after KD introduction, laboratory parameters improved significantly, including lipid levels. Exertion dyspnea disappeared, while capacity for oxygen consumption almost doubled at control examination. Abdominal

ultrasound showed a significant improvement of hepatomegaly. Cardiac MRI was repeated after 16 months, showing a normalization of left ventricular parameters and mass (from 70 g to 35 g), without residual outflow obstruction. **Conclusions:** Cardiomyopathy might be a life-threatening complication in GSD IIIA patients. While on KD, we observed a normalization of a severe progressive obstructive cardiomyopathy and an important clinical improvement in a patient with GSD type IIIa with no hypoglycemias recorded.

442 - Familial Glycogen Storage Disease Type IX Diagnosed by Targeted Exome Sequencing

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Glycogen storage disease type IX (GSD IX) is caused by deficiency of phosphorylase kinase which plays a role in breakdown of glycogen. Mutations in the *PHKA2* are the most common cause of GSD IX. Clinical manifestations of X-linked recessive GSD IX resulting from *PHKA2* mutation include hepatomegaly, growth retardation, fasting hypoglycemia, and fasting ketosis. However, the symptoms overlap with those of other types of GSDs. Here, we report Korean familial cases with GSD IX whose diagnosis was confirmed by targeted exome sequencing. A 4-year-old male patient was presented with hepatomegaly and persistently elevated AST/ALT. Liver biopsy revealed swollen hepatocyte filled with glycogen storage, suggesting GSDs. Targeted exome sequencing was performed for the differential molecular diagnosis of various types of GSDs. A hemizygous mutation in *PHKA2* were detected by targeted exome sequencing and confirmed by Sanger sequencing: c.3632C>T (p.Thr121Met), which was previously reported. The familial genetic analysis revealed that his mother was heterozygous carrier of c.3632C>T mutation and his 28-month old brother had hemizygous mutation. His brother also had hepatomegaly and elevated AST/ALT. The hypoglycemia was prevented by frequent meals with complex carbohydrate, as well as cornstarch supplements. Their growth and development is in normal range. We suggest that targeted exome sequencing could be a useful diagnostic tool for the genetically heterogeneous and clinically indistinguishable GSDs. A precise molecular diagnosis of GSD can provide appropriate therapy and genetic counseling for the family.

443 - Challenges in Monitoring Siblings With Glycogen Storage Disease in a Rural Area

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Background: Glycogen storage disease (GSD) is an autosomal recessive inherited disorder which gives 25% probability to have the disease for each birth. The management of GSD consists of adequate nutrition support with uncooked corn-starch, restriction of monosaccharides and excessive fat. Specific medication is given when laboratory examination reveals abnormal result. The success of management depends on adequate long-term monitoring. **Objective:** To present two cases of GSD in siblings. **Case:** A man, who was diagnosed with GSD at 12 years old, had received standard treatment for 3 months at our policlinic. We referred him back to his hometown, which is located in different island as far as 818 miles from Jakarta. Unfortunately, he failed to receive adequate management and monitoring from the local health professional. After 9 years, he came with his younger brother and still has doll-like face, liver enlargement and short stature with height age of 12-year-old boy. The laboratory results showed hypoglycemia, metabolic acidosis, dyslipidemia (triglyceride 1229 mg/dL, total cholesterol 348 mg/dL), increase in plasma lactate and uric acid level. His echocardiography showed normal systolic and diastolic function, with no thrombus or pulmonary embolism. His younger brother, 3 years old, has liver enlargement since he was one year old, doll-like face and recurrent cold-sweaty body in the morning. The motoric and speech development is delayed. There was no history of jaundice, pale, abdominal pain, or bleeding. Both were born normal from non-consanguineous parents. The nutritional status was good but stature was below −2 SD. The laboratory results showed hypoglycemia, metabolic acidosis, dyslipidemia and increased of plasma lactate and uric acid level. Thus, we diagnosed the younger brother with GSD. Furthermore, we manage both of them with standard treatment and convinced the parents to keep contact with us even after we referred them back. **Conclusion:** Diagnosis of GSD, which can be made by clinical and laboratory examination, must be managed and followed continuously in order to achieve optimal growth and development.

444 - A rare cause of isolated hepatomegaly: Glycogen-storage disease type VI

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Background: Glycogen storage disease (GSD) type VI is a metabolic disorder which develops due to hepatic glycogen phosphorylase deficiency. It has a mild clinical course generally associated with hepatomegaly detected incidentally. However, cases may present with ketotic hypoglycemia and growth retardation. Findings generally decline in adolescence and disappear in adulthood. Plasma lipids and lactic acid might increase, uric acid and CK are normal. Mutation analysis is essential in diagnosis. In asymptomatic cases, treatment may not be given, but cases with fasting ketosis and growth retardation may require frequent feeding and uncooked cornstarch.

Case Report: An 11-month-old male patient was initially diagnosed with hepatomegaly by a pediatrician when he was 6 months old with cough complaint. The case was born full-term as the 1st living baby of the 1st pregnancy of a 21-year-old mother and had no complaints until admission. The liver of the case was palpable and measured 8 cm at midclavicular line, while no splenomegaly was observed. Laboratory findings showed that analysis of blood gases, serum lactate, uric acid, CK and bilirubin levels and coagulation tests were normal, while transaminases (AST = 309 IU/L; ALT = 352 IU/L) and triglycerides (472 mg/dL) were increased. Echocardiogram and detailed ophthalmologic examination were normal. Hypoglycemia was never observed in the follow-up. Glycogen storage type 6 disease was considered and a homozygous mutation of c.978_979delGT (p.F327*) (p.Phe327*) was detected in the *PYGL* gene. The family was given genetic counseling about the disease. **Conclusion:** GSD type VI must be considered in cases with isolated hepatomegaly in the presence of elevated transaminases and triglycerides. Otherwise, it may be omitted because of its rarity.

445 - Endogenous Synthesis of Galactose in Galactosemics With Galactose Restricted Diet

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Background: Type I galactosemia is an inborn error resulting from mutations on both alleles of *GALT* gene which leads to absence or deficiency of galactose-1-phosphate uridyltransferase (GALT). Galactose-1-phosphate accumulates within cells and surplus galactose is reduced to galactitol or oxidized to galactonate. Patients with this condition have substantial motor, cognitive, and psychiatric impairments despite dietary treatment. Classical galactosemia is frequently associated with Q188 R, S135 L and K285 N mutations and N314D is associated with Duarte galactosemia and is wide spread among various worldwide populations. **Objectives:** The objectives of this study are to identify most common mutations Q188 R, S135 L, K285 N, and N314D for patients with classical and Duarte Galactosemia and presence of urinary galactitol to monitor the galactose-restricted diet of patients with galactosemia. **Methodology:** The present study aims at detecting Q188 R, S135 L, and K285 N mutations and N314D variant in the *GALT* gene for 8 galactosemia patients and 190 unrelated normal subjects all of Pakistani origin through ARMS and urinary galacitol by quantitative Benedict's test. **Results:** S135 L and K285 N mutations were present neither in galactosemia patients nor in normal subjects. Only one galactosemia patient carried Q188 R mutation that was in homozygous state. However, N314D variant was frequently found in affected 7 of 16 alleles and normal subjects (55 out of 380 alleles). This finding indicates that Duarte allele D314 might be far common in Pakistani population than in European and North American ones. Unexpectedly, galactitol was found in the urine of all patients receiving a galactose-restricted diet, and it was postulated that this sugar alcohol has its origin in part from endogenously produced galactose.

446 - Fructose 1,6 Bisphosphatase Deficiency: An Important Cause of Hypoglycemia in Children

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Introduction: Fructose 1,6 bisphosphatase (F16BP) is a rare autosomal recessive disorder. The aim of this study was to characterize the clinical and molecular features of patients with F16BP deficiency. **Methods**: A retrospective review of six Saudi patients with genetically confirmed F16BP deficiency who presented to our tertiary center over 10 years was conducted. **Results**: The age of the studied patients ranged between 2 and 10 years. All of the patients were an outcome of consanguineous parents. All patients presented with recurrent hypoglycemia and all except one had associated severe metabolic acidosis. Three patients (50%) presented on the first day of life with respiratory distress, hypoglycemia, and severe acidosis. Two patients presented at the age of one year and one at 5 months. The duration between presentation and diagnosis was 41.5 months. All patients had normal growth and development except one with obesity and another one with speech delay. All patient had an initial working diagnosis of glycogen storage diseases or mitochondrial disorders before confirming the diagnosis. All patients carry homozygous mutations in the *FBP1* gene, 2 patients had c.114-115 ins CTGCAC (p.L38delinsLCT), another 2 patients had c.114-119dup (p. C39-T40dup), one patient had c.841G›T (p.Glu281*) and the last patient carries c.334-2 A›T novel homozygous mutation. **Conclusion:** F16BP deficiency is an important cause of hypoglycemic acidosis which can easily be missed as mitochondrial disease and glycogen storage diseases were suspected initially, which in turn lead to delay in reaching the diagnosis for 3 to 4 years. The age of presentation of this cohort was earlier than the classic weaning age when fructose is introduced to the feeding.

447 - Evaluation of Clinical and Genetic Findings of Patients With Galactosemia

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Background: Galactosemia is a carbohydrate metabolism disorder with autosomal recessive inheritance, which can result in life-threatening complications in untreated infants. **Methods**: We retrospectively evaluated the main clinical findings and genetic characteristics of 65 galactosemia patients from 3 different divisions in Turkey. **Results**: Parental consanguinity was detected in 46 (68.7%) patients and 2 had a history of a deceased sibling with confirmed diagnosis of galactosemia. The median [25-75 percentiles] age of diagnosis was 30 days [18-52 days], where, the median duration from symptom onset to diagnosis was 14 days [5-26 days]. The most frequent complaints at admission were jaundice in 48 (71%), poor feeding and vomiting in 6 (9%), respiratory distress and cyanosis in 2 (3%) patients. Twenty-one (32.8%) patients had cataracts. Cataract regressed with galactose-free diet and surgery did not require in 14 patients during follow-up. Initial total and direct bilirubin were 11.2 [4.2-17.3] and 3.6 [2.0-4.7] mg/dL, aspartate and alanine aminotransferase were 140.0 [55.5-222.0] and 78.0 [40.5-156.0] U/L, respectively. DNA sequencing analysis identified 18 different pathogenic mutations on the GALT gene with most relative allelic frequency was the c.563A> G[p.Q188 R] (32.4%). We also identified a new mutation: c.98_99delGCinsAA. Two patients' diagnosis (who were admitted to hospital with a history of neonatal jaundice) were delayed for their atypical clinical findings. Tests for metabolic screening (included urine-reducing substances test, urine sugar chromatography) were all normal. They were diagnosed at 24 and 21 months of age with whole exome sequencing. One of them revealed abnormal isoelectric focusing pattern consistent with congenital glycosylation defect but then after, she was diagnosed as classical galactosemia with genetic studies. The second patient was investigated for hyperammonemia and epilepsy, and finally classical galactosemia was diagnosed with whole exome sequencing. **Discussion**: This study expands the mutation spectrum in GALT gene and reinforces the importance of early diagnosis. In order to make earlier diagnosis we suggest that galactosemia should be included in the national newborn screening program.

448 - Plasma Deoxysphingolipids as Potential Novel Biomarkers in Glycogen Storage Disease Type I

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Background: Pathologically elevated 1-deoxysphingolipids (1-deoxySL) have been found in a number of neurological and metabolic disorders. 1-deoxySLs are atypical sphingolipids, formed when serine palmitoyltransferase (SPT) condenses palmitoyl-CoA with alanine instead of the usual substrate serine. The object of this study was to investigate the role of 1-deoxySL as biomarkers in glycogen storage disease type I (GSDI). **Methods:** In this observational study, plasma 1-deoxySLs were measured by GC-MS in a cohort of 15 adult GSD1 patients (3 GSDIb and 12 GSDIa), and 30 healthy controls. Blood samples for 1-deoxySLs and standard laboratory parameters for monitoring GSDI were collected longitudinally on the occasion of routine consultations, during a period of two years. **Results:** 1-Deoxysphinganine (1-deoxySA) is clearly elevated in GSDI compared to healthy controls (0.181 ± 0.019 vs 0.035 ± 0.002 mmol/L, $P < .001$). Plasma alanine is higher (626 ± 34 vs 398 ± 16 mmol/L, $p<0.001$), whereas serine is lower (89 ± 4 vs 110 ± 3 mmol/L, $P < .001$) in GSDI than in healthy controls, congruent with the mechanism of 1-deoxySL synthesis. In a multiple linear regression model, the major determinants of plasma 1-deoxySA in GSDIa are plasma serine, alanine and triglycerides ($R^2 = 0.795$, $P < .001$). Accordingly, 1-deoxySA correlate with the quality of metabolic control, that is, the frequency of hypoglycemia. GSDIb patients have a distinct 1-deoxySL profile from GSDIa. **Conclusion:** Disturbed lipid metabolism is a hallmark of GSD1. In addition to the known abnormalities of lipoproteins, GSDI patients also have a disturbed sphingolipid profile with elevated 1-deoxySLs. 1-deoxySA correlates with the frequency of hypoglycemia, and constitutes a potential new biomarker for assessing metabolic control. GSDIa and Ib have distinct 1-deoxySL profiles, highlighting another element of diversity between the two GSD subtypes.

449 - Prevalence of Nonalcoholic Fatty Liver Disease in Fructosemia Patients

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Background: Nonalcoholic fatty liver (NAFLD) has emerged as the most important cause of liver disease in the world. Traditionally, the prevalence and severity of NAFLD in children

has been related to the increase in the incidence of obesity, due, in large part, to dietary transgressions during childhood and adolescence. However, it has been observed that patients with hereditary fructose intolerance with strict control of the intake of fructose and derivatives present such fat infiltration, associated or not with hepatomegaly. **Objective:** The aim of this study was to assess the NAFLD incidence in patients with treated fructosemia and to evaluate the relation of NAFLD with metabolic control and the genetic mutations. **Methods:** We conducted a retrospective study examining clinical, biochemical and genetic characteristics in a cohort of 16 patients diagnosed genetically of fructosemia (5 males and 11 females from the age of 2 to 48). The age at diagnosis varied according to the clinical expression. Some patients were diagnosed through a screening program, some due to affected siblings, and others were not diagnosed until adulthood. **Results:** Sialotransferrins, lipid and hepatic profiles showed values within the normal limits, which have served us to control the nutritional status of our patients. Genetic analyses of the patients revealed 4 different mutations. From all the affected individuals, the mutations p.A150P and c.360_363delCAAA in homozygosis represent 81%, 44% and 37%, respectively (A150P in homozygosis, 44%; c.360_363delCAAA in homozygosis, 37%; p.A150P / P.N335 K in heterozygosis, 13%; p.A150P and c.360_363delCAAA, both in heterozygosis, 6%). As a result of imaging techniques and liver biopsies, 9 of the 16 patients presented fatty liver. This supposes that in 56% of the subjects there was at least a slight and diffuse increase of the hepatic echogenicity, that is to say, a slight degree of steatosis. Of these 9 patients, only 3 had hepatomegaly, and none of them had splenomegaly. **Conclusions:** There is a high prevalence of NAFLD in fructosemia patients in despite of good metabolic control. These are non-obese patients and do not show a significantly altered lipid profile. There is a high prevalence of NAFLD associated with the p.A150P mutation.

450 - Congenit Hyperinsulinism With Response to Diazoxide

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Introduction: Congenital hyperinsulinism is the most common cause of persistent hypoglycemia in the first year of life. **Objective:** To describe the case of congenital hyperinsulinism with positive response to diet with complex carbohydrates and diazoxide. Case report: Newborn at term who presented with symptomatic hypoglycemia, with seizures, blood glucose 22 mg/dL. Insulin, glucagon, cortisol, growth hormone, central glycemia and ketonemia are ordered. Hypoglycaemia is still present after admission. Blood sample is collected during crisis (49 mg / dL of blood glucose). Hypoglycemia persists, pyrodoxin and dietary thickeners are added, normal glycemias are suspended, intravenous fluids are given, breast milk is given on demand and formula is given on demand. Patient evolve well. Insulin reporting of 4.60 Mu / L and growth hormone 3.24 ng / mL; Hypoglycemia is persistent especially in the early hours of the morning. Laboratory tests: day 14: Benedict positive (++), urine carbohydrates with glucose band and galactose ketones in blood negative, galactosemia is not ruled out. On day 15, maternal milk and formula milk are suspended, lactose-free milk is started, galactose 1-phosphate uridyl transferase (GALT) and galactose 1 phosphate (GAL) are injected into the blood, the girl worsens from the start of the lactose-free formula, (Glucometrics of 40 mg / dL). Breast milk was restarted and formulated with lactose. Day 21 levels of glucagon 166 (lower limit 208), with glycemia of 40 mg / dL. Diagnostic test with glucagon is started, and glucometrics of more than 30 mg / dL were obtained on basal glucometries. Formula is maintained and a diet rich in starches is started with good nutritional response. On day 33 diazoxide is started at 15 mg / kg in three doses with good tolerance, glycemia levels of 93 mg / dL, starting with a positive water balance, hydrochlorothiazide is started and the amount of diazoxide is reduced. Blood GALT report: 35.2 mmol / h / mg (normal 24.5 mmol /h / mg). Stable metabolic control and patient leaves hospital. **Conclusions:** Probable diagnosis of hyperinsulinemia with response to diazoxide. Hyperinsulinemia with response to this drug is associated with mutations in the internal potassium rectifier channel, glutamate dehydrogenase, glucokinase, and short chain CoA 3-hydroxiacil dehydrogenase (SCHAD-HI). More confirmatory tests are needed for a definitive diagnosis

451 - Successful use of Diazoxide on Glucose Homeostasis in Three Patients With Glycogen Storage Disease Type Ia

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Main treatment in glycogen storage disease type I (GSD I), caused by glucose-6-phosphatase deficiency, is based on appropriate carbohydrate intake with frequent meals and continuous night feeding via nasogastric tube to avoid hypoglycemia and further complications. Diazoxide, a non-diuretic benzothiadiazine drug, was first used in 1968 by Rennert and Mukhopadhyay with an improvement of the glucose homeostasis in two sibs with GSD I. Since then, to the best of our knowledge, the use of diazoxide in order to obtain a good metabolic control has not been reported again. We describe three young patients with GSD I treated by diazoxide (dose from 10 to 15 mg/kg per day). Diazoxide was introduced because of poor metabolic control with recurrent hypoglycemia despite constant increase of carbohydrate intake, suggesting functional hyperinsulinism. Age at introduction of diazoxide

was 9-month-old for patient 1, 10-month-old for patient 2, and 21-month-old for patient 3 and treatment duration was 12 months, 6 years, and 10 months, respectively. The treatment was well tolerated and hypoglycemic events decreased ($P < .05$ in all patients) whereas daily carbohydrate intake was reduced ($P < .05$ in all patients) without reoccurrence of hypoglycemia. Mean blood lactate concentrations decreased by 36% in the first patient (from 6.5 to 4.2 mmol/L; $P = .025$), by 47% in the second patient (from 7.5 to 4 mmol/L; $P < .001$) and by 26% in the third patient (from 4.3 to 3.2 mmol/L; $P = .003$). Diazoxide treatment, in addition to nutrition regimen, helped to counteract the functional hyperinsulinism. Decreasing the number of hypoglycemia with diazoxide resulted in lower carbohydrate intake requirement to maintain normoglycemia, setting a better metabolic control which may be protective against the risk of adenomas development.

452 - Overweight and Obesity Prevalence in Children With Glycogen Storage Disease From a Single UK Center

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Background: Glycogen Storage Diseases (GSDs) are inherited disorders of glycogen metabolism. Dietary treatment requires a delicate balance between over-treatment leading to obesity and its concomitant complications and under-treatment causing poor metabolic control and growth. In 2013, we audited our GSD patients and showed increased adiposity compared to the UK population. For the past 5 years our center has increased focus on avoiding excessive energy intake whilst maintaining optimal metabolic stability and growth. This has been achieved with a number of changes in clinical practice aimed at limiting administered and dietary carbohydrate to only that necessary for individual metabolic control. **Aim:** To compare current Body Mass Index (BMI) Standard Deviation Scores (SDS) and Height SDS of GSD patients to previous audit findings and to the UK population. **Method:** Review of anthropometric data from last visits for pediatric GSD patients (types I, III, VI and IX). This was compared to previous audits (2015, 2013) and to the Health Survey England 2015 (HSE) data for children 2- 15 years. The World Health Organization classification (2006 & 2007) BMI cut-offs for adiposity were used. **Results:** 52 patients (13 type I, 10 type III, 12 type VI, and 17 type IX) were within 2-15 years (median 8.5 years, 65% male). Of these, 44% were either overweight or obese compared to 51% (2017) and 61% (2013). The UK population incidence was 28%. Comparing 2013 to 2017, the incidence of overweight and obesity reduced from 89% to 69% in GSD I, 80% to 60% in GSD III and from 36% to 29% in GSD VI & IX. The overall patient median BMI SDS reduced from 1.41 to 1.07, assuming nonparametric data, this did not reach significance. Overall and for each type the median height SDS improved at each audit. The overall median height SDS for the total group increased from −1.09, IQR (−1.78 to −0.26) to −0.23, IQR (−0.87 to 0.35). This was statistically significant ($P = .007$ Mann Whitney test). **Outcomes:** Over the last 5 years coincident with changes in our clinical practice the incidence of overweight and obesity in our GSD patients has decreased. Median BMI has decreased but not at the expense of overall control as indicated by improved height growth. This is against UK trends of increasing adiposity (National Child Measurement Program 2016). However, within each group type there are individuals whose measurements remain resistant to improvement.

453 - A Change in Practice: Home Ketone Monitoring in Glycogen Storage Disorders (GSD) 0, III, VI, and IX

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Introduction: Optimizing metabolic control in GSD 0, III, VI, IX with suppression of ketosis can lead to improved growth outcome. Traditionally this required an inpatient stay for biochemical monitoring which may poorly reflect home circumstances and is time consuming and costly to families and the NHS. **Method:** 28 patients were randomly selected to partake in a trial of 48-hour home ketone monitoring using a machine previously validated to accurately record low ketones levels (<1.0 mmol/L). A food diary and patient satisfaction questionnaire were also completed. **Results:** 20 profiles were successfully completed. 9 had suboptimal profiles according to our clinical practice: 8 with ketosis (>0.4 mmol), 1 with poor dietary practice. Dietary changes were discussed by telephone. 9 patients were requested to retest with 4 showing satisfactory control. 2 required further intervention and 3 are yet to return profiles. Significant cost saving was made during the course of the trial. All returned questionnaires had a preference for home ketone monitoring. **Conclusion:** Home ketone monitoring has proved effective in managing this cohort of patients. Patient satisfaction was high with this method of monitoring.

454 - Evaluation of the Thyroid Hormone Receptor Agonist VK2809 in an In Vivo Model of Glycogen Storage Disease Type Ia

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Objectives: The objectives of this study were to determine the ability of VK2809, a small molecule prodrug of a potent TRb agonist, to reduce hepatic steatosis and other metabolic derangements vs. controls in the glucose-6-phosphatase catalytic subunit knockout (G6PC −/−) mouse model of glycogen storage disease type Ia (GSD Ia). Methods: We tested the compound VK2809, which acts as a thyroid hormone beta receptor (TBR) agonist, in the G6PC −/− mouse model of GSD Ia. We analyzed groups of 6-7 G6PC −/− mice treated with drug or vehicle to demonstrate statistically significant differences from vehicle-treated controls. We also treated groups of 6-7 wild type (WT) mice with drug or vehicle. Thus, we needed 4 groups of 6-7 mice, for a total of 26 treated mice (13 G6PC −/− and 13 WT). Daily injection of 0.1 to 0.2 mL 10% dextrose subcutaneously was initiated within 3 days of age for G6PC −/−mice. All G6PC −/− mice continued to receive daily dextrose injections throughout drug or vehicle treatment. Dextrose was not administered on the day of tissue collection. Mice were treated with VK2809 from 5 to 8 days of age. Similarly, G6PC −/− and WT mice were treated with vehicle for 4 days to serve as mock-treated controls for all assays. Groups of 6-7 mice were evaluated. Blood was collected on day 9 at time of euthanasia when tissues were also collected. The fasting serum glucose and triglycerides, hepatic lipid and glycogen content, and GSD-related cell signaling pathways were examined. In addition, genetic markers of autophagy were evaluated. Results: Compared with vehicle-treated controls, mean total liver triglycerides in G6PC −/− mice were significantly reduced by over 60% from 13.550 mg to 4.210 mg following 4 days of drug treatment ($P < .05$). Similarly, mean liver weights were significantly reduced by over 30% from 393.55 mg to 253.11 mg in treated vs. control G6PC −/− cohorts ($P < .05$). Drug treatment also produced a decrease in mean liver triglyceride concentration by over 50% in G6PC −/− mice, from 729.59 mg/dL to 336.58 mg/dL, however this downward trend was not statistically significant ($P = .07$). These preliminary data suggest VK2809 reduces hepatic steatosis in the G6PC −/− mouse model of GSD Ia.

455 - Stimulation of Autophagy With Bezafibrate Reverses Hepatic Steatosis in Glycogen Storage Disease Ia

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Objectives: The objectives were to determine if the pan-PPAR agonist, bezafibrate, induces autophagy in a *G6pc* −/− mouse model of glycogen storage disease Ia (GSD Ia) and whether induction of autophagy can alleviate hepatocellular abnormalities in GSD Ia. **Methods:** We tested the drug bezafibrate, a pan-PPAR agonist, in a *G6pc* −/− mouse model of GSD Ia. The experiment consisted of two treatment cohorts, differing only in duration of treatment. In the first cohort, groups of 3-4 *G6pc* −/− mice were injected intraperitoneally with 10 μL/g of 1.25 μg/ μL bezafibrate or vehicle for five consecutive days starting at 5 days of age. Groups of 3-6 WT mice were subjected to the same treatment with drug or vehicle. For the second cohort treatment was extended to ten days. Daily injection of 0.1 to 0.2 mL 10% dextrose subcutaneously was initiated within 3 days of age for all *G6pc* −/−mice in both cohorts and continued up to the day before tissue collection. Liver, kidney, blood and urine was collected and analyzed. Results: In the 5-day treated cohort, *G6pc*−/−mice treated with bezafibrate had significantly increased levels of liver LC3-II, a protein recruited to autophagosome membranes during autophagy ($P = .008$), as well as decreased levels of p62, an autophagosome cargo protein that accumulates when autophagy is inhibited ($P = .008$). The 5-day drug treated *G6pc*−/− mice also showed significant decreases in mean liver triglyceride concentration ($P = .001$) and liver glycogen concentration ($P = .050$) compared with vehicle treated controls. Together, these results suggest autophagy was induced and achieved some success ameliorating the hepatocellular abnormalities of GSD Ia. Analysis of the 10-day drug treated *G6pc*−/− mice did not reveal changes in LC3-II or p62 protein levels indicative of autophagy induction, however, mean blood triglyceride concentration was significantly decreased in bezafibrate treated mice ($P = .048$). Notably, all *G6pc*−/− mice treated ten days with bezafibrate showed measurable blood glucose levels (>20 mg/dL) compared to the vehicle treated mice (with levels all below the detection limit for the glucometer). This increase is blood glucose levels though unexpected, shows the potential for bezafibrate as a treatment not only for liver abnormalities, but also for hypoglycemia in GSD Ia. Conclusion: Bezafibrate shows promise as a treatment of GSD Ia as a proautophagic drug that achieved partial correction of the biochemical abnormalities underlying this disorder.

456 - A Case Report of the Primary Caregivers' Experience of Diet Therapy of Children With Hepatic Glycogen Storage Diseases in Japan

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Introduction: The children with hepatic glycogen storage diseases (GSDs) can survive by the appropriate diet therapy, and uncooked corn starch treatment. In childhood, their mothers manage the child's diet therapy every day. It can be imagined that the management is burdensome, and they have many

distinctive experiences in daily life. However, there are few reports on children with GSDs, and those contents are limited to diagnosis or treatment. Therefore, the study aim is to report the primary caregivers' experience of diet therapy of children with hepatic GSDs, and consider the suggestion for future support. **Methods:** We conducted a semi-structured interview at outpatient setting in January 2017. The participants were two primary caregivers of a child with hepatic GSDs continuing their diet therapy. We conducted narrative analysis for the interview contents. We coded the interview contents and classified each code with similarity, and generated categories and subcategories. This study was approved by the ethical review boards of each university and hospital. **Results:** The participants were two 40's mothers of the 8-years-old son with GSD I a and the 10-years-old daughter with GSD IIIa. Both mothers were mainly taking responsibility for child's diet therapy in the family units, and fathers were cooperative with parental care. The primary caregiver's experiences of the diet therapy in GSD I a case were classified into 5 categories: "suffering from difficulties of getting used to the diet therapy"; "coordinating the school life with preventing hypoglycemia"; "educating for child's self-care and independence"; "having anxiety for correspondence of medical doctors out of the specialized hospital"; and "having a desire for information provision about variation of recipes with diet therapy". In GSDIIIa case, 8 categories were generated: "feeling relief by the diagnosis"; "being introduced to more specialized hospital at the appropriate timing"; "coordinating the school life with preventing hypoglycemia"; "being with specific cognition of diet and eating"; "having temporary vomiting habits by smells of food"; "attending the physical education class in school with exercise limitation"; "try and giving up the ketogenic diet"; "having anxiety about increasing blood levels of creatinine kinase." **Conclusions:** We suggest that engagement for an early-stage education to the child with hepatic GSDs to achieve the ability of self-care with preventing hypoglycemia is important in both cases.

457 - Hepatic Glycogenoses Among Children and Adolescents—Developing Country Perspective

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Aim: To formulate a protocol for management of Glycogen storage disease (GSD) with hepatic involvement among children and adolescents. **Objectives:** 1. To establish a practical approach to sub-typing and managing hepatic GSD, for use in developing countries. 2. To analyze clinical characteristics of hepatic glycogenoses. **Background**: In resource poor countries hepatic glycogenoses are diagnosed late. As awareness of non GSD1 subtypes seems poor among general practitioners, most patients are advised corn starch and further referred for liver transplant. The importance of aggressive dietary modification for different subtypes, role of physiotherapy for associated myopathy are often ignored. Genetic testing remains elusive and thus establishing a practical approach to diagnosis and management of these subtypes will make a significant difference. **Method:** Prospective cohort study of patients newly diagnosed or presenting for follow to Paediatric Metabolic clinic at a tertiary center from May 2016 to April 2017. **Results:** Total of 11 patients were identified. Two girls had florid rickets suggesting GSD type XI as possible diagnosis and were excluded from analysis as management is different. Among the remaining 9, M: F ratio was 7:2. These 9 presented at an average of 1.9 $\pm$ 1.04 years with abdominal distension noted in 100% from an average age of 1 $\pm$ 0.8 years. Myopathy was present in 33.3% (3/9), hypoglycemic seizures in 44.4% (4/9). Based on clinical features and lab investigations, 1 was sub-typed as type Ib, 3 as type IIIa, 3 as type IIIb, and 2 as type 6. Liver biopsy was suggestive of GSD in 4/9 and 3/9 had genetic testing done. The remaining (2/9) are planned for genetic testing at follow up. Genetic testing differed from clinical sub-type in 1/3. Average follow up was 1.9 $\pm$ 1.04 years. Average fasting glucose (mg/dl) and SGPT (iu/l) improved from 40 $\pm$ 20.6 to 90.1 $\pm$ 21.8 and from 348.6 $\pm$ 149 to 242.4 $\pm$ 149.2, respectively. The lipid profile however has shown marginal worsening, triglycerides (mg/dL) 260.1 $\pm$ 96.7 to 277.6 $\pm$ 113.5. All were on UCCS (uncooked Corn Starch) 2 g/kg/dose 2-5 times daily. Omega-3-fatty acid for hypertriglyceridemia is being used in 4/9 patients. Conclusion-Hepatic glycogenoses present mainly as abdominal distension. Non-GSD 1 subtypes need to be considered during diagnosis. UCCS is mainstay of therapy, but protein, fiber intake, and lipid control is equally important. Identification and management of myopathy is essential.

458 - High Frequency of Glycogen Storage Disease Type IX in Cohort of Russian Patients With Liver Glycogen Storage Diseases

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Introduction: Liver Phosphorylase kinase (PhK) deficiency causing glycogen storage disease type IX (GSD IX) is thought to account for about 25% of all Glycogen storage diseases (GSDs). Due to high clinical and genetic heterogeneity, differential diagnostics of liver GSDs is challenging and the disease may be underdiagnosed. Next-generation sequencing (NGS) technologies are revolutionizing the diagnostic screening for genetic disorders with unspecific features. **Objective:** The aim of our study was determination of frequency of different types

of liver GSDs and mutation spectrum in cohort of Russian patients. **Materials and methods:** A cohort of 170 patients with clinical and biochemical features of liver GSDs were subject of the study. Molecular-genetic analysis of the *G6PC* (GSDIa) and *SLC37A4* (GSDIb) genes were made by direct sequencing. For detection of other GSDs, the targeted NGS panel based on Ampliseq technology was designed. Targeted group consisted of 47 nuclear genes including 10 responsible for GSDs. **Results:** In 56 patients (32,9%) genetic diagnosis of GSD was confirmed. The most frequent is GSD IX (42,9%) and GSDIa/b (34,0%). 16 different mutations were detected in *PHKA2* gene (GSD IXa), 12 of them were novel: c. 226G >A, c.268delC, c.749C>T, c.755C>T, c.772G>A, c.1262G>A, c.1324+1G>T, c.1814C>T, c.2578C>T, c.2635G>T, c.3190C>T, c.3331C>T; also 1 case of *PHKB* gene defects (GSD IXb) and 2 cases of *PHKG2* defects (GSD IXc) were diagnosed. 2 compound-heterozygous mutation alleles in *PHKB* and 1 homozygous allele in *PHKG2* were novel: [c.39G>A]; [c.574A>G] and [c.658G>A]; [c.658G>A] respectively. Other types of GSDs were diagnosed in 23,1% of GSD patients: GSDIII—10,7%, GSD0—7,1%, GSDVI—3,6%, GSDIV -1,8%. **Conclusion:** GSD IXa is the most common type of GSDs in Russian patients. Targeted approach is highly effective as the second line of GSDs diagnostics after excluding of common mutations in GSDIa/b.

459 - Homozygous c.966C>A p.(Phe322Leu) Change in the *G6PC* Gene in a Patient With Mild Glycogen Storage Disease 1a

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We present a patient with a complex clinical history where molecular testing identified a likely pathogenic variant of the *G6PC* gene, but biochemical and enzyme analysis suggested an atypical presentation of glycogen storage disease type Ia. The child was born at term after a concealed pregnancy. There were respiratory problems from birth, swallowing difficulties, failure to thrive, and developmental delay. He was on continuous tube feeds overnight from an early age. Dysmorphic features in infancy were investigated by chromosome and array CGH analysis with no abnormality detected. Metabolic investigations with normal results included urine organic acids, acyl carnitines, chitotriosidase, and lysosomal enzymes. At 5 years of age, he presented with an enlarged liver but normal liver function tests which became abnormal by age 8. Plasma cholesterol and triglycerides were increased. Uric acid was normal. He was fasted for a liver biopsy and became hypoglycemic. A 12- hour fast did not show hypoglycemia, although lactate was consistently but mildly elevated. Next-generation sequencing of 27 genes associated with glycogen storage disease and related disorders was performed using the Illumina TruSight ONE^{TM} sequencing panel kit and a HiSeq (Illumina) analyzer. Analysis of genes of interest was performed using an in-house pipeline. Two variants of interest were identified: an apparently homozygous missense change in the *G6PC* gene, c.966C>A p.(Phe322Leu), and a heterozygous splice site variant in the *GBE1* gene (c.691+2T>C). The *G6PC* variant had been documented in the literature in the heterozygous state (the second mutation was not specified) in a single patient with complete deficiency of hepatic glucose-6-phosphatase. Another study indicates that p.Phe322Leu affects a residue within a transmembrane helix, not the catalytic domain, an in vitro assay showed decreased but not completely absent activity. Glucose-6-phosphatase activity in a liver biopsy from our patient showed an activity of 29 μmol/min/g protein (34-166) with marker enzyme within the normal range. Our patient is unusual in that he has low but not complete absence of *G6PC* activity invariably seen in GSDIa, suggesting an atypical or mild form of disease may be associated with the p.(Phe322Leu) variant.

460 - Evaluation of Our Patients With Fanconi Bickel Syndrome

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Fanconi-Bickel syndrome is a rare autosomal recessive disorder due to mutations in the facilitative glucose transporter 2 (GLUT2 or SLC2A2) gene resulting in excessive glycogen storage predominantly in the liver and kidney. The patients with Fanconi-Bickel syndrome were presented to be evaluated with clinical, laboratory and follow-up findings. Four female patients with Fanconi Bickel syndrome were evaluated. Hepatomegaly, hypoglycemia, and tubular dysfunction were detected in all of the patients. The age range of the patients at diagnosis was between 30 months and 7 years of age. Four of them have failure to thrive and initial high SDS scores were −3.03/−3.47/−3.6, and −5.3, respectively. Clinical and laboratory findings of the rickets were also detected. Liver biopsy performed in two patients and liver fibrosis and bridging necrosis, glycogen storage and steatosis were detected. For preventing hypoglycemia all of them treated with uncooked cornstarch and avoid fasting. *SLC2A2* gene analysis revealed homozygous p.G162Rfs*17(c.482dupC) mutation in one patient and homozygous p.S169*(c.506C>G) in the other patient. Two of the patients have the same homozygous p.V106X(c.135_322delinsGT) mutation. One of the patients

had growth hormone replacement therapy and her final height was 154 cm. In the presence of Fanconi type nephropathy, hepatomegaly, hypoglycemia, Fanconi-Bickel syndrome should be considered in differential diagnosis. Rarely, hypergalactosemia can lead to confusion in the neonatal scan. A similar increase in galactose was detected in one of our patient. Our patients were presented for the rare occurrence and discussion of follow-up data.

461 - Familial Analysis of Clinical Consequences of Galactosemia

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Background: Type I galactosemia is an inborn error resulting from mutations on both alleles of *GALT* gene which leads to absence or deficiency of galactose-1-phosphate uridyltransferase (GALT). Galactose-1-phosphate accumulates within cells and surplus galactose is reduced to galactitol or oxidized to galactonate. Patients with this condition have substantial motor, cognitive, and psychiatric impairments despite dietary treatment. Classical galactosemia is frequently associated with Q188 R, S135 L and K285 N mutations and N314D is associated with Duarte galactosemia and is wide spread among various worldwide populations. **Case Reports:** Twenty cases with age of 2 months to 1 year have been reported with galactosemia, diagnosed by deficiency of galctose-1-phosphate uridyl transferase (GALT) enzyme studies and mutational screening of most common mutations Q188 R, S135 L, K285 N and N314D for patients with classical and Duarte Galactosemia and presence of urinary galactitol to monitor the galactose-restricted diet of patients with galactosemia. **Results:** Familial pedigrees confirmed autosomal recessive inheritance with galactosemia and have been characterized with in its early stages by hepatocellular damage (jaundice, hepatomegaly, abnormal liver function tests), food intolerance (vomiting, diarrhea, poor feeding) and failure to thrive with *Escherichia coli* sepsis was not an uncommon outcome. S135 L and K285 N mutations were present neither in galactosemia patients nor in normal subjects. Only five galactosemia patient carried Q188 R mutation that was in homozygous state and N314D variant was frequently found in affected 14out of 30 alleles. **Discussion:** In Pakistan, there is no reported data available and routine newborn screening is not practiced at all. Galactosemia can be treated with galactose restricted therapy but galactitol was found in the urine of all patients receiving a galactose-restricted diet due to its origin in part from endogenously produced galactose.

462 - Fructose 1-6 Bisphosphatase Deficiency in Three Turkish Infants

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Objective: Fructose 1-6 bisphosphatase deficiency is an autosomal recessive inherited gluconeogenesis deficiency. Hypoglycemia, metabolic acidosis, vomiting, apnea, sleepiness tendency and/or coma were some of the clinical findings. Increased lactate, alanine, glycerol, ketone, and glycerol 3-phosphate may be present during the acute episode. We report three Turkish patients with clinical and molecular findings. **Case reports:** The male to female ratio of the patients was 2/1. There was consanguinity marriage in all three families. Initial age for admission to the hospital ranged from six to 12 months and the first complaint was vomiting, restlessness and rapid breathing. Patients were often admitted to intensive care unit after starvation, infection and fructose intake. On physical examination, all of the patients had dizziness, weakness, acidotic respiration, and hepatomegaly. Laboratory findings were hypoglycemia, metabolic acidosis, ketonemia, hyperuricemia, hypertransaminasemia, mild lactate and pyruvate elevation. Abdominal sonographic examination revealed hepatomegaly and increased liver echogenicity. Metabolic tests revealed increases in glycerol, lactate, ketone in urine organic acid analyses. Molecular genetic examination revealed IVS5 + 1G>A homozygous in one patient with *FBP1* gene and homozygous first exon deletion in other two patients. Fructose, sucrose and sorbitol restricted diet, frequent diets and corn starch recommendations were started. The hepatomegaly of the patients improved on follow-up. **Conclusion:** Metabolic diseases should be considered in differential diagnosis in patients with recurrent intensive care unit admission.

463 - Long Term Outcome of Patients With Medically Treated Congenital Hyperinsulinism: Subtotal Pancreatectomy Still Needed?

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Background: Congenital hyperinsulinism (CHI) is the most frequent disease leading to hypoglycemia during infancy. CHI patients resistant to an oral treatment with diazoxide and octreotide may require a subtotal pancreatectomy. This surgery is controversial because it leads to an insulin-treated diabetes. Conversely, some teams propose an intensive medical and conservative treatment, though little is known about the long-term evolution under this medical treatment. **Objective:** To describe the spontaneous improvement of CHI patients treated medically. **Method:** We report here on the evolution of 160 medically treated CHI patients. All patients were followed at Necker-Enfants Malades Hospital, APHP, Paris France, between 1978 and 2014. **Results:** 109 patients were responsive to diazoxide (DZX-R), whereas 51 were diazoxide-unresponsive (DZX-U). In patients with DZX-R CHI, the treatment was still ongoing in 36/109 (33%) patients. In whom the treatment was stopped (73/109), they were mostly diagnosed during the neonatal period (41/66) vs late-onset CHI, and the treatment was stopped after a shorter period (1.2 vs 5.1 years). The treatment could be stopped at 2.9 $\pm$ 4.3 years old (mean $\pm$ SD), without recurrence of hypoglycemia. Patients DZX-U, were treated with diazoxide, somatostatin analogues (51/51) and enteral feedings (26/51). After 10 years of treatment, 35/51 (68%) of patients were still on treatment. The median/ max age treatments were stopped, were: diazoxide 11.8 months / 6.9 years old; somatostatin analogues 9.4 months/ 6.4 years old; and enteral feedings 2.1 / 5.2 years old. Conclusion: CHI improves with time, permitting a decrease in the medical treatment. Enteral feeding, which is the heaviest burden for children and families, could be withdrawn before 5.2 years old in all our patients. Thus, the relevance of subtotal pancreatectomy in DZX-U CHI patients with a diffuse form should be discussed because a long-term medical treatment of CHI might be less detrimental than the pancreatectomy-induced diabetes.

464 - Presence of Two Homozygous Variants for Fructose Intolerance and Deficiency in Whole Exome Sequencing

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Objective: The aim of this laboratory clinical study is to describe a metabolic profile and whole exome sequencing result from a subject investigating an atypical inborn error of metabolism. **Methods and Results:** The subject was initially tested for urine organic acid study and the results were abnormal profile with an elevated lactic acid (>10 000.0 mmol/mol creatine) and 3-hydroxybutiric acid (3001.0 mmol/mol creatine), typical of a lactic aciduria and ketonuria. This initial test did not elucidate which inborn error of metabolism was the subject's diagnosis, but raised the interrogation of a possible metabolic disarrangement. The investigation continued with a whole exome sequencing and two variants were identified in homozygous state. One variant was in the *FBP1* gene related to Deficiency of fructose-1-6-biphosphatase, the other in the *ALDOB* gene related to Fructose Intolerance, both variants were rare in population's databases such as ExAC and a Brazilian specific population database ABraOM, and were classified as variant of unknown significance (VUS). Further investigation is required to determine which of the variants is causing the clinical outcome in the subject; this step is fundamental to provide correct genetic counseling for the family. **Conclusion:** This case study reflects the importance of a joint investigation of inborn errors of metabolism taking into account the biochemical results and whole exome sequencing as a unity, and the importance to review and discuss the biochemical results facing a molecular result for each subject suspected of an inborn error of metabolism.

465 - Liver Organoids as a Patient-Specific Model to Study Disorders in Energy Metabolism

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Introduction: Energy metabolism is an essential cellular process involving glycolysis, the tricarboxylic acid cycle and the respiratory chain. Diseases in energy metabolism represent a heterogeneous, but generally severe group of diseases with limited treatment options. Currently, patient-specific in vitro models for personalized drug development and testing are lacking. Recently, adult stem cells have been identified in many epithelial organs. Cultured under specific conditions, these cells self-organize into three-dimensional structures—organoids—that resemble the original organ tissue in form and function. Liver organoids would provide a novel in vitro model to study energy metabolism. **Objective:** To investigate the potential of liver organoids as a patient-derived in vitro model for disorders in energy metabolism. **Methods:** Liver organoids were generated from remnants of diagnostic liver biopsies, resection-, or autopsy material. Using specific culturing strategies, liver organoids were expanded and differentiated. Using both untargeted and targeted analyses, we investigated energy metabolism in liver organoids and their potential to model

disorders in energy metabolism. **Results:** We successfully established liver organoids from healthy controls and patients. Organoids could be expanded long-term and passaged weekly on expansion medium (EM). In differentiation medium (DM), gene expression and cellular function altered towards a more mature hepatocyte phenotype. Energy metabolism shifted from high glycolysis during rapid proliferation in EM toward glucose homeostasis with glycogen storage in more mature cells in DM, as evidenced by gene expression and metabolic flux analyses of glucose, lactate, and amino acids. Despite distinct changes in glucose metabolism, oxidative phosphorylation was functional and operated well below maximal capacity in both proliferating and more differentiated cells. **Conclusions:** Liver organoids are easy to maintain in culture, amenable to a vast variety of functional analyses and display changes in energy metabolism that correspond to the proliferative or more differentiated nature of liver organoid cells. Therefore, they are promising as a unique patient-derived in vitro model to increase mechanistic insight and to develop and test novel treatment strategies for patients with disorders in energy metabolism.

466 - Coincidence of Hereditary Fructose Intolerance and Wilms Tumor

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Objective: Hereditary fructose intolerance is an autosomal recessive disorder of fructose metabolism resulting in a defect in the *ALDOB* gene. More than 60 mutations have been reported to date. **Case Report:** A 16-year-old male patient was diagnosed with chronic hepatitis B carrier and remission of opere Wilms tumor. He was consulted for abnormality in his lipid panel. No physical features were detected except one incision scar on the upper abdomen. In the laboratory findings, blood count, kidney and thyroid function tests were normal. Cholesterol, LDL, and liver transaminase levels were mildly elevated. Alpha and prebeta lipoprotein were normal and beta-lipoprotein was high in lipid electrophoresis. Abdominal sonographic examination revealed grade 1 steatosis in the liver, left nephrectomy, and compensatory hypertrophy in the right kidney. Echocardiographic examination was normal. It was learned from the story that there was vomiting especially after the consumption of fruit and sugar. In the urine reduction substance was negative. Molecular genetic analyses revealed, a homozygous c.448G> C;p.Ala150Pro mutation in the *ALDOB* gene. Fructose, sucrose, sorbitol and cholesterol restricted diet were recommended. **Conclusion:** It should be absolutely questioned whether there is any avoidance of nutrients in the story. The first and best physician, the organism, has taken to protect itself by protecting these substances by refusing to receive them. The fact that the results of metabolic testing are normal (eg, urine reduction substance negative in this case) may be misleading because of the lack of precursor intake. For this reason, direct genetic analysis should be requested for definite diagnosis.

467 - The Present and Future of Transaldolase Deficiency Based on the Experience From a French Cohort Study and the Literature

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Objectives: Transaldolase (TALDO) deficiency is an inherited disorder of the pentose phosphate pathway, resulting in a multivisceral perinatal disease. In neonates, the typical features include hepatosplenomegaly, jaundice, cutis laxa, telangiectasia, hypertrichosis, abnormal external genitalia, and a normal neurological examination. Thrombocytopenia, anemia and liver failure are also frequent. In the literature, both the nature and severity of presentations are highly heterogeneous, from a rapid death from organ failure or a progressive disease that will eventually result in cirrhosis or kidney failure. However, the treatments according to clinical features are still mainly elusive. We aimed to precise the clinical and biological phenotype of TALDO deficiency and to identify decision-making criteria that would improve the management of patients. **Methods:** This retrospective study included 12 children. We compared the characteristics of our patients to the 29 children reported in the literature. We divided our cohort into two groups, and compared their characteristics according to the outcome. **Results:** Seven boys and 5 girls from 6 families were followed from 1994 to 2016. The main clinical features were similar to the published cases, except a higher frequency of dysmorphism, and a lower frequency of thrombocytopenia in our cohort: 11/12 *vs* 16/29, $p<0.05$ and 6/12 *vs* 25/29, $P < .05$, respectively. Two groups were defined depending on outcome, dead (group A, $n = 6$) or alive (group B, $n = 6$) at last follow-up. The frequency of severe liver failure was higher in group A: 6/6 vs 2/6 $P = .06$. All patients in group A died before the age of 9 months from liver failure ($n = 3$) or following liver transplantation ($n = 1$), malnutrition ($n = 1$), and multi-organ failure and infection ($n = 1$). At last follow-up, children in group B had a normal development but progressive organ damages, including cirrhosis, ($n = 3$), renal abnormalities ($n = 2$) and gonadal dysfunction ($n = 3$). **Conclusions:** Our results suggest

the presence of two presentations and outcomes of TALDO deficiency: a severe and rapidly fatal neonatal disease that might benefit from early liver transplantation; and a slowly progressive neonatal form with favorable long-term outcome for which close attention should be paid to prevent and treat organ damage, including with liver or kidney transplantation as needed.

468 - Retrospective Study in Glycogen Storage Disease Type I (GSD I): A Cohort From Garrahan Hospital

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Introduction: GSD I is an autosomal recessive inborn error of carbohydrate metabolism caused by defects of the glucose 6 phosphatase complex. The aim of treatment is to prevent hypoglycemia through continuous gastric drip feeding and uncooked cornstarch. **Objective:** This study is a descriptive analysis of diagnosis, management, clinical course and outcome of a cohort of pediatric GSD I patients obtained from the retrospective analysis. **Patients and methods:** Patients were identified from Garrahan hospital records. Retrospective case records were obtained from 31 patients. Data were collected from June 1997 until January 2017. Follow-up was made until 18 years old when they were transferred to adult center. The diagnosis of GSD I was made based on biochemical and clinical features, liver biopsy, glucagon test results or mutation analysis of G6Pase/G6P transporter gene. Results: Median age when first symptoms began was 3.5 months (range 0.1-22). Median age when diagnosis was made was 7 months (0.5-34). Median years of follow-up were 8; 25 patients were type IA and 6 were IB. Diarrhea and neutropenia were the chief issue in type IB. The main presenting features were hepatomegaly 70%, symptoms of acute metabolic derangement 100%, failure to growth 76%, hypotonia 68%, and delayed psychomotor 51%. During daytime, 27 patients used uncooked cornstarch. Overnight 16 patients were on continuous nocturnal gastric drip feeding. Tree GSD IA died because of acute metabolic derangement without adequate treatment. There were reported values of triglycerides, cholesterol, hemoglobin, uric acid, height at different ages. Complications were reported: tree patients had adenomas, one hepatocarcinoma. Two had proteinuria. The number of admission was more than ten (63%). Global survival was 90%. Four patients received liver transplant after 7 years of age. Time of follow-up was 3.75 years (range 1-11). We analyzed numbers of admissions, adenomas, dietary unresponsive as predictors of liver transplant and they were statistically significant ($P = .002$). **Discussion:** We present a descriptive analysis of data of 31 patients with GSD I obtained in a retrospective study in one metabolic center. The numbers of admissions, adenomas, dietary unresponsive are predictors of liver transplantation. This disease has high morbidity, and with adequate treatment, death could be avoided. Complications of liver adenomas and progressive renal disease are major causes of morbidity and mortality.

469 - Classical Galactosaemia is Associated With Decreased Bone Mineral Density (BMD) in Both Males and Females

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Objective: Assess the prevalence of low BMD in male and female patients with Classical Galactosaemia. To review the effect of hormone replacement therapy(HRT) on BMDs in females. **Methods:** Current patients' medical records of our pediatric and adult units were reviewed. 65 patients included had atleast one lumbar spine DEXA scan. BMD age matched Z score in patients <18y was measured. In the adult population, we distinguished between normal BMD (T score −1 and above), osteopenia (T score −1.0 to −2.5), and osteoporosis (T score −2.5 and below). Data on HRT, bone fractures, calcium/vitamin D, and supplementation were monitored. Results: 65 patients were included. Male–female ratio is 28:37, age range 6.2 to 38.9 years (median age 20 years). 29 patients are <18 y and 36 patients are >18 y. In patient cohort <18 y, 7/29 patients had Z scores <−2(age range 5-12.8 years), mean BMD Z score is −1.3 SD. In patients >18 y, 20/36 have osteopenia and 7 have osteoporosis. Of the total cohort, 21 females and 13 males have abnormal BMDs. Prevalence of low BMD is observed in just over half of the study population affecting 56% of females and 46% of males. Four patients (2 males and 2 females) had fractures. One of the four has normal BMD (T score >−1 SD) and three have abnormal BMDs. Of the 37 female patients, 20 are on hormone replacement therapy, started between 10.8 and 14.4 y. Age range of the 20 patients is 16.6 to 30.0y, mean age is 23.7 y. For the youngest patient, the Z score at 15y was −1.2 SD. 10/19 patients >18y have osteopenia and 3/19 have osteoporosis. On longitudinal data, in 10/20 patients BMD improved, BMD in 2 patients deteriorated and in 8 patients BMD is unchanged; Looking at the longitudinal data of males over 18 y, BMD is unchanged or stable in 11 patients, got worse in 3 patients and improved over time in 1 patient; Vitamin D levels in 44 of 65 patients were normal(50-120 nmol/L), 15 had insufficient levels(25-50 nmol/L) and 4 were noted to be vitamin D deficient(<25 nmol/L). All calcium levels were normal; 52 patients received either vitamin D and/or calcium supplements. **Conclusion:** Low BMD is prevalent in over 50% of our Galactosaemia cohort affecting both male and female patients. On HRT, BMD profiles in half of the female cohort improved. Bone health in classical galactosaemia patients should be monitored

470 - Diagnosis and Management of Fructosemia in an Infant—A Case Report

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Fructosemia—also known as hereditary fructose intolerance—is a rare inborn error of fructose metabolism inherited as autosomal recessive disorder due to a deficiency of fructose-1-phosphate aldolase activity resulting in the accumulation of fructose-1-phosphate in the liver, kidneys, and small intestine. Current diagnostic methods for this disease include the fructose tolerance test that measures clinical symptoms upon intravenous fructose challenge and direct assay of aldolase activity in liver biopsy samples. These tests are relatively invasive and not routinely available. A direct DNA analysis that scans for known and unknown mutations has also been reported. This case report is about a 5-year-old child born from a consanguineous marriage that presented multiple episodes of reduced fasting plasma glucose, vomiting, elevated rates on liver enzymes assays and absence of glycosuria since complementary feeding. From the beginning, he was treated as glucogenosis with supplementation of carnitine, B complex, acid folic and administration of cornstarch three times a day. Through his first 3 years he had a few episodes of acute decompensation. His total serum protein, serum alkaline phosphatase, serum gamma GT, coagulation profile, and glycosylated hemoglobin were normal. Ultrasonography of the abdomen revealed mild hepatomegaly without splenomegaly. Gas-chromatographic-mass spectroscopy of urine showed high titles of lactate acid and 3-hydroxibutiric acid. Liver biopsy showed steatosis and intranodular fibrosis. Genetic testing was evaluated and revealed homozygous for fructose 1-6-biphosphate deficiency. Special advice on dietary restrictions on fructose, sucrose and sorbitol was given to the patient and his family. From then on, it was initiated the supplementation of vitamin C and restriction of fructose (fruits, sugars, yogurts, and biscuits) with a good response. Differential diagnosis from galactosemia and tyrosinemia is as important as the histological features may resemble each other. Even in undiagnosed pediatric patients' recurrent inadvertent fructose ingestion exposes them to the risk of hepatic failure.

471 - Allogeneic Bone Marrow Transplant as Treatment of Triosephosphate Isomerase Deficiency: First Case From the United Arab Emirates

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Triosephosphate isomerase (TPI, E.C.5.3.1.1) deficiency is an autosomal recessive multisystem disorder presenting with chronic hemolytic anemia and progressive neuromuscular impairment. To date, most cases are fatal in early childhood as there is no available treatment. We describe two cases, female siblings of Emirati origin, currently 3 years and 19 months, respectively, born from consanguineous parents, with genetically confirmed TPI deficiency. Both are homozygous for the c.315G>C variant in the *TPI* gene. They both presented in the neonatal period with hemolytic anemia requiring frequent blood transfusions. Subsequently, the older sibling had developmental regression at the age of 14 months. She continued to progressively deteriorate neurologically and is currently tracheostomized and ventilator dependent. Both continue to have hemolytic anemia necessitating blood transfusions. However, the younger sibling has only mild hypotonia and developmental delay and therefore her neurological disease has not evolved. Previously, red blood cell transfusion proved to successfully yet transiently increase TPI enzyme activity and reduce DHAP concentration in-vivo in one severely affected patient. In addition to proving the feasibility of increasing TPI enzyme activity, it also suggested that sustained levels are required to achieve continuous delivery of the active enzyme. Additionally, in-vitro studies demonstrated similar results. Therefore, allogeneic bone marrow transplant has been proposed as a means of sustained delivery of functional enzyme in vivo and therefore a potential treatment for TPI deficiency. Our younger patient, was considered a good candidate for bone marrow transplantation as a means of treatment that will deliver functional enzyme continuously and halt the neurological progression as well as normalize the hematological parameters.

472 - Glycogen Storage Disease Patients' Adherence to Treatment Followed in a Service of Excellence in Inborn Errors of Metabolism

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Objective: To characterize patient's adherence to treatment with patients diagnosed with Glycogen Storage Disease followed in a Service of Excellence in Inborn Errors of Metabolism. **Methods:** Cross-sectional analysis with convenience sampling. Data were collected through medical record reviews and interviews with patients or relatives. For the classification of adherence, only patients who performed at least three blood samples in the 18-month period prior to inclusion in the study were considered. Patients with a minimum of 80% of normal

values for at least two out of the three biochemical markers used for GSD types Ia, Ib, III and IX were considered adherent; the others were considered non-adherent. **Results:** 19 patients were included. Out of which, 10 (52.63%) had GSD type Ia, 4 (21.05%) GSD type Ib, 3 (15.78%) GSD type III and 2 (10.52%) type IX. The median age was 12 years and the median age of diagnosis was 9 months (ranging from 3 months to 6 years). The majority of patients were in the B1 economic classification. 10 patients (52.63%) were considered adherents. There was no difference between the two groups regarding the distance between the Service of Excellence and the patient's residence, religiosity, economic classification and age of diagnosis. The mother is the main caregiver between 80% of adherent patients and 44% of non-adherent patients. Regarding the difficulties reported, the most cited were: not being able to eat tasty foods, the Service of Reference being far from the patients' residence, and the special diet being expensive. **Conclusion:** The study has shown so far an unsatisfactory adherence to treatment in approximately 50% of the patients. Some verified factors were not suggestive to being relevant for adherence. The treatment of glycogenosis is complex and requires constant and permanent care regarding the hours of raw starch intake, as well as the conscience with the foods that are allowed and prohibited in the diet. Adherence to treatment is a complex subject that should always be evaluated in hereditary metabolic diseases in which the treatment is basically dietary and nutritional. The difficulties associated with the treatment should be worked together with different healthcare professionals in order to find the most effective interventions for each case.

473 - Clinical and Laboratory Profile of GSD Patients in a Reference Center of Northeast of Brazil

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Glycogen storage diseases (GSD) comprise a group of disorders that involve the disruption of metabolism of glycogen. Clinical and laboratorial characteristics included failure-to-thrive, hepatomegaly, dolls face, hypoglycemia, dyslipidemia, elevated hepatic transaminases, hyperuricemia and hyperlactatemia. Skeletal and cardiac muscle, the kidneys and liver can be affected. **Aim**: We described the clinical, anthropometric and laboratorial data of outpatients accompanied at the Inborn Errors of Metabolism Clinic of Pediatric Nutrition Service, Fima Lifshitz Metabolic Unit, Federal University of Bahia, a low-income region of northeast of Brazil. **Methods**: Descriptive retrospective study of longitudinal clinical and biochemical data. Clinical (included height, weight, BMI, and anthropometric indicators height for age and BMI for age) and laboratorial biochemical parameters (included hepatic transaminases, lactate, uric acid, triglycerides (TG), and cholesterol) were retrieved from the paper and electronic files between January/2012 and December/2016. Age of diagnosis was defined as the date of liver biopsy or enzymatic activity dosage or mutation analyze, which occurred first. Descriptive statistics were used to data analyze. **Results**: Thirteen GSD (8 females) patients were included from 12 families. Median (25th-75th) age at the last visit was 4.4 (3.8-10.1), range 3.5 to 12.8 years. Median age (25th-75th) of diagnosis was obtained at 13.0 (8.0-20.0), range 3.0 to 51.0 months. The majority of patients had GSD Ia (8/13; 61.5%), six confirmed by absent (two children) or low glucose-6-phosphatase (G6Pase) activity on liver biopsy, one by mutations in the G6PC gene and one by clinical and laboratorial characteristics. Short stature (SS) and overweight/obesity were more frequent anthropometric alterations, respectively 53.8% (one girl with severe SS) and 46.2% at the first visit. After median (25th-75th) time of 15.0 (10.0-26.0) months of follow-up, only child remains with SS but five patients increased the BMI/age indicator, changing from adequate to overweight risk (2), adequate to overweight (1), overweight risk to overweight (1) and overweight to obesity (1). Elevated hepatic transaminases, hyperuricemia and hypertriglyceridemia were observed in 12 (92.3%), 4 (30.8%), and 7 (53.8%) patients, respectively. **Conclusion**: The median time of diagnosis remains high and the overweight/obesity represents the major nutritional challenges.

N) Disorders of Fatty Acid Oxidation and Ketone Body Metabolism (474 to 519)

474 - Lipoic Acid Alleviates the Pathophysiology in Medium-Chain acyl-CoA Dehydrogenase Deficiency

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Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency (MCADD) is the most frequent fatty acid oxidation (FAO) defect in humans. Fibroblasts from MCAD deficient patients have been shown to be more resistant to oxidative stress

induced cell death than other FAO defects. The enzymatic defect results in accumulation of octanoic acid, the precursor of lipoic acid (LA). LA is an essential cofactor of the acyltransferase components (E2 s) of α-ketoacid dehydrogenase complexes, comprising pyruvate dehydrogenase (PDC), α-ketoglutarate dehydrogenase (KGDC), and branched-chain α-ketoacid dehydrogenase (BCKDC) multi-enzyme complexes. LA and its reduced form dehydrolipoic acid (DHLA) have a key role in the antioxidant network; they can scavenge various reactive oxygen species (ROS), reduce lipid peroxidation (LPO), chelate heavy metals, and recharge other cellular antioxidant pools, such as vitamins C and E. We hypothesized that LA increases and plays a protective role as an antioxidant in MCADD. To test this hypothesis, the total and lipoylated protein content of LA-dependent enzymes, the level of mitochondrial superoxide, LPO, and manganese superoxide dismutase (MnSOD) were monitored in fibroblasts from MCAD deficient patients and healthy controls. MCAD deficient patient fibroblasts displayed significantly increased PDC-E2 and lipoylated-PDC-E2 protein levels compared to fibroblasts from the control group, whereas the E2 subunits of both KGDC and BCKDC complexes showed no significant difference of protein content. Furthermore, we showed that lipids are less oxidatively damaged in MCAD deficient patient fibroblasts, while higher amounts of mitochondrial superoxide and MnSOD were detected compared to healthy controls. The results suggest that accumulated octanoic acid in MCADD increases the level of LA, keeping the PDC-E2 at an increased steady-state level, to facilitate more synthesis of acetyl-CoA, and thus alleviating the bottleneck in FAO. Additionally, LA may play a role as an antioxidant, and decreasing the level of LPO in MCADD.

475 - Severe Cardiomyopathy in a Child With Vlcad Deficiency Despite Triheptanoin therapy

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Patient AJ was born to consanguineous parents of middle eastern origin. Newborn screening collected at five days of life showed a C14:1 acylcarnitine of 3.930 μM (<0.8 μM). *ACADVL* gene testing showed a homozygous intronic sequence change denominated c.1183-15A>G. She was started on MCT oil and levocarnitine supplementation and was followed over time by cardiology. At 4 years of age she was found to have a severe decrease of her ejection fraction from 40% to 22%. She was admitted with symptoms of congestive heart failure and was switched to Triheptanoin 4 grams/kg/day. There was clinical improvement but her function started to deteriorate further over time. Six months after starting triheptanoin she was admitted to the intensive care unit with severely depressed left ventricular function (EF: 18.1%). Evaluation of previously obtained acylcarnitine profiles showed markedly elevated sums of long chain acylcarnitines (C14+C14:1+C14:2+C16+C18) with elevated free carnitine levels. Patient required intravenous pressors and diuretics and was discharged two months later. Essential fatty acid deficiency was addressed and levocarnitine supplementation was suspended resulting in a decreased sum of long chain acylcarnitines. She was noted to have severe speech delay and perseverant behaviors at the age of 4. A chromosome microarray was requested finding a 128 Mb region of homozygosity across multiple chromosomes and a 751 kb duplication at Xp22.33 involving the genes *CRLF2* and *CSF2RA* which did not correspond to a pathological copy number alteration. A comprehensive cardiomyopathy panel consisting of 75 genes showed a mutation in the gene *DSG2* denominated c.91delA (p.T31QfsX14) associated with arrythmogenic ventricular dysplasia. Further studies showed her cardiovascular phenotype was not associated with ARVD. Cardiology has not considered her to be a candidate for heart transplant due to the possible underlying secondary condition. No in vitro studies or exome sequencing have been completed. She is currently ambulatory and stable medically. Echo on March 2017 with EF: 34%. It is not known if the accumulation of long chain acylcarnitines associated with excessive supplementation could have been associated with the poor clinical course. This is a particularly severe case that illustrates the need to follow plasma carnitines very closely and to refrain from excessive supplementation with levocarnitine in affected patients with VLCAD deficiency.

476 - Clinical, Laboratory Data, and Outcome of 7 Patients Affected of Multiple Acyl-CoA Dehydrogenase Deficiency (Glutaric Aciduria Type II) and Report of *ETFDH* Mutation in Neonatal Form

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Objective: Multiple Acyl-CoA Dehydrogenation Deficiency (MADD)is an autosomal recessive inherited disorder of fatty acid, amino acid and choline metabolism caused by defects of electron transfer flavoprotein (ETF, encoded by *ETFA* and *ETFB* genes or ETF-ubiquinone oxidoreductase, encoded by the ETF dehydrogenase (*ETFDH*) gene, mapped to 15p23-25,19q13 and 4q33 respectively. The severity varies from a fatal neonatal form 0f GAII with congenital anomalies (Type I) or without congenital anomalies (Type II) to a mild late onset form of GAII with muscle weakness and pain (Type III). Mutations in the *ETFDH* is known for the late onset form most often responsive to riboflavin treatment and ETFA, ETFB tend to cause neonatal forms. **Methods:** This is a descriptive study on clinical, laboratory data & outcome of 7 GAII cases on treatment with Riboflavin, low fat & protein diet. Diagnosis was confirmed by Acyl carnitine profile (MS/MS), amino acid assays (HPLC), urine organic acid (GC/MS) and molecular

analysis using next generation sequencing (NGS). **Results:** 7 Patients (4♀, 3♂), 4 consanguineous; 2/7 type I (onset: first day), 2/7 type II (onset: second day), 3/7 type III (onset:6 months and 12 years), diagnostic age: 2 months to 13 years, 4/7: neonatal hypoglycemia, 3/7: seizure, 2/7: congenital anomalies, 2/7: hypotonia, 3/7: late onset, 1/3: muscle weakness, 1/3: abdominal pain, 1/3: recurrent myoglobinuria, 4/7: cortical atrophy in brain MRI, 7/7: high liver and muscle enzymes, and 7/7: elevation of C6- C16 and urine glutaric and dicarboxylic acids. Homozygous mutation in ETFDH was discovered in two cases: a case with recurrent myoglobinuria attacks at 13 years with CPK:14444 IU: c.1130 T>C (p.Leu 377Pro) and c.1141G>C (p.Gly381Arg) in a severe neonatal type II, deceased at 12 months despite vit B2 therapy from 3 months of age. Follow up 1-14 years: 3 other neonatal with impaired development, 3 type III: responded well, only mild high CPK. **Conclusion:** We reported ETFH mutation in a fatal neonatal form of MADD who did not respond to riboflavin. Severity depends on the gene defect, enzyme residual activity, and early treatment.

477 - Pathophysiology of 3-Hydroxybutyrate Dehydrogenase (3HBD) Deficiency in Ketone Body Metabolism Using a Bdh1 Knockout Mouse Model

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Background: 3-hydroxybutyrate dehydrogenase (3HBD; EC 1.1.1.30; gene symbol *BDH1*) is an important enzyme in ketone body metabolism. It converts acetoacetate (AcAc) to 3-hydroxybutyrate (3HB) during ketogenesis in the liver and 3HB to AcAc during ketolysis in extrahepatic tissues. To our knowledge, there is no report about patients with 3HBD deficiency. In this work, we investigated the pathophysiology of 3HBD deficiency using a *Bdh1* knockout (KO) mouse model. **Materials and Methods:** We engineered a *Bdh1* KO mouse by CRISPR/Cas9 system and observed the growth and development. Blood levels of total ketone body (TKB), AcAc, 3HB, glucose, and non-esterified fatty acids (NEFA) and liver tissues were examined before and 16, 24, and 48 h after fasting. We compared data of KO mouse with that of wild-type (W-T) controls. **Results:** We found that *Bdh1* KO mice could survive without major problems and had normal fertility. However, their body weight was low compared with that of W-T. Both KO and W-T survived even after 48 h fasting. During fasting, *Bdh1* KO mice showed a gradual increase in AcAc level, whereas W-T showed a gradual increase in 3HB level. However, the increased TKB level in KO mice was less than that of W-T. Conversely, during fasting, glucose level of KO mice was higher than that of W-T, although they showed similar level during fed states. NEFA level had no significant difference between KO and W-T mice. Nevertheless, KO mice showed fatty liver changes, in response to fasting, earlier than that of W-T. **Discussion:** Herein, we investigated, for the first time, the pathophysiology of 3HBD deficiency using a *Bdh1* KO mouse model. Our results indicate that a defect of 3HBD affects some metabolic pathways, including beta-oxidation and ketogenesis. 3HBD deficiency causes insufficient levels of TKB and higher levels of glucose during the fasting state. The higher glucose level and the early fatty changes in the liver might be due to a compensatory mechanism in response to the insufficient ketone body production. Additional research is still required to further elucidate the pathophysiology of 3HBD deficiency. **Conclusion:** It is likely that 3HBD deficiency is not a lethal disorder, but results in fatty change in the liver during fasting or failure to thrive due to energy deficit by low ability of ketone body production. Blood level of AcAc first in *Bdh1* KO mice increases during fasting, whereas 3HB first increases in W-T mice.

478 - Novel Variant in the *ETFDH* Found in Twins With Glutaric Acidemia Type 2

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CC and JC were product of an uncomplicated fraternal twin pregnancy. Newborn screening collected at 25 hours of life showed elevation of C8, C12, C14 and C14:1 acylcarnitines (1.850 μM (<0.6), 3.450 μM (<2), 1.680 μM (1.680) and 1.940 μM (<0.8)) for twin A and for C8, C10, C14:1 (1.2 μM (<0.6); 1.710 μM (<0.65), and 1.1 μM (<0.8)) for twin B. Plasma acylcarnitine profile on both twins showed at 5 days of life elevation of multiple acylcarnitines specially C12-C18. Comprehensive fatty acid oxidation gene panel showed the presence of a pathogenic variant in the *ETFDH* denominated c.250G>A (A84 T) and a variant of unknown significance denominated c.684G>T (K228 N) in both twins. Parental testing showed they were inherited in trans. Twin A additionally carried an heretozygous likely pathogenic variant in the *ACADS* denominated c.322G>A (p.G108 S) and a variant of unknown significance in the *SLC22A5* denominated c.1043T>C (I348 T).

Baseline echocardiogram and liver function testing was normal. Patients were recommended fasting avoidance and were started on 100 mg of Riboflavin daily and 100 mg of Coenzyme Q daily. The *ETFDH* variant c.250G>A is known to be prevalent in Asian populations but the variant c.684G>T has never been described.

479 - Mitochondrial-Targeted Compounds Improve Mitochondrial Bioenergetics Disturbance in Very Long-Chain Acyl-CoA Dehydrogenase Deficient Fibroblasts

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Background: Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency is an autosomal recessive disorder with an incidence of 1:50 000 to 1:100 000, the most common disorder of mitochondrial long-chain fatty acid oxidation (LCFAO). Catabolic situations contribute to the aggravation of symptoms and induce severe metabolic derangement. Treatment for VLCAD deficiency includes dietary control such as the avoidance of fasting and the replacement of long-chain fatty acids by medium-chain triglycerides. However, the development of new therapeutic strategies is highly desirable to improve the wellbeing of patients. **Methods:** We evaluated oxygen consumption rate, ATP production, mitochondrial mass and membrane potential, as well as proteins involved in mitochondrial function in fibroblasts obtained from VLCAD deficient patients. Cells were cultured in medium without glucose for 72-48 h to shift the energy source to fatty acid oxidation. JP4-039 (40 nM and 200 nM) or XJB-5-131 (40 nM and 200 nM), which are mitochondrial-targeted electron and reactive oxygen species (ROS) scavengers, were added 24 hours prior to analysis. **Results:** VLCAD deficient cells showed decreased basal respiration, reserve capacity and ATP production. We also verified that the mitochondrial mass was significantly increased in the cells cultured in the absence of glucose, while no alterations were seen in mitochondrial membrane potential. While ROS generation was decreased, the oxygen consumption rate (OCR) was significantly improved by JP4-039 and XJB-5-131. **Discussion:** Our findings identify decreased respiratory chain function in VLCAD deficient fibroblasts, indicating that the energy metabolism dysfunction in VLCAD deficiency exceeds that of LCFAO alone. JP4-039 and XJB-5-131 were able to improve OCR in VLCAD deficient cells. These pathophysiological findings could contribute to the development of novel therapeutic strategies for VLCAD deficiency.

480 - Clinical Presentation and Outcome in a series of 32 Patients With 2-Methylacetoacetyl-coenzyme A Thiolase (β-Ketothiolase, MAT) Deficiency

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Beta-ketothiolase deficiency (2-methylacetoacetyl-coenzyme A thiolase deficiency, MATD, OMIM #203750) is an inborn error of ketone body utilization and isoleucine catabolism. It is caused by mutations in the *ACAT1* gene and may present with metabolic ketoacidosis. In order to obtain a more comprehensive view on this disease, we have analyzed clinical and

biochemical data as well as information on *ACAT1* mutations of 32 patients from 12 metabolic centers in Germany, Turkey, the Netherlands, Austria, and Switzerland. Data were collected on-site by two medical students who, supported by the local physicians, extracted data from medical records. Patients were between 23 months and 27 years old, more than half of them were offspring of consanguineous parents. 63% of the study participants presented with a metabolic decompensation while most others were identified via newborn screening or family studies. In symptomatic patients, age at manifestation ranged between 5 months and 6.8 years. Only 7% developed a major mental disability, while the vast majority was cognitively normal. Enzyme data were available for 13 patients, all showing reduced or non-detectable MAT activity. More than one third of the identified mutations in *ACAT1* were intronic mutations which are expected to disturb splicing. Our work further extends the mutation spectrum known for *ACAT1* by two missense mutations (p.Gln101Lys, p.Lys124Glu and c.1253G>A), one frame shift mutation (p.Glu154Asp*fs**4) and three intronic sequence variants, which are expected to affect splice sites (c.334+1G>A, c.826+5_826+9delGTGTT and c.940+1G>T). In agreement with previous reports, no clear genotype-phenotype correlation could be found. Although 81% received a protein-restricted diet at least temporarily, no obvious negative consequences could be found in patients without continuous protein restriction. Our study underlines that the prognosis in MATD is good and MAT deficient individuals may remain asymptomatic, if diagnosed early and preventive measures are applied.

481 - 3-Hydroxy-3-Methylglutaryl-Coenzyme A Lyase Deficiency: Clinical Presentation and Outcome in a Series of 37 Patients

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3-hydroxy-3-methylglutaryl-coenzyme A lyase deficiency (HMGCLD, OMIM #246450) is a rare inborn error of ketone body synthesis and leucine degradation. It is caused by mutations in the *HMGCL* gene. In order to obtain a comprehensive view on this disease, we have analyzed clinical and biochemical data as well as information on *HMGCL* mutations of 37 patients (35 families) from metabolic centers in Belgium, Germany, the Netherlands, Switzerland, and Turkey. Data were collected on-site and extracted by two medical students from the medical records, with support by the local physicians. Patients were between 4 months and 40 years old, and half of them were offspring of consanguineous parents. All patients were symptomatic at some stage with 94% presenting with an acute metabolic decompensation. The most common clinical symptoms were recurrent vomiting, seizures, and impaired vigilance, while the main laboratory findings were hypoglycemia and metabolic acidosis. In 50% of the patients, the disorder manifested neonatally, mostly within the first days of life. Only 8% of patients presented first symptoms after one year of age. Six patients died prior to data collection. Long-term treatment mainly comprised protein- and leucine-restricted diet and administration of L-carnitine. In addition to mental impairment, long-term neurological complications were frequent. Half of the patients had a normal cognitive development while the remainder showed psychomotor deficits. Besides microcephaly in 29% of the patients, physical development appeared overall normal. In more than 50% of the study participants, HMGCL enzyme activity was assessed and confirmed the diagnosis. We identified seven novel *HMGCL* mutations. In agreement with previous reports, no clear genotype-phenotype correlation could be found. This is the largest cohort of HMGCLD patients reported so far, demonstrating that HMGCLD is a potentially life-threatening disease with variable clinical outcome. Our findings suggest that the clinical course of HMGCLD cannot be predicted accurately from *HMGCL* genotype and that the overall outcome in HMGCLD is limited.

482 - Results From a 78-Week Single-Arm, Open-Label Phase 2 Study to Evaluate UX007 in Pediatric and Adult Patients With Moderate to Severe Long-Chain Fatty Acid Oxidation Disorders (LC-FAOD)

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A single-arm, open-label phase 2 study was conducted to evaluate safety and efficacy of UX007 (a highly purified, synthetic seven carbon fatty acid triglyceride) for 78 weeks in 29 pediatric and adult patients with moderate-to-severe LC-FAOD (24 subjects completed 78 weeks). LC-FAOD are autosomal recessive genetic disorders caused by defects in mitochondrial fatty acid oxidation enzymes active with long-chain substrates that lead to a general deficiency of energy intermediates and accumulation of toxic fatty acid intermediates causing a variety of serious clinical manifestations. UX007 is being investigated as treatment for LC-FAOD. The mechanism of action of UX007 in restoring energy metabolism is dependent on its medium chain length and odd-carbon properties. UX007 was titrated to a target dose of 25%-35% of total daily caloric intake (mean 31%). Major clinical events (MCE; hospitalizations, emergency room visits, and emergency home interventions due to rhabdomyolysis, hypoglycemia, and cardiomyopathy) were captured retrospectively from medical records for 18-24 months prior to UX007 initiation and compared with the 18 months post-UX007. Prior to initiating UX007, 93% subjects were on MCT therapy. UX007 treatment significantly reduced the number and duration of MCEs. The overall mean annualized event rates decreased from 1.69 to 0.88 events/year ($P = .0208$) and the mean annualized duration rate decreased from 5.96 to 2.96 days/year ($P = .0284$) following UX007 initiation. Hospitalizations due to rhabdomyolysis, the predominant MCE, also decreased from 1.03 to 0.63 events/year ($P = .1044$) following UX007 initiation. Initiation of UX007 eliminated hypoglycemia events leading to hospitalization (0.30 vs 0 hospitalization events/year; $P = .0667$) and ICU care (0.05 vs 0 ICU events/year; $P = .1609$). Adult subjects reported significant improvements in the SF-12 Physical Component Summary Scale ($n = 5$, $P = .0354$), while pediatric subjects reported significant improvements in the SF-10 Physical Summary Scores ($n = 3$, $P < .0001$). Finally, UX007 treatment reduced cardiomyopathy events by 69.6% (0.07 vs 0.02 events/year; $P = .3090$). The most common related treatment-emergent adverse events (TEAEs) were diarrhea, abdominal or gastrointestinal pain, vomiting, and acne, with most mild to moderate in severity. Conclusion: Major clinical events were significantly reduced following UX007 treatment in contrast to pre-treatment, during which most subjects were treated with MCT.

483 - Bi-Allelic Null Mutations in the SCAD Gene Results in Toxic Levels of Butyric Acids

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Objective: Compare the genotype frequency of bi-allelic null-mutations and monoallelic null-mutation in combination with a missense variation in SCAD and MCAD deficient patients. Our hypothesis is that SCADD is a toxicity disease, due to accumulation of butyric acid, so bi-allelic null-mutations in SCADD are rare compared to patients with deficiency of the homolog protein, MCAD. SCAD is located to the mitochondria where it is responsible for metabolism of acyl-CoA, e.g., butyryl-CoA into acetyl-CoA which enters the citric acid cycle. SCADD leads to accumulation of butyryl-CoA/butyric acid in cells, butyryl carnitine in blood, and ethylmalonic acid in the urine. Numerous variants have been described in the in the SCAD gene (*ACADS*). Two of these c.511C>T and c.625G>A are present in 69% of the SCADD patients, but surprisingly also in 29% of the healthy population. The most common manifestation of SCAD deficiency is developmental- and speech delay. The symptoms differ from what is observed in other acyl-CoA dehydrogenase deficiencies. A possible explanation of the different disease manifestation is the toxic accumulation of butyric acid in SCADD. Butyric acid is a Histone Deacetylase (HDAC) inhibitor, known to maintain acetylation of histones, and thereby modify gene expression. It has been shown that transformed cells exposed to butyrate accumulate ROS, it has been further speculated that ROS accumulation might be important for HDAC inhibitor induced cell death. **Method:** We searched through the patient databases at Research Unit for Molecular Medicine, Department of clinical medicine, Aarhus University Hospital and Aarhus University,

of patients genotyped with SCADD or MCADD, over the last 20+ years. Based on genotype they were divided into four groups: 1) Bi-allelic null mutations; 2) monoallelic null mutation and one missense or wild type variation; 3) Two missense variations 4) Others. In total, 357 SCAD and 405 MCAD patients matched the criteria. **Results**: No bi-allelic null-mutations (group 1) were detected in *ACADS,* and only three patients had a monoallelic null-mutation, with the common variant c.625G>A on the other allele (group 2). In comparison, six and 31 MCADD patients were in group 1 and 2, respectively. **Conclusion**: The high frequency of null-mutations in MCADD patients compared to SCADD patients supports our hypothesis that bi-allelic null-mutations of *ACADS* and the resulting accumulation of butyric acid is toxic, and thereby promote apoptosis.

484 - Carnitine Palmitoyl Transferase Deficiency in Indian Patients

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Introduction: Transport of long-chain acyl Co-As into mitochondria occurs by means of a cycle involving four proteins: plasma membrane carnitine transporter, carnitine palmitoyl transferase-1(CPT1), carnitine acyl carnitine translocase, and carnitine palmitoyl transferase-2. CPT-1 transports from cytosol to inner mitochondrial membrane. Due to lack of NBS in India, patients are detected late when they become symptomatic. They are at higher risk of nervous system damage, liver failure, seizures, coma and sudden death. **Objective:** To study the spectrum and outcome of CPT I deficiency in India. **Methods:** This is retrospective study of biochemically diagnosed 8 patients (6M, 2F) with CPT1 deficiency having hepatomegaly, hypoketotic hypoglycemia, failure to thrive, abnormal liver function tests. DBS acyl carnitine profile by TMS, FFA and βHB calorimetrically and dicarboxylic acids by GC-MS of urine were performed. Molecular studies were available in 5 patients. **Results:** 6 patients had hypoketotic hypoglycemia, 3 had hepatomegaly, 1 had encephalopathy, 1 had failure to thrive, and 1 had macrovescicular steatosis on liver biopsy. None of our patients had myopathy or cardiomyopathy or renal tubular acidosis. We found Significantly elevated free carnitine (229.93 ± 30.50, NR: 24.7- 66.6) in DBS and elevated ratio of C0/(C16+C18) (1687.98 ± 1084.35, NR <100). The CPT ratio was in the range of 305-1970. 2 children expired (25% mortality). Both of them had CPT ratio elevation of 1940 and 1970. One patient had grossly elevated FFA–BHB ratio (14.28; NR<5.0). However other 7 patients did not have elevation of FFA–BHB ratio, probably because they were treated with IV dextrose before sampling. All surviving patients (n = 6) were treated with frequent feeds, high calorie, low fat diet and MCT oil supplementation. Uncooked corn starch was not started for any of the patients because of parental apprehension. All of them recovered well with therapy. However, one of them still gets repeated attacks of hypoglycemia and has failure to thrive. All 5 patients had mutations in *CPT1A* gene. **Conclusion:** CPT1 was identified in 8 patients, with hypoketotic hypoglycemia, hepatomegaly, failure to thrive, encephalopathy and abnormal liver function. Of these 2 expired (25% mortality). Early diagnosis and treatment improves the outcome, hence NBS program should be conducted and it should include CPT I. Frequent feeding and MCT oil supplementation helped some of the patients.

485 - VLCAD Deficiency Related Chronic Inflammation Pattern is Suggestive of Systemic Mediators

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Background and Objectives: Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency is a life-threatening disorder of mitochondrial fatty acid oxidation. Newborn screening with early intervention provides the best opportunity to prevent morbidity and mortality. Anaplerotic energy supplementation therapy has been shown to be effective in treating hypoglycemia; however, rhabdomyolysis episodes and atypical chronic inflammation often persist. We hypothesize that rhabdomyolysis susceptibility is associated with maladaptive systemic inflammation that is independent of energy deficiency. **Materials and Methods:** We analyzed pathways with Ingenuity and Pathway Commons programs to correlate previously observed cytokines and proteins that link inflammation and VLCAD deficiency phenotype. Bone marrow was collected from C57BL/6 and $VLCAD^{-/-}$ mice at 10 weeks of life to create in vitro monocytes/macrophages (Mf) and dendritic cells (DCs). We then stimulated inflammatory pathways in half of the cells with lipopolysaccharide (LPS). Spent media was collected, and cytokine profiles were analyzed by a custom 17-Plex (CRP, IFN-g, IL-1a, IL-1β, IL-4, IL-12p70, IL-13, IL-17A, IL-23p19, IL-33, MCP-1, M-CSF, MIP-1a, MIP-1β, S100A8, S100A9, and TNFa) Luminex Assay (R&D Systems, LXSAMSM17). **Results:** In LPS treated $VLCAD^{-/-}$ Mf, IL-12p70 and IL-23p19 were significantly elevated compared to wild type (WT). Otherwise $VLCAD^{-/-}$ cytokine levels were

lower or not significantly different from WT. **Discussion/ Conclusion:** Our results show that *in vitro* inflammatory changes occur in Mf and DCs from VLCAD$^{-/-}$ mice that distinguish them from WT controls. Increased production of IL-12p70 and IL-23p19 by LPS-stimulated VLCAD$^{-/-}$ Mf cells as compared to WT further supports proposed monocyte-activation related mechanism. Additional differences may be explained by cell-specific production and age-dependent increase of cytokines. Current results were found in primary cell culture from 10-week-old mice in comparison to prior data on 6-month-old mouse plasma. We will explore these postulates through time-course measurements of cytokines in mouse plasma. Considering the hypothesis that inflammation contributes to episodes of rhabdomyolysis; clearly chronic inflammation does play a role in VLCAD deficiency and offers a therapeutic target with optimized immune modulators (eg, infliximab) that could significantly improve patient quality of life.

486 - Primary systemic Carnitine deficiency: Clinical Features, Biochemical Findings, and Treatment Outcome in 14 Patients

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Background: Primary systemic carnitine deficiency (PSCD) is an autosomal recessive disorder caused by biallelic mutations in the *SLC22A5* gene, encoding a high-affinity carnitine transporter. Clinical manifestations encompass a broad spectrum of features, from metabolic decompensation to cardiomyopathy and sudden death. Early carnitine supplementation prevents the primary manifestations and reverses symptoms. **Objective:** To describe the clinical features, biochemical profile and treatment outcome of children with PSCD. **Methods:** We retrospectively reviewed the medical records of PSCD patients treated in a tertiary center over the last 15 years. **Results:** Fourteen patients were identified (11 females/3 males). Initial presentation included failure to thrive, recurrent respiratory tract infections, hypoglycemia, hepatic encephalopathy, cardiomyopathy and sudden infant death. Mean age at diagnosis was 23 months (birth—7 years). Nine patients had cardiac involvement. Cardiomyopathy was present in 8/9 patients (4 hypertrophic, 4 dilated). Heart failure, cardiac arrest and short QT syndrome were also reported. Diagnosis was based on total/free carnitine levels measured in dried blood spots and paired urine carnitine levels in symptomatic patients. Three patients were diagnosed as newborns (1/14 newborn screening, 2/14 previous family history) and were asymptomatic. Diagnosis was post-mortem in 2 patients. The combination of plasma carnitine levels as low as 1-5 μmol/L, with renal losses of 10-59 μmol carnitine/mmol creatinine, was highly suggestive for PSCD. *SLC22A5* gene sequencing was performed in 12/14 patients; for 1 patient, diagnosis was confirmed by measuring carnitine uptake in fibroblasts. Most mutations were previously described but we identified one new homozygous mutation in 3 siblings (c.824+5G>A). All patients received oral carnitine supplementation (100-200 mg/kg/day). Mean follow-up was 7.2 years (0.5-16.5). On treatment, patients with no cardiac findings remained asymptomatic. All patients with cardiac involvement improved, while symptoms resolved completely in 3/9 patients with cardiac involvement. Mild cardiac functional abnormalities persisted if heart failure was present at diagnosis (3/9). **Conclusion:** PSCD represents a treatable metabolic disorder. Early diagnosis is imperative as patients have a good prognosis provided that treatment starts before any irreversible damage.

487 - Multiple Defects in Several Genes Appear to Act Synergistically in a Complex Case of Virally Induced Rhabdomyolysis

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Fatty acid oxidation defects (FAOD) are a group of metabolic disorders that present with a variety of phenotypic features. Hypoglycaemia and myopathy and severe rhabdomyolysis may occur in individuals who have molecular changes in more than one gene resulting in synergistic effects. We describe a case of 27-year-old female patient who presented with 3 episodes of rhabdomyolysis precipitated by viral infections and pneumonia between the age of 17 and 25. Her CK increased to 213 000 U/L and was complicated by acute kidney injury requiring admission to ICU. There was a positive family history for Malignant Hyperthermia Syndrome (MHS). Her BMI was 46 kg/m2 and her intention was to reduce her body weight, but prolonged fasting, low calorie intake, infrequent meals and exercise exacerbated symptoms affecting predominantly her legs. In addition, hot temperatures aggravated her symptoms even more ('shaky legs'). MCT product was introduced prior to mild anaerobic exercises to prevent severe myalgia. Initial investigations in muscle found a susceptibility to MHS. Blood acylcarnitines indicated possible CPT2 deficiency. Genetic testing confirmed compound heterozygote mutations in *CPT2* c.[338C>T(;)1933dup], p.[Ser113Leu(;)Glu645 fs] and one "mild" known pathogenic mutation in *ACADVL* c.848T>C, p.[Val283Ala]. Both mutations are reported to be thermolabile. Skin fibroblasts confirmed she had deficient CPT2 enzyme activity at 14% of controls. Fibroblast fatty acid oxidation flux (FAOF) for [9,10-^{3} H]palmitate at 37 C and 41 C was 10% and 6% respectively. Comparative data in 11 "pure" CPT2 deficient myopathic patients resulting from several different mutations, including homozygous and compound heterozygous p.S113 L mutations, gave mean CPT2 enzyme activity of

14 ± 3.5% and palmitate flux at 37 C and 41 C of 55 ± 24% and 21 ± 9% (n = 7) respectively. Myalgia in combination with recurrent rhabdomyolysis warrants thorough biochemical, genetic and histological investigations. Studies should not be stopped after one abnormality is found, in particular, when the severity of clinical presentation does not fit with the result of genetic analysis. In our case CPT2 activity was similar to 11 other "pure" myopathic CPT2 patients but FAOF was the lowest we have encountered in CPT2 deficiency. We strongly suspect synergistic interaction within the fatty acid metabolome that is contributing to the more severe phenotype. This may also be additionally complicated by a MHS susceptibility.

488 - *FLAD1*, a Recently Described Gene Associated to Multiple Acyl-CoA Dehydrogenase Deficiency (MADD) is Mutated in a Patient With Myopathy, Scoliosis, and Cataracts

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Multiple acyl-CoA dehydrogenase deficiency (MADD) is a defect involved in electron transfer to the mitochondrial respiratory chain. Mutations in eight different genes have been associated to MADD. Clinically, it can be presented in the severe neonatal form to the adult form with metabolic acidosis, vomiting, myopathy, muscle pain, or weakness. Some patients are riboflavin responsive. We present a 6 months old female patient with bilateral cataracts. At 1 year of life, she developed scoliosis and later on myopathy. Metabolic investigations at 6 months ruled out galactosemia, lysosomal and peroxisomal disorders. The urinary organic acid profile showed increased ethylmalonic acid, and medium- and long-chain acylcarnitines were increased in plasma (C6, C8, C10, C10:1, C12, C14, C14:1, C14:2, C16:1, C18, and C18:1). These results were suggestive of MADD. The diagnosis was confirmed by the study of deuterated palmitate oxidation in cultured fibroblasts. The patient was treated with riboflavin and low-fat diet, but we could not observe any improvement on the clinical or biochemical data. Molecular studies by NGS using our own-designed Haloplex panel (Agilent technology) revealed two new heterozygous mutations in *FLAD1* gene: c.1555-3C>G and c.797_798delAGinsT. Both mutations have been confirmed in her parents. Western blot in fibroblasts showed total lack of the full-length cytosolic FADS form (50 kDa), but presence of the truncated 26 kDa protein that may allow some FADS activity to be produced. FADS protein is encoded by *FLAD1* gene and is implicated in the synthesis of FAD. In 2016 nine patients have been published in a single article (Olsen RK et al. *Am J Hum Genet*. 2016; 98:1130-1145), some of them were riboflavin-responsive while our patient was not. Cataracts is a peculiar finding not reported in other patients with FADS deficiency, but it has been reported in the more severe forms of MADD.

489 - Clinical and Molecular Evaluation of 15 Korean MCAD Patients Detected by Newborn Screening

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Objective: Medium-chain acyl-CoA dehydrogenase(MCAD) deficiency is a rare metabolic disorder that prevents the body from converting medium-chain fat to energy during fasting. The MCAD mutation, 985A>G (p.K329E), is not common in Asian population. We studied the characteristics of MCAD deficiency in Korean population. **Method:** Biochemical and genetic tests, including plasma acylcarnitine, urine acylglycine, and urine organic acid analyses, in vitro probe assay(IVP), and sequencing of *ACADM,* were performed. in newborns whose elevated octanoyl(C8) carnitine levels were detected through newborn screening (NBS) program and MCAD was confirmed by biochemical tests. We included five previously reported cases of Korean MCAD patients. Preventive and management protocols have been applied. **Results:** All but one MCAD patients in Korea were detected by NBS. All had mildly elevated blood C8 and C10:1 carnitines, and hexanoylglycine, suberylglycine or 3-phenylpropionylglycine in urine. The common mutations in Korean population are 1189T>A (Y397 N) and 449_452delCTGA. All Korean MCAD patients' families received genetic counseling and dietary management to avoid fasting, and their primary care physicians were provided with emergency management protocols. All patients under care have normal growth and development. **Conclusion:** In Korean population, 449-452delCTGA and 1189T>A(Y397 N) mutations are common. The common mutation in caucasian population, 985A>G (p.K329E), was not observed in our Korean cohort. MCAD emergency management protocol is crucial in prevention of metabolic crisis and subsequent neurological complications.

490 - A Mitochondrial-Targeted Electron Scavenger and a Cardiolipin Binding Peptide Decrease Superoxide Generation and Improve Mitochondrial Respiration in ACAD9-Deficient Fibroblasts

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Acyl-CoA dehydrogenase 9 (ACAD9) is a flavoprotein that catalyzes the first step in long-chain fatty acid β-oxidation and acts as an assembly factor for mitochondrial respiratory chain complex I. Individuals with ACAD9 deficiency present with progressive encephalomyopathy, recurrent Reye syndrome, and cardiomyopathy that can be fatal. Although some patients are responsive to riboflavin therapy, there are limited treatment options for this disorder. We evaluated the effect of potential protective compounds on superoxide generation and mitochondrial respiration in fibroblasts of an ACAD9-deficient patient. Patient fibroblasts were cultured in medium without glucose for 48 to 72 hr to assess the ability of *ACAD9*-deficient cells to accommodate the shift of energy source from glucose, and the effect of JP4-039, a mitochondrial targeting free radical scavenger, as well as a novel cardiolipin targeting peptide on superoxide production and oxygen consumption. Superoxide generation was increased, whereas basal respiration and reserve capacity were decreased in ACAD9-deficient cells, compared to normal cells. While either JP4-039 or the cardiolipin targeting peptide decreased superoxide levels, the antioxidants *N*-acetylcysteine, trolox, resveratrol, and mitoQ, and the pan-PPAR agonist bezafibrate did not reduce superoxide levels. JP4-039 and the peptide increased basal respiration and reserve capacity in deficient cells as well. These findings suggest that some of the presumed damaging biochemical abnormalities caused by ACAD9 deficiency can be alleviated by JP4-039 and the novel cardiolipin targeting peptide, in addition to improving bioenergetics in ACAD9-deficient fibroblasts. This provides the impetus for further evaluation of these molecules as potential therapeutics for this disorder.

491 - Assessments of Exercise Tolerance and Muscle Function in Long Chain-Fatty Acid Oxidation Disorders (LC-FAOD): Results From a Phase 2 Open Label Study of UX007

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LC-FAOD are autosomal recessive genetic disorders caused by defects in mitochondrial fatty acid oxidation enzymes active with long-chain substrates. These defects lead to a general deficiency of energy intermediates and accumulation of toxic fatty acid intermediates resulting in serious clinical manifestations. In a Phase 2 open-label study, the effect of UX007 (a highly purified, synthetic seven carbon fatty acid triglyceride) on exercise tolerance and muscle function was investigated. Exercise intolerance was reported in the LC-FAOD specific medical histories of 21/29 (72%) of subjects enrolled in the Phase 2 study. Tests of exercise tolerance and muscle function were performed in the cohort of subjects $\geq$6 years of age that were able to comply and follow test instructions safely and reliably. Cycle ergometry was included as a measure of exercise tolerance and performed at an intensity and duration conducive to the reduction of glycogen stores and oxidation of fatty acids by exercised muscle to evaluate the effect of UX007 treatment in subjects with inherent defects in fatty acid oxidation. Exercise tolerance was assessed by workload and duration at a fixed heart rate during a 40-minute protocol. A 12minute walk test (12MWT) was incorporated into the study design as a measure of muscle function. The distance walked in meters was assessed at 6 and 12 minutes. In the ergometry test, improvements were observed in both workload (watts) and duration (mins) following UX007 treatment (n = 7). After 24 weeks of UX007 treatment, mean workload increased by 60% from 744.6 to 1191.4 watts (median: 127.5; min, max: −388, +2438; Least Squares (LS) mean change: 423.6, P = .1518). Improvements in duration were also noted with mean test times improving from 9.3 $\pm$ 5.1 mins (range: 5-15) to 20.4 $\pm$ 17.0 mins (range: 10-40) in the 3 subjects unable to complete the 40-minute exercise protocol at Baseline. No further improvements were noted after an additional 48 weeks of treatment however, the interpretation was limited by a smaller sample size. In the 12MWT, the mean distance walked increased 28% from 673.4 meters to 861.4 meters after 18 weeks of UX007 treatment (median: 93.5; min, max: −80, +880; LS Mean change: 181.4, P = .0830) (n = 8). This increase was maintained after an additional 42 weeks of treatment. These data suggest UX007 treatment improves exercise tolerance and muscle function in LC-FAOD patients and supports the use of these assessments in this patient population.

492 - Molecular and Biochemical Characteristics of Patients With LCHADD From Ukraine

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Background and objectives: Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) is rare genetic disorder of fatty acids β-oxidation. It is caused by the mutations in the *HADHA* gene. The most common mutation is G1528C in 15 exons. The main objective of this study was to investigate the presence of G1528C mutation in the *HADHA* gene of patients with LCHADD from Ukraine. **Materials and methods:** Measurement of acylcarnitines in dry blood spots by LC-MS/MS, detection of G1528C mutation in samples of patients with LCHADD and their parents by RFLP analysis. **Results and discussion:** From 2011 to 2016 was hold selective screening of 6158 patients with suspicion of inborn errors of metabolism by tandem mass-spectrometry method. During these screening four patients (0,065%) with elevated levels of LCHADD specific biochemical markers (C16OH, C18OH, C18:1OH) in dry blood spots were detected. Diagnosis confirmation was made by PCR which was followed by RFLP analysis. As a result, it was showed that all four patients had G1528C mutation in both alleles, and their parents had this mutation in one allele and were heterozygous. The LCHAD deficiency prevalence is 1:597 245 live birth in Ukraine. Such a figure could be connected with absence of mass neonatal screening of LCHADD in Ukraine. Our laboratory is the only one place to diagnose LCHADD in Ukraine, that's why the level of underdiagnosis of LCHADD in Ukraine is very high. To sum up, it is necessary to emphasize that the major mutation of LCHADD for Ukrainian population is G1528C, as it was found in homozygous condition in four patients. That's why it is recommended to screen all patients with elevated long chain hydroxyacylcarnitines on presence of G1528C mutation.

493 - Carnitine Deficiency Secondary to Methyl-Tetra-Hidrofolatoredutase Mutation: Case Report

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The aim of this study is to draw attention to the rare association between carnitine deficiency and enzymatic mutation methyl-tetra-hidrofolatoredutase, diagnosis usually thoughtless, resulting in progressive myopathy. We describe a retrospective case report using medical record data collection. Patient, male, 12 years bearer of Takayasu arteritis that after two months of treatment with methotrexate presented progressive proximal myopathy, standard MRI showed inflammatory myopathy and two muscle biopsies with accumulation of lipid and glycogen, suggestive of carnitine deficiency. Heterozygous mutation was detected in the C677 T *MTHFR* gene with elevated serum homocysteine and it was started replacement with L-carnitine with progressive improvement in muscle strength and decrease of muscle enzymes, returning to walk in 15 days. The pattern of inflammatory myopathy is described as a reflex of muscle infiltration by fatty acids. Disorders of fatty acid metabolism generate accumulation of acylcarnitine. This accumulation of lipid in the muscle causes inflammatory lesion and progressive muscle damage, similar to inflammatory myopathy. Muscle biopsies, with absence of atrophy of inflammatory infiltrate fibers and presenting lipid infiltration, suggest carnitine deficiency. Carnitine is a facilitator of transport of long chain fatty acids through mitochondria, modulates the intramitochondrial CoA / acylCoA ratio and alternative metabolic pathways. The clinical manifestations vary from the age of presentation according to the cause, and may present as myopathic or systemic form. In myopathic form, serum carnitine levels may be normal (measurement is not essential for diagnosis). MTHFR is an enzyme involved in the homocysteine pathway, the severity of its deficiency depends on the level of enzymatic activity. The clinical deficiency of MTHFR occurs in homozygotes and heterozygotes usually do not present clinical manifestations except in the case of concomitant folate deficiency, as in the use of methotrexate, an analog of folic acid capable of competitively and irreversibly inhibiting the enzyme in question. **Conclusions:** A deficiency of methyl-tetrahydro-hidrofolatoredutase is reported in literature as a cause of secondary carnitine deficiency. However, reports of patients with clinical disease manifest deficiency resulting from this enzymatic inhibition are rare. It is necessary to subject the approach as regards the diagnostic carnitine deficiency and its causes.

494 - Carnitine Acyl Carnitine Translocase Deficiency With Severe Hyperammonemia and Hypoglycemia

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Introduction: Carnitine-acyl translocase deficiency (CACT) is an autosomal recessive disorder of fatty acid oxidation and affects especially long-chain fatty acids. The disease is usually characterized by severe hypoketotic hypoglycemia, cardiomyopathy or arrhythmia, moderate hyperammonemia, liver dysfunction and muscle weakness. Here a case of CACT deficiency with severe hyperammonemia following inguinal hernia operation was presented. **Case:** A 1-day old boy presented with moderate hyperammonemia (400 µmol / l), hypoketotic hypoglycemia and arrhythmia. The parents were consangious. Urine organic acid analysis showed dicarboxylic acid and octenedioic acid as well as tandem mass spectrometer showed increased levels of C10, C10: 1, C12, C14, 14: 1, C14-OH, C16, C16: 1, C16-OH, C18 carnitines. After inguinal hernia operation in his second months of age, the patient had resistant hypoglycemia and hyperammonemia-induced encephalopathy. Ammonia levels gradually increased even under hyperammonemia lowering treatment. Ammonia level was determined to be 440 µmol/L and immediate hemodiafiltration was initiated. At the first hour of hemodiafiltration, ammonia level was found to be 2551 µmol / L and after hemodiafiltration, ammonia levels decreased to normal. The previously identified Phe 90 fs homozygous mutation in the SLC25A20 gene was detected with next generation sequencing analysis and carnitine- acylcarnitine translocase deficiency was diagnosed **Result:** In the literature, carnitine-acyl translocase deficiency with such high levels of ammonia has not been reported yet, and this case was reported to underline that carnitine-acyl translocase deficiency might be presented with high ammonia levels enough to mimic urea cycle disorders.

495 - Short Chain Fatty Acid Oxidation Defect in an Adult Patient With Refractory Seizures

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Introduction: The short chain acyl-CoA dehydrogenase enzyme (SCAD) is a mitochondrial enzyme involved in the β oxidation of fatty acid. Genetic disorders of SCAD are often asymptomatic, but progressive psychomotor retardation, hypotonia, myopathy and some dysmorphic findings have also been reported. Epilepsy is very rarely defined in these patients. **Case:** A 21-year-old girl admitted to the Pediatric Metabolism Department because of the bad smell of her urine. The patient had a resistant seizure for 10 years, used 2 antiepileptic drugs and also vagal nerve stimulation for control of epilepsy was performed. In her history, she had hypoglycemia associated convulsion. There was a third degree of consanguity between the parents and there was no family history similar to the patient. The patient, who was a university student, was mentally well. There was no problem in her physical examination. Magnetic resonance imaging of the cranium was normal, tandem mass spectrometer showed increased C4 carnitine and repeated urine organic acid analysis had ethylmalonic aciduria. In the *ACAD* gene, c (625G›A) + c (625 G›A homozygous mutation was detected. **Result:** SCAD is usually diagnosed after neonatal screenings, as well as disease that does not cause clinical manifestation but sometimes is associated with psychomotor retardation and hypotonia so that it should also be investigated in adult patients with refractory epilepsy.

496 - Severe Neonatal Manifestation of Long Chain 3-Hydroxyacyl-CoA Dehydrogenase deficiency: First Report After 14 Years of Expanded Newborn Screening at the National Children's Hospital, Costa Rica

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Introduction: Long Chain 3-Hydroxyacyl-Coa Dehydrogenase Deficiency (LCHADD), is a mitochondrial disorder of fatty acid oxidation, a rare condition that prevents the body from converting certain fats into energy. In most patients, onset is in infancy or early childhood. LCHADD is an autosomal recessive disorder, recognized as an important inborn error of metabolism that can cause sudden infant death syndrome particularly at times during prolonged fasting and periods of increased energy needs from a catabolic state (infection, fever, stress) not sufficiently satisfied by caloric intake. **Methodology:** We described the clinical features and biochemical findings in a newborn, who presented in acute decompensation, characterized by: hypoglycemia, lethargy, multisystem organ failure, in the first 48 hours of life who was admitted at the Neonatal Intensive Care Unit. **Results:** Tandem mass spectroscopy result showed an abnormal significant elevation of C14-OH, C16-OH, C18-OH and C18:1-OH acylcarnitine species without other elevations. Infectious and cardiac causes were ruled out. Urinary organic acids profile showed a large lactic acid peak, 3-Hydroxyisobutyrate, 2-Hydroxyisobutyrate and 3-Hydroxydecanoic, consistent with LCHADD. Multisystem organ failure started at third day of life and withdrawal of care was required at fifth day of life. **Conclusions:** Presentation of LCHAD was initially described in infants and children usually with first symptoms by 2 years of age; however, neonatal cases do occur. In newborn cases as the one presented: mortality is high, long-term outcome is uncertain. Urgent metabolic intervention decreases mortality of LCHAD-deficient patients, the survival in acute severe crisis is limited as soon as multisystem organ failure starts. Signs and

symptoms typically include feeding difficulties, lethargy, hypoglycemia, hypotonia. Expanded Newborn Screening using tandem mass spectroscopy will show a characteristic pattern with elevations of the acylcarnitine species: (C16-OH) 3-hydroxypalmitoylcarnitine and/or (C18:1-OH) 3-hydroxyoleoylcarnitine. Early detection by new born screening will lead to accurate therapeutic interventions, maximization of caloric intake preventing severe decompensation and multisystem organ failure that can lead to death. Multisystem organ failure onset is a poor evolution and prognosis finding

497 - Heterozygous Carriers of Succinyl-CoA: 3-Oxoacid CoA Transferase Deficiency can Develop Severe Ketoacidosis

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Succinyl-CoA:3-oxoacid CoA transferase (SCOT, gene symbol *OXCT1*) deficiency is an autosomal recessive disorder in ketone body utilization that results in severe recurrent ketoacidotic episodes in infancy. More than 30 patients with this disorder have been reported and to our knowledge, their heterozygous parents and siblings have had no apparent ketoacidotic episodes. Over 5 years (2008–2012), of the patients that presented with severe ketoacidosis who were suspected as having SCOT deficiency, we identified a heterozygous *OXCT1* mutation in four cases (Case1 p.R281C, Case2 p.T435 N, Case3 p.W213*, and Case4 c.493delG). To confirm their heterozygous state, we performed a multiplex ligation-dependent probe amplification analysis on the *OXCT1* gene which excluded the presence of large deletions or insertions in another allele. A sequencing analysis of subcloned full-length SCOT cDNA showed that wild-type cDNA clones were present at reasonable rates to mutant cDNA clones. Over the following 2 years (2013-2014), we analyzed *OXCT1* mutations in six more patients presenting with severe ketoacidosis (blood pH $\leqq$7.25 and total ketone body $\geqq$10 mmol/L) with non-specific urinary organic acid profiles. Of these, a heterozygous *OXCT1* mutation was found in two cases (Case5 p.G391D, Case6 p.R281C). Moreover, transient expression analysis revealed R281C and T435 N mutants to be temperature-sensitive. This characteristic may be important because most patients developed ketoacidosis during infections. Our data indicate that heterozygous carriers of *OXCT1* mutations can develop severe ketoacidotic episodes in conjunction with ketogenic stresses.

498 - Activation of Protective Systems in Fatty Acid Oxidation Disorders can lead to Deleterious Outcomes

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One consequence of long-chain fatty acid oxidation (FAO) disorders is excessive accumulation of free fatty acids within cells. In a healthy cell or tissue, the peroxisome proliferator-activated receptors (PPARs) and other cellular pathways will be activated by these fatty acids and increase expression and activity of proteins associated with FAO and other aspects of lipid metabolism. This reduces the level of free fatty acids to below cytotoxic levels, by oxidation, export or incorporation into membrane lipids. The PPAR system can also be activated pharmacologically; a classic example of PPAR agonists is the fibrate group. In recent years, the bezafibrate has been proposed as a pharmacological treatment for milder forms of long-chain FAO disorders. *In vitro* studies have shown promising results, but small clinical trials less so. The effect on the cellular redox state of pharmacologically activating the PPAR system is not known. Effects could include potentially deleterious aspects, such as activating mitogenesis of inherently dysfunctional mitochondria and increasing expression of mutant protein. Conversely, PPAR activation also initiates protective systems, such as expression of antioxidant proteins. We set out to investigate cellular protection against oxidative stress and survival under external heat stress after bezafibrate treatment. We found that during heat stress mitochondrial superoxide production increases in very long-chain acyl-CoA dehydrogenase deficient patient dermal fibroblasts treated with bezafibrate ($n = 6$), compared to similarly treated controls ($n = 6$). Superoxide production was also greater in bezafibrate treated patient fibroblasts undergoing heat stress, than in patient fibroblasts undergoing heat stress without prior bezafibrate treatment. Likewise, the capacity of bezafibrate-treated patient fibroblasts to maintain peptide antioxidant (*i.e.* glutathione) levels during deceased, which leads to faster cell death during heat stress. The increased predisposal to stress induced cellular damage may partially or completely counteract the beneficial aspects of bezafibrates ability to increase the baseline FAO capacity.

This points to the importance of supportive therapy, to help maintain organelle integrity, as opposed to simply attempting to increase expression of mutant protein and normalize FAO flux.

499 - Multiple Acyl-CoA Dehydrogenase Deficiency due to a Novel Homozygous and Compound Heterozygous Mutation in the *ETFDH* Gene in 3 South African Patients

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Introduction and objectives: Multiple acyl-CoA dehydrogenase deficiency (MADD, OMIM: 231680) is an autosomal recessive metabolic disorder due to mutations in the *ETFA*, *ETFB* and *ETFDH* genes resulting in the deficient function of alpha or beta subunits of the electron transfer protein (ETF) or electron transferring flavoprotein dehydrogenase (ETFDH), respectively. Dysfunction in either of these two flavoproteins leads to compromised fatty acid and amino acid oxidation as well as choline metabolism. We report on clinical, biochemical and genetic findings of three South African patients with ETFDH deficiency. **Patients and results:** These patients were found to be homozygous (patient 1) for a novel c.1067G>A (p.Gly356Glu) or compound heterozygous (patient 2 and 3) for the described novel and known c.1448C>T (p.Pro483Leu) mutations in the *ETFDH* gene, respectively. Functional studies in muscle and fibroblasts confirmed the deleterious effects of compromised ETFDH expression on the respiratory chain function and fatty acid oxidation. The clinical-biochemical presentation for patient 1 included severe neonatal onset with congenital abnormalities, hypoglycemia, metabolic acidosis, hyperammonemia as well as a characteristic metabolite profile of accumulating mono- and dicarboxylic acids, N-acylglycines and acylcarnitines commonly associated with MADD. Delayed onset was noted for patients 2 and 3 which also presented with the characteristic MADD metabolite profile as well as episodic metabolic acidosis, non-ketotic hypoglycemia, mild hyperammonemia, progressive myopathy and hepatosplenomegaly. Early death in patient 1 occurred whereas the compound heterozygotes responded to L-carnitine and riboflavin treatment. Progressive muscle weakness and severe migraine-like episodes occurred in patient 2 and 3. Patient 3 died at the age of 23 years after a stroke. **Conclusion**: A clear genotype-phenotype correlation was confirmed for the novel mutation present in homozygous and/or compound heterozygous state. These findings may potentially predict the prognosis of MADD due to ETFDH deficiency in the affected South African population.

500 - Epidemiological and clinical features of Medium-Chain Acyl-CoA Dehydrogenase Deficiency in the Pediatric Population of the Republic of Ireland

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Objective: This study aims to investigate the disease frequency of Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCADD) in the Irish setting and inform policy regarding the addition of MCADD into the Irish National Newborn Bloodspot Screening Program. **Methods:** Children (<18 years) with MCADD were identified via the National Centre for Inherited Metabolic Disorders and the metabolic laboratory at Temple Street Children's University Hospital. Central Statistics Office population data was used to calculate epidemiological figures. **Results:** From January 1,1998, to August 30, 2016, 17 children were diagnosed with MCADD in Ireland. The average age at clinical presentation was 1.48 years (range: 0.005 to 2.86 years) with 2 patients diagnosed post mortem. The incidence of MCADD during this period was 1:71 650 with a mortality of 15.38% in the first clinical presentation- no child died post diagnosis. The common c.985A>G mutation accounted for 88% of alleles. The current prevalence of MCADD was calculated to be 1.23 per 100,000 children. **Conclusion:** The incidence of MCADD in Ireland is lower than global estimates (incidence of 1: 10 000-30 000 in most countries where newborn screening is in place). The potential for under-ascertainment and late diagnosis of cases exists in Ireland and is of concern for a treatable condition with a significant risk of morbidity and mortality when undiagnosed. These findings support a previously unpublished study where 1000 newborn bloodspot screening cards were screened for the common mutation and an incidence of 1 in 66 000 births was calculated using the Hardy Weinberg equation.

501 - Pharmacological Inhibition of Carnitine Palmitoyltransferase I Restores Mitochondrial Oxidative Phosphorylation in Human Trifunctional Protein Deficient Fibroblasts

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Background: Mitochondrial Trifunctional Protein deficiency (TFPD) is a severe genetic disease characterized by altered energy metabolism and accumulation of long-chain (LC) acylcarnitines in blood and tissues. This accumulation could impair the mitochondrial oxidative phosphorylation (OxPhos), contributing to the non-optimal outcome despite conventional diet therapy with medium-chain triglycerides (MCT). **Method:** Acylcarnitine and OxPhos parameters were measured in TFPD-fibroblasts obtained from 8 children and cultured in medium mimicking fasting (LCFA) or conventional treatment (MCT), with or without Etomoxir (ETX) an inhibitor of carnitine palmitoyltransferase 1 (CPT1) activity, and were compared to results obtained with fibroblasts from 5 healthy-control children. The effects of various acylcarnitines were also tested on control fibroblasts. **Results:** In the LCFA-condition, TFPD-fibroblasts demonstrated a large accumulation of LC-acylcarnitines associated with decreased O_2-consumption ($63 \pm 3\%$ of control, $P < .001$) and ATP production ($67 \pm 5\%$, $P < .001$) without modification of coupling efficiency. A dose-dependent decrease in O_2-consumption was reproduced in control fibroblasts by addition of increasing dose of LC-acylcarnitines, while it was almost preserved with MC-acylcarnitines. The MCT-condition reduced LC-acylcarnitine accumulation and partially improved O_2-consumption ($80 \pm 3\%$, $P < .01$) in TFPD-fibroblasts. The addition of ETX in both LCFA- and MCT-conditions normalized acylcarnitine profiles and restored O_2-consumption and ATP production at the same levels than control. **Conclusion:** Accumulation of LC-acylcarnitines plays a major role in the pathophysiology of TFPD, reducing OxPhos capacities. These deleterious effects could be partially prevented by MCT-therapy and totally corrected by ETX. Inhibition of CPT1 may be view as a new therapeutic target for patients with a severe form of TFPD.

502 - Case Report: Succinyl-CoA:3-Ketoacid CoA Transferase (SCOT) Deficiency

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Objective: Suspect ketone body disorders when accessing patients presenting severe metabolic acidosis without any other laboratorial findings. **Results**: We describe a 3-month male, second child of related parents (first degree cousins) presenting with acute fever and respiratory distress, severe refractory metabolic acidosis and normal blood glucose (BG), without neurologic impairment. During hospitalization, he presented intermittent metabolic acidosis associated to infections. Ammonia levels, acyl carnitine analysis, urinary organic acid and amino acids chromatography were all normal. The molecular test identified a homozygote mutation in the *OXCT1* (3-oxoacid CoA transferase 1, OMIM #601424) gene, variant chr5:41.861.501 C>T, also known as c.193G>A CCDS3937.1, promoting substitution of glycine on position 65 for arginine (p.Gly65Arg). It is absent in more than 60.000 individuals worldwide, thus considered pathogenic. Ketone body (KB) metabolism defects lead to ketoacidosis due to ketone body accumulation. Acetoacetate (AcAc) and 3-hydroxybutyrate (3HB), the 2 main ketone bodies of humans, are important vectors of energy transport from the liver to extrahepatic tissues, especially during fasting, when glucose supply is low, and with ketogenic stresses[1]. SCOT deficiency and mitochondrial acetoacetyl-CoA thiolase (betaketothiolase or T2, gene symbol *ACAT1*) deficiency are two autosomal recessive inherited defects in ketolysis presenting as intermittent metabolic acidosis. Permanent ketosis is a hallmark for SCOT deficiency; however, patients with "mild" SCOT mutations may have nonketotic periods. T2-deficient patients cannot be detected in a reliable manner by newborn screening using acylcarnitines[2] and the ones with "mild" mutations may have normal blood acylcarnitine profiles even in ketoacidotic crises. Clinical presentation and laboratorial findings are not specific. Once Blood total ketone body (TKB) levels dosage is not available in our Country, diagnose is rarely made by clinical and laboratorial findings. Molecular investigation is the only way to confirm diagnosis whenever defects on ketolysis are suspected. **Conclusion:** Ketolysis defects may be considered when severe metabolic acidemia with no other signs presents as the case above. In our country, the diagnosis is confirmed by molecular testing.

503 - Systemic Carnitine Deficiency: Impact of Treatment on Clinical and Biochemical Features in 32 Patients

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Objectives: The aim was to evaluate clinical and biochemical symptoms at presentation and response to treatment in patients with systemic carnitine deficiency (SCD) (OMIM212140) with relevance to genotype. **Methods**: Clinical features of 32 patients with SCD were evaluated at referral and follow-up and mutation analyses of the patients were evaluated.

Results: Thirty-two patients referred at the ages of 5.7 ± 10.7 years. The current age of the group was 13.3 ± 2.9 years and average follow up 7 ± 1 years. At presentation cardiomyopathy (n = 9), Reye-like syndrome (n = 4), neurologic symptoms (n = 3), asymptomatic patients (n = 12) patients diagnosed due to family history (n = 7) or expanded neonatal screening (n = 4) were recorded. At referral mean free carnitine and mean total carnitine levels were measured as 2.2 ± 0.4 and 1.65 ± 0.4 μmol/L respectively. Cardiomyopathy diagnosed as the major initial pathology had resolved completely in all except two patients after carnitine therapy. Homozygous mutations Leu363Pro (c.1088T>C) and Gly152Arg (c. 454G>C) were prevalent in patients with cardiomyopathy whereas patients carrying R471 H mutation exhibited no cardiomyopathy. **Conclusion:** Since cardiomyopathy is treatable, SCD diagnosis must be considered by performing an acylcarnitine profile in all patients presenting with cardiomyopathy.

504 - Mitochondrial Trifunctional Protein Deficiency Presenting as Recurrent Nonimmune Hydrops in Siblings and Maternal Mirror Syndrome

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Introduction: Mitochondrial trifunctional protein (MTP) and long chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) are important for the oxidation of long chain fats. Mothers pregnant with a fetus with LCHAD deficiency can have acute fatty liver of pregnancy. Mirror syndrome occurs when the mother develops symptoms and signs similar to those in her fetus. These cases support a previous report of MTP presenting as fatal cardiomyopathy and hydrops. **Case:** A nonconsanguineous Caucasian couple with two pregnancies complicated by fatal non-immune hydrops. In the first pregnancy, the mother developed life threatening pulmonary edema at 26 weeks (Mirror syndrome) necessitating emergency delivery. The infant died soon after delivery and was investigated by a hydrops gene panel. The second pregnancy was also complicated by maternal acute chest pain and dyspnea (Mirror syndrome), culminating in preterm delivery at 25 weeks. This infant had metabolic studies. **Results:** The hydrops panel on the first infant failed to identify a cause. The second infant had severe lactic acidosis peaking at 22 mmol/L. Newborn screening acylcarnitine profile identified significant elevations in long chain hydroxyacylcarnitines (C16-OH, C18:1OH and C18-OH with borderline C16:1-OH), compared with modestly elevated long chain acylcarnitines. Plasma acylcarnitine profile on the second infant confirmed significantly increased long chain hydroxyacylcarnitines, long chain acylcarnitines (C18:1-OH, C16-OH, C18, C18:1, C16, C14, C14:1, and C12) and normal C2 and C4-OH carnitines. Urine organic acids showed markedly increased lactate and pyruvate, moderately increased 3-hydroxydicarboxylic acids and dicarboxylic acids but mild increases in ketones, consistent with MTP/LCHAD. This was confirmed by long and short chain 3-hydroxyacyl CoA dehydrogenase activity in fibroblast homogenate. Re-interrogation of the first infant's exome data identified a pathogenic variant in the *HADHB*. Sanger sequencing is in progress to find the second mutation. **Conclusion:** These cases add to the previous report of hydrops with MTP deficiency. We wonder if the Mirror syndrome in this mother may be related to the MTP, similar to liver disease in mothers of infants with LCHAD. We recommend inclusion of *HADHA* and *HADHB* in gene panels for hydrops.

505 - Detection of Allele Frequencies of Common c. 517TC and c.625GA Variants in the *ACADS* Gene in Turkish Population

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Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a rare inborn error of mitochondrial fatty acid oxidation defect and protein misfolding disorder. Our aim is to find out Turkish patients diagnosed SCADD in literature and detect allele frequencies of two common variants (c.571T>C and c.625G>A) in Turkish population. We found five Turkish patients from unrelated four families diagnosed SCADD with metabolic screening and mutational analyses. We also investigated allele frequencies of common variants of c.517T>C and c.625G>A and detected 0% and 13% respectively. Homozygous c.625G>A variant was also found to be high in Turkish population as it is in the general population. These variants thought to be confer the susceptibility to disease different than other benign polymorphisms, therefore urgent precautions should be taken in case of metabolic stress (starvation, infection, fever) in people who has these genetic variations to prevent sequela of disease.

506 - Missed Case of Novel ACADVL Homozygous Mutation in Infant With Severe Cardiomegaly and Pericardial Effusion

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Objective: Very long chain acyl-CoA dehydrogenase deficiency (VLCADD, OMIM #201475) is a rare autosomal recessive inborn error of mitochondrial fatty acid β-oxidation. VLCADD is characterized by cardiomyopathy, hepatic encephalopathy, or severe hypoketotic hypoglycemia. Herein, we report an infant with VLCADD who present with massive pericardial effusion and left ventricular hypertrophy. Patient and **Results:** A 6-month-old girl was referred to our hospital due to severe cardiomegaly due to pericardial effusion. Although older sister suddenly died at three days after birth in normal nursery, this girl had normal result in tandem mass spectrometry during neonatal period. At cardiac presentation, elevation of C-14 to C18 was identified by tandem mass spectrometry and molecular analysis of *ACADVL* revealed a homozygous frameshift mutation (c.103_112dup). This novel mutation was inherited from mother and father and they were not consanguineous. Since then, she received riboflavin and medium chain triglyceride formula and pericardial catheter was removed soon. Despite there were several infections including pneumonia and gastroenteritis after diagnosis, metabolic crisis was recovered rapidly by urgent fluid management. Follow-up echocardiography showed normal cardiac function and mild LVH without pericardial effusion. **Conclusion:** Our report highlights that the repetitive metabolic workup during catabolic state is diagnostic clue and early diagnosis and intervention can prevent the lethal course of VLCADD.

507 - Molecular Characterization of *ACADM* Gene in Argentinean Patients

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Introduction: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most frequent inherited defect of fatty acid oxidation. It is characterized by hypoglycemic crisis under fasting or during stress conditions, leading to lethargy, seizures, brain damage and even death. MCADD is a recessively inherited disorder, caused by mutations in the *ACADM* gene. The most common mutation is c.985A>G, which is observed at the homozygous state in 80% of European MCADD patients, and in heterozygous state in 18%. In cases detected by newborn screening, the frequency of the prevalent mutation is lower than that observed in clinically affected patients, and variants that have never seen before in those patients, are identified. **Objective:** To describe molecular characterization of *ACADM* gene in MCADD patients and newborns with positive screening result for MCADD. Methods: DNA was extracted from peripheral blood or dried blood spot from 12 patients. Six of them were referred because of clinical diagnosis of MCADD (two pair of siblings), five because of abnormal newborn screening result and one was an asymptomatic sibling of an screening positive newborn. c.985A>G mutation was studied by PCR-RFLP. Samples negative or heterozygous for c.985A>G were sequenced by analysis of the 12 exons and the exon-intron boundaries of the *ACADM* gene. **Results:** Taking into account only the 9 unrelated patients, c.985A>G appeared in 5/8 alleles of the 4 symptomatic patients and in 4/10 alleles of the 5 screening -positive newborns. Eight different sequence variations were identified, including 3 novel substitutions: c.119-12A>G was detected in two siblings, along with c.985A>G. The older brother presented with hypoglycemia and seizures, his sister was asymptomatic. In silico tools predicted a possible alteration of splicing. c.608T>C (p.L203 S) was found with c.985A>G in a patient presented with hypoglycemia and fever. Several *in silico* tools predicted this missense variant as deleterious. c.1012C>T (p.Q338X) was detected with c.985A>G in a newborn with an abnormal screening result. This variant is predicted as deleterious as it is a nonsense mutation. **Conclusion:** In this cohort, mutational spectrum differed between clinically diagnosed patients and those detected by newborn screening, as previously reported in other populations. Molecular studies supported by *in silico* analysis proved to be important to confirm the MCADD diagnosis in both scenarios.

508 - Ketonesters as a Possible Novel Therapeutic Option for Patients With Fatty Acid Oxidation Disorders

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Rationale: Exertional rhabdomyolysis is a common symptom in patients with long chain Fatty Acid Oxidation (FAO) disorders such as Very Long-Chain Acyl-CoA Dehydrogenase deficiency (VLCADD). Failing muscle ATP homeostasis, due to impaired FAO and glycogen depletion, is the most likely cause. Therefore, suppletion of an alternative energy substrate that prevents ATP depletion, such as the recently developed synthetic ketone-ester (KE) could be a therapeutic option. Oral ingestion of KE in elite athletes resulted in 1) nutritional

ketosis, 2) fueling of the Kreb's cycle, 3) sparing of glycogen, and 4) improved performance. **Objective:** To investigate in patients with VLCADD whether this KE: 1) is tolerated; 2) boosts muscle ATP homeostasis during exercise. **Methods:** Five VLCAD patients (median age 22 yrs. (range 17-45); 4M/1F) were included in a randomized, blinded, placebo controlled, 2-way crossover trial. Prior to each test patients received either KE containing 395 mg KE/kg), or an isocaloric carbohydrate equivalent, and completed a 45-minute exercise test at their individual maximal fatty acid oxidation rate (FATMAX; $\sim 40\%$ VO_2max) workload. During the first 35 minutes, patients cycled on an upright bicycle, followed by 10 minutes of supine cycling inside a MR scanner. Dynamic 31P-MR spectroscopic data of ATP metabolism were recorded from the vastus lateralis muscle during exercise and post-exercise recovery. Blood samples were collected at different time points during the protocol and muscle biopsies were taken before and after exercise. The protocol was repeated after at least one week. **Results:** KE was tolerated by all patients and induced ketosis (max between 2.2-3.2 mmol/L) in the presence of normal glucose levels (range 3.9-7.8 mmol/L). No adverse effects were observed. VO_2 tended to be higher (18 ± 4 vs 19 ± 5 $mL^{-1}.kg^{-1}min^{-1}$, $P = .21$) and RER lower (0.94 ± 0.05 vs. 0.89 ± 0.04, $P = .09$) after KE, suggestive of glycogen sparing. Subjective exertion did not differ between drinks. Analyses of metabolites in blood, muscle and 31P-MRS are currently processed and will be presented. **Conclusion**: The preliminary results of this study show that KE is well tolerated by patients with VLCADD and induces ketosis. Further results are pending and will show whether KE is a potential therapy for patients with VLCADD.

509 - Medium Chain Acyl-coA Dehydrogenase Deficiency: The Importance of Fasting Blood Acylcarnitines and Urinary Organic Acid Profile

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Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is an autosomal recessive inborn error of mitochondrial fatty acid β-oxidation, caused by mutations in the ACADM gene. Although it is the most commonly inherited disorder of the mitochondrial fatty acid oxidation worldwide, there are few reports of cases and mainly regarding the incidence of the disease in Brazil, once there is not a newborn screening program for MCADD. We report here the case of a 2-year-old female patient with recurrent episodes of hypoglycemia, who was investigated through the Brazilian Network of Inborn Errors of Metabolism (*Rede EIM*) and presented interesting findings of the accumulated metabolites profile. Blood octanoylcarnitine (C8) and octanoylcarnitine /decanoylcarnitine (C8-C10) ratio increase, as well as a decrease in free carnitine (C0) with normal hexanoylcarnitine (C6), decanoylcarnitine (C10) and decenoylcarnitine (C10:1) levels were found in the tandem mass spectrometry (MS/MS) acylcarnitine profile. In a second blood sample (postprandial), the acylcarnitine profile was clearly normal, while in a third sample (4-hour fasting) it was observed a marked C8, C8/C10 ratio and C10:1 increase, besides the presence of hexanoylglycine in the urinary organic acid analysis by gas chromatography/mass spectrometry (GC/MS). These findings were suggestive of MCAD deficiency, even though we do not have yet the molecular analysis of the patient. This case demonstrates the importance of fasting sample collection (if possible) when there is a suspicion of an error of mitochondrial fatty acid β-oxidation. Considering the possibility that this disease is underdiagnosed in Brazil and that MCADD is a well-known cause of sudden death in infants, the adequate time of biological sample collection could be crucial to diagnosis.

510 - Betaketothiolase Deficiency—Use of Low Dose Insulin Infusion: A Case Report

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Clinical Details: 5-month-old male infant presented with history of fever, coryza for 2 days and lethargy for 1 day. At presentation to emergency service, he was breathing fast and blood gas confirmed metabolic acidosis (ph 7.05, bicarbonate of 3 mmol/L, base excess of −25), urine ketones were 4+ with euglycemic. He was suspected to have sepsis and managed appropriately. He also received bicarbonate infusion and was ventilated for poor sensorium. Sepsis markers were negative. Despite being euglycemic on 10% dextrose fluids and improvement of acidosis, ketosis was persistent raising the suspicion of a ketone body utilization disorder. Low dose insulin infusion (0.02 U/kg/h) was started after which ketosis resolved in 24 hours. He is the fourth born child to second degree consanguineous parents. There is history of 2 neonatal deaths at 10 days of age with fast breathing and lethargy. Metabolic workup showed slight elevation in glycine 762.34 μmol/L (ref range 2-745 μmol/L), as described earlier in serum amino acid profile and urinary organics showed abnormal unidentified peaks on GC analysis. Acylcarnitine profile was sent after the infant was well and was normal. DNA has been banked for genetic testing. He was initiated on Carnitine supplements and a sick day plan was devised. On moderate protein-restricted diet, this 8-month-old infant is doing well at follow-up. He is

developmentally normal and thriving well. **Discussion**: Beta-ketothiolase deficiency is caused by a defect in mitochondrial acetoacetyl CoA thiolase. This is a rare, often underrecognized disorder which typically presents with recurrent episodes of ketoacidosis with the child being well between episodes. This case highlights the need for clinical suspicion and the use of low dose insulin infusion in the management of same.

511 - Diagnosis of Fatty Acid Oxidation Disorders: Experience at Quest Diagnostics Nichols Institute Biochemical Genetics Laboratory

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Background: Fatty acid oxidation disorders are a group of autosomal recessive enzyme defects that lead to disruption of the mitochondrial beta-oxidation cycle. Although individually rare, the combined incidence of this group of disorders is significant, especially given that many infants identified through newborn screening might never have developed clinical symptoms and would have remained undiagnosed. Fasting hypoglycemia, without accompanying ketosis, is a classic finding in a majority of these disorders. There is variable age of onset and variable clinical presentation affecting multiple organ systems, although heart and muscle are disproportionately affected. Many of these disorders can now be detected through expanded tandem mass spectrometry-based newborn screening. Because of the overlap in clinical features among this group of defects, diagnosis must incorporate confirmatory biochemical and/or molecular testing. Early recognition and diagnosis of these disorders is critical, as avoidance of fasting and/or initiation of dietary therapy is sufficient to prevent irreversible damage in many cases. With early diagnosis and treatment, most babies with these disorders can lead normal, healthy lives. In the absence of treatment, serious sequelae, and even death, can result. Study Goal: The goal of the current investigation was to examine the prevalence of different types of fatty acid oxidation disorders in patients diagnosed at Quest Diagnostics Nichols Institute. **Results:** Out of 1074 patients that were diagnosed, primarily by acylcarnitine analysis in our laboratory, medium-chain acyl-CoA dehydrogenase deficiency (MCAD) was the most common diagnosis (n = 545, 50.7% of positives), followed by very long-chain acyl-CoA dehydrogenase deficiency (VLCAD) (n = 175, 16.3% of positives), short-chain acyl-CoA dehydrogenase deficiency (SCAD) (n = 165, 15.4% of positives), long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency/trifunctional protein deficiency (LCHAD/TFP) (n = 71, 6.6% of positives), Carnitine palmitoyltransferase II (CPT-II) deficiency (n = 64, 6.0% of positives), carnitine transporter/primary carnitine deficiency (n = 48, 4.5% of positives) and carnitine palmitoyltransferase I (CPT-I) deficiency (n = 9, 0.8% of positives). **Conclusions:** Our results highlight how biochemical genetic testing in a clinical setting can be used to detect and classify fatty acid oxidation disorders.

512 - Gender Differences in the Response to Influenza in Long-Chain Acyl-coA Dehydrogenase Knockout Mice

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The role of the fatty acid oxidation enzyme long-chain acyl-CoA dehydrogenase (LCAD) in humans is not understood. We previously showed that LCAD is expressed in human alveolar type 2 pneumocytes and LCAD knockout mice have altered breathing mechanics. Here, we hypothesized that LCAD knockout mice would be susceptible to influenza infection. Surprisingly, there was a gender difference in the response to influenza. Male LCAD knockout mice did not differ from their wild-type counterparts in terms of lung histology, viral titer, weight loss, or blood glucose following influenza infection. Influenza was associated with severe hypoglycemia regardless of genotype. Female LCAD knockout mice, however, were more sensitive to influenza with greater weight loss and poorer survival than wild-type females. Again, hypoglycemia was severe and similar in both genotypes, as were lung histology and viral titers. Female LCAD knockout mice did have higher blood lactate levels and lower core body temperatures than wild-type controls. We conclude that the cause of influenza-associated mortality in LCAD knockout females was the extreme weight loss rather than the lung infection itself. The degree of weight loss correlated very strongly ($R^2 = 0.97$) with lung histology severity scores in female LCAD knockout mice, but not in wild-type females or in male mice of either genotype.

513 - Health-Related Quality of Life in Patients With Long-Chain Fatty Acid Oxidation Disorders

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Background: Patients with long-chain fatty acid oxidation disorder (FAO) are at risk to develop clinical symptoms as hypoketotic hypoglycemia, rhabdomyolysis and cardiomyopathy. There is still limited knowledge about the impact of these symptoms on the quality of life (QoL) in FAO patients and families compared to their healthy peers. Parents of a chronically ill child are at risk of a lower health-related QoL, experience more posttraumatic stress symptoms, and report higher levels of distress than parents of healthy children. Moreover,

studies show that parental psychosocial problems influence the well-being of the child. Over the past decade, patient reported outcome measures (PRO) targeted at the QoL, have become crucial for assessment of new treatment options. Systematically monitoring QoL in FAO patients will therefore be of great value in the evaluation of upcoming treatment options and furthermore provide more insight in the burden of the disorders. **Objective:** To investigate whether: it is feasible to systematically monitor Health Related QoL and psychosocial functioning of patients with FAO in daily practice; how QoL is compared to healthy peers. **Method:** Implementation of a web-based program KLIK (Quality of Life in Clinical Practice) in the national Dutch FAO center. All patients were invited to join the KLIK program before they visited the outpatient clinic for their regular check-up. After registration digital questionnaires (Basic, LTO, PedsQL, PedsQL fatique) are available for both patients and parents, which have to be filled in before each visit to the clinic. Data are collected on physical, emotional and social wellbeing. Individual outcome on each domain is compared with a healthy norm score. **Results:** Currently 15 patients have participated. Preliminary results show that only a very limited amount of patients and parents continue to fill in the questionnaires. In collaboration with the designers of the program we have adapted the method and now allow patients to fill in the questionnaires when they are in the clinic for their evaluation. The most evident finding thus far in LTO is, as expected, the increased anxiety which parents experience. Additional effort is needed to validate the test for patients above the age of 30. **Conclusion:** Systematically monitoring Health Related QoL in patients with FAO is feasible but not yet routine and additional support is needed to collect the baseline information before the data can be used as PRO.

514 - Ketolysis Defect, Biochemical Peculiarities for Diagnosis in a 4-Year-Old Child

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The emerging field of metabolomics, in which a large number of small-molecule metabolites are detected quantitatively in a single step, promises immense potential for early-diagnosis, monitoring, and understanding the pathogenesis of many diseases. The clinical/ biochemical findings in some inborn errors of metabolism (IEM) are often nonspecific; an early differential diagnosis made in a single urinary sample it gives an important advantage. We present our results and the utility of the NMR spectroscopy method for rapid diagnosis and follow up in a ketolysis defect with high ketones in urine, very high lactic acidosis (in urine lactic acid 86.8546 mmol/mol creatinine). The values for urinary ketones were: 3-hydroxybutiric aciduria (40.4284 mmol/mol creatinine), acetoacetic acid (8.6645 mmol/mol creatinine), acetone (3.9781 mmol/mol creatinine) and have been identified in a 4 years old girl with two acute episodes of hypoglycemia during fasting due to intercurrent viral infections. The level of excretion of the metabolites in this disorder has been well within the range of NMR detection (Bruker Avance 400 MHz-Spectrometer). In the critical care setting, IEM that were not diagnosed through the neonatal screening should be considered as cause of acute neurologic, hepatic/ renal decline, rapid diagnosis being essential. We demonstrate the effective use of NMR-spectroscopic-profiles of urine in differential diagnosis in emergency situations, and the possibility of follow up, as well.

515 - How can we evaluate heart manifestations in Fatty acid oxidation disorders (FAO-disorders)?

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Introduction: Cardiac involvement is frequent in FAO-disorders, manifested by cardiomyopathy, arrhythmias and conduction defects. Severe ventricular arrhythmias might cause sudden infant death syndrome (SIDS) or unexpected death in children. In our FAO-disorders' population, 2 patients died from sudden death, a 22-months-old MADD patient, and a 3-month-old MCAD patient whose autopsy exam identified arrhythmia as possible cause of death due to unidentified structural abnormalities. **Objective:** Identify arrhythmias that may determine the prognosis in FAO-disorders. **Methods**: Randomized, prospective study during 2016, in 19 patients with FAO-disorders followed in our Metabolic Diseases Unit. To evaluate cardiac involvement, enzymes (CK, CK-MB, troponins, and NT pro-BNP), echocardiography, electrocardiography and 24 h Holter were included, orientated by a pediatric cardiologist and additional statistical analysis using SPSSV.24 was performed. **Results:** 19 patients with FAO-disorders submitted to cardiological evaluation (14 MCAD, 1 VLCAD, 1 CPTI, 2 CUD, and 1 MADD). 52,6% males, 63,2% Gypsy and 42% had consanguineous parents. All, but one, were diagnosed by expanded newborn screening. No cardiac abnormalities

were detected by enzymatic profile, echocardiography and electrocardiography. Mild abnormalities in 24 h Holter were identified in 7 patients (3 MCAD with supraventricular extrasystoles, 1 MCAD and 1 VLCAD with 2 supraventicular extrasystoles and 1 ventricular extrasystole; 1 CUD with AV block, *mobitz* 1; 1 MCAD with supraventricular extrasystole with aberrant intraventricular conduction). 3 of the patients that showed abnormalities in Holter had developed acute decompensations throughout life. Additionally, long chain FAO-disorders had a higher number of decompensations per patient than medium chain, mean $3 \pm 2{,}8$ vs $1{,}4 \pm 0{,}5$ ($P = .06$, Pearson χ^2). **Conclusion:** Mild rhythm abnormalities were found in long and medium chain FAO-disorders. Long chain FAO-disorders had a higher number of decompensations per patient comparing with medium chain ($P = .482$, Mann-Whitney Test). SIDS occurrence was experienced in both medium and long chain FAO-disorders. Long time rhythm monitoring may be useful to identify FAO-disordered patients that could be at risk for developing arrhythmias. The number of patients and period of our study limit our conclusions. Prospective cardiology evaluation trials in FAO-disordered patients are needed to ensure a clear understanding of cardiac manifestations.

516 - The Biochemical Basis for Overlap of Clinical Features of LCHAD/TFP Deficiency With Mitochondrial Respiratory Chain Defects: Implications for New Therapeutic Approaches

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Introduction: Patients with long chain 3- hydroxy-acyl CoA dehydrogenase (LCHAD) and Trifunctional Protein (TFP; a 4a4b heteroctamer that includes LCHAD function) deficiencies, respiratory chain complex 1 defects (CI), and Barth syndrome (BS) share overlapping features including cardiomyopathy, fatigue, exercise intolerance, hypoglycemia and lactic acidosis during metabolic decompensation. This underscores the need to understand the functional relationship among mitochondrial bioenergetic pathways. While a multifunctional fatty acid oxidation (FAO) complex in which TFP physically interacts with CI in supercomplexes has been described, a link between FAO and cardiolipin remodeling through TFPα and monolysocardiolipin acetyl transferase (MLCLAT) has also been reported. Cardiolipin plays an important role in sustaining the integrity of the electron transport chain (ETC) by maintaining supercomplex stability in the inner mitochondrial membrane. We hypothesize that mutations in TFP disrupt its interaction with CI, and is associated with alterations in MLCL AT activity and cardiolipin, leading to supercomplex instability, and increased reactive oxygen species (ROS). We also hypothesize that defective cardiolipin in BS alters the TFP-CI interaction due to supercomplex instability. **Methods:** Cells and tissues from patients with confirmed LCHAD, and TFP deficiencies, as well as BS were assessed for cardiolipin, ETC, and TFP. Mitochondrial extracts were subjected to blue native PAGE followed by SDS–PAGE and western blotting. Flow cytometry was used to measure ROS (Mito Sox Red) and mitochondrial proliferation (Mito Tracker Green). Oxygen consumption studies were performed using a Seahorse XF®96 Analyzer. Cardiolipin was studied using liquid chromatography mass spectrometry. TFPα and MLCLAT were quantitated using SDS-PAGE followed by western blotting, and measurement of MLCLAT activity. **Results:** Fibroblasts from patients with LCHAD deficiency and BS both had a reduction in MLCLAT activity compared to control cells. There was evidence of altered cardiolipin content, destabilization of TFPα interaction with supercomplexes, increased ROS production, and mitochondrial proliferation compared to control. **Discussion:** These findings suggest that studying alterations in the FAO- ETC- Cardiolipin interaction increase our understanding of the pathophysiology of these disorders, providing impetus for development of new therapeutic approaches.

517 - Multiple Acyl-coA dehydrogenase Deficiency (MADD): Twenty Years of Follow Up, Long Term Treatment, and Outcomes

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Introduction: MADD is an autosomal recessive disorder of fatty acid and protein metabolism. Patients with neonatal presentation usually have hypotonia and hepatomegaly and develop severe cardiomyopathy that leads to a fatal outcome in the first weeks of life. **Aim**: To report the data of 20 years follow up of a patient diagnosed with MADD through NBS. **Material and Methods**: Review of medical records of a female patient with MADD diagnosed at 6 days and followed since 1997. Clinical, biochemical and dietary treatment data were analyzed. **Results**: There was a positive family history of consanguinity with three dead siblings. Pregnancy and delivery were unremarkable. NBS diagnosis was confirm by mutation analysis which found a homozygous variant c.122T>C (p.Phe41Ser) in the *ETBF* gene. The phenylalanine residue is highly conserved and in silico algorithms predict the variant to be deleterious. Patient was breast-fed for 3 months when she was transitioned to a low-fat (14-20%), low protein (6-10%)

and high carbohydrate (73-80%) formula, avoiding long fasting periods. At 6 months of age she started taking pureed food. At 9 months, uncooked cornstarch (UCS) was introduced resulting in clinical and biochemical improvement and allowing for longer fasting periods (6 h). Pancreatic enzymes were transiently required. During infancy patient received a diet for age with additional UCS + glucose polymers (GP) given twice a day (1,6 g/kg each). During childhood and adolescence diet consisted of frequent meals / snacks during the day maintaining UCS (1,7 g/kg) + GP (0,8 g/kg) supplementation. Currently patient continues following a high carbohydrate, low protein and fat diet with UCS (0,9g/kg) once a day. Additional treatment consisted of carnitine (100 mg/kg/day) and riboflavin (200mg/day). Her growth and development have been adequate with tendency to overweight. She had no hospitalizations for metabolic crises and her physical exam is normal except for mild hypotonia and muscle weakness. Throughout treatment patient's acylcarnitines remained elevated while carnitine remained normal with supplementation. **Conclusions:** This patient illustrates that early diagnosis and treatment with a high carbohydrate and low protein and fat diet can successfully avoid the severe manifestations of the disease.

518 - A Rare Cause of Newborn Hypoglycemia and Hyperammonemia: CACT Deficiency

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Carnitine-acylcarnitine translocase (CACT) deficiency is a rare autosomal recessive disease in the mitochondrial transport of long-chain fatty acids which represent an energy production insufficiency during prolonged fasting, febrile illness, or increased muscular activity. CACT deficiency is caused by mutations of the *SLC25A20* gene. Most patients developed severe metabolic decompensation in the neonatal period and died in infancy despite aggressive treatment. **Case:** A female patient born to unrelated parents of Syrian after normal gestation and delivery with birth weight 3000 gr. The patient presented at two days of life with feeding difficulties and somnolence. She had hypoketotic hypoglycemia and hyperammonemia (500 mmol/L). Her physical examination was unremarkable except hypotonia. Plasma hemogram, amino acid levels were normal. Plasma acylcarnitine (ACC) profile showed marked hypocarnitinemia and high C14 and C18:1. GC-MS analysis of urine organic acids showed increased medium-chain dicarboxylic aciduria. Feeding was initiated with frequent feeding, high-carbohydrate/low-fat diet and medium-chain fatty acid-enriched formula (90% as medium chain triglycerides and 10% as long chain triglycerides). Her clinical condition improved with this therapy. The molecular analysis was performed for long chain fatty acid metabolism disorders or carnitine metabolism disorders. The family made a request for discharge immediately. After discharge, the patient died at home soon after discharge. Mutation analysis of the *SLC25A20* gene revealed homozygosity for deletion on exon 1. **Conclusion:** The most frequent presenting symptoms of CACT deficiency are hypoketotic hypoglycemia, hyperammonemia, hepatomegaly, cardiomyopathy and/or arrhythmia, and respiratory distress. The onset of symptoms is predominantly neonatal in 82% and infantile in 18%. The mortality rate is high (65%), most in the first year of life due to myocardiopathy or sudden death. Although CACT deficiency is very rare cause of neonatal hypoglycemia and hyperammonemia, it' s very important to evaluate metabolic screening test like ACC analysis in such patients.

519 - Beta-Oxidation Defect of Fatty Acids (FAO defects): Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency as a Cause of Non-Ketotic Hypoglycemia: A Case Report

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Introduction: Hypoglycemia is a common problem in newborns, but it can occur at any age. The FAO defects, including LCHADD (Long chain 3 hydroxyacyl-Co A dehydrogenase deficiency) are a rare but serious life-threatening disease. LCHADD is an autosomal recessive disorder with incidence of 1/250000 (by newborn screening programs of Australia, Germany and USA). **Objective:** The aim of our report is to emphasize the importance of an early recognition of fatty acid oxidation (FAO) defects. **Case report**: An 11 months old female child arrived from Arequipa (Perú) to our hospital, she had had a severe non ketotic hypoglycemia episode with metabolic acidosis (after fasting and rate of low glucose infusion), and also one was with hypoglycemic coma. During the transportation from Arequipa to Lima had another hypoglycemia episode; at admission, she had metabolic acidosis with, lactic acidemia, hypoglycemia and increased liver enzyme and creatine phosphokinase. She had been previously healthy without signs of diseases with adequate psychomotor development until 7 months; from that age, she had recurrent episodes of vomiting, diarrhea and fever, some was diagnosed and treated as urinary tract infection. Expanded Newborn Screening suggested LCHADD. LCHADD was confirmed with profile of acylcarnitines in plasma and urine organic acids. She was admitted for 2 months, a formula containing predominately medium-chain triglycerides (MCT) and low in long-chain fatty acids (LCFA) was administered through nasogastric tube. Subsequently, at 17 months of age, a gastrostomy tube was placed.

After discharge, he presented several episodes of rhabdomyolysis (CPK up to 27,714) associated with fever and vomiting, the last one in January 2017. Molecular analysis showed new heterozygous pathogen variant 453 + 1G>A and a new heterozygote variant of uncertain significance c.1688A> C (p.Q563P) in the *HADHA* gene. She is 28 months years old and currently overweight, has adequate height, has no cardiac involvement, walks since 18 months old and speaks 20 words. **Conclusion**: FAO defects are rare but life threatening and we should think about them in patients with non-ketosis hypoglycemia to make an early diagnosis that avoids morbidity and mortality.

O) Mitochondrial Disorders: Nuclear Encoded, Disorders of Pyruvate Metabolism (520 to 551)

520 - Severe Brain Malformations in an Infant With Pyruvate Dehydrogenase Deficiency and Down Syndrome

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A female infant born to a 39-year-old G3P2 at 38 weeks gestation with poor tone, respiratory failure, and dysmorphic features. Postnatally, she was diagnosed with congenital heart disease and Trisomy 21 (T21). In addition, she developed persistent lactic acidosis, which in combination with the severe clinical picture was not consistent with T21. This lead to the suspicion of pyruvate dehydrogenase deficiency (PDH), which was later confirmed by molecular testing (c.858_861dupTTAC). Subsequently, a brain MRI revealed marked reduction in cerebral volume and multicystic encephalomalacia. Treatment with thiamine and carnitine supplementation and low carbohydrate diet was implemented with subsequent stabilization of lactate levels. These findings have not previously been reported in patients with either PDH deficiency or T21, and most likely represent a brain insult due to the combination of both conditions. Therefore, this is the first known case of a patient with both PDH deficiency and T21. Finally, it is important to always consider alternate etiologies when the clinical picture is not consistent with a specific disorder.

521 - Childhood-Onset Neurodegeneration Secondary to Absence of the Autophagy Adaptor SQSTM1/p62: Case Report

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We present a 4 years old girl, from a non-consanguineous family. She has no family history of neurological disorders and no significant past medical history. At 2 years old, the patient began cerebellar ataxia, tremors, abnormality in gait, and speech regression. *The patients progress with* unsteadiness of gait, coordination problems, cognitive decline, r*efractory seizures, and generalized dystonia. An appendicular spasticity was observed at 3 years old.* Brain magnetic resonance showed mild cerebellar atrophy, progressing with generalized cerebral atrophy. *Exome* sequencing identified mutation in the *SQSTM1*. SQSTM1 (sequestosome 1), also known as p62, encodes a multidomain scaffolding protein involved in various key cellular processes, including the removal of damaged mitochondria by its function as a selective autophagy receptor. Nine cases were described previously in literature presenting with gait abnormalities, ataxia mostly of the upper limbs, dysarthria, dystonia, vertical gaze palsy and mild cognitive decline. The course was remarkably similar in all described affected individuals. This patient is the youngest person describe with absence of the autophagy adaptor SQSTM1/p62. Our findings expand the SQSTM1-associated phenotypic spectrum and lend further support to the concept of disturbed selective autophagy pathways in neurodegenerative diseases.

522 - Biallelic Mutations in the *TRAK1* Disrupt Mitochondrial Motility and Cause Fatal Encephalopathy

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Cellular distribution and dynamics of mitochondria are regulated by several motor proteins and a microtubule network. In neurons, mitochondrial trafficking is crucial because of high energy needs and calcium ion buffering along axons to synapses during neurotransmission. The trafficking kinesin proteins (TRAKs) are well characterized for their role in lysosomal and mitochondrial trafficking in cells, especially neurons. Variants in the mouse orthologue of human *TRAK1*

(*hyrt* mutant mice) have been associated with low levels of γ-aminobutyric acid type A ($GABA_A$) receptors, and hence deficiency of GABA-mediated neural inhibition, in the brain of hypertonic mice. Using whole exome sequencing, we identified homozygous truncating variants in *TRAK1* (NM_001042646: c.287-2A>C), in lethal encephalopathic patients from three unrelated families. The pathogenic variant results in aberrant splicing and significantly reduced gene expression at both the RNA and protein levels. In comparison with normal cells, TRAK1 deficient fibroblasts showed irregular mitochondrial distribution, altered mitochondrial motility, reduced mitochondrial membrane potential, and diminished mitochondrial respiration. This study confirms the role of *TRAK1* in mitochondrial dynamics and constitutes the first report of this gene in association with a severe neurodevelopmental disorder.

523 - Heterogeneous Forms of Nuclear Encoded Mitochondrial Diseases in Children

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Background: Nuclear encoded mitochondrial diseases constitute a significant part in the structure of mitochondrial diseases in children. These diseases manifest by different clinical phenotypes and are caused by defects in many nuclear genes. **Patients and methods:** Over the last 5 years, at the specialized clinic for children with developmental delay 26 patients aged from 18 months to 11 years were identified having nuclear encoded mitochondrial diseases. Clinical and biochemical methods were used in the examination of the patients. In all children, the diagnosis was confirmed by molecular genetics analysis, including Next-Generation Sequencing. **Results:** The largest group (½ of cases) consisted of 13 patients with Leigh disease (most of them had *SURF1* gene mutations, one boy had a mutation in the *PDHA1* gene (X-chromosome). The second group consisted of 7 children suffering from leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL), where mutations in the *DARS2* gene were detected. Two boys were diagnosed with an X-linked Barth syndrome (*TAZ* gene mutations). *POLG*-related phenotype was identified in two cases: Alpers disease with MELAS-like manifestation in a girl and autosomal recessive progressive external ophthalmoplegia (PEO) in a boy. Another two children suffered from mitochondrial encephalomyopathy, one of them with severe delay in physical development (NDUFB9 gene mutations) and the other with mitochondrial DNA depletion syndrome 7 (C10ORF2 gene mutations). **Conclusion:** Observations revealed a variety of clinical phenotypes of nuclear encoded mitochondrial diseases in children with developmental disorders. *SURF1* and *DARS2* mutations are the most frequent causes of the disease in our patients.

524 - MitoEpilepsy Map: A Novel Computational Resource for the Diagnosis of Mitochondrial Epilepsy

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Objective: Epilepsy is a feature of approaching 60% of childhood-onset mitochondrial disease. The extreme clinical, biochemical and genetic heterogeneity of these disorders leads to considerable diagnostic challenges. We aimed to create a comprehensive gene-to-phenotype interaction network which can be used as a diagnostic resource for mitochondrial epilepsy. **Methods**: Genetic and phenotypic data pertaining to mitochondrial epilepsy disease genes were obtained via a systematic literature review of more than 1500 publications and facilitated by automated text mining. The data were then integrated into a computational gene-to-phenotype interaction network using the Molecular Interactions NEtwoRk VisualizAtion (MINERVA) platform. **Results**: The MitoEpilepsy Map is a computational network which features data from 180 mitochondrial disease genes and greater than 250 clinical features expressed in human phenotypic ontology (HPO) terminology. The genetic and clinical elements of the map can be queried to elucidate candidate genes from sets of query phenotypes and vice-versa. Blinded validation of the network using anonymized case studies demonstrated that the map has the ability to accurately predict sets of causative mitochondrial epilepsy genes. In addition, the data from the MitoEpilepsy Map was used to create expression heatmaps and pathway analysis to hypothesize novel gene-to-phenotype correlations in mitochondrial epilepsy. **Discussion**: The success of the MitoEpilepsy Map demonstrates the aptitude of computational resources as diagnostic aids for mitochondrial diseases when used in combination with whole-exome sequencing, while also providing important insights into the pathophysiology of mitochondrial epilepsy.

525 - Application of Next Generation Sequencing to Mitochondrial Disorders: From Clarifying the Pathophysiology to Creating the New Therapy

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Background: The most important function of mitochondria is biosynthesis of ATP. Mitochondrial disorders are nearly synonymous with mitochondrial respiratory chain disorder (MRCD), as respiratory chain complexes serve a central role in ATP biosynthesis. MRCD have the highest incidence among congenital metabolic diseases, and are thought to occur at a rate of 1 in 5000 births. About 25% of the diseases diagnosed as mitochondrial disorders in the field of pediatrics have mitochondrial DNA abnormalities, while the rest occur due to defects in genes encoded in the nucleus. Mitochondrial diseases comprise a diverse set of clinical disorders that affect multiple organ systems with varying severity and age of onset. Due to their clinical and genetic heterogeneity, these diseases are difficult to diagnose and treat. **Scope of Review:** Here we describe firstly how to make correct diagnoses of MRCD patients using both in vitro isolated enzyme assay and blue native polyacrylamide gel electrophoresis. After the correct diagnosis, we performed comprehensive genomic analyses. The approach includes whole mtDNA and exome analyses using high-throughput sequencing, and chromosomal aberration analyses using high-density oligonucleotide arrays. Finally, our understanding their pathomechanisms have led to new and rationale therapies. **Major Conclusions:** We identified 11 novel causative gene mutations (*QRSL1*, *NDUFB11*, *MRPS23*, *KARS*, *COQ4*, *GTPBP3, SLC25A26*, *PNPLA4, TNNI3, MECP2, IARS*) by complementation assay. We are also working with siRNA knockdown to validate if the other candidate genes could potentially be causative using patients' fibroblasts. Among several candidate drugs in our study, I introduce 5-aminolevulinic acid hydrochloride in combination use with sodium ferrous citrate. **General significance:** Studies of MRCD patients illustrate how understanding the pathogenic mechanisms of mitochondrial diseases can lead to meaningful therapies. In the future, it will be necessary to increase the case number or search for patients with similar symptoms and similar gene mutations in collaboration with researchers throughout the world.

526 - Heterogeneous Forms of Nuclear Encoded Mitochondrial Diseases in Children

Ekaterina Nikolaeva[1], Maria Yablonskaya[1], Polina Tsygankova[2], Tatiana Krylova[2], Tatiana Proshlyakova[2], Petr Shatalov[1], and Malvina Kharabadze[1]

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Background: Nuclear encoded mitochondrial diseases constitute a significant part in the structure of mitochondrial diseases in children. These diseases manifest by different clinical phenotypes and are caused by defects in many nuclear genes. **Patients and methods:** Over the last 5 years, at the specialized clinic for children with developmental delay 26 patients aged from 18 months to 11 years were identified having nuclear encoded mitochondrial diseases. Clinical and biochemical methods were used in the examination of the patients. In all children, the diagnosis was confirmed by molecular genetics analysis, including Next-Generation Sequencing. **Results:** The largest group (½ of cases) consisted of 13 patients with Leigh disease (most of them had *SURF1* gene mutations, one boy had a mutation in the *PDHA1* gene (X-chromosome). The second group consisted of 7 children suffering from leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL), where mutations in the *DARS2* gene were detected. Two boys were diagnosed with an X-linked Barth syndrome (*TAZ* gene mutations). *POLG*-related phenotype was identified in two cases: Alpers disease with MELAS-like manifestation in a girl and autosomal recessive progressive external ophthalmoplegia (PEO) in a boy. Another two children suffered from mitochondrial encephalomyopathy, one of them with severe delay in physical development (NDUFB9 gene mutations) and the other with mitochondrial DNA depletion syndrome 7 (C10ORF2 gene mutations). **Conclusion:** Observations revealed a variety of clinical phenotypes of nuclear encoded mitochondrial diseases in children with developmental disorders. *SURF1* and *DARS2* mutations are the most frequent causes of the disease in our patients.

527 - Clinical Features and Natural History of Childhood Onset POLG Disease

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Objective: *POLG* mutations are recognized to be a relatively frequent cause of mitochondrial disease, with heterogeneous presentations ranging from Alpers syndrome to adult-onset progressive ophthalmoplegia. This heterogeneity leads to challenges in clinical recognition, particularly in childhood. We aimed to study the natural history of early onset *POLG* disease in a large multinational cohort. **Methods:** A multinational retrospective case notes review was performed to obtain clinical, histological, biochemical and genetic features and natural history of patients with childhood-onset biallelic *POLG* mutations. Patients presented at <12 years to two centers in Norway and one in the United Kingdom. **Results:** Twenty-seven patients with biallelic *POLG* mutations (including two novel mutations) presented between 0.6 and 80.4 months (median 11 months). The most frequent presenting features were global developmental delay (100%), hypotonia (96%) and faltering growth (89%). Epilepsy was present only in 65% at presentation and 73% at any time during the disease course, and was notably absent in a subgroup with the myocerebrohepatopathy phenotype. **Conclusion:** We provide the most complete clinical information pertaining to childhood *POLG*-related disease. Based on our findings, we propose that the classification of early-onset *POLG* disease can be simplified into subgroups with or without epilepsy. Further we show that the possibility of *POLG* disease must be considered in any child presenting with diffuse neurological symptoms. Targeted *POLG* sequencing may miss mutations, thus complete *POLG* sequencing is recommended in suspected cases.

528 - Mitochondrial Dysfunction is Comorbid With Susceptibility to Post-Traumatic Stress Disorder in Mice

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Psychopathology is frequently associated with mitochondrial dysfunction in patients. Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder that affects millions of people worldwide. Induced by exposure to a traumatic event, the presentation of the disease in terms of duration and symptomatology is highly heterogenous, and the severity of the inducing trauma does not predict the severity of the disorder. This suggests the possibility of an underlying genetic metabolic factor. We therefore investigated whether susceptibility to PTSD is associated with mitochondrial dysfunction. We exposed 48 wildtype male mice of the FVB.129P2 ("sighted" FVB) background to a PTSD-induction paradigm previously shown to reliably induce PTSD-like symptomatology with a similar presentation to that of PTSD in humans. The inducing trauma was inescapable electric foot shock, administered in two decontextualized sessions over two consecutive days. PTSD-susceptible animals were diagnosed through a series of behavioral tests to identify some physical symptoms of PTSD: hyperarousal, hypervigilance, and sleep cycle disruption. Those 12 animals most and least clearly displaying PTSD-like symptomatology were identified, brain tissue was collected, and mitochondria were isolated. The activities of the electron transport chain (ETC) complexes I, II, III and IV, as well as citrate synthase, were measured spectrophotometric assay using a Konelab autoanalyzer to determine mitochondrial activity and density, respectively. Comparing, ETC activity and mitochondrial density to PTSD-symptomatology reveals a statistically-significant inverse relationship between PTSD susceptibility and mitochondrial capacity ($P = .016$). Our results indicate that susceptibility to PTSD in mice is comorbid with reduced mitochondrial capacity. To demonstrate that mitochondrial dysfunction is sufficient to induce PTSD susceptibility, we are currently inducing PTSD in a strain of transgenic mice with genetically-induced suboptimal mitochondrial function, and assaying for a concordant increase in PTSD susceptibility. We are also investigating how reduced mitochondrial capacity affects activation of large scale brain networks and endocrine functions involved in stress response.

529 - CMPK2, a Mitochondrial Enzyme in the Nucleotide Salvage Pathway, Might be Related to a MNGIE-Like Phenotype

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Objectives: Mitochondrial DNA depletion syndromes (MDS) are caused by genetic defects involving different pathways (Neuromuscul Disord 2010;20(7):429-37). Mitochondrial neurogastrointestinal encephalopathy (MNGIE, MIM 603041) is a MDS caused mainly by mutations in TYMP (thymidine phosphorylase, MIM 131222), an enzyme involved in the nucleotide salvage pathway (NSP). Here, we describe a new case of MNGIE-like phenotype with mutations in another enzyme of the NSP, CMPK2 (cytidine monophosphate kinase 2, MIM 611787, NM_1256478), never reported before. **Case:** An 11 years old boy was referred with severe myopathy, failure to thrive, abdominal pain, gastrointestinal dysmotility, global developmental delay, gait instability, neuropathic pain, neurogenic bladder and dysautonomic signs, progressing since age 5. Physical exam revealed cachexia, muscle atrophy, proximal weakness, and signs suggestive of a peripheral neuropathy. Thymidine testing was negative for TYMP deficiency. Mitochondrial complexes study by Blue Native-PAGE on muscle revealed a generalized diminution of all 5 complexes.

Results: Two heterozygous CMPK2 mutations were found by WES, c.59G>C (p.Arg20Pro) and c.1027C>T (p.Leu343Phe). Both mutations were confirmed by Sanger sequencing. Parental analyses confirmed that mutations were in trans. The c.59G>C and c.1027C>T have a prevalence in ExAC of $3.4X10^{-4}$ and $9.3X10^{-5}$, respectively. CMPK2 is a UMP-CMP kinase located in the mitochondria and involved in the NSP, phosphorylating dUMP, dCMP and CMP (J Biol Chem 2008;283(3):1563-71). **Conclusion:** Biallelic mutations in CMPK2, a mitochondrial enzyme involved in the NSP, might be related to a MNGIE-like phenotype. More patients and functional studies are needed to understand the pathophysiology of the disease. This abstract has been presented at Garrod Symposium, Montréal, QC, May 4-6th 2017

530 - Mutations in the *NDUFAF4* Impairs Mitochondrial Supercomplexes Formation and Causes Dysmorphia, Cardiomyopathy, and 3-Methylglutaconic Aciduria

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We present two siblings with convulsions, irritability, facial dysmorphia, leukodystrophy and hypertrophic cardiomyopathy since the neonatal period. Metabolic studies showed high plasma levels of lactate and α-alanine. The organic acid profile showed increased excretion of lactate, succinate, fumarate, 2-ketoglutarate and 3-methylglutaconate (3-MG). The excretion of 3-MG together with the dysmorphic features made the rationale for the analysis of possible mutations in genes associated to 3-MGA-uria, which were excluded using a self-designed gene panel. Whole-exome sequencing identified a homozygous nonsense mutation in the *NDUFAF4* (c.558G>T; p.Glu160*), an assembly factor of the mitochondrial respiratory complex I. BN-PAGE performed in patient's fibroblasts showed an isolated deficiency in the assembly of complex I, that was consistent with the genetic data. Since the first description in 2009 (Saada et al. Am J Hum Genet. 2009) no other patients with *NDUFAF4* mutations have been reported. Moreover, the pathophysiological mechanisms underlying NDUFAF4 deficiency have not been well documented so far. To further characterize this disorder, we have analyzed mitochondrial membrane potential by TMRM labeling and demonstrated a severe loss of membrane potential in patient' cells. We have also studied the impact of NDUFAF4 deficiency in the mitochondrial function by analyzing the formation and the activity of respiratory chain supercomplexes. We have demonstrated a complete absence of the higher molecular-weight forms of respiratory supercomplexes in NDUFAF4 patient' cells together with an abnormal accumulation of complexes containing exclusively CIII and CIV. Our results provide new insights on the pathophysiological mechanisms underlying NDUFAF4 deficiency and highlight the importance of precise biochemical and functional characterization for the elucidation of the genetic causes of mitochondrial energy metabolism disorders using next generation sequencing.

531 - A Lethal Neonatal Phenotype of Mitochondrial Short-Chain Enoyl-CoA Hydratase-1 Deficiency

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Short-chain enoyl-CoA hydratase (SCEH) is a mitochondrial enzyme involved in the oxidation of fatty acids and the catabolic pathway of valine and, to a lesser extent, isoleucine. Deficiency of this enzyme was recently shown to cause an early childhood Leigh syndrome phenotype. The few reported patients were compound heterozygotes for two missense or missense with truncating variants in the *ECHS1* that encodes SCEH. We describe two siblings with severe refractory lactic acidosis and death within the first 2 days of life. Following negative clinical whole-exome and whole-genome sequencing, we resorted to autozygome/exome analysis on research basis and identified a homozygous splice site mutation (c.88+5G>A) in the two cases. Analysis of cDNA confirmed complete replacement of the normal transcript with an aberrant transcript (r.88_89ins 88+1_88+11) predicting premature truncation of the protein [p.(Ala31Glufs*23)]. Furthermore, quantitative reverse transcriptase polymerase chain reaction (RTPCR) showed marked reduction in ECHS1, most likely nonsense-mediated decay (NMD)-mediated. This is the first report of homozygosity for a truncating mutation in the *ECHS1*, which may explain the severe phenotype. Our report highlights the need to consider SCEH deficiency in patients with lethal neonatal lactic acidosis, and the potentially limited sensitivity of untargeted genomic sequencing toward noncanonical splicing mutations, which may explain at least some of the "negative" cases on clinical exome/genome sequencing.

532 - Developing a Tissue-Specific ACAD9 Deficient Mouse Model Using Cre-lox Recombination

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Introduction: Acyl-CoA Dehydrogenase 9 (ACAD9) is a member of the family of acyl CoA dehydrogenases that catalyze the α, β dehydrogenation of acyl-CoA esters during fatty acid β-oxidation and branched chain amino acid metabolism. ACAD9 is unique in that it also has a second function as an assembly factor in the formation of complex I in the electron transport chain. Impairment in both functions contribute to the phenotype in human ACAD9 deficiency. Variable symptoms include cardiomyopathy, hypoglycemia, and liver dysfunction. We have established ACAD9 deficient mice to better understand the pathophysiology of this defect in patients. **Methods:** A complete ACAD9 deficiency appeared to be embryonic lethal. To circumvent this problem, Cre-lox technology with tissue specific and inducible promotors was used to construct heart, skeletal muscle, and total body inducible ACAD9 deficient animals missing exon 4 of the *ACAD9* gene. Genotyping was performed using PCR and pre-designed primers. Cardiac MRI scanning was used to characterize *in vivo* heart function in cardiac-specific mutants. Hang tests were performed on skeletal specific mutant animals. Tissue samples from all animals were collected for immunologic studies and biochemical analysis. **Results:** Cardiac mutant mice were live born, but died between the ages of 14 to 21 days. Cardiac MRI scans of the mutant animals at the age of 14 days confirmed severe cardiomyopathy. The hearts were enlarged with a greatly thickened ventricular wall and reduced left ventricular ejection fraction. Routine histology on tissue from sacrificed animals confirmed cardiomyopathy. Of note, immunostaining revealed ACAD9 antigen localized to mitochondria, probably representing a low level of unexcised inserts. Skeletal muscle deficient animals showed a significantly reduced hang time compared to controls. Histologic studies are in progress. **Conclusion:** ACAD9 deficiency in heart and skeletal muscle in separate mouse models leads to viable animals that mimic a tissue-limited phenotype of the human deficiency. These models will provide a valuable platform to test potential therapies and better understand the interplay of the fatty acid oxidation and respiratory chain complex 1 deficiencies in determining phenotype in humans. A temporally inducible model has now been created and will provide further insight into full body deficiency.

533 - Extending the Phenotypic Spectrum of Sengers Syndrome: Severe Case Presenting With Cardiogenic Shock and Synthetic Liver Dysfunction in a Newborn

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Sengers syndrome is a rare autosomal recessive mitochondrial disease characterized by lactic acidosis, hypertrophic cardiomyopathy and bilateral cataracts. It is caused by mutations in the *AGK* gene which codes for acylglycerol kinase, a mitochondrial transmembrane enzyme that can participate in phospholipid synthesis or act as signaling molecule regulating a number of cell processes including the OXPHOS. Sengers syndrome has two distinct clinical forms, a severe neonatal form and a later onset form with survival into the fourth decade of life. To date there have been less than thirty molecularly diagnosed patients with Sengers syndrome, with the majority consisting of later onset disease. We present here a case of neonatal demise within the first day of life, found to have a severe lactic acidosis, in the setting of both chorioamnionitis and cardiomyopathy with signs of cardiogenic shock. Clinical exam was notable for profound encephalopathy and bilateral opacification of the corneas. Metabolic labs were concerning for significantly elevated lactate refractory to resuscitation, and a lactate to pyruvate ratio of 91 suggestive of mitochondrial disease. Low albumin, total protein, and prolonged PT, PTT with normal bilirubin and elevated transaminases were noted. Plasma amino acids showed elevation of methionine, phenylalanine, and branched chain amino acids, indicating liver dysfunction and catabolism. The patient did not receive TPN. Blood, urine, and CSF cultures were negative. Given metabolic results and consanguineous family history, a nuclear mitochondrial gene panel was sent revealing a homozygous pathogenic variant in *AGK*, c.979A>T p.K327*. A novel finding in our patient was synthetic liver dysfunction which has not been previously noted in Sengers syndrome. To our knowledge, there is only one patient reported in the literature with primary liver involvement showing cytosolic granules in liver cells after autopsy. Some authors have even suggested that the neonatal form of Sengers syndrome should be described as a mitochondrial encephalomyopathy due to CNS involvement but there is no mention of liver compromise which is not unusual to find in mitochondrial disorders. We present a severe case of Sengers syndrome reported with novel characteristics. This case highlights the importance of a wide differential diagnosis for congenital lactic acidosis even in cases with apparent common neonatal conditions.

534 - Novel Role for Store-Operated Calcium Entry in Mitochondrial Gene Expression, Energy Production, and Beta-Oxidation

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Store-operated Ca^{2+}entry (SOCE) is a pathway for increasing intracellular Ca^{2+} levels regulated by stromal interaction molecule 1 (STIM1), STIM2, and the Ca^{2+} channel ORAI1. SOCE-deficient patients suffer from **C**alcium **R**elease-**A**ctivated **C**alcium (CRAC) channelopathy characterized by immunodeficiency, autoimmunity, myopathy, and anhidrotic ectodermal dysplasia. Several mitochondrial enzymes/complexes depend on Ca^{2+} but the source of Ca^{2+} required for their function are not entirely clear. We recently showed a cell-intrinsic role of SOCE in human mitochondria (Maus M et al. *Cell Metab.* 2017;25(3):698-712). MitoView Green showed reduced mitochondrial volume in fibroblasts of patients with ORAI1/STIM1 loss-of-function mutations. mtDNA copy numbers and mRNAs expression of selected mitochondrial transcription factors were normal. SDS-PAGE/Western blot analysis showed reduced expression of NADH ubiquinone oxidoreductase subunit-B8, Cytochrome b-c1 complex subunit-2, Cytochrome c oxidase subunit-I, Cytochrome C, Mitochondrial porin and permeability transition pore, etc. Blue native PAGE of isolated mitochondria confirmed reduced expression of CI, CIV and supercomplex CICIII2. SOCE-deficient fibroblasts had reduced mRNA and protein expression of uncoupling protein 2, higher basal mitochondrial membrane potential (MMP) and higher numbers of damaged mitochondria as suggested by increased co-localization of mitochondria and lysosomes and increased MitoKeima reporter activity indicative of lysosomal mitophagy. Oligomycin-induced ATP-synthase inhibition revealed decreased electron transport and proton pumping rates measured as MMP hyperpolarization rates and reduced superoxide production assessed by MitoSOX. Maximal O_2 consumption rates in SOCE-deficient cells were decreased. Skeletal myocytes had reduced CI and CIV function in 2 out of 3 ORAI1-deficient patients. Gene expression of very long chain acyl-CoA dehydrogenase and long-chain fatty acid transporter carnitine palmitoyltransferase 1B was reduced in patient fibroblasts cultured in either high glucose medium or oleic acid (OA) medium followed by starvation in 2 mM glucose medium. Furthermore, SOCE-deficient fibroblasts were lacking a starvation-induced increase in etomoxir-sensitive mitochondrial respiration in OA medium and showed reduced rates of OA beta-oxidation when cultured in ^{14}C-OA-medium with or without subsequent starvation. Our findings indicate an important new role of SOCE in mitochondrial function.

535 - Next Generation Sequencing Improves Mitochondrial Diseases Diagnosis

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Introduction: Mitochondrial diseases are a group of rare inherited disorders characterized by extreme phenotypic heterogeneity that can be transmitted by any mode of inheritance, with hitherto no effective therapy options. The recent evaluations of Next Generation Sequencing for mitochondrial disorders have shown that this methodology is more likely to provide a diagnosis, being quicker and cheaper. Since 1993, our group, pioneer in the study of these disorders in our country, has been studying more than 2500 patients with suspicious diagnosis of mitochondrial disorders. The biochemical and molecular approach used, allowed the characterization of many of these patients; however, some of them still remain without molecular diagnosis. **Objectives:** The overall aim of our research project* was to develop a Next Generation Sequencing strategy to identify nuclear disease causing-mutations in patients suspicious of mitochondrial disorders but without molecular etiology. **Methods:** Next Generation Sequencing was performed in a MiSeq Illumina instrument** using i) a custom mitochondrial gene panel with around 200 genes involved in mitochondria metabolism. Libraries were prepared using SureSelect QXT target enrichment system from Agilent; and ii) the entire human mitochondrial genome enriched by a single amplicon long-range PCR followed by nextera XT workflow. **Results:** Until now we analyzed 56 patients, identifying disease related mutations in 22 of them. These mutations were confirmed by Sanger Sequencing in the index cases and in their relatives. Our project is ongoing and the patients undiagnosed after this first approach will be further selected for Whole Exome Sequencing. **Discussion and Conclusion:** This study is contributing to i) identify the pathogenic mutations in the studied patients (22/56—39%), ii) expand the mutational spectrum in the etiology of these disorders, and iii) propose an accurate genetic counseling. Custom design panels have been widely used for molecular heterogeneous disorders however, the development of this panel will be innovative in our country strengthening our center as a national reference for the study and research of

mitochondrial disorders. *This Research Project is support by FCT (Fundação da Ciência e Tecnologia) (PTDC/DTP-PIC/2220/2014). **This Research Project is support by NORTE2020 (NORTE-01-0246-FEDER-000014 DESVENDAR "DEScobrir, VENcer as Doenças rARas")

536 - Novel Hemizygous Mutation of *TAZ* Gene in a Boy With Atypical Barth Syndrome

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Background: Barth syndrome (BTHS; MIM 302060) is widely considered a rare disorder, characterized by cardiomyopathy, neutropenia, skeletal myopathy, and growth delay. The *TAZ* gene encodes tafazzin, a component of the mitochondrial membrane that has been shown to play an essential role in heart development and cardiac function. However, genotype-phenotype correlation has not been clearly defined yet. Although, clinical symptoms are usually present early in infancy, the age of presentation and features of BTHS varies significantly among patients. **Methods and results:** We describe the case of a 6-year-old male with a dilated cardiomyopathy and increased left ventricular trabeculation, dysmorphic features, speech impairment, normal growth, and the absence of elevation of 3-MGA. The boy manifested neither neutropenia nor recurrent infections. The genetic test of genomic DNA from the venous peripheral blood sample was performed by NGS Illumina Tru Sight One Sequencing Panel. No mutations in genes known to cause dilated cardiomyopathy were identified. Hemizygous missense mutation NM_000116.3: c.608G>T (p.Cys203Phe) in exon 8 of the *TAZ* gene for proband was identified. Multiple sequence alignment of tafazzin revealed that mutation is in a conserved region and is located at a functionally relevant site (in a phosphate acyltransferase domain). The in silico analysis of the identified variant shows it to be as disease causing variant (PolyPhen—probably damaging (0.998), Mutation Taster—disease causing, Provean—Deleterious (4.426)). Detected mutation has been proved by Sanger sequencing using specific primers for exon 8 of *TAZ* gene. His mother was proved to be the carrier of this mutation. **Conclusions:** We describe a patient with dysmorphic features, dilated cardiomyopathy, and speech impairment with novel hemizygous missense mutation in the *TAZ* gene. The investigation for BTHS must be considered in male babies and young boys with dilated cardiomyopathy even if other characteristic features are absent.

537 - Diagnosis of Mitochondrial Disorders: The Perspective of a Biochemical-Genetics Testing Laboratory

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The irruption of high-throughput sequencing techniques in the genetic analysis of human Mendelian diseases has dramatically improved the rate of diagnostic success of diseases with complex and expanding spectrums of clinical phenotypes and genotypes such as the primary mitochondrial disorders. The 55 patients (males and females) included in this study are a representative sample of the broad spectrum of symptoms of the patients' population referred to our center from different Hospitals with clinical suspicion of congenital lactic acidosis (CLA), mitochondrial disorder (MD), or even of a less specific -inherited metabolic disorder-. Most of them showed a metabolite profile suggestive of CLA or MD with elevated lactate and/or alanine in blood, and urine and all were analyzed according to our *in house* genetic screen cascade. The framework included a first analysis of nuclear genes by using a Mendelioma massive parallel sequencing (MPS), and a subsequent entire mitochondrial genome (mtWGS) by MPS. Rare variants were filtered and prioritized, and for those patients with only one mutation identified in strong nuclear candidate genes, a functional genomic study based in a case-by case analysis of aberrant transcript present in patients' cells and absent in control samples was applied. For mtDNA variants, we targeted those changes reported in MITOMAP. With this approach, we identified the genetic cause in 24 out of the 55 patients analyzed. Of these, 21 patients harbored 30 disease-causing changes, 12 of them news, in 16 known nuclear genes. Other three patients carried known point mutations in mtDNA: G8719A (*MT-ATP6),* T10158C (*MT-ND3*) and G13513A (*MT-ND5*). In silico predictions supported the pathogenic role in each case. Segregation analysis in parents' DNA confirmed their carrier condition. Finally, cDNA analysis of other three patients with only one change detected in *GFM1*, *ARALAR* or *MRPL3* concluded with the identification of the deep intronic c.689+908G>A in *GFM1* gene and left undiagnosed the other two. Up to now, our pipeline has allowed to diagnose a 44% of the patients analyzed. Beyond the use of genetic diagnosis strategies based in whole exome or RNA sequencing, the identification of specific metabolic signatures lying downstream of the primary mitochondrial lesions and the unification of criteria to classify clinical symptoms, will contribute to improve the MD diagnosis and treatment. PI12/02078; PI16/00573; MINECO-FEDER. Fundación Isabel Gemio

538 - Novel Mutation in the *MT-ND5* Gene as the Cause of a Multisystemic Mitochondrial Disorder—Expanding the Phenotype

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Introduction: Mutations in the mitochondrial-encoded nicotinamide adenine dinucleotide dehydrogenase 5 gene (*MT-ND5*) have been implicated as an important genetic cause of childhood mitochondrial encephalomyopathies namely MELAS or LHON syndromes and Leigh-like disease. We present a pediatric case with a *de novo* mutation in the *MT-ND5* gene, multisystemic manifestations and minimal neurological involvement although Leigh-like syndrome and red-ragged fibers are present. **Case Report:** We report a 7-year-old child who, following an episode of otitis and respiratory infection, presented at the age of 22 months with lethargy and signs of severe dehydration and hydroelectrolytic imbalance as well as short stature. Initial investigations revealed a proximal and distal tubulopathy panhypopituitarism, hyperlactacidemia, increased alanine in plasma and lactic, fumaric, and malic acids in urine. Further studies were performed and magnetic resonance imaging suggested Leigh syndrome. Muscle biopsy revealed several "ragged-red fibers" and decreased activity of complex II+III of the respiratory chain. At the age of five years mild hypoacusia was detected, and a year later pigmentary retinopathy affecting rod and cone cells. Just before seven she presented persistent macrocytosis and more recently transfusion dependent sideroblastic anemia. Regarding the psychomotor development and neurological examination, she presents very mild delay mainly in verbal speech, and coordination difficulties. Molecular studies of mitochondrial DNA showed a novel m.13115 T>C mutation in the *MT-ND5* gene in heteroplasmy (82% of the molecules). **Comments:** We are describing a child carrying a *de novo MT-ND5* mutation with an unusual phenotype. She presents a multisystemic disorder with renal, endocrine, ocular and hematological involvement and very mild neurological symptoms. She presents red ragged fibers, very rare in children, in a precious age and no Complex I deficiency. We therefore hope to contribute to expand the knowledge of this deficiency.

539 - Mitochondrial Cavitating Leukoencephalopathy: Phenotypes and Novel Mutations in the *NDUFA5* and *APOPT1*

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Objectives: To report two patients presenting with white matter disease who early evolved to cavitating leukoencephalopathy due to mutations in the *OXPHOS*. **Case Report:** Patient 1: A 22-month-old boy, first child from unrelated parents, born at term, presented laryngeal stridor one month before admission. Perinatal history was uneventful. First brain MRI (Magnetic Resonance Image) was suggestive of acute demyelinating disease. He received methylprednisolone. The stridor solved. Four weeks later while on steroid tapering he presented ataxia, swallowing difficulties, progressive encephalopathy and tetraparesis. Control brain MRI showed cavitating lesions in the white matter. Patient 2: A 30-month-old girl, first child from unrelated parents, who presented with an acute encephalopathy, was diagnosed with ADEM, received methtylprednisolone and improved clinically except for persisting ataxia. She had received flu vaccine seven days before the admission. One month later ataxia worsened. She presented neurological regression, aphasia and quadriparesis. Brain MRI presented cavitating leukodystrophy and an increased lactic peak. **Methods**: Nuclear encoded mitochondrial proteins and complete mitochondrial genome was sequenced in DNA. **Results:** PATIENT 1: Sequencing identified two unreported heterozygous mutations in the *NDUFAF5* gene coding for NADH ubiquinone oxidoreductase subunit A5. Pathogenic variants are associated with autosomal recessive isolated complex I deficiency. Patient 2: Sequencing identified two unreported homozygous mutations in the *APOPT1* gene which is known to cause mitochondrial complex IV deficiency. **Conclusion:** Our patients expand the clinical spectrum for mitochondrial cavitating leukoencephalopathy. Radiologic and clinical presentation leads the suspicion of respiratory chain complex mutations. More studies are needed to understand the transient positive response to steroids.

540 - NAD(P)HX Dehydratase (NAXD) Deficiency: A Novel Neurodegenerative Disorder Exacerbated by Febrile Illnesses

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Background: Stress or high temperatures may damage essential nicotinamide metabolites (NAD(P)H), generating toxic metabolites (NAD(P)HX). The highly conserved enzyme NAD(P)HX dehydratase (NAXD) is essential for metabolite repair. Here we present molecular investigations on 3 unrelated individuals who suffered episodes of febrile-induced neurodegeneration or cardiac failure and early death. All individuals carried pathogenic variants in the *NAXD*. **Methods**: Genomic DNA was isolated from patient blood and subjected to whole exome analysis (WEA) for potentially pathogenic variants. Candidate variants were confirmed by Sanger sequencing in probands and parental samples. Fibroblasts from patients 1 and 2 were used for molecular analysis (RT-PCR, response to metabolic stress, and mitochondrial protein expression) and compared to four pediatric control fibroblasts. Statistical analysis was performed with GraphPad Prism 5.01 using one-way ANOVA with Bonferroni correction. **Results**: WEA identified compound heterozygous variants in the *NAXD* in patient 1 (a splicing variant and a missense change) and patient 2 (a frameshift and a missense change), and a homozygous variant in patient 3 (frameshift). In silico analysis predicted these to be pathogenic. RT-PCR confirmed altered splicing in patient 1. There was no significant growth defect in patient fibroblasts in response to metabolic stress (galactose or serum withdrawal); however, there was a significant growth impairment in patient 1 cells under regular growth conditions. Interestingly, there was a significant reduction of OXPHOS complexes-I and -IV expression and decreased complex-IV activity in patient fibroblasts. **Conclusion**: This is the first study to identify pathogenic variants in the *NAXD*, which are likely to have devastating effects in tissues such as the brain, which are critically dependent on efficient energy metabolism and sensitive to abnormal metabolite accumulation.

541 - The Utility of Quantitative Proteomic and RNA Analyses to Aid in Diagnosis of Exome-Unsolved Cases and in the Identification and Characterization of Novel Disease Genes

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Objective: Mitochondrial disorders can be caused by mutations identified in over 250 genes. Whole exome sequencing has transformed diagnosis of these disorders but up to 50% of cases remain unsolved. This can be due to failure to detect or interpret mutations in genes of interest and because some genomic regions remain refractory to analysis or interpretation. We aimed to identify novel mitochondrial disease genes and to exploit quantitative proteomic and RNA analyses to understand the underlying pathogenic mechanisms. **Results:** We and others recently identified large deletions, gene conversions and de novo point mutations in the *ATAD3* locus encoding 3 highly homologous tandemly arrayed genes (*ATAD3C, ATAD3B* and *ATAD3A*). We identified 5 subjects from 4 unrelated families with congenital pontocerebellar hypoplasia who carried deletions or gene conversions in the *ATAD3* locus (Desai, Frazier et al., *Brain*, in press). In these subjects, 2 similar deletions of ~38 kbp resulted in an *ATAD3B/ATAD3A* fusion gene that produced an mRNA encoding a protein 99% identical to ATAD3A, but under the control of the ATAD3B promoter. Immunoblotting identified a striking reduction in the amount of ATAD3 in cells and tissues. Quantitative proteomics confirmed this decrease and showed that peptides unique to ATAD3B were found only in the N-terminal region. RNA-Seq identified increased expression of genes involved in cholesterol metabolism in ATAD3 deficient fibroblasts and we confirmed increased cholesterol levels together with aberrant mtDNA organization. Although high homology within the *ATAD3* locus complicates genomic analyses, putative deletions can be identified by targeted re-analysis of existing SNP and exome data. RNA analyses also allowed us to identify the genetic cause in two patients with likely pathogenic single heterozygous variants identified by exome sequencing (in the *ACAD9* and *NDUFAF6* genes), both of whom had a deep intronic variant in trans that resulted in incorporation of a

cryptic exon. Finally, we describe a novel mitoribosomal protein (MRP) defect, in which quantitative proteomics of fibroblasts identifies instability of most MRPs of the small ribosomal subunit and respiratory chain complexes I and IV with little impact on MRPs of the large ribosomal subunit. **Conclusion:** Quantitative proteomic analyses of patient cell lines have much potential for identifying and proving causation in mitochondrial and other inherited metabolic diseases.

542 - *TMEM70* Mutation in an Turkish Infant With Volvulus, Hypertrophic Cardiomyopathy, Wolff Parkinson White Syndrome, Hypotonia, and Dysmorphism

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Objective: Mutations in the *TMEM70* gene are the most common cause of nuclear encoded ATP synthase deficiency and responsible for the clinical symptoms and signs of lactic acidosis, cardiomyopathy, and encephalomyopathy. The other features of this mutation are oligohydroamnios, intrauterine growth retardation, hypospadias and genital abnormalities, growth retardation, hernias, cataracts, dysmorphism, microcephaly, persistent pulmonary hypertension, and hyperammonemia. Wolff Parkinson White Syndrome is seen only 13% of patients and volvulus has not been reported before. **Methods:** The clinical features of an Turkish infant with *TMEM70* mutation were evaluated and compared with the other frequently reported signs and symptoms of the literature. **Results:** A 7 months old male patient, the second child of a consanguineous Turkish parents referred to our hospital with complaints of lactic acidosis and the suspicion of a metabolic disorder due to the family history. The first female child was died at the age of 7 months with respiratory and cardiac deficiencies without a specific diagnosis. The index case was born at the 29th weeks of pregnancy with a low birth weight due to oligohydroamnios and intubated for 10 days. He had a volvulus operation when he was 4 months old. The family noticed neuroregression and hypotonia at the age of 6 months. Physical examination revealed severe hypotonia, mild dysmorphism, growth retardation, and microcephaly. Ophthalmic examination was completely normal. He did not have lactic or metabolic acidosis on admission. Organic acid analysis showed 3-methylglutaconic aciduria. Electrocardiography and echocardiography was compatible with Wolff Parkinson White syndrome and hypertrophic cardiomyopathy. A probable diagnosis of TMEM70 deficiency was proven with the mutation (c.238C›T) detected in *TMEM70* gene. At the age of 16 months he was living with mechanical ventilation support. **Conclusions:** With this case, for first time in the literature we report volvulus in *TMEM70* mutation. Although the other clinical features of our patient are similar with the frequently seen signs and symptoms, Wolff Parkinson White syndrome is also rare.

543 - Molecular and Clinical Spectra of FBXL4 Deficiency

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F-box and leucine-rich repeat protein 4 (FBXL4) is a mitochondrial protein that plays significant roles in mitochondrial bioenergetics, mitochondrial DNA (mtDNA) maintenance, and mitochondrial dynamics. Biallelic *FBXL4* pathogenic variants have been associated with an encephalopathic mtDNA maintenance defect (MDMD) syndrome characterized by early onset lactic acidemia, developmental delay, hypotonia, feeding difficulties, growth failure with microcephaly, hyperammonemia, seizures, cardiomyopathy, liver dysfunction, recurrent infections, variable distinctive facial features, white matter abnormalities and cerebral atrophy in neuroimaging, combined deficiencies of multiple electron transport complexes, and mtDNA depletion. Since its initial description in 2013, 36 different pathogenic variants in *FBXL4* have been reported in 50 affected individuals. Herein we present 37 additional affected individuals and 11 previously unreported pathogenic variants. We summarize the clinical features of the total 87 individuals with *FBXL4*-related MDMD, review FBXL4 structure and function, map the 47 pathogenic variants onto the gene structure to assess the variants distribution, and investigate the genotype-phenotype correlation. Finally, we provide future directions to understand the disease mechanism and identify treatment strategies.

544 - Infantile Mitochondrial DNA Depletion Syndrome due to *RRM2B* mutation: A Natural History Study of Four New Cases

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Objective: Mitochondrial DNA depletion syndrome encompasses a group of disorders affecting mitochondrial DNA maintenance and energy metabolism. Mutation of the gene encoding ribonucleotide reductase TP53 inducible subunit M2B (*RRM2B*) is an uncommon cause which may present with a range of phenotypes spanning neonatal-onset encephalomyopathy to adult-onset progressive opthalmoplegia. We aimed to characterize the natural history of the early-onset form. **Methods:** Multicenter retrospective case note review. **Results:** Four new cases of infantile onset RRM2B deficiency, all from unrelated families (one consanguineous), presented with a characteristic clinical phenotype, including truncal hypotonia (detected from birth-7mths) and hyperlactatemia (peak lactates 3.2-5 mM). Generalized seizures evolved later at 2-7mths. Other neurological features included loss of tendon reflexes (4), generalized weakness (3), poor suck (3), poor visual contact (2), ptosis (2), progressive ophthalmoplegia (2), and dysphagia (2). Early MRI brain scans were normal (3). Non-neurological manifestations included: respiratory distress/failure (4), faltering growth due to poor feeding (3), recurrent vomiting (2), renal tubulopathy (2), and left ventricular hypertrophy (1). Muscle biopsies for respiratory chain enzyme assays performed in all 4 cases showed (average % of normal reference range): complex I 33% (4-83), complex II 122% (16-228), complex III 17% (16-19), complex IV 16% (7-35), and citrate synthase 392% (234-550). Histology revealed lipid deposition, ragged-red fibers and COX-negative fibers. Residual mitochondrial DNA content was 5%, 6%, and 20%, respectively, in 3 cases. All cases had proven biallelic pathogenic variants in the *RRM2B*. Management included long term respiratory support via tracheostomy (3), long term NG/gastrostomy feeds (4), coenzyme Q_{10} (4), riboflavin (4), thiamine (2), carnitine (2), vitamin E (1), and medium chain triglycerides (1). No response to treatment was seen. Mean age of death was 10.9 m (2.5-22 m). The cause of death included status epilepticus (2), respiratory failure (2), overall neurological deterioration (1), or cardiac arrest (1) leading to a redirection of care in 3 cases. **Conclusion:** Infantile onset mitochondrial DNA depletion due to *RRM2B* mutation is a severe and progressive disorder with characteristic symptomatology and laboratory features, and extremely poor prognosis. Management is supportive as there is no effective treatment. Novel treatments are urgently needed.

545 - Mitochondrial Disorders Associated With High Percentage of DNA Damage in Infantile—Pediatrics Patients Compared To Adult Patients—A Pilot Study From North India

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Background: High oxidative stress increases the pathogenicity of Mitochondrial disorders by increases the production of ROS/Free radicals, results in compromised the DNA (mitochondrial & nuclear DNA) stability. **Aim:** This study was aimed to decipher the DNA damage in patients with mitochondriopathy. **Method:** Human leukocytes isolated from whole blood of 34 patients samples (20 pediatric cases + 14 adult onset) and 14 controls, mixed in PBS, and processed for the alkaline comet assay. More than 500 nuclei in controls and upto 1000 nuclei in cases, scanned using Florescent microscope (Metafer 4), calculated automatically via Meta-Cyte Comet Scan system for tail length, tail movement, olive tail movement and percentage DNA in tail. **Results:** 20 pediatric cases (10 of fatal infantile lactic acidosis, 4 mitochondrial myopathy, 3 MELAS, 2 Leighs, and 1 PEO) shows 3.12 times high percentage of DNA damage compare to controls & adult cases respectively. **Conclusion:** This study reveals the significantly high percentage of DNA damage (3.12 times) in early age group compared to patient with adult onset of mitochondriopathy, correlating severity of mitochondrial disorders in infantile–pediatrics population. Screening for DNA damage in mitochondriopathy at early age groups can helps us to amend the natural course of disease by minimizing the morbidity and mortality through better preventive medication and therapy.

546 - Whether a Frequency of p.E140 K Substitution in the *SCO2* Gene is Underestimated in Children With Infantile Mitochondrial Encephalomyopathy?

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Mitochondrial disorders present a heterogeneous group of inherited disorders with wide clinical manifestations. As age of onset and symptoms considerably vary, NGS technologies

become the main diagnostic approach for searching a genetic cause of mitochondrial pathology and could be effective to expand knowledge about genotypic and phenotypic spectrum in this group of diseases. During the last 2 years we apply a target sequencing of mitochondrial nuclear genes using NGS technology on IonTorrent PGM to define a molecular defect in patients suspected for mitochondrial disease. Here we present a group of 8 patients (6 from Russia, 2 from Ukraine) with revealed homozygous c.418G>A (p.E140 K) mutation in the *SCO2* gene with a clinical diagnosis of infantile mitochondrial encephalomyopathy/ Leigh syndrome. These children have varieties in disease manifestations but there are some important common clinical features: progressive psychomotor development delay, acute hypotrophy, muscle hypotonia with decreased reflexes, respiratory insufficiency and stridor, oculomotor abnormalities, lactic acidosis and first symptoms developed at age 2-8 months. Our data demonstrates that p.E140 K mutation in the *SCO2* gene is a second frequent cause of infantile mitochondrial encephalomyopathies after 2 bp deletion c.845_846del in *SURF1* gene among Russian and Ukranian patients with nuclear DNA mutations. So far, we have been diagnosed about 60 *SURF1* patients with c.845_846del, however all of them have been revealed on a first step of routine diagnostic by SSCP analysis but simple methods have not been applied for searching frequent mutations in the *SCO2* gene. Interestingly that fist description of this mutation was in 1999 in a patient with fatal cardiomyopathy, however first Russian patient with *SCO2* mutation was found in 2015. According to ExAc database for European (non-Finnish) population, the allele frequency of c.845_846del in *SURF1* is about 1 per 7000 and the allele frequency of c.418G>A in *SCO2* is about 1 per 5500. Therefore, we speculate that contribution of *SCO2* gene p.E140 K substitution in infantile mitochondrial encephalomyopathy is underestimated in Russian and Ukranian patients.

547 - Exome Sequencing Reveals Mutations in the Mitochondrial Phosphodiesterase PDE12 in an Infant With Lethal Neonatal Lactic Acidosis

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The mitochondrial (mt) phosphodiesterase PDE12 removes mt-RNA poly (A) tails, plays a role in mt-RNA turnover and translation and is involved in immune response. Studies to establish the effects of decreased or overexpressed *PDE12* suggest species and tissue specific outcomes. In mice, *Pde12* knockout results in an embryonic lethal phenotype. No disease has been reported in association with mutations in the *PDE12*. **Aim:** We describe clinical, biochemical, and MRI abnormalities in the first patient with mutations in the *PDE12* and their effects in patient fibroblasts. **Methods:** Exome trio was performed as compassionate study by GeneDx. Protein levels of PDE12 and oxidative phosphorylation (oxphos) complexes were assessed by western blot. O_2 consumption and extracellular acidification were measured via Seahorse Bioanalyzer. **Results:** An infant was born with 2.3 kg (SGA) at 39 weeks via C-section to nonconsanguineous parents. Mother reported decreased fetal movements and prenatal ultrasound showed brain anomalies. At birth he was limp, apneic and acidotic (pH 6.9) and required intubation. Lactic acid was 20 mmol/L (<2.1) and pyruvate 0.33 mmol/L (0.03-0.08) with L/P ratio of 60.6 (<20). Alanine was elevated (1966 μmol/L, rr 131-710) and organic acids showed increase in lactate, pyruvate, 4-OH-phenyllactate and mild ketosis. MRI was striking for lissencephaly, dysgenesis of the corpus callosum and extensive periventricular and subcortical cysts. Lactic acidosis persisted despite aggressive treatment. On day 2 life support was discontinued and patient died. Pyruvate dehydrogenase complex activity was normal. Exome trio revealed a homozygous novel mutation in *PDE12*, inherited from both parents. The c.1115G>A; p.Gly372Glu change affects a highly conserved residue in the C-terminal catalytic domain. In silico tools deem the variant as highly pathogenic in 9/9 algorithms interrogated. The mutation leads to almost absent mitochondrial PDE12 protein levels. Protein levels of oxphos complexes were found to be either normal or differentially up-regulated, depending on tissue culture conditions. Electron transport chain studies in fibroblasts were normal while oxygen consumption was decreased and extracellular acidification rate was significantly increased, suggesting preferential use of glycolysis for energy generation. **Conclusion:** We report the first patient with mutations in the *PDE12*, and a phenotype consistent with severe mitochondrial disease.

548-A Novel *ETHE1* Mutation Produces Multifocal Epilepsy and Diffusion Restriction on Brain MRI in Ethylmalonic Encephalopathy

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Ethylmalonic encephalopathy is a metabolic disorder, in which the neurological involvement is characterized by neurodevelopmental delay and regression, pyramidal and extrapyramidal signs. Brain MRI demonstrates necrotic lesions in deep gray matter structures. Here, we report on a patient with Ethylmalonic encephalopathy with a severe phenotype, which

includes multifocal epilepsy and diffusion restriction on brain MRI, who also carried out a novel mutation in the *ETHE1.* The proband presented with developmental delay, seizures, diarrhea, acrocyanosis and petechiae. The EEG showed multifocal epilepsy. Brain MRI findings included injuries in deep basal ganglia and brain stem with diffusion restriction. The metabolic work-up demonstrated metabolic acidosis and high urine levels of ethylmalonic acid. The molecular analysis showed a novel mutation in the *ETHE1*. The identification of this novel mutation in the *ETHE1* in a proband with a severe neurological involvement which includes multifocal epilepsy and restriction to the diffusion allows to expand the known phenotype of this lethal disorder.

549 - A Novel ETHE1 Mutation Produces Multifocal Epilepsy and Diffusion Restriction on Brain MRI in Ethylmalonic Encephalopathy

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Ethylmalonic encephalopathy is a metabolic disorder, in which the neurological involvement is characterized by neurodevelopmental delay and regression, pyramidal and extrapyramidal signs. Brain MRI demonstrates necrotic lesions in deep gray matter structures. Here, we report on a patient with Ethylmalonic encephalopathy with a severe phenotype, which includes multifocal epilepsy and diffusion restriction on brain MRI, who also carried out a novel mutation in *ETHE1.* The proband presented with developmental delay, seizures, diarrhea, acrocyanosis and petechiae. The EEG showed multifocal epilepsy. Brain MRI findings included injuries in deep basal ganglia and brain stem with diffusion restriction. The metabolic work-up demonstrated metabolic acidosis and high urine levels of ethylmalonic acid. The molecular analysis showed a novel mutation in *ETHE1*. The identification of this novel mutation in ETHE1 in a proband with a severe neurological involvement which includes multifocal epilepsy and restriction to the diffusion allows to expand the known phenotype of this lethal disorder.

550 - "Neuronal Ceroid Lipofuscinosis Like" Phenotype Masking RARS2 Mutations

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Aims: To describe two patients with Neuronal Ceroid Lipofuscinosis (NCL) like phenotype where investigation pointed to the *RARS2* mutations. **Methods:** Clinical history assessment associated to charts reviews. **Results:** The first patient is a 4-year old girl, third child born to consanguineous parents. She had a normal pregnancy and delivery. Patient started with motor regression and epilepsy by 2 years old, progressing with severe developmental regression and epileptic encephalopathy. At physical examination, she presents hypotonia and dystonia. After extensive biochemical and metabolic investigation, she only had elevated lactate. Electroencephalogram showed epileptic encephalopathy with theta rhythm. It was proceed cranial tomography and electroneuromyography that were normal. Brain MRI showed diffuse cerebral volumetric reduction, severe reduction of the cerebellum and discreet of brain stem, suggesting Neuronal Ceroid Lipofuscinosis ou mitocondriopathy. Karyotype Sequencing and MLPA of *SLC2A1* and mitocondrial DNA were normal. Whole Exome Sequencing (WES) was proceed and found a pathogenic mutation at gene *RARS2*, c.-2A>G. The patient showed seizures improvement and gain of motor development with canabidiol and L-carnitine. The second patient is a one old year boy, son of non consanguineous parents, that had normal pregnancy and delivery. At the second month of life, he started with epilepsy and motor regression, losing cervical tonus. He presents hypotonia and hyperreflexia, at examination. Biochemical and metabolical screening were all within the normal range. Sequencing of *SCN1A* and *SCLN2A* were normal. Brain MRI showed diffuse brain atrophy and also suggested Neuronal Ceroid Lipofuscinosis. WES detected pathogenic a mutation at *RARS2*, c.772-1G>A in heterozygosis. **Conclusion:** Early onset epileptic encephalopathies are a group of diseases manifesting in the first year of life with frequent seizures and/or prominent interictal epilept form discharges, development delay/regression and usually a poor prognosis. *RARS2* gene mutations were the first nuclear encoded defects of mitocondrial DNA translation that were associated with early onset intractable epilepsy and a neurodegenerative course. The presence of neuronal ceroid lipofuscinoses in brain MRI does not exclude others diagnosis with similar presentation. Next generation sequencing, especially WES, is becoming a cost-effective mean to elucidate the molecular bases of neurological disease.

551 - Pyruvate Dehydrogenase Deficiency: New Missense Mutation Due to Classic Phenotype

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Aims: We describe a patient with seizures and psychomotor development delay with pyruvate dehydrogenase (PDH) deficiency diagnosis caused by uncommon mutation. **Methods:** Clinical history assessment associated to laboratory and radiology investigation. **Results:** Our subject is a 15-year-old female, the first child out of three of a non-consanguineous couple. There were no pathological events during pregnancy, and the patient was delivered after 39 weeks of gestation through a C-Section due to a transverse presentation. She first presented at our service as an 8-month old whom had started having seizures since 2 months old, with psychomotor development delay. The preictal was characterized by loss of contact and would evolve into symmetrical tonic hand movements of a minute, with no postictal period. With 3 years old, the optimal treatment was reached and seizures stopped. With 15 years old, the patient has a dysarthric speech, forms phrases, and has interpersonal contact. Her morphology shows a low anterior hair implantation, short forehead, almond-shaped eyes, tented mouth, malocclusion of the teeth, joint hypermobility, hirsutism in arms and trunk. Also, her right hemi-body muscular strength has a discrete reduction and the ocular movement is slower and the upside vertical eye movement is paralyzed. Biochemical and metabolic investigation was performed and showed only mild lactate elevation. Electroencephalograms was all normal and brain MRI revealed the epileptic encephalopathy as a parenchymal volume loss with supratentorial ventriculomegaly. *PDHA1* gene amplification was performed by PCR, and genetic sequencing revealed a missense mutation in exon 4, c.380g>T (p. Arg127Leu), causing PHD deficiency, mostly inherited in X-linked recessive manner. **Conclusion:** PDH deficiency is one of the most common cause of primary congenital lactic acidosis, most caused by defects in the E1 alpha subunit gene on Xp21.3. Clinical symptoms can vary considerably with developmental lethality in some males with severe mutations and the pattern of X-inactivation in females. The mutation found in this patient is new in literature and it is responsible for a classic phenotype in a female, even if it is a missense mutation, whereas the most common is meet deletion at female DNA.

P) Mitochondrial Disorders: Mtdna (552 to 561)

552 - Mitochondrial DNA Variation Among Emiratis

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This study investigated the mitogenome haplogroups and variants in 232 healthy Emirati citizens. Its main purposes were to study phylogenetic relationships in the tribal population, and describe novel mitochondrial DNA (mtDNA) variants in the region. Whole mtDNA sequences were used for these purposes. Major haplogroups with >10% frequency each were **R** (West Eurasia), **U** (Central/ South Asia, North/ the Horn of Africa, and the Near East), **M** (South Asia), **K**, and **J** (Western Asia for both). African gene flow was detected through the presence of subclades L0-L4 in 20 (8.6%) of the 232 studied samples, reflecting earlier migrations. There were significant variations in haplogroup composition among the seven emirates. For example, the **K** haplogroup was clustered in Ras al-Khaimah and Fujairah, demonstrating groups rarely migrated or married outside. Genetic similarities and variations were present among the studied tribes. Some subclades were present only in certain kinships. Ninety novel variations, not previously reported in PhyloTree (Build17), were found. These matrilineal descents are relevant to our population genetic structures, genealogy, medical genetics, and forensic medicine.

553 - Different Clinical Phenotypes in Two Children With m.8362TG and m.8363GA MTTK Mutations

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Background: Respiratory chain disorders feature clinical polymorphism. The mitochondrial DNA mutation in different patients may result in different clinical presentations even within a family. This is usually explained by distribution of the mitochondrial DNA mutations in cells and tissue. We observed two girls with different manifestations and different courses of systemic mitochondrial diseases caused by mitochondrial DNA mutations in the neighboring nucleotides of the *MTTK* gene, which encodes the mitochondrial transfer RNA for lysine. **Case reports:** In the first case (m.8363G> *MTTK* mutation), the debut of the disease occurred at the age of 5 years. The first signs were intolerance to physical exertion, weakness, palpitations, and impaired vision and hearing. In the clinic, the girl was examined at the age of 14 years. Encephalomyopathy, ataxia, ophthalmoparesis, dilated cardiomyopathy, sensorineural hearing loss, and retinal dystrophy were detected. In the blood, severe lactic acidosis (pH 7.21, lactate - 15 mmol/l (N being upto 2.2), as well as moderate increase in lactate dehydrogenase, creatine kinase, and aspartate amine transferase were determined. In the second case (m.8362T> G *MTTK* mutation) the initial signs of the disease such as progressive weakness, muscle hypotension, and ataxia appeared at the age of 18 months. At the age of 9 years, when the girl was exposed to clinical examination,

encephalomyopathy, ataxia, dilated cardiomyopathy, and lactate acidosis (pH 7.3, BE-7.6, lactate - 6.7 mmol/L) were detected. In the examined children with mitochondrial DNA mutations in neighboring nucleotides of the *MTTK* gene, the similarity of core manifestations such as encephalomyopathy, ataxia, cardiomyopathy, and lactate acidosis were revealed. At the same time, there were significant differences, i.e., ophthalmoparesis, neurosensory hearing loss, and retinal dystrophy which were revealed only in the first girl. **Conclusion:** Clinical polymorphism of diseases caused differences in differentiated diagnosis in the patients: in the first case, mitochondrial DNA mutations were searched first, in the second case, neurodegenerative diseases and metabolic diseases were first excluded.

554 - Reticular Dysgenesis and Mitochondriopathy Induced by Adenylate Kinase 2 Deficiency Identified in the Amish Population

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Background: Reticular dysgenesis (RD) is autosomal recessive form of severe combined immunodeficiency (SCID) caused by defects in adenylate kinase 2 gene (*AK2*). It is the only known immunodeficiency syndrome causally linked to energy metabolism and therefore is classified as mitochondriopathy. AK2 catalyzes reversible transfer of a phosphoryl group from ATP to AMP and is located in the mitochondrial intermembrane space. AK2 is the only adenylate kinase isoenzyme from the ADK family that is expressed in bone marrow and its dysfunction leads to immunodeficiency. RD has not previously been reported in the Amish population. **Methods:** Whole exome sequencing (WES) was performed on a 3 years old Amish male with SCID. Genetic testing for the known SCID syndromes in the Amish was negative. Protein expression was assessed using western blot, immunohistochemical and immunofluorescent staining on patient's (pt) fibroblasts and bone marrow, and compared to wild type (wt). Studies to confirm mitochondriopathy were performed on fibroblasts from pt and wt. These include measurement of 1) Mitochondrial respiration and oxygen consumption rate (OCR) using Seahorse XF^{e}96 Flux Analyzer 2) Superoxide production using MitoSOX Red 3) Mitochondrial membrane mass and membrane potential using MitoTracker Green and Red 4) ATP production using a bioluminescence assay. **Results:** WES analysis revealed a novel homozygous mutation [c.622T>C; p.Ser208Pro] in the *AK2* gene in the pt. AK2 protein was absent in fibroblasts and bone marrow of the pt confirming AK2 deficiency. OCR in fibroblasts, reflected in measured basal respiration and reserve capacity of the pt, was decreased compared to wt. Additionally, superoxide production was increased dramatically in pt with or without glucose. Significant increase in mitochondrial mass concurrent with a relatively higher increase in mitochondrial membrane potential in pt indicates disruption in the proton gradient crucial for ATP synthase ATP production. This is consistent with significantly lower ATP measured in pt. **Conclusions:** RD is a novel genetic disorder finding in the Amish population and should be considered in Amish patients with SCID. Functional studies confirmed the pathogenicity of the mutation and provided more evidence of the mitochondrial dysfunction associated with RD. While there are no reports whether patients with RD benefit from supplementation with CoQ10, hematopoietic stem cell transplantation remains the only effective treatment.

555 - High-Coverage NGS Sequencing of Complete MT-DNA as Diagnostic Tool for Mitochondrial Disease

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We established a high-coverage next generation sequencing (NGS) procedure for mtDNA. The entire mtDNA was enriched by a single amplicon long-range PCR and analyzed by massive parallel sequencing. Sequence alignment to NC_012920 sequence with subsequent variant calling was performed. Technical sensitivity was 1% heteroplasmy for SNVs and allowed determination of heteroplasmy with high precision. For 297 samples, we achieved an average coverage of 6801 reads/sample. 27.6 variant per sample were detected. For diagnostic purposes, we set a heteroplasmy threshold of 10% (blood samples) and 20% (urine or muscle samples) for sequence variants being evaluated, to exclude likely insignificant variants. mtDNA deletions were detected by both, long-range PCR and the coverage analysis, the latter providing breakpoint information. In 2015 and 2016, we performed diagnostic high-coverage NGS of mtDNA for 189 index patients. Most of the patients were analyzed because of suspected mitochondrial disease (146/189, 17 among them MELAS), 22/189 for LHON and 21/189 for other indications. For patients with LHON and MELAS suspicion, the common mutations had been excluded in most cases by other methods before using high-coverage NGS. In one family, we could identify the most common MELAS mutation m.3243A>G after it had been missed by Sanger sequencing.

Overall, we got 72 clinically relevant results. Half of them were deletions (36), many deletions associated with suspected CPEO (12/18) or other suspected mtDNA-deletion syndromes (3/10). In 16 cases, we found confirmed pathogenic point mutations, in 20 cases yet unreported variants of unknown significance with probability of relevance. Deletions were found in a higher proportion in adult samples than in pediatric samples as opposed to point mutations. In 29 cases with negative result of mtDNA high-coverage NGS we performed additional whole exome sequencing (WES). We found pathogenic relevant nuclear sequence variants in 14 cases (48.2%). *GTPBP3* (3/14) and *AARS2* (2/14) were detected in unexpected high number. 4/14 nuclear genes found in patients with suspected mitochondrial disease are not associated with mitochondrial function so far. In conclusion, high coverage mtDNA sequencing is an efficient tool for diagnosing mtDNA mutations in mitochondriopathies, especially in adult samples.

556 - Mitochondrial Dysfunction in Infantile Acute Liver Failure due to *TRMU* Gene Defect

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Introduction: Acute liver failure is a life-threatening condition in childhood. Mitochondrial DNA depletion is an important cause of liver failure in infancy and accurate diagnosis becomes critical since liver transplant is often contraindicated. **Case:** A 3 months old boy, first child of not consanguineous parents presented with vomiting after feeding. Initial clinical evaluation showed hepatomegaly and hypertonia. The laboratory disclosed acute liver failure with severe coagulopathy, low albumin, direct hyperbilirubinemia, metabolic acidosis, high alpha-fetoprotein, and hyperlactacidemia. Blood ammonia level was normal, and plasma amino acid profile showed high tyrosine, methionine, and valine. Succinylacetones negative. Urinary organic acid analysis revealed massive excretion of lactate. Bacterial, viral and immunologic causes were ruled out. Abdominal ultrasound disclosed enlarged homogenous liver. The following diagnoses were excluded by metabolic workup: galactosaemia, tyrosinemia, FAO disorders, UCD, organic acidurias, haemochromatosis, alpha1antitrypsin deficiency, CDG, NPC, and bile acid synthesis disorder. The patient required intensive care for 8 weeks, with supportive nutrition and blood products given for coagulopathy and active bleeding. Liver transplantation was considered but dismissed because of high index of suspicion of mitochondrial disease. Brain MRI showed basal ganglia abnormalities (Leigh-like syndrome) with lactate peak in the MRs. The patient developed multiorgan failure and died at age 5 months with suspected diagnosis of mitochondrial hepatopathy due to mtDNA depletion. A mitochondrial gene panel by next generation sequencing (NGS) identified a homozygous point mutation in exon 8 of the *TRMU* gene c.835G>A: p.V279 M. **Discussion**: Our patient showed typical clinical findings of acute liver failure due to mitochondrial dysfunction. Mitochondrial DNA depletions induced by mutations of the nuclear genes POLG, DGUOK, and MPV17 are the three main causes of mitochondrial hepatopathy in infants. More recently, mutations in the *TRMU* gene encoding the mitochondrial tRNA-specific 2-thiouridylase were found in infantile hepatopathy related to mitochondrial translation defect. The NGS panel for mtDNA depletion syndrome is a useful tool that confirmed the diagnosis in our patient. Further evidence is needed to define If liver transplantation could be an option in the *TRMU* gene defects

557-Endothelial Dysfunction and the Effect of Arginine and Citrulline Supplementation in Children With Mitochondrial Diseases: Interim Results

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Background: In addition to inadequate energy production, nitric oxide (NO) deficiency can occur in mitochondrial diseases. Vascular endothelium produces NO which causes smooth muscle relaxation needed to maintain the patency of small blood vessels. NO deficiency can contribute to the pathogenesis of several complications observed in mitochondrial diseases including stroke-like episodes, myopathy, diabetes, and lactic acidosis. NO synthase converts arginine to citrulline and NO. Citrulline can be converted back to arginine. Therefore, both arginine and citrulline are NO precursors. In response to reperfusion, normal endothelial cells release NO leading to vasodilatation. Endothelial function can be assessed using peripheral arterial tonometry which utilizes finger plethysmograph sensors to record the pulse wave amplitude (PWA) at baseline and during reactive hyperemia which is induced by inflating then deflating a pressure cuff on the upper arm. The reactive hyperemic index (RHI) is the post-occlusion to baseline PWA ratio. Lower RHI index reflects endothelial dysfunction. Due to NO deficiency in mitochondrial diseases, we hypothesized that children with mitochondrial diseases have endothelial dysfunction (lower RHI) that will improve after arginine or citrulline supplementation. **Methods:** We aim to study 10 children with mitochondrial diseases and 10 healthy control children. RHI is determined once in control children, and before and after 2-week supplementation of arginine or citrulline in children with mitochondrial diseases. **Interim results:** Six children with mitochondrial diseases and five control children have completed the study. RHI is lower in children

with mitochondrial diseases when compared to control (1.1 ± 0.1 vs 1.6 ± 0.7, $P = .07$). RHI is higher after arginine ($1.12 \pm 0.20 \rightarrow 1.23 \pm 0.23$, $P < .05$) and citrulline ($1.00 \pm 0.20 \rightarrow 1.16 \pm 0.27$, $P = .09$) supplementation. **Interim conclusions:** Lower RHI in children with mitochondrial diseases supports the hypothesis that children with mitochondrial disease have endothelial dysfunction which can be due to defective NO production. The improvement in RHI after arginine or citrulline supplementation suggests that NO precursor supplementation can improve endothelial function through enhancing NO production. When this study is completed, its results can provide evidence for endothelial dysfunction in mitochondrial diseases and support the potential therapeutic utility of arginine and citrulline in mitochondria diseases.

558-A Neurophysiologic Model of MELAS Disease, Using Inducible Pluripotent Stem Cell Derived Excitatory Neurons

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Most individuals diagnosed with the common mitochondrial phenotype of MELAS carry the pathogenic variant 3243A>G in their mitochondrial DNA (mtDNA). Patients show a broad range of symptoms, involving mainly the skeletal muscle and the central nervous system. The mutation being present in the maternally inherited mtDNA, makes generating a knockdown/knockout animal model problematic. In order to increase our understanding on the neuropathobiology of mt.3243A>G we created human cortical neurons (iNeurons), derived from inducible pluripotent stem cells (IPSCs) and studied the impact of mt.3243A>G on mitochondrial- and neuronal (including synaptic) function. Patient-derived fibroblasts were reprogrammed to IPSCs, which were tested for heteroplasmy by sanger sequencing; three clones from the same patient, with different heteroplasmy levels (0%, 72%, and 83%) were expanded. These IPSC's were differentiated into excitatory iNeurons by lentiviral rtta- and Neurogenin 2 (Ngn2) expression. Here we report on the characterization of the iNeurons both at the single-cell and neuronal network level. We describe whole-cell patch clamp recordings assessing spontaneous excitatory post-synaptic currents (sEPSC's), combined with Mitotracker, MAP2- and Synapsin immunocytochemical staining. Additionally, we report on multi-electrode arrays (MEA's) used to study the effects of the 3245A>G mutation on the network activity of the iNeurons. Our study illustrates the relevance of our patient-specific in vitro neurophysiologic model of mitochondrial involvement, which will enhance our understanding of the role of mitochondrial dysfunctions in neurological manifestations of MELAS disease.

559 - MELAS Syndrome: A Descriptive Study About 55 Patients From a French Department of Endocrinology, Diabetology, and Metabolism

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Introduction: MELAS syndrome (Mitochondrial Encephalomyopathy, Lactic Acidosis, Stroke-like episodes) is characterized by a wide range of phenotypical disorders: neurological, muscular, neurosensorial (macular pattern dystrophy, hearing impairment), cardiac, endocrine, renal and digestive impairment. It is associated with mutations of the mitochondrial DNA, the most frequent being the m.3243A>G. The aim of our study was to analyze the clinical, metabolic and hormonal features of patients with MELAS syndrome who were genetically diagnosed. **Patients and Methods:** We retrospectively collected the data of 55 patients who were referred to the department of endocrinology, diabetology and metabolism of Lille University Hospital. Among them, 18 were the propositus. **Results:** The average age is 44 years old (± 13) and sex ratio is 18M/37F. All patient present with neurological disorder (central, peripheral or both, disturbed brain MRI), 35 (63%) with diabetes (including 24 with insulin treatment), 18 of 53 of the tested patients (34%) have a potential pituitary disorder, responsible for one or several partial pituitary hormone deficiencies (mainly affecting the corticotropic and somatotropic axes but also concerning those gonadotropic and thyreotropic). None of them has hypoparathyroidism. Concerning other organs, 47/53 (89%) of the patients suffer from peripheral hearing loss, 13/48 (27%) from a mild retinal pigmentary defect, 29/50 (58%) from ventricular hypertrophy and/or electrocardiographic abnormalities, 11/53 (20%) from renal failure. The average postprandial serum lactate dosage is higher than the average preprandial dosage (2.19 mmol/L versus 1.98 mmol/L, $P = .0020$), even though they are in the normal range (0.63-2.44 mmol/L). The diabetic patients present an average postprandial serum lactate/pyruvate ratio higher than the nondiabetic patients ($P = .004$). Patients with stroke-like episodes and early age at the diagnosis of diabetes are associated with higher heteroplasmy levels in the blood leucocytes ($P = .0004$ and 0.0009) and in urinary epithelial cells ($P = .0029$ and $P = .0175$). **Conclusion:** Our study confirms the heterogeneity of the phenotypical presentation of MELAS syndrome. We found that the endocrine disorders are not uncommon. The heteroplasmy level is associated with the severity of the neurological disorders and the early age at the diagnosis of diabetes.

560-A Case Report of MELAS Syndrome With Early Diagnosis

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The goal is to demonstrate the importance of the early diagnosis of Melas syndrome. Information was obtained through interview with the patient and reviews of the literature and medical chart. ABSS, 4 years old, born full-term by C-section, normal blood spot test, started with 1 month of inconsolable crying, was prescribed infant formula, causing episodes of vomiting and dehydration. Started lactose-free amino acid formula, with no improvement. He presented hypoglycemia, high lactate dehydrogenase, normal abdominal ultrasound, MRI of the brain with signs of delay in the myelination and important lactate peak in the spectral analysis of the nuclei of the base on the right and of the ventricular system, electroencephalogram suggestive of West syndrome, metabolic acidosis, high anion gap, increased lactic and pyruvic acids, normal plasma, and urine amino acid chromatography. He had several hospitalizations due to worsening acidosis. Suspected of lactic acidemia and Leigh syndrome. Diagnosis made at 2 years by exome. He presented developmental delay, seizure, diffuse appendicular hypertonia with axial hypotonia. He evolved with malnutrition, then performed gastrostomy. He made use of carnitine, coenzyme Q10, complex B, and lamotrigine. Hypertonia and development improved, interacting better, smiling, gaining weight, decreased hospitalizations, reduced spasticity, and absence of seizures. MELAS syndrome is a rare mitochondrial disorder that begins in childhood, that affects especially the nervous system and muscles. Clinically, there are stroke-like episodes, encephalopathy, seizures, dementia, and mitochondrial myopathy evident by lactic acidosis or ragged-red fibers. A genetic study shows a mutation in the leucine transporter RNA gene, leading to the exchange of A by G at nucleotide 3243 of mtDNA, accounts for about 80% of cases. There is no specific approach for the treatment of individuals with MELAS syndrome, which is largely symptomatic. Comprehensive assessment, nutritional support and rehabilitation are necessary. Several supplements are being used based on limited clinical trials, like those used on this study, and they have been helpful in individual patients but further studies are needed to prove their efficacy. Although MELAS remains a largely untreatable condition, early diagnosis, prevention and management of medical complications may come to symptomatic relief and improvement of the quality of life, adding to the benefit of establishing a diagnosis.

561-Review, Clinical Presentation, Laboratory, and Genetical Findings on Seven Cases of Kearns Sayre Syndrome

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Introduction: Kearn Sayre syndrome (KKS) is a mitochondrial disorder characterized by the emergence before age 20 of progressive external ophthalmoplegia, pigmentary retinopathy with other clinical signs including cardiac conduction defects, cerebrospinal fluid protein concentration higher than 100 mg/dL and cerebellar ataxia. Other signs, frequent but not always present, are short stature, hearing loss, dementia, limb weakness and endocrinopathies. **Method:** Retrospective and longitudinal study based on the review of clinical histories of seven patients diagnosed at Dr. N. A. Chamoles Neurochemistry Laboratory. **Results:** Clinical findings: All seven patients showed symptoms before age 20 (in one case at age 9) with palpebral ptosis and progressive external ophthalmoplegia. All had low stature and muscular hypotrophy at the time of diagnosis. Five patients required pacemaker due to right bundle branch blocks. Four patients developed endocrinopathies such as glucose intolerance, hypothyroidism and hypogonadism. Four patients presented cognitive disorder at some time, three patients had intolerance to exercise and sensorineural hearing loss. One had ataxia and one had intentional tremor. All patients had increased CK; in six patients, a muscle biopsy showed COX deficient ragged red fibers. Brain MRI performed on six patients showed subcortical white matter disorders, three associated brain stem involvement, one cerebellum and one basal ganglia. Molecular diagnosis confirmed mit DNA deletion in four patients. Two of the patients were identical twins presenting a single deletion of equal size. **Evolution:** One of the twins, who did not have a pacemaker, died suddenly at age 29. The rest of the patients evolved gradually and slowly. **Discussion:** Kearn sayre syndrome is a slow progressive disease that severely impacts quality of life and shows high mortality rates due to cardiac conduction defects. Therefore, it requires early diagnosis and strict multidisciplinary follow up to reduce morbidity and mortality.

Q) Disorders of Purines, Pyrimidines, Nucleic Acids, and Porphyrias (562 to 568)

562 - Dihydropyrimidine Dehydrogenase Deficiency: Lack of Phenotype/Genotype Correlation and Ethical Perspectives of Termination of Affected Pregnancy

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Introduction: Dihydropyrimidine dehydrogenase deficiency (DPD deficiency) is an autosomal recessive disorder caused by a deficiency of Dihydropyrimidine dehydrogenase due to mutations in the *DPYD* gene. **Clinical Report:** We here report on an unfortunate family with two siblings and a fetus affected by this disease with variable severity. The proband is a 2-year-old Saudi boy born to consanguineous parents. The antenatal scan showed a discrepancy between femur length and biparietal diameter and oligohydramnios. He presented with intractable seizures in the neonatal period followed by a global developmental delay and severe failure to thrive in infancy. Examination showed dysmorphic facial features (micrognathia, low-set ears, and high arch palate). He was marasmic with all growth parameters below the 3 rd percentile. He had hypotonia with decreased reflexes and global developmental delay. His Urine organic acid showed increased secretion of thymine and uracil. Whole exome sequencing confirmed the diagnosis of DPD deficiency with a previously described homozygous mutation in the DPYD gene, c.1651G>A p. (Ala551Thr). Family screening revealed a 16-year-old female sibling with mild speech delay and cognitive delay and history of infrequent seizures in infancy controlled by anticonvulsant therapy. Carrier testing revealed the same mutation in a homozygous state. Also, the mother was found to be pregnant at 16 weeks gestation. A chorionic villous sampling confirmed that the fetus is homozygous for the same mutation carried by the two siblings. This raised an ethical dilemma regarding offering termination of pregnancy for a disease with lack of phenotype/genotype correlation and variable phenotype within the same family. Also, there were religious concerns about termination. Parents requested termination of pregnancy which was performed at 17 weeks of gestation. **Conclusion:** In conclusion, this report illustrates the lack of phenotype/genotype correlation in DPD even in the same family that led to an ethical dilemma regarding offering termination of an affected pregnancy.

563 - Effect of Intrastriatal Hypoxanthine Administration on Cellular Bioenergetic in Striatum of Young Adult Rats

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Lesch-Nyhan disease (LND), an inborn error of purines metabolism, is characterized by oxypurine accumulation, mainly hypoxanthine. Purine represents an important role in cellular metabolism since it is substrate to the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) in purine salvage pathway in the brain. The deficiency of HGPRT activity results in high concentrations of hypoxanthine, being that LND patients present 5 times the normal concentration of this purine in cerebrospinal fluid. Symptomatology of LND are cerebral palsy, cognitive and behavioral disturbances, motor dysfunction (spasticity, dystonia), self-mutilation behavior, as well as hyperuricemia. Although mechanisms of brain dysfunction in LND are poorly understood, it is believed that the accumulation of hypoxanthine contributes to neurological damage of this disease. Since this purine is closely related to ATP formation, in the present study we investigated the effect of this purine on cellular bioenergetic in striatum of young adult rats submitted to the intrastriatal hypoxanthine injection model. We evaluated Cytochrome c oxidase activity and immunoconent by Western Blotting, intracellular ATP levels, mitochondrial mass and mitochondrial membrane potential by MitoTracker. Sixty-day-old Wistar rats were divided into two groups: group 1 received hypoxanthine (10 μM) injection or saline (0.9%) injection. Thirty minutes after the administration, animals were decapitated and cerebral striatum were dissected. Results showed that hypoxanthine diminished cytochrome c oxidase activity, as well as its immunocontent (P <b.001 and P < v.01, respectively). Hypoxanthine administration also was able to decrease significantly ATP levels ($P < .05$). Further, hypoxanthine injection decreased the percentage of cells with mitochondrial membrane label ($P < .01$) and increased mitochondrial mass potential labeling ($P < .05$). These findings suggest that hyper-hypoxanthinemia, with levels similar to those found in LND patients, promotes energetic misbalance that might be related, at least in part, with the pathophysiology of Lesch-Nyhan disease. Supported by CNPq/Brazil.

564 - Phase 1, Randomized, Placebo Controlled Study of Givosiran, an Investigational RNA Interference (RNAi) Therapeutic, in Patients With Acute Intermittent Porphyria: Interim Study Results

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Acute hepatic porphyrias (AHP) are a family of rare, serious and life-threatening metabolic diseases predominantly caused by a genetic mutation in one of the enzymes required for heme synthesis in the liver. Acute intermittent porphyria (AIP) is the most common subtype of AHP. AIP is characterized by disabling neurovisceral attacks and chronic disease symptoms, that are secondary to the upregulation of the first and rate-limiting step in heme synthesis, ALA synthase 1 (ALAS1), and the accumulation of the neurotoxic heme intermediates aminolevulinic acid (ALA) and porphobilinogen (PBG). With prolonged and severe attacks, paralysis, respiratory failure and death can occur. Givosiran (ALN-AS1) is an investigational RNAi therapeutic targeting ALAS1 mRNA in the liver in order to decrease the accumulation of the neurotoxic heme intermediates ALA/PBG, and thereby potentially reduce subsequent porphyria disease activity. We are conducting a phase 1 randomized, placebo-controlled, study in 3 parts to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of subcutaneously (SC) administered givosiran in patients with AIP (Part A, single ascending dose; Part B, multiple ascending dose; and Part C, multiple dose). In addition, Part C examines the impact of givosiran treatment on porphyria disease activity (ClinicalTrials.gov Identifier: NCT02452372). Interim data from Parts A, B, and C of the study suggest givosiran was generally well tolerated with no serious adverse events or clinically significant laboratory abnormalities related to study drug, and no discontinuations due to drug-related adverse events. Givosiran treatment also led to significant dose-dependent and sustained reductions in ALAS1, ALA and PBG with single and multiple doses. In Cohort 1 of Part C, significant clinical activity was demonstrated in givosiran treated patients as evidenced by a 74% decrease in annualized attack rate, a 75% decrease in annualized hemin usage, and a mean maximum attack-free interval in the treatment period that is approximately 10.5 times that observed during the run-in period (up to 6 months). Interim progress and data from subsequent cohorts of Part C will be presented. ENCORE presentation.

565-EXPLORE: A Prospective, Multinational Natural History Study of Patients With Acute Hepatic Porphyria (AHP) With Recurrent Attacks

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The acute hepatic porphyrias (AHPs) are characterized by chronic disabling disease symptoms and potentially life-threatening neurovisceral attacks, often requiring urgent medical care and/or hospitalization. Three types of AHPs are acute intermittent porphyria (AIP), variegate porphyria (VP), and hereditary coproporphyria (HCP), which are rare diseases caused by a genetic mutation in one of the enzymes required for heme biosynthesis, resulting in accumulation of neurotoxic heme intermediates. EXPLORE is a prospective, international, observational study characterizing the natural history and clinical management of patients with AHP with recurrent attacks (>3 attacks/year) or who receive prophylaxis to prevent attacks. Patient medical history, physical examination and porphyrin precursors, along with questionnaires on porphyria activity, quality of life and health-care utilization were collected. A total of 112 patients with AHP enrolled from 13 countries, and have been followed for 6 to 12 months. Mean patient age is 39 years, with 89% female, and 93% with AIP, 4% VP, and 3% HCP. Patients reported 9.5 attacks in the 12 months prior to the study, with pain being the most common symptom in 99% of attacks. Chronic symptoms were also reported by 64% of patients in between attacks, with pain being the most frequent symptom. While on study, the annualized attack rate in patients was 4.4 attacks/person, of which 76% required treatment at a healthcare facility or with intravenous hemin. Patients reported diminished quality of life (EQ-5D-5 L), with the greatest impact seen in the domains of pain/discomfort, usual activities and anxiety/depression. EXPLORE is the first international natural history study in patients with AHP and recurrent attacks and demonstrates that patients suffer from both acute attacks, and chronic symptoms (most commonly pain) in between attacks that together result in a diminished quality of life. Updated 12-month data will be presented from the study. ENCORE presentation.

566 - Analysis of the *HPRT1* Gene Mutations in 6 Russian Families With Lesch-Nyhan Syndrome

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Lesch-Nyhan syndrome is an X-linked inborn error of purine metabolism caused by mutations in the *HPRT1* gene encoding the purine recycling enzyme hypoxanthine phosphoribosyltransferase (OMIM 308000). Urate hyperproduction and nephropathy in this disease can be combined with neurologic impairment, which leads to three overlapping clinical phenotypes: (1) the classical form of the Lesch-Nyhan disease characterized by cognitive impairment, dystonia, spasticity, self-injurious behavior, and urate nephropathy; (2) intermediate phenotype with hyperuricemia and variable neurological manifestations with no self-injurious behavior; and (3) hyperuricemia alone. The purpose of the study was to identify pathogenic mutations in the *HPRT1* gene in a cohort of patients with the Lesch-Nyhan syndrome in the Russian Federation. During the period 2012 to 2017, seven boys aged 16 months up to 14 years with different clinical variants of Lesch-Nyhan disease from six unrelated families have been identified and genetic analysis of the *HPRT1* gene has been performed by direct automatic sequencing of the entire coding region of the gene, including exon-intron boundaries. All of the patients examined in this research had hemizygous variants in the *HPRT1* gene; three of them have not been previously described. The deletion of three nucleotides within the reading frame c.24-26delCTG (CD070472), missense mutation c.610C> G (CM880047) and the new missense mutation c.464C> T (p.155Pro>Leu) were detected in three unrelated patients with the classic phenotype of the Lesch-Nyhan disease. In three probands with neurological dysfunction and hyperuricemia with no self-mutilating behavior we have detected two *HPRT1* gene variants: the new missense-mutation c.599G>A (p.200Arg>Lys) in one patient and c.635G> A (CM002634) in two siblings. In the 14-year-old boy who had hyperuricemia with no neurological dysfunction we detected a novel single-base substitution: c.359T> G (p.122Leu>Arg). The phenotypic manifestations of Lesch-Nyhan syndrome variants in Russian patients with previously described mutations did not differ from those of patients described in the world research. The exception was the child with mutation c.610C> G (CM880047), who had an extremely early formation of chronic renal failure at the age of 16 months. Three mutations in the *HPRT1* gene that have been probably unknown before were identified. According to the PolyPhen2 algorithm, these amino acid changes are probably pathogenic.

567 - Subacute Partially Reversible Leukoencephalopathy Expands the Phenotype of Aicardi-Goutières Syndrome

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Aicardi-Goutières syndrome (AGS) is a genetic encephalopathic and immunologic disorder that results from mutations in *TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1,* and *IFIH1* genes. It usually presents in infancy with basal ganglia calcification (mimicking congenital infection), white matter abnormalities, chronic CSF lymphocytosis, and increased serum levels of alpha-interferon. Despite the delineation of clinical, radiological, and biochemical criteria for the diagnosis of AGS, the range of phenotypic presentations associated with mutations in AGS related genes might be wider than previously claimed. We report five unrelated patients (four males) with subacute loss of developmental milestones, pyramidal signs and regression of communication abilities, with onset at ages ranging from 14 to 20 months. Recurrence of demyelination occurred in one patient at age of 5. Currently, age of patients varies from four to nineteen years. Pregnancy, labor and initial development was unremarkable for all patients and parental consanguinity was present in one individual. One patient received a yellow fever shot 2 weeks before disease onset. CSF at active phase of disease was normal in four patients (one had mild lymphocytosis in both events) and, on brain CT-scan, a single patient had a very subtle basal ganglia calcification. At onset, brain MRI showed in all patients, asymmetric demyelination of white matter with centrum semiovale involvement without contrast enhancement. All them were diagnosed and/or treated as acute demyelinating encephalomyelitis (ADEM) during the acute phase, despite the atypical presentation. At one year of follow-up, brain imaging was markedly improved but patients remained with residual spasticity and dysarthria. Diagnosis was established by whole exome sequencing or leukodystrophy genes panel, and three patients were found to carry biallelic pathogenic variants on *RNASEH2B* (2 homozygous p.Ala177Thr and 1 compound heterozygous p.Ala177Thr/p.Gln58*) and two in *RNASEH2A* (homozygous p.Ala249Val). This report expands the phenotype of AGS to include subacute developmental regression with partial recovery clinical and radiological reversible leukoencephalopathy. These clinical features might be misdiagnosed as ADEM.

568-Patients With Hypoxantine-Guanin Phosphoribosyltransferase Deficiency

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Hypoxantine-guanin phosphoribosyltransferase (HGPRT) takes part in purine metabolism and it is recessively inherited based on X with *HPRT1* gene. Clinical spectrum is extensive in enzyme deficiency and extends from hyperuricemia related to HGPRT, neurological dysfunction and classic Lesch-Nyhan disease. It was aimed to present two patients displaying two extreme clinical tables. A four-month old male infant had no active complaint and he was evaluated with increased serum uric acid level. His development and growth were normal. His serum uric acid increased as; 14.4 mg/dl (N:2–5.5 mg/dl). Hemizygote p.Y72C mutation was determined in HPRT1 gene. There was a phenotype- genotype correlation and he had no additional finding other than hyperuricemia concerning HGprt. A three-year old male patient was referred with tyrosine hydroxylase pre-diagnosis. Delay in the development steps, biting himself, repetitive urinary tract infections and uneasiness were present in the history. In the physical examination, there were conditions including growth and developmental delay, biting marks on hands and lips, dystonia, and uneasiness. Serum uric acid increased 11.8 mg/dl (N:2-5.5), medullary nephrocalcinosis was identified in the urinary ultrasonography. In the examination performed in epicenter, Homovanilic acid (HVA) was low (52 nmol/L) in brain cerebrospinal fluid (according to age N: 211-871), 5- hydroxyindol acetic acid (5–HİAA) was normal 310 nmol/L (N:105-299) and HVA/5-HIAA level was low 0.16 (N:1.5-3.5). However self-mutilation, repetitive urinary tract infection, hyperuricemia led to Lesch Nyhan disease pre-diagnosis. Hemizygote p.R51P mutation was established in the *HPRT1* gene. In the presence of unexplained hyperuricemia and/or unexplained neurological findings, growth deficiency, behavior issues, movement disorder and self-mutilation evidence; HGPRT deficiency should take part in the definitive diagnosis. It should be kept in mind that clinic spectrum is extensive.

R) Peroxisomal, Sterol, Bile Acid, Lipid and Lipoprotein Disorders (569 to 584)

569 - Glutathione and Sulfhydryl Levels in X-Linked Adrenoleukodystrophy Patients: The Effect of N-Acetyl-L-Cysteine

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X-linked adrenoleukodystrophy (X-ALD) is an inborn error of peroxisome metabolism. Hexacosanoic acid (C26:0) and tetracosanoic acid (C24:0) are saturated fatty acids that accumulate in tissues and body fluids. The exact mechanisms underlying brain damage in X-ALD are poorly elucidated, but some researchers have proposed that oxidative stress represents a hallmark in the pathogenesis of X-ALD. The antioxidant capacity of N-acetyl-L-cysteine (NAC) has been proposed based on data from *in vitro* studies, in which this compound has been shown to reduce oxidant induced cell damage, acting as a cysteine prodrug and a glutathione (GSH) precursor. GSH plays a wide variety of physiological roles and its antioxidant effects depend on the presence of the free sulfhydryl group as a ready source of reducing equivalents to quench radical species. NAC can also reduce disulfide bonds in proteins, scavenge free radicals and bind metals to form complexes. Considering that NAC has been proposed as a GSH precursor, the aim of this study was to analyze the in vitro effect of NAC on GSH and sulfhydryl levels in X-ALD patients. A total of eight X-ALD patients were included in this study (four heterozygotes women, one Addison only and three patients with cerebral form). Venous blood was collected under sterile conditions in heparinized vials, and blood cells were incubated with NAC (2.5 and 5 mM) for 6 hours. Then, the blood was centrifuged, GSH content was analyzed in erythrocytes and sulfhydryl levels were performed in plasma. All subjects or parents gave written informed consent. A significant reduction of GSH and sulfhydryl content was observed in X-ALD patients compared to the control group. Furthermore, 5 mM of NAC, in vitro, led to an increase in GSH content and sulfhydryl groups in these patients. The present results confirm former reports showing the role of oxidative stress on X-ALD and demonstrate the in vitro protective effect of NAC on GSH and sulfhydryl content in this disease. Therefore, this study underscores the pertinence of using antioxidants as an adjuvant therapy for X-ALD to improve oxidative imbalance in X-ALD patients.

570 - Expanding Our Understanding of Cerebral Adrenoleukodystrophy and Interim Phase 2/3 Results of an Autologous Hematopoietic Stem Cell Gene Therapy

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Objective: Adrenoleukodystrophy (ALD) is a rare X-linked metabolic disorder caused by mutations in the *ABCD1* gene that result in a deficiency in peroxisomal ALDP. The most severe form of ALD, the inflammatory cerebral phenotype known as cerebral ALD (CALD), is a rapidly progressive neurodegenerative disease that is often fatal. Allogeneic hematopoietic stem cell transplant (allo-HSCT) is an effective treatment when performed at an early stage of cerebral disease but is associated with serious and life-threatening complications. Lenti-D is an investigational autologous gene therapy designed to restore functional ALDP, without the risk of alloimmune-mediated complications. **Methods:** Study ALD-101 was designed to understand the natural history of CALD and outcomes after allo-HSCT. It was a multi-institutional, retrospective case review of 137 boys with CALD: 72 untreated and 65 allo-HSCT treated. Starbeam (ALD-102) is an international, single-arm study of Lenti-D in boys $\leq$ 17 years old with CALD. Outcome assessments include mortality, major functional disabilities (MFDs), changes in Neurologic Function Score and MRI lesion severity score, and rates of GVHD. The primary efficacy endpoint is the proportion of patients who are alive and have no MFDs at 2 years. **Results:** In ALD-101, estimated 5-year overall survival was 55% in untreated and 94% for allo-HSCT treated patients with early disease. Two-year MFD-free survival estimates for patients with active cerebral disease at baseline were 29% for untreated and 84% for allo-HSCT treated patients with early disease. Mortality rates post allo-HSCT were 8% (100 days) and 18% (1 year). Infections were common (29%) and despite prophylaxis, the incidence of GVHD was 59%. Initial results from Starbeam with all patients at 6 months of follow-up were promising, and updated safety and efficacy results will be presented. **Conclusions:** Retrospective data confirm the potential efficacy of allo-HSCT but highlight the need for alternative treatments for CALD. Lenti-D may offer an alternative to allo-HSCT, particularly for patients without HLA-matched-sibling donors. Additional follow-up is ongoing to fully assess long-term efficacy and safety.

571-Preliminary Results of Our Laboratory for Bile Acid Metabolism Disorders

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The measurement of plasma sterols is an important biomarker for diagnostic purposes in cholesterol and bile acid metabolism disorders. In this study, we aimed to analyze plasma sterol levels by GC-MS in our laboratory and diagnose bile acid and cholesterol metabolism disorders. This method is based on the principle of alkaline hydrolysis of sterol esters, extraction of free sterols with organic solvent and derivatization with BSTFA 1%TMCS + pyridine. After derivatization, we analyze the sterols on the GC-MS with SIM mode. Quantitative analysis of cholesterol, 7-dehydrocholesterol, cholestanol levels, as well as several sterols such as lathosterol, campesterol, and sitosterol are possible with this method. The measurement of unconjugated bile acids and alcohols with GC-MS is the most valid method. Good separation by gas chromatography and increased specificity and sensitivity by "selected ion monitoring" (SIM) on the mass spectrometer provides the advantage of this method. The reproducibility (CV) of the method for cholestanol and 7-dehydrocholesterol was 8.94% and 6.92%, respectively, and recovery was 95.63% and 99.3%. We analyzed plasma sterols of 95 healthy person for the determination of reference range in our laboratory. Reference range of cholestanol levels were determined as 3-16.9 μmol/L and this value was found compatible with the literature. External quality control of this analysis was provided by ERNDIM quality control program. Plasma sterol analysis was performed on 10 suspicious patients with bile acid metabolism disorders and we found that 2 patients had high cholestanol levels (105.17 and 114.18 μmol/L, reference range 3-16.9 μmol/L). Molecular genetic analysis was planned for these patients. The measurement of plasma sterols is an important parameter in the diagnosis of bile acid metabolism disorders and following treatment.

572-Next Generation Exome Sequencing in the Molecular Diagnosis of Primary Hypobetalipoproteinemias: Report of Four Turkish Cases

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Primary hypobetalipoproteinemias (HBLs) include three genetic disorders characterized by reduced plasma levels of total cholesterol, low density lipoprotein cholesterol (LDL-C), apolipoprotein B (apoB) and associated with a wide spectrum of clinical findings. Abetalipoproteinemia (ABL) and Chylomicron Retention Disease (CMRD) are two autosomal recessively inherited, rare pathologies of lipid metabolism, defined with a severe picture related to intestinal lipid malabsorption. However, familial Hypobetalipoproteinemia (FHBL) is a relatively frequent, autosomal dominant inherited metabolic disorder, often coexist with fatty liver and fat malabsorption

(in some homozygotes). We investigated four Turkish patients who were referred to our clinic for etiological evaluation of severe intestinal lipid malabsorption, hepatic steatosis and growth retardation, together with very low levels of total cholesterol, LDL-C and apoB, and suggesting the diagnosis of ABL or CMRD. To define the molecular defect, we performed a parallel sequencing (targeted next generation sequencing) of 16 primary HBLs related genes, including the main candidate genes (*APOB, MTTP, SAR1B* and *ANGPTL3*). Genomic DNA sequencing of the candidate genes showed that three patients, all born from consanguineous parents, were homozygous for pathogenic mutations in *MTTP* gene: i) a single nucleotide substitution in intron 7 (c.909+1G>A) which involves the donor splice site of this intron, causing a splicing defect with the formation of an abnormal mRNA predicted to encode a truncated protein devoid of function; this mutation has not been reported previously and found in two siblings; ii) a 17 nucleotide deletion in exon 1 (c.57del17) predicted to cause a frameshift with the formation of a premature stop codon at position 26 resulting in a truncated MTP protein of 25 amino acids, devoid of function. This mutation has been reported previously in an ABL patient. In the fourth patient, no definite pathogenic variants were found. This patient was found to be heterozygous for a rare missense variant in ANGPTL4 (p.Phe248Leu) which was found to be damaging in "silico". However, its clinical significance is uncertain. In conclusion, targeted next generation sequencing allows the rapid molecular diagnosis of rare genetic heterogeneous disorders like HBLs.

573 - Molecular Analysis of Hyperoxaluria Type I in Two Southwestern Colombian Patients Reveals the Same Mutation on the Alanine-Glyoxylate Aminotransferase Gene

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Primary hyperoxaluria (PH) type 1 is a rare autosomal recessive disorder characterized by an accumulation of calcium oxalate in various bodily tissues, especially the kidney, resulting in renal failure. Type I PH (MIM 259900) is caused by absent, deficient, or mistargeted activity of the liver-specific peroxisomal enzyme alanine-glyoxylate aminotransferase (AGT; MIM 604285). This enzyme catalyzes the transamination of L-alanine and glyoxalate to pyruvate and glycine. The enzyme defect has been attributed to mutation in the *AGXT* gene located on chromosome 2. The overproduction of oxalate results in the accumulation of nonsoluble calcium oxalate in various body tissues, with pathologic sequelae. The prevalence of PH1 is approximately 1-3 cases per million on world population. At least 1% of the End Stage Renal Disease seen in the pediatric population is attributable to PH1 in European and Japanese studies. Two infant female patients non-consanguineous from Colombian Southwestern region with clinical history of global neuro-developmental delay, convulsions, myoclonus, hypotonia, optic atrophy, Raynaud phenomenon, Hematuria per episode of renal stone resolved without Renal failure, pathologic fractures, and osteosclerosis were reported. Exome sequencing was performed on the iIlumina platform finding the same single nucleotide variant in both patients (C> T) on gene *AGXT*, registered as rs34116584, with heterozygous inheritance mode, an missense effect, and previously associated with pathogenicity. Hyperoxaluria continues to be a challenging disease and appropriate treatment requires a high index of suspicion and timely diagnosis. Prompt clinical recognition and distinction between these disorders is essential not only for timely intervention but also prognosis impacts in patients with hyperoxaluria.

574 - A Metabolomic Map of Mild Peroxisome Biogenesis Disorders Reveals Sphingomyelins as Novel Disease Biomarkers

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Peroxisomal biogenesis disorders in the Zellweger spectrum (PBD-ZSD) are pediatric metabolic and multisystemic diseases ranging from severe forms with seizures, neuronal migration defects, hypotonia, and death within 1 year of life, to milder forms with developmental delay, retinopathy, and hearing loss. Patients with PBD-ZSD exhibit impaired peroxisomal biochemical functions and have abnormal levels of peroxisomal metabolites. We ascertained a cohort of 19 individuals with clinically and molecularly characterized mild to moderate PBD-ZSD who presented with hearing loss, developmental delay, and varied observations of microcephaly, retinopathy, and movement disorders. Analysis of plasma samples included quantitative peroxisomal biochemical diagnostics in parallel with untargeted small molecule metabolomic profiling with detection of >650 named molecules. The cohort represented a mild subset of PBD-ZSD with mild peroxisomal biochemical alterations on targeted analysis. Untargeted metabolomic profiling showed anticipated alterations in pipecolic acid, long

chain fatty acids, long chain lysophosphatidylcholines, several bile acids, and plasmalogens. In addition, multiple sphingomyelin species were reduced in every patient sample. These perturbations in the plasma metabolomic profiles are unique, specific, and not previously seen in >1000 other samples analyzed as normal controls or for other indications. Reduced sphingomyelin was one of the strongest effects observed in these samples and detected only by untargeted metabolomic profiling. Interestingly, all of the metabolic abnormalities in PBD-ZSD were more pronounced in younger subjects, suggesting studies earlier in life reveal larger biochemical changes. As the clinical presentation of mild PBD-ZSD broadens and as clinical sequencing identifies novel genetic variants, the need for assessment of peroxisomal function increases. The use of untargeted metabolomics in the analysis of PBD can provide insight into pathogenesis, identify novel biomarkers of disease, and may provide novel indicators for therapeutic monitoring. Untargeted metabolomic screening identified several specific biomarkers that taken together allow for effective detection of these mild cases of PBD-ZSD, ending the diagnostic odysseys for many of these patients.

575 - Universal Population Screening of Cholesterol in Slovakia—Experiences of East Slovakia Center

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Background: Cardiovascular diseases (CVD) are the main reason of the mortality of an adult population in Slovakia. Risk factors begin to manifest and the process of atherosclerosis starts in childhood. At the same time, it is possible to influence this process and prevent its clinical manifestation. Significant influenceable risk factor is hypercholesterolemia. Childhood as a period of life with less number of comorbidities is the best period of identifying the genetic hypercholesterolemias. **Objective:** Authors present Slovak system of complex care for children and adolescents with high cardiovascular risk based on the screening of cholesterol and other atherosclerotic risk factors. **Report:** Principles are defined in the Regulation of Slovak Ministry of Health (49-51, vol. 52, 2004 year) for primary prevention of cardiovascular diseases in childhood and adulthood. Universal population screening of total cholesterol (TCH in mmol/L) and other atherosclerotic risk factors (family history- FH of CVD and/or dyslipoproteinemia, hypertension, obesity, smoking, life style, and so on) is performed in the age 11 and 17 years during regular preventive visits. Three levels of diagnostic, therapeutic and preventive care providing complex care based on TCH levels: • Primary care pediatricians: screening, rescreening if TCH is above 4.85; • Pediatric specialists (cardiologists and endocrinologists): TCH between 4.85 and 5.4 and positive FH or TCH between 5.4 and 6.5; • 3 specialized centers at university children's hospitals for individuals with TCH above 6.5 or below 2.85, and/or combination of risk factors, and/or serious FH. The tertiary center provides also molecular analysis and pharmacological therapy of familial hyperlipoproteinemias; The screening program, subsequent examinations and CVD risk individuals follow up is financed by health insurance. Although participation of families in the screening is voluntary, more than 900 000 of children and juveniles were screened in Slovakia from 2004. In East Slovakia Center, more than 2000 individuals with high CVD risk have been followed up, approximately 220 new per year. The alarming fact is that almost 20% of children with familial hypercholesterolemia are obese or overweight. **Conclusion:** Slovakia with Slovenia are the only countries with universal screening of cholesterol in childhood. No doubt it is a strategy with the highest chance for identifying the most risk individuals, though many questions are still opened and need next study.

576-Alpha-Methylacyl-CoA Racemase deficiency: Atypical Presentation in Four Patients

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Background: α-Methylacyl-CoA-racemase (AMACR) deficiency (OMIM 604489) is a rare peroxisomal disorder resulting in accumulation of the pristanic acid, DHCA, THCA and bile acid deficiency, with a variable age of onset from infancy to late adulthood. It has previously been reported in one neonate with cholestasis and coagulopathy, one adolescent and 9 adults presenting with neuropsychiatric disorders such as sensorimotor neuropathy, epilepsy, relapsing encephalopathy, thalamic lesions, type 2 diabetes, cataract, pigmentary retinopathy and tremor. Objectives: AMACR deficiency is usually described as an adult onset disorder but better definition of clinical phenotypes and natural history may support earlier diagnosis and treatment. **Case reports:** 12-year-old girl presented at the age of 10 months with coagulopathy, hepatic dysfunction, elevated pristanic acid, DHCA, THCA levels and homozygous c.59G<A mutation. She has been on follow-up since 11 years,

compliance to dietary treatment is poor, hepatic dysfunction still continues, no neurologic pathology but attention deficit hyperactivity disorder and subnormal IQ. Her brother, 20 years old university student, diagnosed at family screening with elevated pristanic acid level had hepatic dysfunction and hepatosteatosis but no neuropsychologic pathology. 19-year-old male patient referred recently with rhabdomyolysis following strenuous exercise, genetic evaluation revealed homozygous c.1006G>A mutation compatible with AMACR deficiency. Elevated pristanic acid level was correlated with the diagnosis. His 23-year-old sister also has the same mutation and pristanic acid elevation. They both have normal neuropsychologic evaluation. **Conclusion:** Adult onset neurometabolic disorders as AMACR deficiency may be underdiagnosed due to overlapping clinical symptoms with neuropsychological disorders. Early diagnosis is important as patients with this disorder may benefit from restricted dietary phytanic and pristanic acid intake.

577 - Hypertriglyceridemia Secondary to Congenital Generalized Lipodystrophy in a Four Months Old Baby

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Introduction: Lipodystrophy is a group of rare heterogeneous disorders characterized by the complete or partial loss or absence of subcutaneous adipose tissue. Lipodystrophy can be congenital or acquired, generalized or partial. Congenital generalized lipodystrophy (CGL) is a group of at least eight different genetic disorders characterized by a generalized lack of adipose tissue at birth and is accompanied by prominent muscularity and subcutaneous veins. Diagnosing CGL early is important as there are a number of associated metabolic derangements like insulin resistance, diabetes mellitus, hepatic steatosis and dyslipidemia that may be amenable to dietary and therapeutic interventions. **Case report:** Here we reported a four months baby boy HY who presented with feeding problem and failure to thrive since two weeks of age. Physical examination revealed an active baby with a pointed chin, triangular face and prominent eyes. He appeared generally "muscular" with almost absent subcutaneous fat. Abdomen appeared distended with mild hepatomegaly. Blood was noted to be lipemic during venipuncture. HY's pre-meal serum triglyceride was 13 mmol/l (<1.7). Total cholesterol, HDL cholesterol and LDL cholesterol were within the normal range. The diagnosis of Congenital generalized lipodystrophy due to mutation in 1 -acylglycerol-3-phosphate-O-acyltransferase 2 (*AGPAT2*) was confirmed by molecular genetic testing with two heterozygous mutations identified. c.646A>T. p. (Lys216*) was a known pathogenic mutation resulting in premature termination of protein translation. c.396_398delCAT. p.(Ile132del) was a likely pathogenic mutation resulting in an in-frame deletion of an isoleucine residue at position 132. HY was switched to a semi-elemental formula pepti junior which has 50% of the fat content in the form of medium chain triglyceride. Serum triglyceride level fell promptly to 1.9 mmol/l two weeks after starting pepti junior and has remained within the normal range since. **Conclusion:** The cardinal feature of lipodystrophy is the selective loss of subcutaneous adipose tissue giving rise to an apparent "muscular" appearance. It is important to recognize this feature clinically as this is often the first step towards diagnosing congenital lipodystrophy disorders. The associated metabolic complications are often amenable to treatment which may improve the long-term outcome for this group of patients.

578 - Investigation of *LDLR* Gene Mutations in Turkish Patients With Familial Hypercholesterolemia

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Familial hypercholesterolemia (FH) is characterized by severely elevated LDL (low-density lipoprotein) cholesterol levels that lead to an increased risk for cardiovascular disease. An estimated 70% to 95% of FH results from a heterozygous pathogenic variant in one of three genes (APOB, LDLR, and PCSK9). Many people have mutations in the *LDLR* (low-density lipoprotein receptor) gene that encodes the LDL receptor protein, which normally removes LDL from the circulation. The aim of our study was to examine the genetic background of Turkish patients suspected of FH. **Material and Methods:** In this study, we characterize the spectrum of mutations causing FH in 48 Turkish probands suspected to have FH. Next-generation sequencing was performed in all subjects for *LDLR* gene. **Results:** A total of 25 mutations (5 novel mutations) were detected in the LDLR of 40 subjects. For the patients (8 patients) who did not have a mutation in LDLR gene, sequencing analysis for ApoB, PCSK9 has been started. **Conclusions:** FH diagnosis have been achieved with a high success rate by using a combination of clinical criteria and targeted next-generation sequencing.

579 - Case Report of a LAL-D Infant Presentation: Going Beyond Known Boundaries

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The early onset lysosomal acid lipase deficiency (LAL-D) phenotype is usually fatal in the first year of life. Recently, enzyme replacement therapy (ERT) for LAL-D became available. The main goal of this report is to present a successful case of an LAL-D infant presentation on ERT since she was born. S.R., female, consanguineous parents, brother not treated deceased with LAL-D, 98 days old. She was born in good conditions: Wt = 3210 g, Ht = 48 cm. A new homozygous mutation was detected prenatally: exon 4 - gene LIPA, c266>c (p.leu89pro). In silico analysis suggested a pathogenic mutation. Hospital discharge on second day of life and ERT with Sebelipase Alfa, on fourth day of life: 3 mg/kg/every week (EW). Increased dose of ERT to 5 mg/kg/EW (3-6 mg/kg/EW during 3 months) due to persistence of the symptoms. Feeding with a polymeric formula with no lipids, designed to fatty acid β-oxidation defects. Baby was not growing, presenting severe undernutrition and intestinal malabsorption (12%) and diagnosed with heterologous protein allergy. Exchanged to amino acids formula. Abdominal ultrasounds showed hepatomegaly and adrenal calcifications. 3 months old: admitted at ER with severe abdominal distension, pain, sepsis and went to laparotomy. Surgery findings: fat deposition on lymph nodes and bowel distension. Post-op: satisfactory evolution, but initially with total parenteral nutrition (TPN). Presented hyperthermia during ERT: premedication (antihistaminic and antipyretic drugs) before ERT. 7 months old: increased frequency of ERT to 5mg/kg/dose twice a week. Using central venous access for TPN, had 3 septic episodes. Afterward, she improved intestinal absorption, allowing the advance of enteral nutrition (76% absorption). Last nutritional assessment (1 y 19 d): Wt = 6.265 g; Ht = 61.5 cm; IMC = 16.45kg/m^2. Laboratories are getting better from the beginning (AST, ALT, gamma-GT; total cholesterol and fractions; triglycerides and hemoglobin). Diet: used modulated amino acids formula until development of tolerance of intact protein. Now she is receiving a skimmed modulated formula: 185 kcal/kg/d, 4,9 g ptn/kg/d, 10% lipids; complimentary food; multivitamins. Diet goals: catch-up growth and neurodevelopment. To the best of our knowledge, she is one of the 6 children alive from all over the world with LAL-D on ERT. The very early diagnosis and treatment (ERT) combined with an appropriate management of the serious complications allowed a survival in good conditions.

580 - Diagnosis of Peroxisomal Storage Disorders by Very Long Chain Fatty Acids analysis: Experience at Quest Diagnostics Nichols Institute Biochemical Genetics Laboratory

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Background: Peroxisomal disorders are a group of inherited defects caused by either the absence of normal peroxisomes or impaired peroxisomal function. Peroxisomal disorders are subdivided into two major categories: peroxisome biogenesis disorders (PBD), which result from a failure to form intact, normal peroxisomes and lead to multiple metabolic abnormalities, and those disorders resulting from the deficiency of a single peroxisomal enzyme or transporter. The clinical and biochemical findings between the two groups of disorders are not discrete, and there can be significant overlap between them; however, PBD disorders are generally more severe. For some disorders, early recognition can enable diagnosis and/or treatment before the onset of symptoms or before irreversible damage is sustained. Once a biochemical diagnosis is established, familial mutation studies can be helpful in identifying carriers or other affected family members, as well as in prenatal diagnosis for future pregnancies. **Study Goal:** The goal of the current investigation was to examine the prevalence of different types of PBD in patients diagnosed at Quest Diagnostics Nichols Institute. **Results:** Out Of 432 patients that were diagnosed primarily by very long chain fatty acids (VLCFA) testing in our laboratory, X-linked adrenoleukodystrophy (X-ALD) was the most common diagnosis (n = 214, 50% of positives), followed by X-linked adrenomyeloneuropathy (X-AMN) (n = 130, 30% of positives), Zellweger spectrum disorders (n = 10, 2.3% of positives), Refsum disease (n = 3, 0.7% of positives) and α-methylacyl-CoA racemase deficiency (n = 3, 0.7% of positives). In addition, we were able to diagnose 73 X-ALD carriers through VLCFA analysis. **Conclusions:** Our results demonstrate how biochemical genetic testing in a clinical setting can be used to detect and classify peroxisomal disorders. Although individually rare, the number of positive cases we identified suggests that the prevalence of these disorders may be higher than previously reported, especially since clinical symptoms can be very mild in some cases. If specific clinical symptoms are recognized, diagnoses of peroxisomal disorders can be easily confirmed by VLCFA analysis.

581 - *PEX16* Mutations Presenting as a Mild Peroxisome Biogenesis Disorder

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Background: PEX16 plays an important role in the peroxisomal assembly and is associated with severe clinical phenotype of Zellweger syndrome. However, patients with milder phenotypes have also been described. The milder form of PEX16 is a progressive disorder, characterized by lower limb spasticity and ataxia resulting in wheelchair dependence in the first decade but the cognition is relatively preserved. **Objective:** To expand the phenotype of PEX16 by describing the clinical, biochemical and molecular characteristics of an Arab family with two affected children who presented with relatively mild but progressive disease. **Patients:** The index case is an 11-year-old male born to consanguineous parent after uneventful pregnancy. At birth, the physical examination was unremarkable except for bilateral

deformed thumb. Development in the first year was normal. Around two year of age patient was noted to have developmental regression and progressive spastic diplegia in addition to midthoracic syrinx which was treated with syringo-pleural shunt. At 7 years of age he became wheelchair bound. He has difficulties with coordination, progressive dysarthria with relatively normal intelligence. The brain MRI shows progressive demyelination. The younger sibling, one-year-old female has similar bilateral thumb deformities and missing phalanges in great toes with normal development, neurological examination and normal brain MRI. **Result:** The plasma very-long-chain fatty acids showed mildly elevated C26 and C26:0/C22:0 ratio with normal plasmalogen levels. Genetic analysis demonstrated a novel homozygous variant in the *PEX16* (c.859C>T). The immunofluorescence microscopy analysis with antibodies against the peroxisomal matrix enzyme catalase in fibroblasts of index case showed that most cells have slightly enlarged peroxisomes. The biochemical profile of the younger sibling was similar to the index case and the same genotype was confirmed by mutation analysis. Further analysis of the family revealed that sibling who don't carry the genotype had the same skeletal manifestation. **Conclusion:** Although *PEX16* plays a major role in peroxisome assembly and is anticipated to result in severe disease, a relatively mild phenotype was observed in our patients. Based on the family testing, the skeletal manifestations do not seem to be caused by *PEX16* mutation. Taken together, this highlights the possibility of occurrence of more than one autosomal recessive conditions in highly inbred families.

582 - Serum of VLCFA Levels in the Diagnosis of X-ALD/AMN Heterozygotes

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X-linked adrenoleukodystrophy, X-ALD (MIM 300100), is a neurodegenerative peroxisomal disorder caused by mutations in *ABCD1*. X-ALD is clinically heterogeneous, and can present at a variety of ages. Biochemically, it is characterized by the accumulation of very long-chain fatty acids (VLCFAs), particularly lignoceric (C24:0) and cerotic (C26:0) acids in blood and tissues. **Subjects:** Sixteen patients with adrenomyeloneuropathy (AMN) aged 28–51 years were diagnosed based on the clinical, biochemical, radiological, and genetic findings. Serum VLCFA levels in eighteen asymptomatic X-ALD/AMN patients' daughters, aged 2.5 to 23 years were investigated. **Result and conclusion:** In all samples from the patients' daughters, the presence of increased VLCFA levels was proven (C24:0/C22:0 = 1.102 $\pm$ 0.090 and C26:0/C22:0 = 0.019 $\pm$ 0.016 mg/mL). Mean VLCFA levels in patients' daughters were elevated at heterozygote levels (60 females, range 8-59 years). No statistically significant differences were found between the VLCFA level of the patient's daughters, obligatory heterozygotes, and the X-ALD / AMN heterozygous group. These results underline the usefulness of the VLCFA assay as a reliable biomarker in the diagnosis of X-ALD / AMN heterozygotes.

583 - A Case Report of a 6 Years Old Turkish Patient With Neonatal Adrenoleukodystrophy and a Mutation in the *PEX26* Gene

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Background: Peroxisome biogenesis defects are divided into 16 subgroups due to the clinical heterogeneity. Neonatal adrenoleukodystrophy (NALD) is a very rare autosomal recessively inherited milder form of this spectrum. The mutations in *PEX26* gene causes NALD. The exact disease-causing mechanism of *PEX26* mutation is not fully understood. Clinical findings are; hypotonia, failure to thrive, severe psychomotor retardation and seizures in the first weeks of life. **Case Presentation:** Here, we report a case with neonatal adrenoleukodystrophy. A 6-year-old male admitted to our clinic for etiological evaluation of mental-motor retardation and deafness. He was the fifth child of a consanguineous Turkish parents, born at term without a complication. Psychomotor retardation became evident at the age of 8 months, when the family recognized the deafness and blindness of the patient. He was never able to talk or walk. His physical examination revealed mild dysmorphism, growth retardation, failure to thrive; height: 101 cm (<5. P), weight: 15,5 kg (<5.P), nystagmus, hypotonia and 6 cm hepatomegaly. Laboratory results were compatible with elevated levels of serum ACTH and very long chain fatty acids (VLCFA). MRI showed cerebellar atrophy and focal nonspecific signal intensities in the cerebellum and midbrain. Adrenal insufficiency with neurological problems and the increased levels of VLCFA led us to the suspicion of a peroxisomal disease. A homozygous mutation p.R98 W (c.292C>T) in the *PEX26* gene was detected. **Conclusion:** Neonatal adrenoleukodystrophy is a very rarely seen peroxisomal disease. The patients with neuromotor regression, hepatomegaly, deafness, blindness, and adrenal insufficiency must be evaluated for a peroxisomal disorder for both genetic counseling and treatment options.

584 - NV1205 for the Treatment for X-Linked Adrenoleukodystrophy (X-ALD)

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X-ALD is a genetic disease due to mutation of the *ABCD1* gene on the X-chromosome with a worldwide incidence of 1:17,000. *ABCD1* encodes a peroxisomal membrane protein that facilitates transport and degradation of very long chain fatty acids (VLCFA) in the peroxisome. *ABCD1* mutations result in an inability to degrade VLCFA. VLCFA accumulates in all tissues, and accumulation in the neuronal white matter and adrenal cortex leads to Addison's disease and varying degrees of neurological symptoms. Approximately 40% of affected young males develop a cerebral form of the disease, or childhood cerebral ALD (CCALD), characterized by cerebral inflammation, rapidly developing white matter lesions, severe neurological symptoms, vegetative state, and death in a few years. Adrenomyeloneuropathy (AMN) is the adult form of the disease manifested by spinal cord axonopathy leading to slowly progressive neurological symptoms such as paraparesis, gate disturbances, and fecal and urinary incontinence. The only available treatment for CCALD is hematopoietic stem cell transplant that must be performed early in the course of the disease to be effective. There is no therapy available for AMN. *ABCD2*, a gene encoding a homologous peroxisomal transporter to *ABCD1,* is an attractive therapeutic target with the possibility of correcting the underlying biochemical defect with potential disease modifying properties. NV1205 is an orally bioavailable small molecule that upregulates the *ABCD2* gene. Pharmacological studies in *ABCD1* knock-out mice treated with NV1205 have shown that long-term systemic administration of NV1205 results in VLCFA reductions in the central nervous system, adrenal cortex, and blood. The results support the upregulation of *ABCD2* by NV1205 to complement the genetic defect as a treatment modality for all phenotypes of X-ALD.

S) Lysosomal Disorders: Mucopolysaccharidoses, Oligosaccharidoses (585 to 676)

585 - Improvement of Diagnostic Technique of MPS VII in Dried Blood Spots

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The mucopolysacharidosis VII is caused by deficiency of the β-glucuronidase enzyme (GUSB). The aim of this work is to miniaturize, correlate and validate the method for measuring the activity of GUSB in 1.2 mm DBS and then determine the biochemical parameters of this enzyme. The technique for the measurement of GUSB activity in 3 mm DBS was adapted to 1.2 mm and the reagents were reduced 4-fold. Correlation tests were performed and after we used for the validation tests: Interassay, Interpersonal and Intraassay. For the optimum pH determination, we used the values of pH 2.0 to 5.4. The substrate curve was performed with 1.25 to 12.5 mM of substrate and according to this analysis, the concentrations 0.25 to 1.50 mM were chosen to determine Km and Vmax (Lineweaver-Burk plot). Comparing the 3.0 x 1.2 mm DBS, we had a significant correlation (Pearson $r = 0.7908$). This method was validated and the coefficients of variation were: intraassay 9.3%, interassay 11.9%, and interpersonal 9.4%. We observed that pH 4.4 showed higher levels of enzyme activity. Km of GUSB in DBS from healthy subjects was calculated as 1.77 mM and Vmax found was 460.94 nmol/h/mL. These results show that it is possible to measure the activity of GUSB with a smaller DBS diameter and a 4-fold reduction in the volume of the reagents, as has been done with other lysosomal hydrolases (chitotriosidase, alpha-iduronidase, and beta-glycosidase), obtaining the same results from former method. The miniaturization of the MPS VII diagnostic method allows sample saving, which can then be used to screen for other inborn errors of metabolism, and reagents, reducing the cost of the analysis. The results obtained in the validation of the miniaturized method indicate that the three coefficients of variation are within the acceptable value. This demonstrates the accuracy and reproducibility of the method used. The pH of 4.4 was then determined to be the optimum pH of the enzyme. The former technique, with a 3 mm DBS, uses 4.8 pH for the enzymatic assay, the same pH used in the measurement technique of GUSB activity in plasma. For the first time, optimal pH studies were performed to measure enzymatic activity in blood collected on filter paper. The Km of GUSB in leukocytes from healthy subjects was calculated as 1.77 mM and the Vmax found was 460.94 nmol/h/mL. There are no other papers in the literature citing kinetic data for this enzyme, so we cannot compare them. Supported by CNPq.

586 - Ocular Changes in Patients With Mucopolysaccharidosis of Different Forms: 5-Year Experience

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Objective: To describe ocular changes in patients with mucopolysaccharidosis of different forms. **Materials and Methods:** We analyzed the results of 78 children examination (156 eyes) at the age from 1,5 to 18 years, of them 11 children-with MPS I type, 38 children- with the MPS II Hunter, 18 - with the MPS III Sanfilippo, 4 patients with IV type and 6 children with the MPS VI Maroteaux-Lamy. The ophthalmic investigation included visometry, IOP measurement (noncontact air-puff tonometry), biomicroscopy, ophthalmoscopy, retinoscopy **Results**: 78 patients were identified with a diagnosis of MPS. The anomaly was bilateral in 78 children. The ocular management of patients with MPS of different forms were conservative

because of their intellectual impairment. Vision remained stable in 10 patients. Deterioration in other 68 patients was related to progressive corneal clouding and refraction errors, amblyopia. Three patients were diagnosed with having intraocular pressure-related optic nerve damage. 5 patients had symptoms of ocular hypertension. Other 70 patients had no previous studies of the incidence of ocular hypertension or glaucoma. Biomicroscopy in 36 children with megalocornea, the cornea diameter reached 13 mm and dilation of conjunctiva vessels and thickening of deep layers of cornea were detected. In 16 patients, marked corneal opacity bullous regeneration was seen. Minor changes in pigmentary epithelium and chorioids were detected 24 patients with scleral defect walls and edges. Patients with MPS I were hypermetropic and three patients were myopic. Three patients with MPS III and one with MPS II had severe difficulties in speech, their vision was measured with cards In 26 children, the retina vessels had dilation, wall thickening, and normal pathway. In 68 cases on retinoscopy, astigmatism was detected, high-degree hypermetropia, and high-degree amblyopia. **Conclusion:** In MPS I macrocornea, corneal clouding, conjunctiva vessels dilation, retinal dystrophy and increase in intraocular pressure were detected. In the MPS-II megalocornea, cornea opacity localizing in the cornea deep layers and stroma thickening were observed. The excavation of optic disc in MPS II was much higher than in other types of MPS. MPS III is characterized by hypertelorism and proptosis, no changes in retina. In the MPS-IV corneal deposits like "dust", fundus changes in the form of optic disk edema and secondary optic atrophy were seen.

587 - Adult-Onset Diagnosis of Mucopolysaccharidosis (MPS) Type IIIA

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Mucopolysaccharidosis IIIA (Sanfilippo A) is an autosomal recessive disorder caused by the *SGSH* gene mutation. Clinical features include severe neurologic degeneration, developmental delay and behavioral problems between 2-6 years of age, with death by the age of 30 years. The Genetics of Learning Disability (GOLD) Study performed whole exome sequencing (within UK10 K study) in patients ascertained through UK Genetics Centers having Intellectual Disability of unknown etiology. 41-year old man who presented with mild learning difficulties was diagnosed with MPS IIIA at 40 years of age. His brother, 43 years old, is severely affected with the progressive course of the condition; disabled, nonambulant, and nonverbal. The GOLD study revealed that both brothers were compound heterozygous for the c.220C>T p.(Arg74Cys) and c.1063G>A p.(Glu355Lys) variants. Sulfaminidase activity was deficient at 0.2 nmol/mg/17 hrs. Urine GAGs were raised at 13.6 mg/mmol creatinine. In childhood, his problems started from ear infections at the age of 5. Despite the need for some support in primary and secondary school, he completed his education without major problems. He had remained physically active until his late 30s when he started requiring some support from carers. He was diagnosed with retinitis pigmentosa following impairment of night vision in his late teens. He also suffers from hearing impairment. On examination, he walked independently (height: 179 cm, BMI 22.8 kg/m2). There were no obvious skeletal abnormalities (apart from some mildly broad wrists) or behavioral disturbance. MPS IIIA may present only with learning disability with no significant clinical morbidities. Patients affected with classical mutations are able to live to their 40s. Both brothers are some of the longest living siblings with MPS IIIA. The patient was mildly affected while his brother who has the same pathogenic variant has followed a more typical course. This case shows the importance of considering ultra-rare disease diagnosis in nonclassical patients.

588 - Development of a Scoring System in the Diagnosis of Mucopolysaccharıdoses

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Objective: Mucopolysaccharidoses (MPS) are disorders characterized by a wide variation in symptoms and progression rates. Usually patients with nonspecific symptoms are not recognized by the physicians and diagnosis is often delayed. In severe forms, the disease progresses very quickly and goes to death before 10 years of age. Therefore, early diagnosis and starting enzyme replacement therapy before the irreversible defects are crucial. Enzyme analysis of every suspected case is very expensive. For this purpose, selective clinical diagnostic methods (scoring system) are needed to ensure the recognition of pediatric patients with MPS. This paper discusses a scoring system based on the clinicopathologic features and their potential usefulness in case finding studies. **Methods:** 107 Patients attended to 3 metabolic clinics from different regions of Turkey were enrolled in this retrospective study. The MPS Physical Symptom Scoring System (PSSS) based on the general clinical features of the disease was applied to all patients. This scoring system was based on literature review and clinician feedback. A standardized testing protocol and scoring rules were created. Major clinical features such as; skeleton anomaly, mental retardation,

psychomotor retardation were scored "2." Minor clinical features such as; visual disorder, dermatological manifestations and progressive hearing impairment were scored "1." Six points and over were accepted as a risk for MPS. **Results:** Out of 64 patients with score 6 and over, 59 (92.18%) diagnosed a type of MPS by enzyme and/or mutation analysis. On the other side, 9 of 59 patients confirmed diagnosis of MPS had scoring 5 or less. Sensitivity, specificity, positive, and negative predictive values of the scoring system were 86.7%, 90.5%, 92.2%, and 84.2%, respectively. **Conclusion:** Recently developed MPS specific PSSS seems to be reliable and could be useful in future investigations and case finding studies.

589 - Clinical, Biochemical, and Molecular Features of Iranian Families With Mucopolysaccharidosis

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Background: Mucopolysaccharidoses (MPS) are a group of rare inherited lysosomal storage diseases (LSD) caused by impaired degradation of the glycosaminoglycans (GAG) in the lysosomes. This metabolic disorder is characterized by abnormalities in multiple organ systems and reduced life expectancy. This study aims to ascertain genetic variants that may cause the most common type of MPS in Iranian families. **Methods:** Eight patients from Iranian families with MPS were investigated for the genetic defect. Clinical, biochemical and genetic data were examined to identify suspected patients with MPS. Peripheral blood samples were obtained from all family members after obtaining informed consent. Based on clinical findings, to identify causative variants three different genetic tests including Sanger sequencing for two genes (IDUA and IDS), targeted Next Generation Sequencing (10 genes) and Whole Exome Sequencing (WES) techniques were applied. **Results:** A total of nine different mutations were identified in four genes (*IDUA*, *IDS*, *SGSH* and *NAGLU*), including seven novel mutations, and two previously reported missense mutations. All identified mutations were confirmed by Sanger sequencing and segregated within the family. One patient out of nine was a compound heterozygote carrying two mutations in the *NAGLU* gene. **Conclusions:** This study identified novel mutations in MPS related genes determining the type and subtype of the disease by molecular approaches. The result of this study positively contributes to the mutation spectrum of *IDUA, IDS, SGSH and NAGLU* genes in Iranian families. It may also enrich genetic counseling for rapid risk assessment and disease management.

590 - Clinical and Genetic Characteristics of Mucopolysaccharidosis Type IV A (Morquio A Syndrome) in Russian Patients

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The purpose of the study is to reveal clinical and genetic characteristics of patients with Morquio A syndrome based on the DNA diagnostics method which was first developed in Russia for this orphan disease. We examined 14 patients with Morquio A syndrome aged from 2 to 34 years (the sex ratio is 1:1). All of the patients had typical signs of the "Morquio-like" phenotype such as dwarf growth, keeled thoracic deformation, valgus knee joint, short neck, increased volume and stiffness of large joints and hypermobility of small (interphalangeal) joints, rough features, moderate hepatosplenomegaly, corneal opacity, and hearing loss. The DNA diagnostics was performed in 8 patients. In 3 probands the second mutation in the *GALNS* gene has not yet been determined. The character of 13 identified mutations was as follows: 7 missense mutations; 2 splice site mutations and 4 deletions found in 2 siblings in the homozygous state (*del ex 14/del ex 14* and *c.1516 delA/c.1516 delA*). Clinical symptomatology: out of 8 children examined, 2 had severe symptoms of muscular hypotonia, which was most likely formed due to the narrow cervical canal caused by deposition of keratan sulfate. These children were capable of moving only with a wheelchair. The *GALNS* gene analysis revealed missense mutations p.Gly44Val/p.Trp230Arg and P.Cys165Tyr/p.Asp447His. In two patients with the *GALNS* gene deletion, the disease was characterized by severity and decreased intelligence, which, as a rule, is not typical of patients with Morquio A syndrome. The child with *GALNS* gene deletion *c.1516 delA/c.1516 delA* died suddenly at the age of 2.5. According to the anamnesis, his older sister died also suddenly at the age of 10 with the same symptomatology. The autopsy was not conducted due to religious beliefs of the family. Thus, this research allowed us to detect clinical and genetic relationships in patients with mucopolysaccharidosis type IV A.

591 - Natural History and Clinical Assessment of Taiwanese Patients With Mucopolysaccharidosis III

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Mucopolysaccharidosis type III (MPS III), or Sanfilippo syndrome, is caused by a deficiency in one of the four enzymes involved in the lysosomal degradation of heparan sulfate. Four MPS III types have been recognized, characterized by a large phenotypic heterogeneity. MPS III has a variable age of onset and variable rate of progression. A retrospective analysis was carried out of 28 patients with MPS III (3 with IIIA, 23 with IIIB, and 2 with IIIC; 15 males and 13 females; mean age, 10.1 $\pm$ 5.7 years; median age, 8.2 years; age range, 2.7-26.5 years) seen in 6 medical centers in Taiwan from January 1996 through March 2017. The diagnosis of MPS III was confirmed by measurement of enzymatic activities of particular lysosomal hydrolases in leukocytes or skin fibroblasts and/or two-dimensional electrophoresis of urinary glycosaminoglycans. The mean age of confirmed diagnosis was 4.7 $\pm$ 2.1 years. The mean z scores for height, weight, and BMI at the time of the latest medical records were -0.47 ± 1.97, 0.02 ± 1.49, and -0.17 ± 0.95, respectively. Both z scores for height and weight were negatively correlated with age ($r = -0.693$ and -0.718, respectively; $P < .01$). The most prevalent clinical manifestations were speech delay (100%), mental retardation (100%), hirsutism (93%), hyperactivity (79%), coarse face (68%), sleep disorders (61%), and hepatosplenomegaly (61%). Ten patients (36%) had epilepsy, and the mean age of the first time of seizure attack was 10.7 $\pm$ 4.8 years. Thirteen patients (46%) experienced at least one surgical procedure. The most prevalent surgical interventions were adenoidectomy (11%), tonsillectomy (11%), supraglottoplasty (11%), inguinal hernia repair (11%), percutaneous endoscopic gastrostomy (11%), ear tube insertion (7%), and epiphyseal surgery (4%). The most prevalent cardiac valve abnormalities were tricuspid regurgitation (72%) and mitral regurgitation (67%). At the time of the present study, 7 of 28 patients have passed away at the mean age of 13.0 $\pm$ 3.0 years. An understanding of the natural history involved in MPS III may allow early diagnosis of the disease. All affected patients experienced significant functional limitations, and adequate evaluations and timely management may improve their quality of life. These findings and the follow-up data can be used to develop quality of care strategies for patients with MPS III.

592 - The Levels of Urinary Glycosaminoglycans in Patients With Mucopolysaccharidoses and the Effects of Enzyme Replacement Therapy Detected by Liquid Chromatography-Tandem Mass Spectrometry

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The Mucopolysaccharidoses (MPSs) are a group of rare lysosomal storage disorders characterized by the accumulation of glycosaminoglycans (GAGs) in various tissues and organs. The dimethylmethylene blue (DMB) spectrophotometric method commonly used in most biochemical genetics laboratories relies on a non-specific total GAG analysis which has led to false positive results, and even false negative results (mainly for MPS III and IV patients). The purpose of this study was to validate a reliable liquid chromatography/tandem mass spectrometry method (LC-MS/MS) for the urine quantitation of three GAGs [dermatan sulfate (DS), heparan sulfate (HS), and keratin sulfate (KS)] to help make correct diagnosis of MPS and evaluate the effectiveness of enzyme replacement therapy (ERT). Sixty-six patients with MPS (age range, 0.7 to 49.3 years; 14 with MPS I, 14 with MPS II mild form, 9 with MPS II severe form, 11 with MPS III, 10 with MPS IVA, and 8 with MPS VI) were evaluated of their three urinary GAGs (DS, HS, and KS) by LC-MS/MS method, as well as non-specific total urinary GAG by DMB spectrophotometric method. The mean levels of DS (reference < 1.68 μg/mL) were 194.6, 118.0, 253.8, and 108.9 μg/mL for MPS I, MPS II mild form, MPS II severe form, and MPS VI, respectively. The mean levels of HS (reference < 3.88 μg/mL) were 6.2, 7.3, 446.6, and 260.7 μg/mL for MPS I, MPS II mild form, MPS II severe form, and MPS III, respectively. The mean level of KS (reference < 17.8 μg/mL) of MPS IV was 401.0 μg/mL. The mean DMB ratios were 601.0, 367.0, 913.8, 654.4, 142.0, and 853.7 for MPS I, MPS II mild form, MPS II severe form, MPS III, MPS IV, and MPS VI, respectively. Among these 64 patients, 20 patients with mental retardation had higher levels of HS, compared with 44 patients without mental retardation (328.8 vs. 4.1, p)

593 - Mutation in the *VPS33A* Affects Metabolism of Glycosaminoglycans: A New Type of Mucopolysaccharidosis With Severe Systemic Symptoms

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Mucopolysaccharidoses (MPS) are a group of genetic deficiencies of lysosomal enzymes that catabolize glycosaminoglycans (GAG). Here we report a novel MPS-like disease caused by a specific mutation in the *VPS33A* gene. We identified several Yakut patients showing typical manifestations of MPS: coarse facial features, skeletal abnormalities, hepatosplenomegaly, respiratory problems, mental retardation, and excess secretion of urinary GAG. However, these patients could not be diagnosed enzymatically as MPS. They showed extremely high levels of plasma heparan sulfate (HS, one of GAG); 60 times the normal reference range and 6 times that of MPS patients. Additionally, most patients developed heart, kidney, and

hematopoietic disorders, which are not typical symptoms for conventional MPS, leading to a fatal outcome between 1 and 2-years old. Using whole exome and Sanger sequencing, we identified homozygous c.1492C>T (p.Arg498Trp) mutations in the *VPS33A* gene of 13 patients. VPS33A is involved in endocytic and autophagic pathways, but the identified mutation did not affect either of these pathways. Lysosomal over-acidification and HS accumulation were detected in patient-derived and VPS33A-depleted cells, suggesting a novel role of this gene in lysosomal functions. We hence propose a new type of MPS that is not caused by an enzymatic deficiency.

594 - Enzyme Replacement Therapy Recovers the Testicular Interstitium With Progressive Damage Observed in the Mucopolysaccharidosis Type I (MPS I) Animal Model

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Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disease caused by a dysfunction of alpha-L-iduronidase (IDUA), a lysosomal hydrolase that degrades glycosaminoglycans: heparan and dermatan sulfate. MPS I is a progressive and multisystemic disorder that affects many organs and tissues as a consequence of the unsuitable storage of substrates inside lysosomes. In order to evaluate the effect of MPS I progression on male reproductive tract, we performed testicular histological sections of *Idua*−/− C57BL/6 J mice with different ages: 3, 6, 8, and 12-month-old. Six and 8-month-old *Idua*−/− mice were submitted to enzyme replacement therapy (i.v. injection of 1.2 mg/kg of Laronidase® every 2 weeks): one group treated since neonatal period and other group from 6-8 months old. We also treated an *Idua*−/− group with cathepsin B inhibitor (10 mg/kg/day i.p.) from 2-6 months old, since high levels of cathepsin B are associated to the MPS I histopathology, such as cell death and inflammation. We performed testicular histological sections in all groups, counted Leydig and interstitial vacuolated cells, when present. Interstitium is the most damaged testicular compartment of *Idua*−/− mice, with numerous vacuolated cells, that are more frequent in 6-month-old compared to 3-month-old *Idua*−/− ($P = .008$) and in 12-month-old compared to 6-month-old *Idua*−/− mice ($P = .023$). We found less vacuolated cells in groups submitted to enzyme replacement therapy started at either neonatal period and adult age, compared to 6-month-old untreated mice ($P = .009$; $P = .014$, respectively). No difference was found in *Idua*−/− mice treated with cathepsin B inhibitor. Leydig cells, the major source of testosterone, were not quantitatively altered among all groups. Plasma testosterone and progesterone, previously accessed in 6-month-old *Idua*−/− untreated mice, were similar to those from same aged *Idua*+/+. We concluded that the absence of IDUA progressively influences on the vacuolization of a specific cell type located in testicular interstitial compartment and does not influence on Leydig cell number. Under enzyme replacement therapy, the interstitial compartment is recovered, regardless the period of treatment beginning. **Financial support**: AFIP, CAPES and CNPq.

595 - Individual Heat Map Assessments Demonstrate ERT Treatment Response in Highly Heterogeneous MPS VII Study Population

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Sly syndrome (mucopolysaccharidosis, MPS VII) is an ultra-rare, progressively debilitating, life threatening lysosomal disease in which patients are deficient in the enzyme, beta-glucuronidase (GUSB). Vestronidase alfa (UX003) is a recombinant human GUSB being developed as a potential enzyme replacement therapy for MPS VII. The Phase 3 Study was a randomized, placebo-controlled, Blind-Start study of 4 mg/kg vestronidase alfa given every-other-week for 24-48 weeks which incorporated several innovative elements to address drug development challenges associated with ultra-rare, life-threatening diseases. Because of extreme disease rarity, eligibility criteria were broad to be inclusive of all available patients thus resulting in a highly heterogeneous study population with variable clinical manifestations and physical +/or cognitive limitations. To provide an integrated view of the diverse data, the changes from baseline in study assessments for each subject were grouped into 3 functional categories for evaluation: Mobility, Fatigue, Fine Motor + Self-care, and analyzed as subject-level heat maps. Mobility included the 6-minute walk test, 3-minute stair climb test, BOT-2 gross motor function subtests, and patient reported outcome (PRO) data related to movement, pain, and ambulation. Fatigue included the PedsQL Multidimensional Fatigue Scale scores and additional PRO results related to fatigue and energy. Fine-Motor + Self-care domains of the PRO instruments included eating, dressing, hygiene, and caregiver assistance and grouped with BOT-2 fine motor function subtests because improved use of arms and hands is expected to improve activities such as self-grooming and feeding. Overall, 10 of 12 subjects showed improvement in at least one functional category: 4 improved in Mobility, 5 improved in

Fatigue and 3 improved in Fine Motor + Self-care. Two subjects improved in 2 or more categories and 2 did not show clear improvement in any functional category. Both severely and mildly affected subjects improved in clinical tests, PRO results or both. By grouping related clinical domains including functional test results and PRO assessments and displaying over the entirety of the study in a heat map, both worsening and improvement on placebo and active treatment could be visualized and an assessment of treatment response made for each subject. This comprehensive per-subject approach may be useful in other rare diseases with complex, highly variable clinical presentations.

596 - Oligosaccharidurias Profiles by MALDI-TOF Mass Spectrometry and Post-Analytical Interpretation Using Multivariate Pattern Recognition Software

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Oligosaccharidurias occurs in many lysosomal storage diseases involving enzyme deficiencies causing an accumulation of oligosaccharides in tissues and increased excretion in urine. In addition to glycoproteinoses, mucolipidoses, mucopolysaccharidoses, and some congenital disorders of glycosylation can also be identified by the analysis of urine oligosaccharides. Due to the broad spectrum of clinical presentations and biochemical phenotypes, the clinical significance of complex oligosaccharide profiles (60 species and 1223 calculated ratios) generated by MALDI-TOF mass spectrometry is achieved by automated post-analytical interpretation using a custom-made multivariate pattern recognition software, Collaborative Laboratory Integrated Reports (CLIR; https://clir.mayo.edu). Oligosaccharides are prepared by permethylation (modified from Xia et al, 2013). Validation included specimens from confirmed patients and additional cases have been detected and confirmed prospectively in patient samples submitted for diagnosis affected with α-mannosidosis (N = 4), β-mannosidosis (N = 1), fucosidosis (N = 2), Pompe disease (N = 7), GM1 gangliosidosis (N = 5), MPSIVB, (N = 1), GM2, Sandhoff disease (N = 2), Schindler disease (N = 1), aspartylglucosaminuria (N = 2), and NGLY1-CDG (N = 11), all shown to have a specific profile pattern leading to the biochemical diagnosis of a single condition and hence recommendation of targeted confirmatory analysis. Mucolipidoses (MLII, N = 7; MLIII, N = 5), sialidosis (N = 4), and galactosialidosis (N = 3) showed abnormal overlapping profiles, yet disease specific patterns. Overlapping oligosaccharide profiles within mucopolysaccharidoses (MPSI, N = 3; MPSII, N = 6; MPSIII, N = 2; MPSIVA, N = 3; MPSVI, N = 3) lead to less accurate resolution of diagnostic options via this platform, but it is recommended to be confirmed by glucosaminoglycan (GAG) analysis. CLIR has been updated with >1100 reference data and assists to quickly identify abnormal specimen patterns to aid the clinician in further testing. Our multiplex approach allows us to diagnose oligosaccharidoses, mucopolysaccharidoses, CDG-IIb, NGLY1-CDDG, and sulfatiduria in a small urine sample (<2 mL).

597 - Design and Rationale of Ongoing Observational and Treatment Studies for BMN 250, a Novel Enzyme Replacement Therapy for Sanfilippo Syndrome Type B (MPS IIIB)

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Sanfilippo Syndrome Type B (MPS IIIB) is a lysosomal storage disease characterized by rapidly progressing neurological decline, due to deficiency of the α-N-acetylglucosaminidase (NAGLU) enzyme. To determine whether treatment effects are clinically meaningful, it is critical to understand Sanfilippo B disease progression, particularly cognitive decline, in untreated patients. Development quotient (DQ) derived from scores on either one of two cognitive tests (BSID or KABC) has been validated as a cognitive measure in children with Sanfilippo A. While studies have demonstrated marked changes in DQ in younger MPS IIIA patients, information regarding DQ trajectory in young Sanfilippo B patients is scarce. BMN 250-901 (NCT02493998) is an observational study designed to quantify the progression of Sanfilippo B over time in affected children primarily aged 1-5 years at baseline and with relatively preserved cognitive function, and to correlate changes in clinical features of the disease, in particular changes in DQ, with MRI characteristics and biochemical markers of disease burden. BMN 250-902 is an additional observational study designed to capture disease trajectory across the spectrum of Sanfilippo B, including patients of all ages and all degrees of cognitive impairment. Concurrently, the BMN 250-201 treatment study (NCT02754076) is also underway using BMN 250 (NAGLU-IGF), a novel enzyme replacement therapy designed to restore functional NAGLU activity to the brain. Part 1 of BMN 250-201 is a dose-escalation period to establish safety; Part 2 is a dose-expansion period and consists of patients from participating sites rolling over from Part 1 and the BMN 250-901 observational study. Efficacy will be assessed by comparing changes in disease progression in the BMN 250-901 study vs. changes observed in Part 2 of BMN 250-201. Studies BMN 250-901, BMN 250-201 and BMN 250-902 are currently enrolling. Data from the BMN 250 clinical development program will provide valuable information on the natural history of untreated Sanfilippo B patients and the efficacy and safety of BMN 250. This abstract was previously presented at the 2017 LDN WORLD Symposium.

598 - Preliminary Safety and Pharmacodynamic Response Data From a Phase 1/2 Study of ICV BMN 250, a Novel Enzyme Replacement Therapy for the Treatment of Sanfilippo Syndrome Type B (MPS IIIB)

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Sanfilippo Syndrome Type B (MPS IIIB) is a lysosomal storage disorder caused by deficiency of the α-N-acetylglucosaminidase (NAGLU) enzyme and subsequent heparan sulfate (HS) accumulation in the brain. Sanfilippo B patients display progressive neurocognitive decline and typically do not live past the second or third decades of life. BMN 250 (NAGLU-IGF2) is a novel enzyme replacement therapy (ERT) for Sanfilippo B consisting of NAGLU enzyme fused to insulin-like-growth factor 2 (IGF2) to enhance lysosomal targeting. This report presents preliminary results from the first human study of BMN 250 (BMN 250-201; NCT02754076). BMN 250-201 is a phase 1/2, open-label study with two parts. Part 1 consists of 2 dose-escalation periods (≥4 weeks) of intracerebroventricular (ICV) BMN 250 administered as an isovolumetric bolus infusion QW. Subjects from Part 1 and from an ongoing observational study of Sanfilippo B patients (BMN 250-901; NCT02493998) will continue to Part 2, a 48-week treatment period to examine efficacy/safety at the maximum tolerated tested dose. For enrollment into Part 1, 1- to 10-year-old subjects with Sanfilippo B must have deficient NAGLU activity at screening. Three subjects have received ≥8 30 mg BMN 250 doses QW and ≥3 100 mg doses QW. All subjects demonstrated marked and sustained decreases in cerebrospinal fluid (CSF) total and disease-specific HS levels relative to baseline. BMN 250 was well tolerated with no treatment-emergent serious adverse events. These findings demonstrate that BMN 250 can be safely administered into brain ventricles via isovolumetric bolus infusion and that this treatment approach leads to a marked pharmacodynamic response in the CNS of Sanfilippo B patients. High levels of HS clearance are associated with improvement in markers of neuronal damage in preclinical models, suggesting potential translation into clinical benefit. Completion of BMN 250-201 will provide further information on the efficacy and safety of BMN 250. This abstract was presented at the 2017 LDN WORLD Symposium.

599 - Twelve novel *GUSB* Mutations and genotype-phenotype correlation in Mucopolysaccharidosis VII (MPS VII) patients

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MPS VII is a clinically heterogeneous, ultra-rare, autosomal recessive lysosomal disease in which patients are deficient in beta-glucuronidase enzyme (GUS). Nearly fifty unique MPS VII-causing mutations in *GUSB* gene have been reported. In this study, we present twelve novel *GUSB* mutations and provide potential genotype-phenotype correlation. Twenty-three MPS VII patients were enrolled in early access programs and clinical studies investigating recombinant human GUS, vestronidase alfa (UX003), as a potential enzyme replacement therapy (ERT). *GUSB* testing results were available on 74% of patients (17/23). 65% (11/17) were male, 35% (6/17) female; 35% (6/17) were Hispanic, 29% (5/17) Caucasian, 18% (3/17) Asian, and 6% (1/17) each for African American, native Brazilian and unknown ethnicity. 65% of patients (11/17) were unrelated, in addition to three sets of non-twin siblings. All tested patients harbored at least two mutations. Overall, twenty different *GUSB* mutations were identified, all being missense. 60% (12/20) were novel mutations (c.1A>G, p.G97A, p.E141A, p.H142Y, p.W288 L, p.N379 S, p.M430 T, p.S485F, p.V496 L, p.G512 R, p.W587 S, p.I608 M). 59% of patients (10/17), including two of three sibling pairs, were heterozygous for at least one novel mutation, and 30% of these patients (3/10) were compound heterozygous for two novel mutations. 82% of patients (14/17) harbored at least one previously published mutation. To assess potential genotype-phenotype correlation, an individual clinical assessment of disease severity at baseline was done, considering dysmorphic, clinical, diagnostic and functional assessments including walking ability and patient reported outcomes. All patients (4/4) heterozygous for these novel mutations: c.1A>G, p.G512 R, p.W288 L, p.S485F, p.V496 L, p.W587 S and p.I608 M exhibited an overall severe phenotype. All patients (4/4) heterozygous for these novel mutations: p.M430 T, p.G97A, p.E141A and p.H142Y exhibited an overall mild phenotype. One sibling pair harbored p.N379 S novel mutation, and overall phenotype was mild for one sibling but severe for the other. Our results add twelve novel *GUSB* mutations to previously reported genotype data, and provide further evidence that genetic heterogeneity could contribute to MPS VII clinical heterogeneity.

Genotype-phenotype correlation will benefit from future studies aimed at understanding possible role of various genotypes in potential response to investigational ERT in MPS VII patients.

600 - First Report of an Exclusively Japanese GUSB Mutation in a Non-Japanese MPS VII Patient

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Mucopolysaccharidosis VII (MPS VII) is an ultra-rare, autosomal recessive lysosomal disease characterized by deficiency in beta-glucuronidase enzyme. Over sixty different mutations distributed along *GUSB* gene have been reported to date. Mutation spectrum includes missense, nonsense, deletions and splice site mutations. Population specific *GUSB* mutations have been reported, with Pro408Leu and Pro415-Leu being observed only in Mexican patients and Ala619Val only in Japanese patients. We present a severely affected MPS VII patient from Thailand with no reported Japanese ancestry, who harbors Ala619Val *GUSB* mutation. This patient is a female product of nonconsanguineous Thai parents from a rural area and G2P1 mother, who was born prematurely at 32 weeks of gestation. Prenatal history was significant for hydrops fetalis detected by ultrasound. Post-natal, neonatal and childhood history was significant for short stature, poor weight gain, mild coarse facial features, macrocephaly, corneal clouding, hypertelorism, obstructive sleep apnea, respiratory failure, cardiac arrest, mild hepatomegaly, umbilical hernia, kyphoscoliosis, hip dysplasia, congenital club feet, cervical cord compression, global developmental delay and wheelchair confinement. Comprehensive diagnostic workup including the following was obtained: Karyotype showed 46, XX; skeletal survey showed dysostosis multiplex mainly involving hip and cervical spine; echocardiograms showed malformed atrio-ventricular annulus and aortic root with resolution of pulmonary stenosis and atrial septal defect; MRI spine showed cervicomedullary and C7-T2 compression for which the family declined neurosurgical intervention. At age of 9.5 years, further diagnostic workup confirmed MPS VII. Leukocyte beta-glucuronidase enzyme was deficient (4.45 nmol/hr/mg, normal 31.4-224 nmol/hr/mg). *GUSB* sequencing revealed homozygous c.1856C>T (p.Ala619Val) mutation. Literature review revealed only two other MPS VII patients, both Japanese, harboring Ala619Val mutation which was considered a founder mutation. Follow-up with patient's family affirmed their Thai but not Japanese ancestry. This report represents a severely affected non-Japanese MPS VII patient carrying the Japanese-specific *GUSB* Ala619Val mutation.

601 - The Trafficking and Enzymatic Activity Defects of the Beta Galactosidase D151Y Mutant Causing GM1-Gangliosidosis are Correctable by Chemical Chaperones and Reduced Temperature

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Missense mutations affecting protein stability and folding properties are common in many genetic disorders resulting in low protein half-life, premature degradation, aggregation or retention in the endoplasmic reticulum. GM1-Gangliosidosis, a lysosomal storage autosomal recessive neurological disorder, is associated with more than 115 missense mutations in the *GLB1* gene. These mutations cause beta-galactosidase (β-Gal) enzyme deficiency in lysosomes. Pharmaceutical chaperones (PCs) have been proposed to restore the lost function of β-Gal affected by certain missense mutations. PCs are small molecular weight compounds that specifically bind and stabilize the mutated enzymes to promote trafficking and maturation. In this study, an Emirati child has been clinically characterized with GM1-Gangliosidosis who had lost two siblings with the same disorder. Segregation analysis by Sanger Sequencing confirmed the presence of the homozygous c.451G>T mutation resulting in a missense mutation (p.D151Y). Biochemical analysis using the patient's fibroblasts showed that the mutant protein has retained less than 1.3% of the wild type enzymatic activity. In addition, our data showed that the mutant protein trafficking is defective as evidenced by the exclusive presence of the ER immature in these cells. Initial rescue experiments using glycerol and culturing cells at reduced temperatures promoted the mutated β-Gal maturation and trafficking to lysosomes and increased its residual activity by up to 6 folds. Furthermore, the enzymatic activity of D151Y β-Gal mutant increased to 2-3% of wild type using two different chemical chaperones. These findings raise the possibility of using pharmaceutical chaperones as possible personalized treatments for this disorder.

602 - A Temporal Cluster of Mucopolysaccharidosis Type I in the Region of Sucre and Cordoba, Colombia

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Introduction: Mucopolysaccharidosis type I (MPS I) is the representing syndrome of the classic phenotype of MPS,

consisting of coarse facies, corneal opacities, visceromegaly, multiple dysostosis, short stature and mental retardation. MPSI is an orphan disease with an estimated incidence of 1 in 10 000 to 22 500 and an estimated carrier frequency of 1 in 81. **Objectives**: To describe a series of confirmed cases of MPS I concentrated in the departments of Sucre and Córdoba (Colombia, South America). To establish the parameters of a geographical genetics study (Time and space clustering) and the clustering conditions for this group of lysosomal diseases in the departments of Sucre and Cordoba Colombia. **Methods**: Clinical, enzymatic, and molecular analysis of 11 cases of MPS I at five towns in Cordoba and Sucre (Colombia). A spatial and temporal analysis was carried out, looking for the geographic grouping of MPSI cases, using a discrete Poisson model (SatScan v9.4.2). Cases were considered by date of birth, the city of origin of the probands and by the town of origin of their parents. Geographic coordinates of the towns are in decimal degrees. The number of inhabitants of the region was extracted from the 2005 national population census, using the projection of the total population by the municipality for 2010. **Results**: We describe 11 patients (4 pairs of siblings) with confirmed diagnostic of MPS1 in 7 unrelated families. All of the probands have low activity of α-L-iduronidase on peripheral blood leukocytes and molecular confirmation of gene mutations (5 compound heterozygous). The most frequent mutation was c.144_146delGAG (Allelic frequency of 13 alleles), present in all subjects but two brothers. Using a purely temporal retrospective analysis of MPS cases, from 2015 to 2017, we found that the 11 patients with diagnostic of MPS1 in the region establish a temporal cluster in the zone (Log likelihood ratio: 5.4, *P* value: .007). Analyzing the obligated carriers of the families (16 subjects), we found another temporal cluster in the region (Log likelihood ratio: 6.5, *P* value: .002). **Conclusions**: the presence of a temporal cluster in the area, in an apparently no related group of families, suggests a founder effect. However, there is a high number of compound heterozygous, suggesting a more complex dynamic of the involved variants of the gene. Further directions of the research are discussed.

603 - A Long-Term Extension Study Evaluating Intrathecal Idursulfase-IT in Children With Hunter Syndrome and Cognitive Impairment

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Objective: To evaluate the long-term safety and clinical outcomes of idursulfase intrathecal (IT)—administered in pediatric patients with neuronopathic mucopolysaccharidosis II (MPS II, Hunter Syndrome). **Methods:** Eligible patients who completed a 6-month phase 1/2 study (NCT00920647) with idursulfase-IT were enrolled in an on-going open-label extension (NCT01506141). Patients receive monthly doses of intrathecal idursulfase-IT (10 mg or 30 mg) via an intrathecal drug delivery device (IDDD) and weekly doses of idursulfase 0.5 mg/kg via IV infusion. The study monitors adverse events (AEs), the presence of an anti-idursulfase antibody response, the concentration of glycosaminoglycans (GAGs) in cerebrospinal fluid (CSF), and standardized neurodevelopmental assessment scores. **Results:** As of January 2017, 15 patients, age range 3.6-11.2 years at baseline, were enrolled in the extension study; 10 patients received 10 mg and 5 patients received 30 mg idursulfase-IT. Patients received monthly idursulfase-IT injections (with weekly intravenous idursulfase infusions) for a median of 257.7 (range 78-361.4) weeks. One patient discontinued from the extension following intrathecal device failure and CSF infection that resolved without sequelae, and one patient was withdrawn by the investigator for behavioral issues. Of 54 serious adverse event types observed during the trial and extension, two were causally related to idursulfase-IT (pyrexia and vomiting). Anti-idursulfase antibodies were not detected at any time in seven patients. Five patients tested positive for antibodies in serum or CSF, but their titers were similar over time. In three patients, we observed increasing or high antibody titers. A mean decrease of $\sim 90\%$ in CSF GAG level was observed over the study population. Serial post-baseline General Conceptual Ability (GCA) scores, available for five patients, stabilized in three patients, one patient became untestable, and one patient experienced cognitive decline. **Conclusion:** Phase 1/2 and extension study results in patients with MPS II provide encouraging signs of clinical efficacy and stabilized cognitive function. Phase III study results are anticipated in late 2017. **Note:** This abstract is being submitted as an encore and has been previously presented at the 13th Annual WORLDSymposium, February 13-17, 2017, San Diego, CA, USA.

604 - Prenatal Diagnosis Experience of Hunter disease in Argentina

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Prenatal diagnosis of Hunter syndrome (mucopolysaccharidosis type II; MPS II) is preferably achieved in uncultured chorionic villi (CV), as this allows early, rapid and reliable results. The objective of this study is to report the prenatal diagnosis tests carried out in Argentina for Hunter disease. Chorionic villi samples were obtained from heterozygote women (n = 6) for Hunter disease. Samples were processed to carry out enzymatic

assays for Iduronate sulfatase and karyotyping to establish fetal sex. Whenever the mutation was previously known molecular genetic test was performed. Pregnant patients from families with MPSII disease from different places of Argentina were referred for genetic counseling and prenatal testing. A total of 6 CV samples were obtained between 12-15 weeks of gestation, 3 fetuses were male and 3 females. Two of the male cases showed deficient IDS activity, and in one, the diagnosis was also confirmed by genetic molecular test. Among female fetuses two were heterozygous and the other one did not carry the family mutation. **Conclusion:** To our knowledge this is the first report of prenatal diagnosis of Hunter disease in Argentina. In developing countries, accessibility to these procedures becomes the most effective assistance to families at risk for MPS II disease. Prenatal testing should be offered to all these heterozygous patients during the process of genetic counseling.

605 - Evaluation and Impact on the Quality of Life of Patients With Mucopolysaccharidosis IV-A (Morquio A) at the Colombian Southwestern

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Mucopolysaccharidosis IVA (MPS IVA; Morquio A syndrome) is a rare autosomal recessive lysosomal storage disorder. The disease is caused by a deficiency of the enzyme N-acetylgalactosamine-6-sulfatase (GALNS), which is responsible for the degradation of the glycosaminoglycans: keratan sulfate and chondroitin-6-sulfate. The Morquio A population is characterized by significant genotypic and phenotypic heterogeneity. There is a wide variability in clinical presentation, disease severity, and rate of progression of symptoms among patients. The clinical manifestations generally result in progressively worsening gait abnormalities, impaired mobility / wheelchair use, limitations in performing daily living activities (ADL), and premature death in the classical phenotype. A study of 28 patients with clinical, enzymatic and molecular diagnosis of MPS-IVA at the Colombian Southwestern (age 3.5-27.3 years). ADL was evaluated using the MPS-HAQ, a 52-question instrument originally developed to assess self-care and mobility in patients with MPS I. MPS-HAQ self-care (27 questions related to eating / drinking, dressing, bathing, grooming, tooth brushing, and toileting): 68% without any difficulty, 11% with some difficulty, 7% with much difficulty, and 14% unable to do. Mobility (12 questions related to dexterity, mobility, walking, stair climbing and gross motor skills): 53% without any difficulty, 11% with some difficulty, 7% with much difficulty, and 29% unable to do. And the extent of required caregiver assistance in the performance of these activities (13 questions): 18% patients perform activities without difficulty, 14% with some difficulty, 25% with much difficulty, and 43% unable to do. A significant (-0.75 $P < .01$) correlation was found between age and total score on the MPS-HAQ. Our study showed that the greatest deviations from a healthy population were seen in domains of pain / discomfort and mobility. Problems with self-care, wheelchair use, or usual activities were also critical factors affecting HRQoL. Regular assessments of QoL and ADL are recommended in order to assess the risk of morbidity and mortality attributed to the disease.

606 - Reactive Nitrogen Species and Inflammatory Biomarkers in Mucopolysaccharidosis Type II Patients Treated With Enzyme Replacement Therapy

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Mucopolysaccharidosis II (MPS II) is an X-linked lysosomal storage disorder caused by a deficiency of iduronate-2-sulfatase activity. This leads to an abnormal storage of glycosaminoglycans (GAG) in tissues and body fluids. It is known that GAG and oligosaccharides fragments can act as direct activators of pathological cascades, increasing proinflammatory cytokines production. Moreover, reactive nitrogen species (RNS)—such as nitric oxide (NO), nitrogen dioxide radical, and peroxynitrite—can cause a variety of cellular damages, and also lead to the activation of inflammatory signaling pathways. In this work, we evaluated the RNS and proinflammatory cytokines production in long-term idursulfase-treated MPS II patients. Urinary nitrate/nitrite, and plasmatic NO, IL-1β, and TNF-α were measured in 8 MPS II patients [mean age: 17.1 years-old; mean time on ERT: 5.2 years (range: 1.5-7.0 years)] and in 10 healthy age-matched individuals (control group). Nitrate/nitrite and NO contents were assessed by Griess reaction methods. IL-1β and TNF-α plasmatic concentrations were measured using ELISA assays. Our results showed that treated MPS II patients present higher levels of RNS in both plasma and urine, compared to the control group. Furthermore, proinflammatory cytokines concentrations were found increased in these patients. It was also observed a significant positive correlation between plasmatic IL-1β and NO concentrations. These results evidence that, despite the long-term treatment with enzyme replacement therapy, MPS II patients present a disruption of inflammatory and nitrative status, and possibly these pathophysiological mechanisms are associated. Considering the deleterious systemic effects of chronic inflammation and redox imbalance, our results suggests that the use of anti-inflammatory and antioxidant drugs may be an important complementary strategy in MPS II treatment. **Financial support:** CAPES; CNPq; FIPE-HCPA.

607 - Neuroradiological Findings and its Correlation With the Cognitive Status and Phenotype in Patients With Mucopolysaccharidosis I and II

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Aim: To determine correlation between neuroradiological findings and cognitive status and phenotype in MPS I and II. **Materials and Methods:** Observational, ambispective, transversal, and analytical study was carried out. We included 10 patients, 6 with MPS I and 4 with MPS II. We analyzed neuroimaging findings: cribiform alterations (perivascular spaces), degree of atrophy, white matter hyperintensity, ventricular dilatation, were assigned a score of 0-3 and total score (0-12), as well as IQ, phenotype, age, gender, type of MPS, and mutation. We used U of Mann Whitney and chi square and Spearman test for correlation. **Results:** There was no significant difference in neuroimaging and IQ findings between MPS I and MPS II. Comparing phenotypes there was a difference in neuroradiological findings, with the exception of cribiform spaces. Comparing patients with mental retardation and without delay, there was significant difference in white matter hyperintensity and total score. There was a correlation between IQ and mental retardation with white matter hyperintensity and total score in imaging studies. There was correlation between phenotype, white matter hyperintensity, degree of atrophy, ventricular dilatation, and total score. **Conclusions:** Neuroradiological findings cannot differentiate between MPS I and II; however, they may help to determine the phenotype or severity of the disease (attenuated and non-attenuated). There is correlation between IQ and mental retardation with white matter hyperintensity and total score. Likewise, there is a correlation between white matter hyperintensity, degree of atrophy, and ventricular dilatation with phenotype.

608 - Efficacy of Genistein in MPS III Patients From India and Pakistan

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Background: MPS III is autosomal recessive disorder for which ERT is not available so far. Substrate reduction therapy is one of the possible treatments that could be effective in management of MPS III. Genistein (natural or synthetic) has been demonstrated to be inhibitor of GAG synthesis and effective in MPS types I, II, and III. **Objective**: To evaluate efficacy and safety of oral natural Genistein (>98% Purity) in patients with MPSIII **Method:** This is a prospective observational study of patients receiving Genistein therapy. 13 patients diagnosed with MPSIII were provided with information regarding possibility of Genistein Therapy. Of these, 9 patients started oral Genistein therapy. Parents obtained natural Genistein (>98% purity) through pharmaceutical company of their choice. Initial starting dose was 5 mg/kg/day and later it was gradually escalated to 30 mg/kg/day. Biochemical and clinical response was monitored periodically. **Result:** MPSIII was diagnosed genetically and biochemically in 13 patients (9 M and 4F). Genistein was opted by 9 patients. There has been a significant improvement in the biochemical parameters and clinical observations of 2 patients with MPS IIID. Both of them show a reduction of HS and great improvement in texture of skin & hair, reduced hyperactivity, improved cognition and reduction of upper respiratory infections. These 2 patients did not show any adverse effect to the therapy. Both are now on Genistein for more than 3 years. 4 patients with MPS IIIB have shown some response- there is improvement in skin and hair texture; however, hyperactivity and behavioral problems remain. 1 patient with MPS IIIC had stabilized with improvement in behavior and skin and hair. However, parents opted to stop therapy after 2 years. 2 patients with MPS IIIA did not tolerate therapy initially, developed gastrointestinal upset with vomiting and loose motions however later they tolerated and now have good response. Now they are continuing with treatment. **Conclusion:** Natural Genistein used in powder form of >98% purity in cases of MPS III showed varying degrees of results or improvements in most of the patients. 4 patients (2 MPS IIIA and 2 of MPS IIID) showed very satisfactory response (44.44%), 4 of MPS IIIB had improvement in skin & hair but not much in behavior & cognition (44.44%), 1patient of MPS IIIC had stabilized but they decided to stop the therapy. Acceptance by parents was good. 8 of 9 patients are still continuing the therapy.

609 - Spectrum of LSDs: An Experience of 158 Affected Individuals From India

Anil Jalan[1], Ketki Kudalkar[1], Rishikesh Jalan[1], Durga Shinde[1], Monal Borugale[1], Rasika Tawade[1], Neelam Yadav[1], Gauri Gaikwad[1], Claudia Cozma[2], Sabrina Eichler[2], Arndt Rolfs[3], and Susanne Zielke[3]

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Background: Lysosomal storage disorders represent a group of at least 55 genetically distinct, biochemically related,

inherited diseases. High prevalence has been reported in some populations; however, there is a limited data on prevalence of these disorders in Indian population. **Objective:** To determine the spectrum in patients detected to have LSDs from a single diagnostic facility with referrals from all over India. **Materials and method:** A retrospective study on the data of patients with LSDs, for the period from 2011 to 2017. Patients clinically and biochemically suspected to have any LSD were selected for this study. Common clinical features for referral included bone abnormalities, organomegaly, central nervous system dysfunction, and coarse facies. **Result:** For the period January 2011 through April 2017, there were 268 individuals suspected to have LSD because of clinical feature or biochemical parameter. These diagnoses represent 27 different LSDs. Of these 158 (58.96%) were confirmed to have particular LSD by means of molecular analysis. No molecular diagnosis is available in 110. Mean age at presentation was 5 years. The commonest clinical features were growth retardation (failure to thrive, short stature), coarse facies, anemia, hepatomegaly, splenomegaly and neuroregression. Gaucher's Disease was the most common LSD found in our cohort with 32 confirmed cases. Other LSDs with high incidence included MPS IVA (19), MLD (15), Niemann Pick Disease A/B (16) and Niemann Pick C (12). Few common mutations were seen in our cohort: c.1448T>C (p.L483P) in exon 10 of GBA gene (23/32 patients with Gaucher's Disease), c.1624C>T (p.R542X) in exon 6 of SMPD1 gene (5/14 patients with Niemann Pick A/B) and c.1469T>C (p.L490P) in exon 10 of IDUA gene (6/12 patients with MPS I). We also found pseudo deficiency alleles in 3 patients with MLD- c.1055A>G (p.N352 S) and c.1178C>G (p.T393 S) which resulted in low enzyme activities in vitro. We did not encounter any patient with MPS IV-B, MPS VII, Farber Disease and Wolman's Disease. **Conclusion:** Overall, the study demonstrates that Gaucher's, MPS IVA, MPSI, NP A/B, NPC, and MLD are the most common LSDs in India and can be used as a part of screening programs.

610 - A Case Series of Twins With Mucopolysaccharidoses

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Introduction: Mucopolysaccharidoses (MPS) is a group of hereditary lysosomal storage disorders. Mucopolysaccharidosis type II is a rare X-linked recessive disorder which means 50% of the male offspring is at risk, whereas, MPS IIIB is an autosomal recessive disorder, which means 25% of the offspring are at risk. Three boys from 2 pairs of twins with MPS in Indonesia are reported. **Case 1:** A 6-year-old boy is one of the non-identical twins who suffer from Hunter Syndrome (MPS Type II). Patient has a younger twin and an older brother, and none of his siblings show signs and symptoms for MPS. With a 2-year history of delayed growth and development, recurrent upper respiratory tract infections and snoring; at 6 years old, patient was referred to our hospital with suspected MPS. His physical examinations are according to MPS, however, mother states that she has never suspected any disorder as patient looks like his father. The supporting examination reveals mild mitral and tricuspid regurgitation, obstructive sleep apnea, hydrocephalus and sinusitis. Patient is currently a candidate for enzyme replacement therapy at the hospital. **Case 2:** Two 7-year-old identical twin boys suffer from Sanfilipo Syndrome (MPS Type IIIB). With a 6-year history of delayed growth and development, recurrent upper respiratory tract infections and several foreign consultations, patients were then suspected with MPS in India and were referred to our hospital for further management. Cerebral MRI reveal minimal periventricular diffuse white matter disease. Twin A has cerebral atrophy and Twin B has hypoperfusion on the bilateral parietal and occipital lobes. Twin A's intelligence and developmental quotients is comparable to an 18-month old, whereas Twin B's to a 42-month old. Epigenetics is the most probable cause for this significant difference. Patients' mother had a history of 3 miscarriages. They were born through surrogacy at 32 weeks gestational age. At birth, Twin A and B were 1900 g and 1600 g, respectively. Twin A was under intensive care for 5 weeks and intubated for 2 weeks. Twin B was admitted for 3 weeks with no history of intubation. Patients are currently waiting for gene therapy trials. **Conclusion:** During genetic counseling, patterns of inheritance should be a vital part of the discussion to be able to explain why two pairs of twins with MPS end up having 2 different presentations. In addition, parents should be explained that epigenetics also play a factor in patient's prognosis.

611 - Interest of Early Treatment With Laroniadase in MPS I Moroccan Experience of the Military Hospital of Rabat

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It is difficult to evaluate the efficacy of the enzyme replacement therapy in MPS type 1 because of the heterogeneity of the phenotypes. However, the follow-up of siblings under treatment allows us to conclude the importance of early treatment at the beginning of the treatment. Our work aims to share our experience in the therapeutic management of MPS1. We collected 7 patients over a period of 8 years, benefiting from full coverage social security, all Hurler-Schei phenotypes, under treatment with the replacement enzyme (Laronidase: Aldurazyme®) except one patient who died at the age of 9 just before treatment. Four patients come from two families and therefore two brothers and then one brother and one sister. The follow-up of these patients and especially of siblings allowed us to conclude that the earlier the treatment is started the better the results are as well on the stability of the initial lesions especially skeletal, as on the improvement of the clinical symptoms.

612 - High Incidence of Alpha-Mannosidosis in the State of Rio de Janeiro—Brazil?

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α-Mannosidosis is a rare autosomal recessive, lysosomal storage disorder caused by deficient activity of lysosomal α-mannosidase (MAN2B1), with an estimated prevalence of 1: 500 000. Lack of this enzyme results in accumulation of undigested oligosaccharides and patients accumulate mannose-containing oligosaccharides in tissues and body fluids. LABEIM (Laboratory of Inborn Errors of Metabolism) in Rio de Janeiro state received in 25 years, 11.500 patients with suspected metabolic disease, having diagnosed 6 cases of α-mannosidosis. This number of cases in relation to total cases reported in Brazil suggest that this disease may not be so rare in the state. Of these six cases, five are female and one male, from four different families. In common, all patients are alive, manifested recurrent infections, coarse facies, skeletal abnormalities, cognitive, and motor and hearing impairment. Two families are consanguineous, with two affected in each of them. Symptoms appeared in early age and were gradually progressive. Four patients and their families come from northeastern Brazil, from 2 counties in Paraiba and Bahia, with Portuguese ancestry. The other patients were born in Rio de Janeiro, a Portuguese colonization state, and each of them has a grandfather who migrated from the Northeast, also from Paraiba and Bahia. These data suggest a possible founder effect in the northeastern region of Brazil, probably of Portuguese origin. The molecular study of our patients, not yet undertaken, will be needed to prove this hypothesis. **Acknowledgements:** Rede EIM and HCPA.

613 - Coexistence of Mucolipidosis Type III With Trimethylaminuria in a 4.5 Iranian Boy

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Mucolipidosis type III α/β (ML III α/β) (OMIM252600) has been shown to be caused by a reduced level of uridine diphosphate (UDP)-N-acetylglucosamine-1-phosphotransferase enzyme (EC 2.7.8.17) activity. This disorder is caused by mutations in the *GNPTAB* gene and is inherited in an autosomal recessive manner. Trimethylaminuria (OMIM 602079), fish-odor syndrome, is caused by homozygous or compound heterozygous mutation in the gene encoding flavin-containing monooxygenase-3 (FMO3) on chromosome 1q24. Here we report a 4.5-year-old boy with coexisting of both mucolipidosis type III and trimethylaminuria. He is suffering from a, coarse face, corneal opacity, hearing loss, short stature, hirsutism, umbilical and inguinal hernias, clue hand, developmental delay, and an offensive decaying fishy odor of sweat, breath, and urine since his infancy. Other findings include dysostosis multiplex in skeletal survey, significant increases of multiple lysosomal enzymes' activities in plasma, and not detectable GAG but huge an amount of TMA in urine. Whole exome sequencing in this patient revealed one novel mutation in the *GNPTAB* gene and three compound heterozygote mutations in the *FMO3*. We confirmed these findings by his parents' and Sanger gene sequencing analysis. Coexistent different inborn errors of metabolism in a patient make the clinical diagnosis and management of the diseases complicated.

614 - Early ERT for MPS IV A (Morquio A) in Under 5-Year-Old Pediatric Patient: 3 Years Follow-Up

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Introduction: Mucopolysaccharidosis IVA (MPS IVA) is an autosomal recessive disorder caused by a deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS), leading to accumulation of keratan sulfate (KS) and chrondroitin-6-sulfate. Enzyme replacement therapy (ERT) has been approved in 2014. Patients under 5 years were not evaluated in the phase-III study for ERT in MPS IV A. **Objective:** to describe the evolution of a Morquio A pediatric patient who was diagnosed at 11 months old and started ERT at 25 months for the next 36 months. **Case:** G: A is a 5 years old Argentinian girl diagnosed of MPS IVA at age 11 m. At time of diagnosis the patient shows typical dysostotic signs of the disease including dorsal kiphosis, and history of lower respiratory obstruction episodes, but her overall health condition was good. At 25 months of age Elosulfase alfa (Vimizin) in standard dosing (2 mg-kg-week IV) was initiated, initially as compassionate treatment. The ERT appeared to reduce her urinary excretion of glycosaminoglycans (GAGs). The girl experienced only one moderate ERT-associated reaction in the 8th infusion consistent in fever and dyspnea that was solved using antihistamines as premedication from the on. Under treatment, the height of this patient increased during the first 2 years of the ERT on percentile 97th for Morquio A although decrease in the growth rate is observed thereafter for last 12 months. Moreover, despite of ERT, his bone deformities and join hyperlaxity became marked after age 4. Improvements in lower respiratory obstruction episodes is seen; however upper respiratory obstruction associated to tonsillar hypertrophy increased during the last year of treatment with ERT. **Conclusion:** Early ERT treatment seems to have limited

effect on the skeletal outcome in this pediatric MPS IV A patient after 36 months-long treatment. A longer term follow-up is required to further assess the efficacy of ERT on other clinical aspects. Lack of consistent biomarkers to evaluate treatment efficacy is a special challenge in young children with MPS IVA disorder. **Financial support:** CNPq (141552/2015-8); FIPE-HCPA; CAPES.

615 - Oxidative Biomarkers in Morquio A Patients

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Morquio A disease is an autosomal recessive inborn error of glycosaminoglycan catabolism due to a defect in N-acetylgalactosamine-6-sulfatase enzyme. This enzyme deficiency is responsible for keratan sulfate and chondroitin 6-sulfate accumulation mainly in the cartilage, cornea and heart valves of Morquio A patients, as well as in blood and urine. As the pathophysiology of this LSD is not completely understood, the aim of this study was to investigate oxidative stress parameters in Morquio A patients at diagnosis. It was studied 15 untreated Morquio A patients, compared with healthy individuals. Damage to lipids was evaluated by urinary 15-F2t-isoprostane levels. To determine oxidative damage to proteins, plasmatic concentration of sulfhydryl groups and urinary di-tyrosine levels were measured. Basal DNA damage was investigated by comet assay and we assessed DNA repair using endonuclease III enzyme which recognizes oxidized pyrimidines bases and converts them into breaks reflected in comet tail. We also determined the urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels, a product of DNA oxidative damage. Reduced glutathione (GSH) levels, the main non-enzymatic intracellular antioxidant, were measured in erythrocytes, as well as the activity of antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutatione reductase (GR). Finally, urinary keratan sulfate was measured by a LC/MS/MS technique. The affected individuals presented higher lipid peroxidation, and no protein damage. Furthermore, Morquio A patients showed DNA oxidative damage in both pyrimidines and purines bases, being the DNA damage positively correlated with lipid peroxidation. In relation to antioxidant defenses, affected patients presented higher levels of GSH and increased activity of GPx (showing a possible response to oxidative injury), while SOD and GR activities were similar to controls. The urinary keratan sulfate levels were greatly increased in Morquio A patients, and it is important to emphasize that differently of blood samples, urinary KS levels remained high in Morquio A patients even after 20 years of age. Considering the expose, our findings indicate that Morquio A patients at diagnosis present redox imbalance and oxidative damage to lipids and DNA, reinforcing the idea about the importance of antioxidant therapy as an adjuvant treatment in this

616 - Fabry Disease (FD) Screening Among Females With Kidney Disorders Suggests Absence of the Common Asian Late-Onset or Cardiac *GLA* Variant c.640-801GA (rs199473684) in the Mexican Population

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Introduction: FD is an X-linked lysosomal storage disease due to deficiency of alpha-galactosidase resulting from mutations in the *GLA* gene. Life-threatening FD conditions include hypertrophic cardiomyopathy (HCM), end-stage renal disease (ESRD) and stroke. Several FD screening studies performed among high-risk ESRD or HCM populations, have not included the search of the deep-intronic pathogenic allele c.640-801G>A (rs199473684) commonly identified in Asian populations (1 in 1,460 Taiwanese newborn males) and responsible of the late-onset/cardiac form of FD with variable renal involvement. In Mexico, full *GLA* mutational spectrum and c.640-801G>A allelic frequencies (AF) are still unknown. Moreover, genotype databases have not reported the AF for rs199473684 in any population. Thus, we included the rs199473684 genotyping to increase the FD detection yield and to determine its AF within a FD screening initiative. **Methods:** A nationwide FD screening program included 2,126 unrelated Mexican females with ESRD or diverse kidney disorders recruited during 2013 to 2017. They were referred mainly by a nephrologist or medical geneticist whom considered seeking FD such as the underlying etiology of renal disease. Prior written informed consent, genomic DNA obtained from dried blood spot samples was subjected to direct Sanger sequencing of the *GLA* gene (NM_000169.2), along with an intron 4-derived amplicon encompassing the c.640-801 site. The c.640-801G>A was searched by direct visual inspection in each alignment. **Results:** Successful amplification and sequencing of intron 4-derived fragments were obtained in all samples included. However, the c.640-801G>A variant was absent in the analyzed 4,252 *GLA* alleles. Detailed analysis for patients bearing other heterozygous *GLA* variants is currently ongoing. **Discussion:** The preliminary results suggest that c.640-801G>A is a very rare or absent FD-causing allele in the Mexican population. As far as we know, our study represented the largest number of non-Asian individuals in which directed search of the c.640-801G>A has been done and these figures could be real, since the employed DNA-based testing strategy is more accurate than enzyme-based assays for identification of c.640-801G>A heterozygous females. This information might be relevant to design DNA-based, second-tier confirmatory assays applicable to FD screening programs in our country. We thank Sanofi Genzyme Mexico for its scientific and bibliographic support.

617 - Morphology of Sinus of Valsalva in Patients With Mucopolysaccharidosis

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Background: In patients with mucopolysaccharidosis (MPS) the cardiovascular system is frequently impaired. In clinical studies, aortopathy is common (up to 35%), and is a leading cause of aortic aneurysm and dissection with a high risk of mortality. Objective: To study the morphological features of aortic root anatomy in children with MPS. Materials and methods: 32 children and young adults with MPS (8 with MPS type I, 14 with MPS type II, 4 with MPS type IVA and 6 with MPS type VI). Transthoracic echocardiography was used to assess proximal aortic morphology and function in accordance with the EACVI and ASE standards. Measurements were corrected for BSA calculated using the DuBois formula. Results: The mean size of the aortic root was 16.7 $\pm$ 6.4 mm. Only 2 patients, both with MPS II, had evidence of aortopathy. Patient M., 4 years old, height 87 cm, weight 14 kg, BSA = 0.59 m^2, the size of the atrioventricular valve is reduced (z-score-1.3). Signs of left ventricle (LV) dilatation (EDI 90 mL/m^2). Patient R, 27 years old, height 147 cm, weight 68 kg, BSA = 1.15m^2, normal dimensions of the LV cavity (EDI 61.9 mL/m^2). In both cases, aortic valves dilatation is significant (z-score 3.0 and 3.6, respectively), especially the sinus of Valsalva (z-score 3.5 and 4.3, respectively). Moreover, the patient M. had an aneurysm of the right coronary sinus. Additionally, the valves of the aortic valve in both children were slightly thickened and there was a failure of the valve function with mild regurgitation (PHT 720 ms and 460 ms, respectively). Hypertrophy of all LV walls was detected only in patient M, the relative thickness 0.46 cm, the LV mass index 173.2 g/m^2. While the patient R. had isolated hypertrophy of the posterior wall of the LV. The function of the LV was normal in both patients, with cardiac index 4.7 L/min/m^2 and 4.3 L/min/m^2 respectively, without calculating the error for aortic insufficiency. Conclusion: Thus, among observed children with MPS in Republic of Kazakhstan aortic root impairment was found only in 2 patients with MPS type II, that is 6,25% of all observed children. It is mandatory to follow up morphology of aortic root in children with dilated sinus of Valsalva.

618 - Molecular Analysis of MPS VI Patients in Brazil

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Mucopolysaccharidosis type VI is a lysosomal autosomal recessive disorder due to *ARSB* gene mutations which leads to impairment of N-acetylgalactosamine-4-sulfatase(ARS-B)enzyme causing a broad spectrum of clinical manifestations. The MPS VI world estimated incidence varies between 1:248 000 and 1:300 000 newborns depending on the region studied. So far, around 200 different mutations in the *ARSB* gene have been described worldwide. The aim of this study was to characterize the genotype and calculate the frequency of mutations in MPS VI Brazilian patients. The study was conducted at *Medical Genetic Service of Hospital de Clínicas de Porto Alegre* using PCR followed by Sanger sequencing of the 8 exons and intron boundaries comprising the *ARSB* gene. The analyses of 116 patients (232 alleles) resulted in 36 different mutations: 21 already described in literature (12 missense, 3 nonsense, 4 deletions, 2 splicing and 1 insertion) and 13 new (8 missense, 1 nonsense, 2 deletions and 2 insertions). The most frequent mutation was c.1143-1G>C in intron 5 with 19,39% (45 alleles), followed by p.Leu72Arg in exon 1 with 16,37% (38 alleles) and c.1143-8G>C also in intron 5 with 13,36% (31 alleles). Exon 1 presented the highest number of detected mutations (n = 14). Six different polymorphisms (SNPs) were found in exons 4,5 and 6 and intron 5, 73% of patients have at least 1 of the 6 SNPs, being the most frequent IVS5-27A>C, in intron 5, present in 40% of patients, from which 54% were homozygous and 46% were heterozygous. Most of the patients included in this study originated from the Northeast (48%) and Southeast (36%), which is in agreement with previous studies showing, that the majority of medical genetic services are located in Southeast and South regions, making it difficult to access for a huge parcel of the Brazilian population. The lack of genetic services, cannot exclude the possibility of the occurrence of patients with genetic diseases, including MPS VI, in those regions. In addition, the large number of patients in the Northeast region is due to the presence of the founder effect of the p.His178Leu mutation in the county of Monte Santo, located in the countryside of Bahia. The observed results throughout the years confirm the broad genetic heterogeneity among MPS VI Brazilian patients. This study will contribute to estimate the real prevalence of MPS VI mutations which is important for epidemiological studies and genetic counseling.

619 - Analysis of *Neu1* Regulation by microRNAs From miR-17 Family Differentially Expressed in the Cerebellum of Mucopolysaccharidosis Type I Mice

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In a previous study from our group, three microRNAs (miRNAs) from miR-17 family (miR-20b-3p; miR-20b-5p and miR-106b-5p) were found to be differentially expressed in the cerebellum of mucopolysaccharidosis type I (MPS I) mice,

along with a reduction in *Neu1* expression. Since those miRNAs are predicted to bind to *Neu1*, the aim of this study was to analyze the direct binding of miRNAs from miR-17 family to the 3' UTR of *Neu1*, which could explain the reduction of *Neu1* expression observed in the MPS I mouse model. This binding analysis was performed through luciferase assays. *Neu1* 3' UTR was inserted in the cloning site downstream of the luciferase gene in a pMIR-REPORT™ miRNA Expression Reporter, generating *pMIR-Neu1-3'UTR* plasmids which were transfected to *COS-7* cells. MiR-20b-3p, miR-20b-5p and miR-106b-5p mimics were separately transfected to those cells, to evaluate their possible effect on *Neu1* expression. Three different miRNA concentrations were tested and the effect of a miRNA scramble transfection was also evaluated. Experimental conditions were analyzed in triplicates and results were confirmed through three independent experiments. No statistically significant differences through Kruskal-Wallis test were observed in luciferase luminescence in the presence of neither miRNAs tested, suggesting that miR-20b-3p, miR-20b-5p and miR-106b-5p do not regulate *Neu1* expression by direct binding to its 3'-UTR region. However, these results do not exclude the hypothesis of *Neu1* regulation by these miRNAs since they may also act through different mechanisms, including by interaction with other miRNAs and gene networks. **Financial support:** FAPESP (2014/14230-0)

620 - Management Experience of a Cohort of Patients With Mucopolysaccharidosis Type IV A in the Western Center Region of Colombia

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Objective: Report the management experience of a Series of Patients with mucopolysaccharidosis type IV A in the Western Center Region of Colombia. **Methodology:** Information was collected between 2013 to 2017 in the Clinica Comfamiliar Risaralda. Diagnosis confirmation was done by detection of chondroitin and keratan sulfate urine bands, the measurement of galactose-6-sulfate sulfatase blood low levels, and in some patients the identification of the molecular defect. We analyzed the data from the electronic medical records of the institution. For each patient, the following variables were reviewed: age, sex, genotype, antecedent of consanguinity of the parents; age of onset of malformations; height; and the next clinical manifestations: respiratory compromise, muscle skeletal dysplasia, myelopathy, and associated cardiac, ocular, or ear compromise. Current medical treatment: enzyme replacement therapy and adverse reactions. Other genetic associated disease. **Results:** 18 families were identified with 21 patients affected with mucopolysaccharidosis type IV A, 3 of them with history of consanguinity. The molecular defect has been identified in 13 people, 12 had 901G>T p. (Gly301Cys) mutation in the *GALNS* gene. 12 patients were women; 8 were over 18 years old, 2 have died. The average height was 99 cm. The malformations were variable according to age: prenatal stage (hydrops fetalis); at birth (thoracolumbar humps, pectus carinatum, cafe au lait and Mongolian spots; and older (skeletal dysplasia). Respiratory symptoms were present in 19 of 21 patients, myelopathy in 17, corneal opacity in 20, all chronic otitis media but just 4 severe hearing loss. By echocardiogram 5 patients had valvular disease. Two patients had other genetic diseases such as cystic fibrosis and neurofibromatosis. Two patients had seizures. 14 patients are under enzyme replacement therapy and physical therapy. Subjective improvement in quality of life is reported by patients after 3 months of treatment. **Conclusions:** The most common mutation found has not been described in European people, so it is probably due to a founder effect in the region favored by the geographic isolation, with a calculated frequency of 1:90 000. Phenotype expression is variable among patients with the same mutation. The high frequency of cases found in this region of our country, emphasize the importance to implement a newborn screening as a public health policy and specific molecular defect detection.

621 - Genetic Diagnosis of Mucopolysaccharidosis in Cuban Patients Through Next Generation Sequencing

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Introduction: Mucopolysaccharidoses are caused by deficiencies of genetic origin in the lysosomal hydrolases that degrades the glycosaminoglycans or mucopolysaccharides (MPS). Eleven different genetic defects have been described, all with autosomal recessive inheritance except for one with X-linked inheritance. The determination of mucopolysaccharides in urine allows the identification of six different clinical entities (MPSI-VII). **Objective:** Genetic characterization of 19 Cuban patients with clinical and biochemical suspicion of mucopolysaccharidosis. **Methodology:** Next generation sequencing using TruSightOne® clinical panel combined with a virtual capture of genes related to clinical and biochemical findings, Mendelian segregation analysis and bioinformatic analysis of variants (Alamut Visual Software). **Results:** Seventeen patients were genetically diagnosed: seven with hemizygous mutations in the *IDS* (MPS II-Hunter) and ten with biallelic mutations: three in the *IDUA* (2 MPS I-Hurler and 1 MPS I-Scheie), four in the *NAGLU* (MPS IIIB-Sanfilippo B), one in

the *HGSNAT* (MPS IIIC-San Filippo C), and two in the *GALNS* (MPS IVA-Morquio A). Six of these ten patients were heterozygous compounds of two mutations. The mutational spectrum includes: 17 single nucleotide described variations (10 missense, 4 nonsense, and 3 splicing), 1 small insertion, 1 small deletion, and a genetic rearrangement. Eight variants were not described, four of them predictably severe (c.103+1G>A and p.Trp109Ter in IDS, p.Leu214ProfsTer59 in NAGLU, and c.852-2A>G in HGSNAT) and four of uncertain clinical significance (VUS) (p.Leu18Pro in IDUA and p.Met1Leu, p.Gly82Arg, and p.Trp404Cys in NAGLU), although bioinformatic analysis predict that they could be pathogenic. **Conclusions:** The use of massive parallel exome sequencing has allowed to identify the genetic defect responsible for the disease in 17 Cuban patients with mucopolysaccharidosis, providing essential information for adequate genetic counseling and clinical management of the patients.

622 - Mucopolysaccharidosis Type I, Scheie Type: Trigger Finger can be a Diagnostic Clue!

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The Mucopolysaccharidosis I (MPS I) is a lysosomal disorder caused by deficiency of the enzyme alpha-L-iduronidase required for the degradation of glycosaminoglycans (GAGs). MPS I is inherited as an autosomal recessive condition, and it has been divided into three syndromes—Hurler (severe form), Hurler–Scheie (intermediate), and Scheie (milder form). Typical symptoms include corneal clouding, organomegaly, cardiac valve abnormalities, joint contractures, and dysostosis multiplex. Treatment includes enzyme replacement therapy (ERT), hematopoietic stem cell transplantation and symptom-based and palliative care. **Case report:** An 8-year-old male patient, first child of healthy non-consanguineous parents. After an uneventful pregnancy, the child was born at term. At the age of two, he was investigated for Kawasaki syndrome due to joint edema and restrictions. He underwent a surgery for trigger finger (left thumb) at 3 years of age and for umbilical/inguinal hernias and postectomy at 4 years old. At 5 years of age, the mother noticed restriction of the interphalangeal joints in hands and feet, in addition to toe walking. At this time, he had rheumatological workup without a definitive diagnosis. At 6 years of age, the patient was referred to the Genetics Unit. At the initial evaluation, the patient did not show neither coarse facies nor hepatosplenomegaly but presented joint restrictions in hands and feet. Radiological investigation revealed diaphyseal widening mainly in metacarpal bones, mild paddle-shaped ribs, and fibrous defect in the left femoral medial metaphysis. Baseline abdominal ultrasound and Echocardiogram were normal. The diagnosis of MPS I was confirmed by urinary GAGs, enzymatic assay and molecular test. Interestingly, his healthy younger brother also initiated a trigger finger and had borderline alpha-L-iduronidase levels, with molecular investigation detecting mutation in only one allele (heterozygous). After the diagnosis, the patient started ERT with laronidase. **Conclusion:** MPS I-Scheie might present as a subtle phenotype and represents a challenging diagnosis. Due to the large number of differential diagnoses, it may take a long period until MPS is suspected. Since it is a treatable disorder, an early detection allows multidisciplinary management, improving the quality of life and decreasing the progression of the disease.

623 - Neonatal Form With Severe Cardiac Manifestation in an Infant With Rapidly Progressing Phenotype of Mucopolysaccharidosis Type VI

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The Mucopolysaccharidosis type VI (MPS VI), also known as Maroteaux-Lamy Syndrome, is an autosomal recessive lysosomal disorder caused by the deficiency of the enzyme N-acetylgalactosamine 4-sulfatase. It is described a phenotypic spectrum ranging from mild to severe. Here we report an MPS VI patient whose manifestations started in the prenatal period, rapidly evolving valvular insufficiency with congestive heart failure before one year, which ended up requiring surgical repair. **Case report:** male patient, 1 year 2 months, only child of healthy non-consanguineous parents with no reports of

cardiopathies in the family. Second trimester ultrasonography observed fetal ascites, hydrocephalus and bilateral hydrocele. Prenatal screening for infectious diseases was negative. The child was born at term with bilateral hydrocele, diastasis recti, patent foramen ovale, mild dysmorphic features but without hepatosplenomegaly. Abdominal ultrasound revealed a small amount of free anechoic intra-abdominal fluid. At two months of age, the patient was admitted in the hospital due to upper respiratory tract infection. A deformity in the lumbar spine was noticed and the patient was investigated with image studies. At the age of six months, the patient was evaluated at the Genetics Unit and the diagnosis of MPS was suspected. Urinary GAGs and enzymatic assay confirmed the diagnosis of MPS VI. After the diagnosis, a new echocardiogram was performed and reported significant mitral insufficiency with hemodynamic compromise. The patient evolved with congestive heart failure and eventually underwent surgical mitral valvuloplasty. Enzyme replacement therapy (ERT) with galsulfase was initiated at one year of age. Cardiac involvement in MPS VI is common and progressive. Nevertheless, congestive heart failure and valvular surgical repair are not frequently seen, and if so, they are usually observed mainly in adult age (from 21 to 40 years with a mean 29 years). In contrast with most previous reports, our patient has a severe valvular disease with onset in the first year of life with minimal aortic valvular involvement. This case reinforces the importance of thorough baseline investigation of newly diagnosed MPS patients in order to evaluate the extent of this progressive and potentially severe disease. Despite the existence of ERT for some types of MPS, the valvular compromise may still be progressive and require surgical intervention in some cases.

624 - The Family Quality of Life: A Fundamental Element for the Care of People With Morquio Syndrome

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Purpose: For 18 years, the Colombian Association of Patients with Lysosomal Depositoid Disease (ACOPEL) facilitates the access of its patients to the health system through legal counseling, emotional and economic support. However, the transcendence of their actions in the quality of life had not previously been questioned. **Objective:** To characterize the Quality of Life of the Families of People with Morquio IVA (SM-IVA) that are linked to ACOPEL. **Methodology:** A Key Family Informants were surveyed in which at least one member has the SM-IVA Diagnosis. The Family Life Quality Scale (ECVF) Adapted for Colombia (Verdugo, 2011) was used and the Family Quality of Life Map (MCVF) was defined. It identifies two areas (Critical and Strong). **Results:** By 2015, ACOPEL had identified 121 patients in the Colombian Territory, of whom 102 were interviewed corresponding to 81 families. The MCVF Results showed that Families do find themselves satisfied with all the factors. However, the proportion of families satisfied with various factors of one to another: familiar interaction (91%), parental role (90%) and health and safety (77%), family resources (56%) and support to PDCs %). Families reported lower satisfaction in the factors: family resources (29%) and support to PDCs (35%). These areas were considered critical in the MCVF. Conclusion: Families relate the dissatisfaction of their quality of life immediate consequence of the health condition of their familiar with Morquio syndrome; situation which in turn reinforces the presence of the biomedical model in the care provided and limits the capacity of the family to transform their reality.

625 - School Inclusion and Morquio Syndrome IVA: A Case Study in Two Educational Institutions in Cali, Colombia

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Introduction: Accessing and participating in Educational Contexts in Decent Conditions is one of the problems that students with disabilities face. **Objective:** This study aimed to analyze the UN School Inclusion Process in two Institutions with similar characteristics in Cali, Colombia. **Methodology:** Develop a qualitative research, with a case study design. To collect the information, a semi-structured interview, the Short List of the International Classification of Functioning and Disability, and a participant observation strategy were used. The group followed for six months three families, and the case of an 11 years old student, with a genetic disease—Morquio syndrome—in two Educational Institutions of Santiago de Cali is described. **Results:** The analyzed institutions shared characteristics related to the UN socio-demographic make, but with a significant difference in their Institutional Educational Project. Such a difference defined the success or failure of the processes of school inclusion in each of them. **Conclusion:** The evaluation of two institutions made it possible to demonstrate that there are two limitations to the process of school inclusion: the conception of disability and the pedagogical models that orient the institutional practices.

626 - Mutational Profile of Mucopolysaccharidosis Type I Patients Worldwide

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Mucopolysaccharidosis type I (MPS I) is a rare disorder caused by mutations in the *α-L-iduronidase (IDUA)* gene. More than

200 pathogenic mutations have been described so far, but their frequencies were not yet analyzed in a worldwide scale. In this study, we analyzed the mutational profile of MPS I patients from different regions of the globe in order to identify the differences between populations. Data were collected from published papers and inclusion criteria used were: information about the country of origin of patients and the absolute number of alleles in the study. Thirty-five papers were selected and used in this analysis. We also interrogated for the most common mutation from each country in Genome Aggregation Database (gnomAD) to evaluate its allelic frequency in the general population. The most common mutation observed among patients was p.Trp402*, being the major allele in Spain, Colombia, Germany, North America, United Kingdom, the Netherlands, Brazil, Australia, Czech Republic, and Slovakia, with frequencies up to 63%. The second most frequent mutation is p.Gln70*, present mainly in the North and East of Europe, in countries as Norway (50%), Russia, Poland, and Austria, ranging from 30% to 50% of alleles. North African countries Morocco, Algeria, and Tunisia have p.Pro533Arg as the most frequent mutation, corresponding to 92%, 81%, and 54% of alleles, respectively. This missense mutation is also present in other Mediterranean countries, such as Turkey, Spain and Italy—mainly in the Sicily region (42%)—and Latin America (Mexico and Brazil), though it is very rare in North Europe, North America, and Australia. Mutations frequently observed in East Asians were not found in Western populations, as c.1190-1G>A, p.Ala79Val, p.Leu346Arg and c.613_617dupTGCTC. Conversely, the mutations p.Trp402* and p.Pro533Arg were not found in patients from East Asia. In gnomAD, allelic frequency among individuals without the disease mirrors the data found in patients. For example, the p.Trp402* allele was observed in Europeans (0.0014) and Latinos (0.0004), but it was not present in Asians or Africans. In conclusion, the most common *IDUA* mutations in MPS I patients are p.Trp402*, p.Gln70* and p.Pro533Arg, but each country has its own mutational profile. The knowledge of the genetic background of MPS I for each population is essential for developing new therapies that depend on the genotype, as well as provide fast diagnosis and improve the management of patients.

627 - Clinical and Molecular Diagnosis in a Patient With Mucopolysaccharidosis Type IIIC

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Introduction: The Mucopolysaccharidosis type IIIC (MPS IIIC, OMIM 252930) is due to impaired degradation of heparan sulfate that produced a lysosomal storage disease characterized by severe central nervous system degeneration together with mild somatic disease. This disorder is caused by mutations in gene that code for heparan acetyl-CoA: alpha-glucosaminide N-acetyltransferase (HGSNAT). **Materials and methods:** The index case is an 8 years old which was born from healthy non-consanguineous parents, pregnancy and delivery were normal. He had psychomotor, developmental and speech delay and frequent upper respiratory infections and an umbilical hernia surgical correction. The fascies was coarse face, synophrys, broad lips and big tonsils (grade III), hepatomegaly and splenomegaly were recorded. Behavioral abnormalities as hyperactivity and anxiety which required treatment with methylphenidate. **Results:** Previous paraclinics reported total abdominal ultrasound reported within normal limits, audiometry reported mild conductive hearing loss, and echocardiogram reports structurally normal heart with adequate biventricular function. During evaluation and follow-up by the specialty of metabolic diseases and genetics MPS electrophoresis analysis is performed showed chondroitin sulfate and heparan sulfate bands. Subsequently, DNA analysis from HGSNAT (sequencing) gene analysis showed compound heterozygous mutations: c.607C>T/c.1700 G>A. Both mutations have not been reported, however, they produce nonsense mutations thus are considered pathogenic. **Conclusions:** The MPSIIIC is characterized by severe and rapid progression of central nervous system degeneration usually onset between 2 and 6 years with death typical during the second or third decade of life. This case highlights the relevance of diagnosis of MPSIIIC and identification healthy carrier in the related family to prevention of new cases through genetic counseling.

628 - Characteristics of Patients With Mucopolysaccharidosis Type II (MPS II) Diagnosed Aged ≥ 5 Years: Data From the Hunter Outcome Survey (HOS)

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Individuals with the severe form of mucopolysaccharidosis type II (MPS II; Hunter syndrome) are rarely diagnosed before

1–2 years of age, with patients with the attenuated form typically diagnosed later. Using data from the HOS global observational registry (Shire, Lexington, MA, USA), clinical characteristics and surgical history of prospective patients aged ≥ 5 years at diagnosis ('late-diagnosis') were compared with those aged < 5 years ('early-diagnosis'). As of January 2016, 204/947 patients were aged ≥ 5 years at diagnosis and 609/947 < 5 years (median ages [P10; P90]: 6.6 [5.0; 15.3] and 2.7 [0.7; 4.1] years, respectively). Median ages at symptom onset were 3.0 (0.5; 7.0) and 1.3 (0.3; 3.3) years. Joint stiffness, hernia and facial features consistent with MPS II were common in both groups (189/200 [95%] vs 537/602 [89%]; 174/198 [88%] vs 475/599 [79%]; 189/200 [95%] vs 563/602 [94%]). However, cognitive impairment and behavioral problems were less common in the late-diagnosis than the early-diagnosis group (81/196 [41%] vs 398/597 [67%]; 73/196 [37%] vs 375/598 [63%]). Median numbers of surgeries per patient were similar in both groups (4 [1; 9] and 5 [1; 10]), but first surgery occurred later in the late-diagnosis than the early-diagnosis group (4.0 [0.2; 16.8] vs 2.3 [0.2; 7.6] years). Hernia repair, carpal tunnel decompression and cervical decompression were more common in the late-diagnosis (n = 204) than the early-diagnosis group (n = 609; 58% vs 48%; 36% vs 26%; 5% vs 2%); conversely, ear tube insertions and adenoidectomies were less common in the late-diagnosis than the early-diagnosis group (46% vs 55%; 42% vs 56%). 14% (28/204) of patients in the late-diagnosis group and 18% (111/609) in the early-diagnosis group had died. Patients diagnosed later in life may have different clinical characteristics than those diagnosed earlier, although with significant morbidity. Further analysis is required to delineate these differences and explore whether longer follow-up time is a factor. Previously presented at WORLDSymposium 2017. **Funding information:** Shire sponsors HOS and funds medical writing support.

629 - A Novel Mutation (Met1Ile) of a Cuban Patient in the *NAGLU* Gene With Mucopolysaccharidosis IIIB

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Mucopolysaccharidosis type III (MPS III), also known as Sanfilippo syndrome, is an autosomal recessive lysosomal storage disorder characterized by the accumulation of heparan sulfate. These disorders are classified in four types (A-D). MPS IIIB is caused by mutations in the *NAGLU* gene. The aim of this study is to describe the clinical, biochemical and molecular findings of a patient with MPS IIIB. The diagnosis was performed by clinical findings suggestive of MPS, qualitative chemical tests in urine of the first urination and Thin Layer Chromatography (TLC) for MPS in 24-hour urine. We quantified the enzymatic activity of N-α-acetylglucosaminidase (NAGLU). The pathogenic variants were identified by mutation analysis. The patient studied was a 4-year-old boy, with a prenatal history of small stomach and oligoamnium in the second and third trimesters of gestation, respectively. He presented with weight and normal height, discrete facial dysmorphia and recurrent respiratory infections from 4 months of age, accompanied by severe episodes of sleep apnea requiring adenotonsillectomy at 2 years. He had hyperactive behavior with signs of aggressiveness and bruxism, associated with bilateral hearing impairment. In the qualitative tests, we detected an increment of GAG in urine. TLC showed urinary heparan sulfate excretion. NAGLU enzyme activity was deficient in leukocytes. In the molecular study of the *NAGLU* gene we detected 2 mutations: 3G>A (associated with severe phenotypes) and Met1Ile (allelic variant). The Met1Ile mutation was a novel finding. The carrier study in the parents suggested the parental origin of each variant found. The clinical manifestations, the heparan excretion, the decrease in NAGLU activity allowed the diagnosis of a Sanfilippo syndrome B type in this patient. Besides, the molecular study confirmed the presence of a novel mutation associated with this disorder.

630 - A Novel Mutation (c.852-2A>C) of a Cuban Patient in the *NAGLU* Gene With Mucopolysaccharidosis IIIC

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Mucopolysaccharidosis type III (MPS III), also known as Sanfilippo syndrome, is an autosomal recessive lysosomal storage disorder characterized by the accumulation of heparan sulfate. These disorders are classified in four types (A-D). MPS IIIC is caused by mutations in the *HGSNAT* gene. The aim of this study is to describe the clinical, biochemical and molecular findings of a patient with MPSIIIC. The diagnosis was performed by clinical findings suggestive of MPS, qualitative chemical tests in urine of the first urination and Thin Layer Chromatography (TLC) for MPS in 24-hour urine. We quantified the enzymatic activity of Heparan-α-glucosaminido-N-acetyltransferase (HGSNAT). The pathogenic variants were

identified by mutation analysis. We studied a 7-year-old patient with a history of multiple admissions for respiratory infections and surgeries of inguinal-scrotal hernia and adenoiditis from the first year of age. He had attention and hyperactivity disorder with rapid evolution to a significant deterioration of their behavior with implications in family management, educational and social insertion. This child has had focal convulsive seizures since the age of 6, associated with bilateral hearing impairment. We detected an incremented GAG excretion in urine by qualitative chemical tests. TLC showed urinary heparan sulfate excretion. HGSNAT enzymatic activity was deficient. In the molecular study, we confirmed two mutations: c.493 + 1G> A (previously described) and c.852-2A>C (allelic variant). The last one is a novel mutation and severe variant. The carrier study in the mother suggested the parental origin of each variant found. The neurological and psychiatric manifestations, the heparan excretion and the decrease in HGSNAT activity allowed the diagnosis of Sanfilippo syndrome C type in this patient. Besides, the molecular study confirmed the presence of a novel mutation associated with this disorder.

631 - Profile of MPS IVA Patient Sample Followed at the Medical Genetics Service of Hospital de Clínicas de Porto Alegre, Brazil

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Objective: The purpose of this study was to evaluate a Brazilian cohort of MPS IVA patients followed at the Medical Genetics Service of the Hospital de Clínicas de Porto Alegre, Brazil, regarding selected characteristics, including sociodemographic, clinical and therapy related data. **Methods:** Retrospective study based in chart reviews of Brazilian MPS IVA patients. The variables ascertained were: date of birth, age at diagnosis, quantification of urinary glycosaminoglycan (GAG) level at diagnosis, cardiological manifestations, enzyme replacement therapy (ERT), spinal cord compression, corneal opacity, age at death, genotype information. **Results:** Fifteen patients were included, with a mean age of 14.8, being 10 females and 05 males (67%/33%). The mean age at diagnosis was 4.4 years (range: 1 to 10). The mean urinary GAG level at diagnosis was 169.7 μg/mg creatinine (RV: 44-106). From 15 patients, 8 (53%) were on ERT, with an average age at onset of treatment of 6.25 years (range: 1 to 18). Eight patients show cardiac complications, such as valvopathy (aortic and/or mitral valve stenosis). Four (26%) patient have spinal cord compression. Ophthalmological examinations showed a significant number of patients (11/15) have corneal clouding. The majority of patients (11/15, or 73%) are able to walk, being that 7 of them improved their performance after starting therapy. Regarding genotype information, all patients had their genetic data known, and the most frequent alleles were: p.Gly116Ser (53%), p.Asn164Thr (46%) and p.Gly301Cys (40%). **Conclusion**: MPS IVA patients show wide variation regarding age of presentation, symptoms presented and disease course. It is important to monitor cardiac, ophthalmological and neurological signs, and laboratory tests, as well as monitor the performance under ERT, in order to improve the quality of life and reduce disease related risks. The involvement of a multidisciplinary team is strongly recommended for the follow-up of these patients.

632 - Clinical, Medical, and Social Aspects of a Brazilian Series of 5 MPS I-Scheie Patients

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Mucopolysaccharidosis I (MPS I) is a rare inherited, autosomal recessive disorder, caused by deficiency of the lysosomal enzyme, α-iduronidase. MPS I has been classified into a severe (Hurler syndrome) and attenuated types (Hurler-Scheie and Scheie). We are describing the clinical aspects of five MPS I-Scheie female patients followed at the Medical Genetic Service of Hospital de Clínicas de Porto Alegre, Brazil. All patients were diagnosed by this service through specific biochemical tests (high levels of urinary glycosaminoglycans and deficiency of α-iduronidase activity) and molecular analysis (two pathogenic variants in separate alleles of the *IDUA* gene). The mean age at diagnosis was 12 years (from 5 to 20) and currently the average is 32.4 years (29 to 35). The current mean urinary glycosaminoglycan level is 66.4 (RV 13-45 μg/mg creatinine). All patients are under enzyme replacement therapy with 1.2 mg/kg of laronidase every 2 weeks. Three patients have spinal cord compression; one of them performed two decompression surgeries at 16 and 21 years of age. Four had carpal tunnel syndrome, with onset from 15 to 27 years. Valvulopathy (aortic and/or mitral valve stenosis) is present in all patients. One patient presented moderate aortic stenosis and severe mitral stenosis with presence of thrombi in the left atrium at 35 years old. She was submitted to mitral valve replacement, aortic commissurotomy, and removal of thrombus, without complications. Ophthalmological examination showed corneal clouding in all patients. One out of five has also hypermetropia, astigmatism and glaucoma. Another patient went through two cornea transplantations at 13 and 14 years of age, and one patient performed corneal transplantation at 32 years old (at 27 yo she presented retinal detachment and glaucoma). One patient became pregnant at 27 years old, and remained on enzyme replacement therapy during pregnancy, having delivered a normal baby. She presented no signs of spinal cord compression and there was no change in valvulopathy, but as she had worsening of respiratory symptoms a pre-term caesarean section was recommended. **Conclusion:** cases of attenuated MPS I show wide variation with respect to age of presentation, symptoms, and disease course. It is

important to monitor cardiac, ophthalmologic and neurological signs to improve the quality of life and reduce risks. In adult female patients is it possible to consider the pregnancy with multidisciplinary follow-up.

633 - Early Initiation of Elosulfase Alpha is Associated to Better Outcomes in Mucopolysaccharidosis IVA (MPS IVA)

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We report 2 patients with MPS IVA disease from the same family, consanguineous parents, and analyze their differences regarding disease severity considering the different ages at ERT start. Patient 1: male, 8 years old. He started walking at the age of 1 year and 4 months. At 2 years of age, prominent skeletal abnormalities were noticed. He was diagnosed with MPS IVA at the age of 6 years, quantitative GAGs 335 μg/mg (VR 53-115 μg/mg of Cr), GAGs electrophoresis showed keratan sulfate, galactose-6-sulfate sulfatase indetectable and started elosulfase alpha infusions when 8 years old. Molecular exam *GALNS gene* showed c.Gly301Cys (p.Gly301Cys) in homozygosis. He is currently well, but with prominent skeletal abnormalities and a very short stature (Z-score −6.4). His MRI shows cervical stenosis, without cord compression. Patient 2: a first cousin of patient 1, male, 2 years old. He was diagnosed with MPS IVA at the age of 8 months, Quantitative GAGs 232 μg/mg (VR 133-274 μg/mg of Cr), GAGs electrophoresis showed Keratan Sulfate, Galactose-6-sulfate sulfatase 4.4 nmol/h/protein (VR 58-242 nmoles/h/protein) and started the ERT at 18 months of age. Molecular exam *GALNS* gene showed c.Gly301Cys (p.Gly301Cys) in homozygosis. His current length Z score is −0.9. Moreover, in spite of having signs of cord compression, he had a marked improvement of his mobility after ERT and physical therapy. **Conclusion:** While individual differences may also be important even in related patients, these cases are in accordance to the concept that early initiation of ERT is associated to better outcomes in MPS IVA. Additional material: patient's pictures, hip and spine X-ray, and brain and spine MRI.

634 - Systemic and Oral Manifestations in Maroteaux-Lamy Syndrome: Case Report

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Maroteaux-Lamy syndrome (MPS VI) is a rare metabolic disorder caused by the deficiency of the N-acetylgalactosamine 4-sulfatase enzyme, responsible for the degradation of glycosaminoglycans, resulting in its accumulation within the lysosomes, resulting in multiple systemic and oral changes. The prevalence of this disease is 1.3-4.5: 100 000 live births. The objective of this study was to describe the medical history, the systemic and oral manifestations of a MPS VI male, 8 years old patient (ESB) assisted at the Genetics Clinic of the Department of Pediatrics of the Federal University of Rio de Janeiro (UFRJ). ESB was the first son of a young and no consanguineous couple; GII/PII, normal pregnancy with spontaneous vaginal delivery, at term (38 weeks) with no asphyxia. Birth weight = 4330 g; birth length = 55 cm, large for gestational age. Developmental milestones (months): held his head at 3, sat without support and first word at 9 and walked at 11. At 14 months, he showed umbilical hernia and macrocrania. He was diagnosed with MPS VI at the age of 2 years old, presenting also short stature, dolycocephaly, coarse face, noisy breathing, skeletal deformities (*pectus carinatum*, thoraco-lumbar kyphosis), hepatomegaly and Mongolian spots on the back. Enzyme Replacement Therapy with galsulfase was initiated at the age of 2 years 10 months. So far, his cognition is normal. He was referred to the Pediatric Dentistry Clinic / UFRJ for dental evaluation. Anamnesis, clinical examination (evaluation of soft tissues, occlusion, periodontal evaluation, and decayed missing filled surfaces/teeth (DMFT/dmft) index) and computed tomography (CT) of the craniofacial region were performed. Several oral alterations were observed: macroglossia, anterior open bite, high dmft (4), and poor periodontal conditions evidenced by high bleeding probing index (41.1%) and dental biofilm index O'Leary (61.7%). The CT revealed condyles with morphological alterations and severe bilateral hypoplasia, absence of the articular fossae and eminences of the temporomandibular joints, bilateral reduction in mandibular body height in the posterior region, mucosal retention cyst in the right lateral wall of the right sinus, anterior open bite, delayed eruption of the first permanent molars and increase of the periocoronary space of the lower first molars and canines suggesting dentigerous cysts. The systemic alterations hamper the dental treatment and the general prognosis of the patient.

635 - Oxidative Stress Assessment by Glutathione Peroxidase Activity and Glutathione Levels in Response to Selenium Supplementation in Patients With Mucopolysaccharidosis I, II and VI

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Objective: To assess the status of plasma selenium (Se), the level of oxidative stress by the activity of the enzyme glutathione peroxidase (GPx) and the response to Selenium supplementation in individuals with Mucopolysaccharidosis (MPSs) I, II e IV. **Method**: Cross-sectional study including patients with enzymatic diagnosis of MPS type I, II, and VI, which perform enzyme replacement therapy (ERT). The Se supplementation was based on the Recommended Dietary Allowance (RDA) and was made during 12 months with following plasma analysis: Se, total glutathione (GSHt), oxidized (GSSG), reduced (GSH), and GPx. Plasma concentrations of Se were determined by hydride generation atomic absorption spectrometry (HG AAS). The GSHt and GSH were analyzed by high performance liquid chromatography (HPLC) through fluorescence detection and isocratic elution. GPx activity was performed by colorimetric analysis. All data were presented as mean $\pm$ standard deviation. Comparisons of Se, GPx, GSH, and GSSG before and after supplementation were determined using the paired Student's *t*-tests and a 5% significance level was adopted. **Results:** Plasma concentrations of Se from 30 patients were analyzed: 13 patients with MPS I (eight males and five females), eight patients with MPS II (seven males and one female) and eight with MPS VI (6 males and 2 females). The mean was 13.1 $\pm$ 8.3 years old (mean $\pm$ 2 standard deviation SD). The analysis of Se status showed that 28 patients (93.33%) were deficient with the mean 35.7 $\pm$ 10.0 aeg/L (mean $\pm$ 2 SD). After the first supplementation, 21 patients (87.5%) reached the normal Se levels and the mean was 59.20 $\pm$ 15.6 aeg/L. Three patients (12.5%) maintained values below normality (mean 33.67 $\pm$ 6.3). The GSHt before supplementation was 7.90 μmol/g Hb and after 5.76 μmol/g Hb ($P < .05$). GSH/GSSH before supplementation were 2.3 μmol/g Hb and after supplementation 0.58 μmol/g Hb. The value of GPx was 16.46 U/g Hb before supplementation and after 4.53 U/g Hb ($P < .05$). **Conclusion**: Although the pathophysiology and oxidative stress in MPSs patients are not yet completely understood, our study has demonstrated in an accessible way that selenium supplementation improved the oxidative stress parameters related to GPx, and glutathione in patients with MPS I, II e VI.

636 - Subdural Hemorrhages in a Boy With Mucopolysaccharidosis IIIB

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Subdural hematomas are most common in infants and elderly adults, result from bridging veins' tears and usually arise as head trauma outcome. The soft consistency of the unmyelinated brain and cerebral atrophy are likely to be a pathophysiological basis of such age predisposition. Subdural hemorrhages, occurring spontaneously or after head trauma, have been described in several neurometabolic diseases with cerebral atrophy and myelination disturbance, viz glutaric aciduria type I, pyruvate carboxylase deficiency, Menkes disease, D-2-hydroxyglutaric aciduria, methylmalonic aciduria, homocystinuria, Hermansky-Pudlac syndrome, infantile neuronal ceroid lipofuscinosis, MPS II and IIIB. There are scant descriptions of impaired hemostasis in MPS I, II presumably due to anticoagulant activities of glycosaminoglycans. An 8-year-old boy was admitted to neurological department for systematic workup. At 2 years 10-months he was diagnosed with MPS IIIB. During last 2 years, his developmental regression progressed, he completely lost his speech, his motor activity decreased significantly, and in recent months he became fatigued and sleepy and started to experience dysphagia. Brain MRI revealed cerebral atrophy, chronic subdural hematoma of right hemisphere, compression of right hemisphere and right lateral ventricle. 3 weeks later the boy presented with a single episode of adverse seizure with tonic posturing of ipsilateral arm, he started to have hemiparetic gait, got drowsy. Brain KT displayed acute hemorrhage in the region of the chronic subdural hematoma. Laboratory studies demonstrated fluctuating thrombocytopenia, impaired platelet aggregation to ADP and arachidonic acid, decreased level of plasma fibrinogen. He was treated with platelet concentrate transfusion, etamsylate, folic acid and closed external drainage of subdural hematoma was performed. Three months after subdural hematoma surgery the boy presented without apparent neurologic regression compared to previous examination, he still had hemiparetic gait, laboratory workup showed thrombocytopenia, elevation of APTT, impaired platelet aggregation to ADP, arachidonic acid, and thrombin. We conclude that regular neurovisualization and coagulation tests should be included in the standard workup for patients with neuronopathic mucopolysaccharidoses, as constellation of abnormal hemostasis and progressive cerebral atrophy may present a risk of intracranial hemorrhage.

637 - Estimating the Epidemiological Profile of Mucopolysaccharidoses (MPS) in the State of Rio Grande do Norte (RN) in the Period From 1995 to 2015

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MPS are characterized by the intralysosomic accumulation of glycosaminoglycans (GAGs), secondary to a deficient activity of one of the enzymes involved in the degradation of GAGs, being classified in MPS I, MPS II, MPS III-A, MPS III-B, MPS III C, MPS III-D, MPS IV-A, MPS IV-B, MPS VI, MPS VII, and MPS IX. The worldwide incidence of MPS ranges from 1.9 to 4.5 cases in 100 000 births. In Brazil, the prevalence is still

unknown, but, according to a survey by the MPS Brazil Network, it is known that there are about 250 patients with MPS in the whole country. Comparing the epidemiological profile of the MPS in the RN in a period of 20 years in relation to the prevalence in Brazil and in the world, we performed a descriptive study carried out with SESAP/RN from the analysis of medical records of patients attended at the medical genetics outpatient clinic and diagnosed with MPS in the RN from 1995 to 2015. Data regarding the type of disease, sex and City of origin of the affected. **Results:** In the RN, from 1995 to 2015, 24 individuals (1: 132 000 inhabitants) were diagnosed, of which 14 (58.33%) were MPS VI, 4 (16.66%) MPS I, 4 (16.66%), MPS IV, and 2 (8.33%) MPS II. The prevalent sex was female (58.33%). The cities with the highest prevalence were Apodi (3 cases = 1: 11 557), Ipanguaçu (2 cases = 1: 6602), Tibau do Sul (2 cases = 1: 5692) and Riacho da Cruz (2 cases = 1: 1582). In Brazil, few States have reported or published MPS prevalence, however, with no distribution reported by municipalities of origin. **Conclusion:** It is difficult to estimate the prevalence of MPS, due to the lack of population studies and epidemiological data. For this reason, isolated studies in different States become of great value, since they allow to quantify cases in the country, even so partially. Another important point is that, once the reality of the various microregions has been defined and the knowledge of those with the highest prevalence known, it is possible to plan screening strategies and population orientation, as well as adequate multidisciplinary assistance to ensure greater effectiveness in State policies and a better quality of life for those affected and their families. These results will be presented as a proposal to the Government of the RN that, despite being a small State in terms of population, presents a high prevalence in some cities, which are not being properly observed.

638 - Mucopolysaccharidosis Type IV A in a Patient With Congenital Glaucoma

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The Mucopolysaccharidose (MPS) type 4A (Morquio) is an autosomal recessive inherited lysosomal storage disorder characterized by the accumulation of keratan sulfate and chondroitin-6-sulfate in cornea and bone and associated with N-acetyl galactosamine-6-sulfate sulfatase deficiency. Major clinical findings are short stature, skeletal dysplasia, dental anomalies and corneal clouding. These can also be accompanied by nerve-type hearing loss, heart valve diseases, joint laxity, and cervical myelopathy. This disease can treat with enzyme replacement therapy and gene therapy due to absence of central nervous system involvement. Typical ocular manifestations in many children with MPS type 4A include corneal clouding, retinal degeneration, reduced electroretinogram wave amplitude, optic nerve atrophy, papillary edema, and glaucoma. Herein we described a MPS type 4A patient with bilateral congenital glaucoma. **Case:** A 34-day-old girl admitted to our clinic for preoperative evaluation due to bilateral congenital glaucoma. Parents were second degree cousin marriages. She had term, 2430 gr birth history. Her 7-year old sister and aunt were MPS type 4, but no congenital glaucoma. On physical examination, body weight was 3320 gr, height was 52 cm, and head circumference was 36 cm. Maculopapular rashes was detected on the upper chest and back but no organomegaly. The patient was diagnosed with MPS type 4 due to a low detection of leukocyte-galactose-6-phosphate sulfatase enzyme activity (4.9 nmol / 17 h / mg protein [normal: 41-331]). Ocular disorders can also be seen in patients with MPS type 4. Glaucoma is one of these conditions. However, we didn't observe an MPS type 4A patient with congenital glaucoma in the literature. Therefore, for the early detection of etiology, MPS should be considered in differential diagnosis in infants with congenital glaucoma.

639 - Computational Analysis of Mouse Brain Interactome for the Lysosomal Enzyme Iduronate-2-Sulfatase

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Mucopolysaccharidosis type II (MPS II) is a lysosomal storage disease caused by the deficiency of iduronate-2-sulfatase (IDS), which leads to the subsequent storage of undegraded glycosaminoglycans heparan and dermatan sulfate. There is limited information about the IDS interactome in brain or other tissues. In this study, we used bioinformatics tools to study an IDS proteome previously isolated from a whole cellular extract of wild-type mouse brain. The proteome, which comprises 217 proteins, was analyzed with PPI Spider and GENEMANIA, to predict an interaction network and identify potential protein-protein interactions. The networks were visualized by Cytoscape. The analysis of networks allowed to predict 32 proteins with potential protein-protein (physical) interactions with IDS. This protein dataset was investigated regarding their functional categories, biological pathways and functional localization by using PANTHER and DAVID database. Proteins were divided into the following groups based on their functional annotations: 1) Formation and trafficking of vesicles, 2) Scaffold proteins, 3) Proteins related to ATPase activity, 4) Signaling cascades, 5) Transcriptional factors, 6) Cell adhesion, 7) Intermediate metabolism, 8) Neurofilaments and cytoskeleton, and 9) Anchorage proteins and receptors. According to this information, we selected 10 proteins to validate their physical interaction with IDS: SYT1, ALDOA, 1433Z, 1433G, CAMKV, PRDX2, HSP7C, ALDOC, LSAMP, and EAA1. These results represent valuable information in the characterization of protein-protein

interactions for IDS in brain, which will allow us to increase our knowledge about the role of IDS under physiological conditions, as well as the implications of its deficiency in MPS II patients.

640 - Macular Edema Reduction After Long Term Enzyme Replacement Therapy in Mucopolysaccharidosis Type II (Hunter Syndrome): A Case Report

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Ocular abnormalities in Hunter syndrome include exophthalmos, disk swelling, optic atrophy, and retinopathy. Accumulation of GAGs in retinal tissue induced retinal degeneration and pigmentary retinopathy. Macular edema was occasionally described in MPSII. Spectral Domain Optical Coherence Tomography (SD-OCT) is a useful tool for detecting retinal pathology, particularly macular changes. **Purpose:** To show the evolution of macular edema in a patient with Hunter syndrome after 5 years of enzyme replacement therapy. **Methods:** A 17-year-old patient diagnosed with non-neuronopathic Hunter syndrome was referred for ophthalmologic evaluation after six months of having started IV enzymatic replacement therapy (ERT) with idursulfase. Annual follow-up was performed with visual acuity, biomicroscopy, intraocular pressure, fundus and corneal retinography, computerized visual field, and SD-OCT. Electroretinogram (ERG) was performed at the first examination and after five years. **Results:** At baseline, the patient presented hyperopia, macular edema and pigmentary retinopathy, evidenced clinically by reduced best-corrected visual acuity and arcuate visual field loss. After 5 years of treatment, a significant reduction of macular edema was evidenced by SD-OCT (Right eye: 675 μm at the center shifted to 469 μm. Left eye: 589 μm to 508 μm). Nevertheless, spectacle-corrected visual acuity declined from 6/10 to 2/10 in both eyes. Visual field progressed from an arcuate scotoma to a bilateral ring scotoma. ERG: Deterioration of photopic and 30 Hz responses after 5 years. **Conclusions:** Long term ERT with IV idursulfase appears to be related with a reduction of macular edema in Hunter syndrome although clinical manifestations of retinal degeneration have shown no significant improvement

641 - The Limitations to Enzyme Replacement Therapy in a 14-Year-Old Boy With MPS II Who is Morbidly Obese

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Mucopolysaccharidosis type II (MPS II; Hunter Disease) is a rare inborn error of metabolism resulting from a deficiency in the Iduronate 2-sulfatase enzyme. MPS II affects numerous systems including the central and peripheral nervous systems and the cardiovascular, gastrointestinal, respiratory, and musculo-skeletal systems. In Australia, ERT for MPS II is funded by the Federal Government through the Life Saving Drug Program (LSDP). A 14-year-old boy from rural Victoria was diagnosed with MPS II at 7 years of age but started ERT only at 12 years of age due to the family's reluctance to access treatment. Currently the patient is attending the hospital for weekly ERT treatment and the many sub specialty appointments required for the provision of continued ERT treatment, as per the LSDP. Despite the long distance between home and the hospital and challenging social issues, there has been very good compliance with attendance at all required medical appointments (weekly ERT and 48 sub-specialty face-to-face encounters between April 2016 and April 2017). Like many patients with MPS II, our patient requires many surgical procedures such as C-spine stabilization, Tonsillectomy and Adenoidectomy, carpel tunnel release surgery and insertion of a permanent central venous access device (CVAD) for the provision of ERT. This patient is morbidly obese (currently, at 14 years of age: weight 107 kg; height 143.5 cm, body mass index 49.87 kg/m^2). The patient is becoming increasingly difficult to cannulate and without a long-term CVAD line for the intravenous infusions to be administered through, there may come a time when it will be impossible to administer the ERT. However, his obesity poses an additional high risk for anesthesia. It was made clear by the anesthetic team that before any intervention, he must lose weight. Weight management is multifactorial. It requires the child and family to comprehend the risk of obesity. Consideration must be given to the family's financial abilities and social support. Current strategies to control and manage his weight, including dietetic advice and monitoring have been unsuccessful due to poor compliance. The lack of local professional psychological and family support and the need to manage his weight through long distance consultation adds to the difficulties. The possibility of reaching an endpoint with no ability to provide on-going ERT is a difficult issue to address with a patient and family.

642 - Clinical Trial of Intracranioventricular Enzyme Replacement Therapy for Hunter Syndrome

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To evaluate efficacy on neurocognitive function of patients with mucopolysaccharidosis type II (MPSII), we have started a 52-week, multicenter, single arm, open-label phase I/II clinical trial. The participants first receive implantation of CSF Reservoir System. Idursulfase beta (supplied by Green Corss) is injected into cerebroventricular space via the device monthly

for 48 weeks. Weekly i.v. idursulfase treatment is continued throughout this study without overlapping 24 hr period before or after ICV injection. The primary endpoint is to detect declining from base line heparan sulfate in cerebrospinal fluid at 52 weeks. The secondary endpoint is to assess change of developmental age after 12 months of treatment at visit week 52, as obtained by Kyoto Scale of Psychological Development and other scales if available. Six patients with genetically and enzymatically diagnosed on MPSII have been enrolled in this study. This study is going without any serious adverse reactions. We observe significant decrease of heparan sulfate concentration in cerebrospinal fluid in all six patents.

643 - Mucopolysaccharidosis—Is There a Link Between Disease Severity and Nutritional Status? A cross-sectional Portuguese study

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Background: Mucopolysaccharidosis (MPS) patients may be susceptible to malnutrition due to disease progression, and/or inadequate food habits. **Aim:** to assess nutritional status of MPS patients and relate it with disease severity. **Patients and methods:** A cross-sectional study including patients from four national Centers was developed. Anthropometric, body composition and laboratorial data were collected. The Health Assessment Questionnaire was answered by all patients/caregivers. The study was approved by Ethical Committees and other legal parties required. A written informed consent was obtained. **Results:** 31 patients (5 MPS I; 4 MPS II; 9 MPS III; 3 MPS IV; 9 MPS VI; 1 MPS VII; 17 males), aged between 1.7 and 32.7 years, were included. The lowest weight, height and BMI z-score values were observed in MPS VI, MPS IV and MPS I groups, respectively. Mean phase angle varied from 3.9° to 5.0°, in MPS I and MPS VI groups, respectively. Pre-albumin, retinol binding protein (RBP), creatinine and HDL-cholesterol were low in 59.3%, 75%, 77.4% and 48.4%, of the patients, respectively. Vitamin D insufficiency was present in 38.7% and deficiency in 48.4%. MPS III group showed significantly higher plasma pre-albumin RBP and vitamin A levels and MPS VI group exhibited lower RBP, vitamin A and vitamin E than the other groups. Mean total HAQ score (part I) was 6.6 (SD = 3.8) (11 = total inability), highest in the oldest. MPS III patients had higher scores in "eating and drinking", "dressing" and total HAQ than the other MPS groups. Patients with lowest plasma creatinine showed higher scores in "walking and climbing stairs" domain. Caregiver assistance total score (part II) presented a mean of 3.0/4.0 (SD = 1.1). **Discussion and conclusion:** This set of MPS patients showed a high inability associated with elevated disease severity. The oldest patients and the MPS III group showed the highest levels, probably caused by disease progression and/or cognitive impairment. In a substantial number of patients (namely MPS VI and the oldest ones), nutritional status was also found to be impaired. The degenerative character of MPS, as well as an unhealthy pattern of living may lead to nutritional deficits. Nutritional status monitoring and improvement, by correction of deficits, may contribute to a better prognosis and, possibly, to a better quality of health during the reminiscent years of life.

644 - Pre-Post Treatment Urinary Glycosylated Lysine in Mucopolysaccharidoses Patients

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Collagens are subject to post-translational modifications including hydroxylation and subsequent glycosylation of lysines and prolines. We have developed a simple LC-MS/MS method to monitor the modification and hence metabolism of glycosylated collagen in urine. Collagen IV is highly glycosylated and is the main collagen found in basement membranes. Extracellular matrix disruption is known to be an early pathological feature in the mucopolysaccharidoses (MPS). Elevated glycosylated collagen is observed in other bone disorders and free glycosylated lysine is elevated in MPS patient urine. **Objectives:** To explore whether modified urinary lysine is a better biomarker of bone pathology in MPS than glycosaminoglycan (GAG) levels by assessing the effect of enzyme replacement therapy (ERT) treatment. **Methods:** Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to quantitatively characterize lysine and its modified forms; hydroxylysine, galactosyl hydroxylysine and glucosyl-galactosyl hydroxylysine in the urine of MPS patients and controls. All analytes measured in this study were normalized to urinary creatinine levels. **Results:** Using the new method we performed a preliminary study looking at paired pre-post treatment samples of 19 MPS patients (2 MPS I, 5 MPS II, 1 MPS VI, and 11 MPS IV) and 41 controls. We found that the MPS samples exhibited a significant correlation between modified lysine and total GAGs pre-treatment ($r^2 = 0.3474$-0.6605, $P < .0001$). Modified lysine expression levels were age-dependent which is similar to GAG levels. We confirm that galactosyl

hydroxylysine and glucosylgalactosyl hydroxylysine were significantly higher in MPS patients compared to age-matched controls. The correlation between changes in glycosylated lysine and GAG expression levels post treatment was found only in 10 out of 19 patients. Conclusion: Whilst modified lysine expression was increased similarly to GAG expression pre-treatment, only in approximately half of the patients, post-treatment modified lysine was reduced. As MPS bone disease has limited response to ERT, our findings suggest that the level of free glycosylated collagen could assist in understanding the underlying mechanism, and in the search for new therapies. Further longitudinal studies are needed to confirm if this marker can be used in prognosis and treatment monitoring.

645 - Quality of Life of a Group of Patients With Mucopolysaccharidosis Type IVA and VI of COLOMBIA and Argentina in Enzyme Replacement Therapy

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Objective: To assess the quality of life of a group of patients with Mucopolysaccharidosis type IVA and VI of Colombia and Argentina in enzyme replacement therapy. **Materials and methods:** A cross-sectional descriptive study was carried out in Pereira Colombia and Rosario Argentina. All the patients with clinical and biochemical diagnosis of Morquio and Maroteaux Lammy syndrome were included in this study. A trained assistant applied the following instruments: for adults, the WHOQOL-BREF questionnaire, developed and validated by the Group WHOQOL of the World Health Organization (WHO). This questionnaire accounts for 4 domains that are: Physical, Psychological, Social, Environment. For children (under 18 years of age), their quality of life was assessed using the Stanford Health Assessment Questionnaire (SHAQ). The SHAQ measures eight domains as follows: dressing and grooming, getting up, walking, eating, hygiene, reaching, grabbing, and activities. **Results:** A total of 18 patients, 12 children and 6 adults were evaluated. Children: Consolidated results of the CHQ according to their domains, presents significant affectation in dressing, hygiene, followed by walking domains and other activities. A negative relationship between age and quality of life was evidenced. In relation to the total score of the quality of life, it has a rather important affectation (mean score 2 of a score of 1-4) accompanied by an assessment on a visual analog scale of pain (1-10 points) On average is 3 Points, showing a slight of slight intensity pain. In some patients, however, there was a maximum range of up to 7 points in pain, unrelated with age. **Conclusions:** Patients with mucopolysaccharidosis have an evident effect on the quality of life due to their mobility, as evidenced by the difficulty in dressing, walking, and activities for personal hygiene. This affectation increases with age. There is a moderate correlation between the age and the degree of pain reported by the patient $r = 0.56$. Having specific instruments for Mucopolysaccharidosis to measure quality of life is necessary to better objectify it, since the available WHO surveys are implemented for patients with rheumatoid arthritis and other chronic diseases. However, the instrument used was easy to apply, logical responses were obtained in correlation with the clinically state of the patients, and the results are similar to those obtained in cohorts of patients with other chronic pathologies. However further research is required.

646 - Use of Methylphenidate for Treatment of Hyperactivity in Children With MPS II (Hunter Syndrome)

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Hunter syndrome (MPSII) is an X-linked chromosomal storage disorder due to deficiency of the lysosomal enzyme iduronate-2-sulfatase. It is clinically divided into severe or "neuronopathic" form (about 2/3 of cases) and attenuated or "non-neuronopathic" phenotype. Hyperactive behavior is a very common clinical feature in MPSII during childhood. However, literature evidence about treatment with psychoactive agents for hyperactivity in MPSII is lacking. This study evaluates methylphenidate (MPH) safety and efficacy in a cohort of pediatric patients with Hunter syndrome. **Methods:** Seven MPS II patients (mean age 5 years, range 3 to 9 years) were put on MPH between 2010 and 2016. Mean duration of treatment was 28 months (range 3 to 50 months) All the patients received weekly infusions of IV idursulfase. Regular cognitive assessments were done in all the patients. A retrospective chart review obtained comorbid symptoms, concomitant medication, vital signs, side effects, and parent-reported MPH efficacy. **Results:** Five of seven MPS II patients had neuronopathic involvement defined for the progressive cognitive decline over time, all of them still with IQ above 50. Additional comorbidities included seizures (1) and supraventricular arrhythmia (1). Four patients reported one or more MPH side effects: appetite suppression (2) anxiety / worsening of obsessive behaviors (2) marked rebound effect (1), insomnia (1). There were no statistically significant changes in weight, EKG or blood pressure 12 and 24 months after medication initiation. Hyperactivity improvement was subjectively reported by parents in 4/7 patients for at least 18 consecutive months. Risperidone was used as concomitant medication in 3/7 patients. Three of seven patients remained on MPH after 2 years of treatment. **Conclusions:** This study evaluates empirical symptomatic MPH use in a small group of hyperactive children with Hunter syndrome. Methylphenidate was tolerated and effective in 4 of 7 subjects. Side effects were common but

mostly minor. MPH could be a potential therapeutic tool for MPSII children with hyperactivity disorder at some stage of the disease. Further controlled studies with larger sample size are needed to better evaluate safety and efficacy of psychiatric agents for behavioral symptoms of MPS II.

647 - A Novel Variant at Exon 9 of Iduronate 2-Sulfatase Gene in an Indonesian Patient With Mucopolysaccharidosis Type II

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Mucopolysaccharidosis type II or Hunter syndrome is one of the lysosomal storage diseases caused by the absence or malfunctioning of iduronate 2-sulfatase (I2 S) enzyme in the lysosome due to alteration of iduronate 2-sulfatase (IDS) gene. Normally, I2 S enzyme works in the degradation of heparan and dermatan sulfate. An alteration of IDS gene will cause the abnormality of this process and cause the accumulation of both glycosaminoglycans (GAGs) in tissues or organs which will lead to the occurrence of symptoms. This case report aimed to demonstrate our first MPS type II patient which tested for genetic analysis using Sanger sequencing method. A 3-year-old boy, diagnosed as MPS type II based on a very low I2 S enzyme activity. This patient was first brought to Department of Neurology due to speech delay and facial changes since 1.5 years old. There was no motor delay, but mother noticed stiffness of both hands. He is the first child from Indonesia non-consanguineous parents. He was born spontaneously with unremarkable history. He has normal growth and development until 1-year-old. Physical examination showed coarse facies, frontal bossing, enlarged adenoid and joint arthropathy. Supporting examination revealed "bullet shaped" of cervical vertebrae, trivial mitral regurgitation from echocardiography, normal liver size from abdominal ultrasound, narrowing of foramen magnum from brain CT-scan, and severe sinusitis. This patient is our first case that receives genetic testing to find pathogenic variant. Pathogenic variants are frequently reported being located at exon 9, especially in Asian population. Due to limited resources, we first tried to do Sanger sequencing at exon 9. A pair of primers reported in one study from Taiwan are being used to amplify exon 9. Using Sanger sequencing, this exon being sequenced. One single-nucleotide alteration variant in exon 9 was found. This alteration has never been reported elsewhere. This novel variant (c.1506G>C) lead to the alteration of amino acid, from tryptophan to cysteine (p.Trp502Cys). A novel variant (c.1506G>C) is a candidate disease-causing mutation which might affect the normal structure and function the of I2 S enzyme. In silico analysis using bioinformatics approach might help to predict protein structural and function alteration. Sequencing other exons is our next step to be done. Considering the wide variety of mutation in the *IDS* gene, the need to check the possibility of other variants in other exons is a must.

648 - ZFN-Mediated In Vivo Genome Editing Results in Phenotypic Correction in MPS I and MPS II Mouse Models

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Mucopolysaccharidosis (MPS) types I and II result from the deficiency of lysosomal enzymes α-L-iduronidase (IDUA) and iduronate 2-sulfatase (IDS), respectively, and the accumulation of glycosaminoglycans (GAGs) substrates, with severe patient morbidity and shortened lifespan. Although severe MPS I (Hurler syndrome) can be treated by hematopoietic stem cell transplantation, this carries significant morbidity and mortality risks. Additional treatment for MPS I & II consists of enzyme replacement therapy, which slows disease progression but requires lifelong weekly infusions and is a severe hardship on patients. We have developed a ZFN-mediated genome editing strategy to permanently modify patient liver cells through insertion of a corrective hIDUA or hIDS gene at the *Albumin* locus. Insertion into this locus and co-opting its high-level transcriptional activity potentially provides long-term expression of the corrective transgene in stably modified hepatocytes. This avoids potential issues associated with non-integrating gene therapy approaches; particularly for liver-directed treatment of pediatric disease where significant hepatic cellular division during growth and development create a potential loss of episomal vector genomes. In MPS I and MPS II mouse models, treatment with AAV2/8 vectors comprising the *Albumin*-targeting ZFNs and the respective corrective human transgene donor, results in supraphysiological hIDUA or hIDS hepatic enzyme levels, secretion of active enzyme into the plasma, and uptake by secondary tissues at levels sufficient for complete clearance of GAG substrate. Histological observations show reduced disease-related cellular vacuolation in the liver and other target tissues. Specific Mass Spec analysis of heparan and dermatan sulfate in the brain demonstrated systemic reduction of GAG accumulation. ZFN+Donor treatment prevented cognitive deficits exhibited in both animal models in the Barnes maze at 4 months post-treatment, where treated and wild-type animals exhibited similar cognitive behavior. Biochemical characterization of expressed hIDS and hIDUA proteins demonstrated the expected glycosylation patterns, and we found mannose-6-phosphate-dependent cellular uptake in vitro. In summary, our results provide proof-of-concept for ZFN-mediated targeting of the *Albumin* locus in hepatocytes to express therapeutic amounts of hIDUA and hIDS for the treatment of MPS I & II. We currently have open clinical studies for both diseases.

649 - Presenting Signs and Symptoms of MPS: Results of an International Physician Survey

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Background: Mucopolysaccharidosis (MPS) disorders are rare genetic conditions which result in progressive cellular damage. The benefits of early intervention make prompt diagnosis of paramount importance. Unfortunately, due to lack of awareness and heterogeneity of clinical presentation, MPS diagnosis is often delayed. The objective of this study was to characterize how, when, and to which specialties individuals with MPS first present. **Methods:** A multilingual, international, online survey was utilized to capture data relating to physicians' experience with MPS. Physicians were recruited from an online research panel that had previously consented to participate in surveys. Screening questions were used to identify physicians who had previously diagnosed, referred, or managed a patient who had confirmed or suspected MPS. Subsequent questions explored the presenting signs and symptoms among physicians' MPS patients, referral patterns, and diagnostic testing, based on the physician's recall. Responses were tabulated overall, and stratified by physician specialty, MPS subtype, and patient age at presentation, where applicable. **Results:** The 209 eligible survey respondents were distributed across 14 countries, had a median of 16 years of experience post-residency, and represented the following fields: orthopedics, rheumatology, neurology, internal medicine, ophthalmology, cardiology, pediatrics, metabolic disease/metabolic genetics, otorhinolaryngology, and general practice/family medicine. The most frequently reported signs and symptoms at presentation involved skeletal/muscular abnormalities and developmental delays. Forty-one percent of physicians reported signs and symptoms they had not previously known were associated with MPS, including behavioral and gastrointestinal presentations. Respondents' patients were predominantly referred from general practice physicians and pediatricians and most frequently referred to metabolic specialists, geneticists, and pediatricians. Seventy-three percent of respondents said they would refer patients to another physician for diagnostic testing. **Conclusion:** The collective physician perspective obtained in this global survey could potentially be applied to the development of referral tools, to facilitate earlier diagnosis of MPS and improved patient outcomes.

650 - Surgical Procedures in MPS Pediatric Patients

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Objectives: New therapies in mucopolysaccharidosis have improved prognosis and patient's lifespan over the last 15 years. This has brought to an increase in the needs of procedures to optimize patient's quality of life, among which, surgeries are one of the most important. We aim to report the prevalence of different surgeries in our cohort of pediatric MPS patients. **Methods:** Retrospective study of 28 pediatric genetically confirmed MPS patients from 2005 to 2017. **Results:** The study includes 9 MPSI patients (all but one with BMT in the first years of life), 5 MPS II, 8 MPS III, 4 MPS IV, 1 MPS VI, and 1 MPS VII with ages from 2 to 18 years. All groups except MPS IV patients underwent ENT surgery in the first five ages of life (18/24). All groups except MPS III underwent some type of orthopedic surgery (16/20); being carpal tunnel surgery the most frequent in all but MPS IV (12/16) followed by genu valgo correction which was performed in MPS I, MPS IV, and MPS VI children; 6 patients were operated with spine fixation and 2 older ones had hip replacement. 6 patients with MPS II, VI, and VII had ventriculo-peritoneal shunting for hydrocephalus. 6 MPS patients underwent hernia repair and 3 MPS I and VI children had a corneal transplant. 6 patients had a porth-a-cath implantation for ERT. Regarding the type of MPS, patients with MPS III only had ENT surgery (4/8) while MPS VI and VII had the larger number of surgeries (6-8) during the first 12 years of life. All surgeries were done with an experienced team and no complications were seen in airway management. All patients were under monitoring for several hours after extubation. More than a surgery at a time was done in 6

occasions. **Conclusions:** MPS patients undergo a large number of surgeries in the first decades of life, especially those with skeletal involvement. ENT and orthopedic surgery are globally the most frequent. ENT, hernia repair and genu valgo surgeries are done mostly in the first 5 years. When performed in an experienced environment, anesthetic complications can be avoided.

651 - Pheno-Genotypic Features and Long-Term Enzyme Replacement Treatment Results of 18 Turkish Mucopolysaccharidosis Type II Patients From a Single Center

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Objective: Mucopolysaccharidosis type II (Hunter syndrome, MPSII) is an X-linked recessive lysosomal storage disorder caused by the deficiency of iduronate-2-sulfatase (IDS), an enzyme encoded by the *IDS* gene. Clinical manifestations include short stature, coarse face, recurrent ear infections, hepatosplenomegaly, inguinal and/or umbilical hernias, joint contractures, skeletal changes, and variable cognitive impairment due to the accumulation of dermatan and heparan sulfates. We evaluated the clinical, biochemical, molecular data and the results of long term follow-up with enzyme replacement treatment (ERT) of 18 patients with MPSII from the South part of Turkey. **Methods:** In this study, we reviewed the medical records of 18 Turkish patients with MPSII in a single center. **Results:** The admission symptoms were; coarse face (8/18), recurrent respiratory infections (4/18), cognitive impairment (3/18), macrocephaly (2/18), and joint contractures (1/18). The mean age at onset of symptoms was 27.8 $\pm$ 13.5 (7-53) months. The activity of IDS was markedly low in all patients. The duration between the first symptom and the diagnosis was 8.1 months (1-83). The clinical findings were; joint stiffness, dysostosis multiplex, and coarse face (18/18), airway obstruction (17/18), cardiac involvement (16/18), umbilical hernia (16/18), hepatomegaly (15/18), developmental delay (10/18), recurrent ear infections (10/18), kyphoscoliosis (9/18), inguinal hernia (7/18), hearing loss (5/18), splenomegaly (4/18), and papular rash (1/18). Analysis of *IDS* gene revealed eleven different mutations. p.S87 R, p.R468Q, p.C21* and p.H138Y were novel mutations. The duration of ERT was 49.6 $\pm$ 30.9 months. One patient died without ERT at the age of 9.5 years. The mean current age of the patients was 89.6 $\pm$ 41 (18-162) months. **Conclusion:** Here we present the clinical and molecular features of a large group of Turkish MPSII patients and the results of long term follow-up with ERT. Increased mobility and walking capacity together with greater independence in daily activities are the most striking effects of ERT. Also, the frequency and the severity of respiratory infections and the number of hospitalization were significantly decreased. So, ERT is an effective and a safe way of treatment in milder-moderate forms of MPSII especially when initiated early. It improves the quality of life and decreases the comorbidities of the disease. This study also adds four novel mutations to the literature.

652 - Recombinant N-Acetylgalactosamine-6-Sulfate Sulfatase (GALNS) Enzyme Produced in Pichia Pastoris: In Vitro and In Vivo characterization

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Mucopolysaccharidosis IV A (MPS IVA, Morquio A disease) is a lysosomal storage disease produced by mutations in N-acetylgalactosamine-6-sulfate sulfatase (GALNS) enzyme. Currently, the treatment for MPS IVA disease is based on enzyme replacement therapy (ERT) by using of elosulfase alfa. Although ERT with elosulfase alfa is a therapeutic option for MPS IVA patients, it has limitations including i) a limited effect on skeletal, corneal, and heart valvular issues, ii) a short half-life of the enzyme, iii) immunological issues, and iv) the high cost. Previously, we had reported the production of the recombinant GALNS enzyme in the yeast *Pichia pastoris* as an alternative for Morquio A ERT. In this study, we present the results of the in-vitro characterization and biodistribution of this recombinant enzyme. The cell up-take was evaluated in HEK293 cells, Morquio A fibroblasts and wild-type mouse chondrocytes. Intracellular traffic was evaluated in HEK293 cells using an AlexaFluor 568 stained enzyme. Reduction of Keratan Sulfate (KS) was evaluated in Morquio A fibroblasts by using HPLC-MS/MS. In-vivo biodistribution was studied in wild-type C57Bl/6 mice using an AlexaFluor 568 stained enzyme. The cell uptake results showed that the internalization process is mediated by both mannose (MR) and mannose-6-phosphate (M6PR) receptors. However, in chondrocytes, this process was mainly mediated by MR than for M6PR. Cell uptake assays using Morquio A fibroblasts, showed that the enzyme was taken up reaching intracellular enzyme activity levels similar to those observed in fibroblast from healthy volunteers; while the intracellular traffic assay, using HEK293 cells, showed that this enzyme was targeted to the lysosome. It is noteworthy that this recombinant GALNS allowed the

reduction of KS in Morquio A fibroblasts. For the biodistribution assay in wild-type mice, intravenous doses of 5 mg/kg were administered and tissue samples were taken at 12 and 24 hours. At 12 hours postinjection, it was observed the enzyme was primarily recovered in the liver, spleen, kidney and heart; while at 24 h post-injection the enzyme was only detected in liver and spleen. In conclusion, these results show the ability of the enzyme to reach several tissues and to be up taken by cells through different pathways, which confirm the potential of these enzyme towards the development of a new ERT for Morquio A disease.

653 - Evaluation of Cardiac and Vascular Involvement in Patients With Mucopolysaccharidosis

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Cardiovascular involvement is common in patients with Mucopolysaccharidoses (MPS). In this study, we investigated the effects of the markers involved in vascular endothelial injury pathogenesis (transforming growth factor β-TGF-β, interleukin-IL 6, IL 10, high sensitive C reactive protein-hs CRP, vascular endothelial growth factor-VEGF, N-terminal pro-Natriuretic peptide-NT-proBNP) and clinical, laboratory and echocardiographic findings of the patients. The same age and gender were compared with the healthy control group. A total of 37 patients (5 MPS I, 4 MPS II, 2 MPS IIIa, 4 MPS IIIb, 14 MPS IVa, and 8 MPS VI) were included in the study and 32 controls with similar age and sex were included in the study. All of the patients had evidence of dysostosis multiplexes. Corneal clouding was seen in 29 patients. 2 patients were transferred to the cornea. There were 23 patients with organomegaly, and 28 patients with hearing loss. When the groups were compared in terms of NT-proBNP, hsCRP, TGF-β, IL6, IL10 and VEGF levels, there was a statistically significant increase in the patient group ($P = .04$, $P = .022$, $P = .032$, $P = .026$, $P = .037$, $P = .025$, respectively) . The carotid intima media thickness was statistically significantly higher in the patient group ($P < .01$). Left ventricular diastolic diameter was significantly higher in the patient group ($P = 0.09$). Intraventricular septum thickness was significantly higher in the patient group ($P < 0.01$). E / A ratio was significantly lower in the patient group ($P < .01$). Percentage of right ventricular ejection fraction and right ventricular fractional shortening were significantly lower in the patient group ($P = .01$ and $P = .029$, respectively). Cardiac involvement in MPS patients is a major cause of mortality and morbidity. It is thought that cytokines, proinflammatory markers are elevated in patients with vasculature damage similar to other lysosomal diseases. There is a need for further studies to determine biomarkers for vascular involvement.

654 - Analysis of the Founder Effect of the Nonsense Variant in the *HGSNAT* Gene, in the Runta, Boyacá Population

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Objective: To determine the founder effect of a clustering of MPS III in Boyacá, Colombia. **Methods:** Study of a great pedigree in Runta, Boyacá; Analysis of the pathogenic variant responsible for MPS III; biochemical analysis for MPS IIIA/B; sequencing of the *HGSNAT*; typing of 6 STRs adjacent to the variant found in 69 Runta and 78 healthy controls of Tunja; haplotype analysis; calculation of WHE; F of Wrigth; study of carriers on 198 settlers; calculating the age of origin of the mutation by ESTIAGE and DMLE v3.1. **Results**: There were 7 related live affected individuals on a large pedigree of 14 patients (7 deceased), detecting in them the 1360C> T nonsense variant in the *HGSNAT* confirming MPSIIIC diagnosis. The ancestral haplotype around the mutation was present in 88.9% of Runta individuals, with an excess of heterozygotes and differences in Fst between Runta and Tunja people. A very high frequency of heterozygotes (34%) was evidenced in the study of carriers. The estimated age of the ESTIAGE mutation was 41 (CI 95% 35-48) and 39 (CI 95% 35-46) generations for DMLE v 3.1. **Conclusions**: We identified an ancestral haplotype not found in a control population that supports the existence of a founder effect for the variant c.1360C> T in the Runta village and an origin of the mutation of approximately 1000 years, so that it can be treated as a mutation of pre-Hispanic origin possibly Muisca that has managed to remain until the present.

655 - Prenatal Diagnosis for Lysosomal Disorders: A Reference Center Experience

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Objective and methods: This work highlights the experience of a reference center with Prenatal diagnosis (PND) of 123

pregnancies at risk for LD. Enzyme assays were performed according to the Lysosomal diseases (LD) type. Molecular analysis was performed in those cases where the mutation was established in the index case. The samples used for the PND were chorionic villi, amniotic fluid and fetal blood. **Results**: From 1988 to 2017, 123 high risk pregnancies for LD were investigated. PND from 121 cases originated from different Brazilian centers and two from Argentina and Peru. A positive diagnosis was found in 37/123 cases (30,08%). Only in 16 cases the mutation was known, and allowed the combination of molecular and biochemical investigation of 6 MPS I; 5 MPS II, 2 metachromatic leukodystrophy,2 gangliosidosis and 1 LAL-D. Biochemical confirmation of the result was obtained in a new sample in 46 cases (17 affected prenatally and 29 normal postnatally). **Discussion:** The biochemical analysis when combined with molecular analysis allows a higher confidence in the final result, and it is especially important in metachromatic leukodystrophy to discard pseudodeficiency. We suggest postnatal confirmation of the diagnosis in positive cases. PND allows a new perspective for couples at risk, and it can be very important for genetic counseling. Once there are new specific therapies for several LD, PND allows for early treatment, with a better prognostic, especially for those couples who want to follow with the pregnancy.

656 - Correlation of CSF Flow by Using Phase-Contrast MRI With Ventriculomegaly and CSF Opening Pressure in Mucopolysaccharidoses

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Objective: Very little is known about the incidence and prevalence of hydrocephalus in MPS patients. The biggest challenge is to distinguish communicating hydrocephalus from ventricular dilatation secondary to brain atrophy, because both conditions share common clinical and neuroradiologic features. Ventriculomegaly, a cardinal feature of hydrocephalus, can be measured by the Evans' index, the width of the III ventricle and the callosal angle. Phase-contrast (PC) MRI has the ability to measure the aqueductal CSF stroke volume, the elevation of which is associated with hydrocephalus. CSF opening pressure measured by lumbar puncture and higher than 25 cm H_2O can be considered indicative of hydrocephalus. Our objective is to assess the relationship between hyperdynamic aqueductal CSF flow, ventriculomegaly and increased intracranial pressure in MPS patients. **Methodology**: Forty-three MPS patients (12 MPS I, 15 MPS II, 5 MPS III, 9 MPS IV A, and 2 MPS VI) performed clinical and developmental tests and PC MRI followed by a lumbar puncture with the CSF opening pressure assessment. The mean age of the patients was 13.7 years (age range 0.9-36 years). Twenty-five patients were male (58.1%). Severe forms of the disease were found in 13 patients (30.2%). Macrocephaly was present in 32.6% of the patients. Based on IQ and development testing, 41.9% of the patients had cognitive impairment. This study (13-0252) was approved by the Scientific Committee and the Research Ethics Committee of Hospital de Clínicas de Porto Alegre. **Results**: All the scores used to assess the supratentorial ventricles enlargement and the ventricular CSF volume presented a moderate association with the CSF aqueductal flow. The CSF opening pressure did not correlate either with the three measures of ventriculomegaly or with the ventricular CSF volume. Also, no association was found between the CSF opening pressure and the CSF aqueductal flow. Dilated perivascular spaces and the white matter lesions load showed a large association with the ventricular CSF volume. **Conclusions**: Ventriculomegaly is associated with a severe phenotype, increased neurological impairment, white matter lesion severity and enlarged perivascular spaces. The addition of the CSF flow MRI to the other measures already used to characterize the ventricular enlargement helps to distinguish atrophy from communicating hydrocephalus in MPS patients; however, the CSF opening pressure has not been shown to be reliable.

657 - Mucopolysaccharidoses Type III: Case Series From Turkey

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Objective: Mucopolysaccharidosis type 3 (MPSIII) is a group of lysosomal storage disease caused by the lack of enzymes to break down the specific mucopolysaccharide called heparan sulfate. There are four subtypes coded by different genes, all are autosomal recessively inherited. Cardinal clinical findings are neurologic; such as psychomotor retardation, hyperactivity, seizures, behavioral disturbances, and intellectual decline. Skeletal findings are subtle and occur late. Hirsutism and synophrys are frequently seen characteristic dysmorphic findings. Here, we aimed to describe the features of our MPSIII patients. **Methods:** Medical records of 39 MPSIII patients were evaluated retrospectively. Demographic features, clinical data, and molecular analyses were summarized. **Results:** 39 patients were included in this report. 20 of the patients were female. The mean age of the first symptom was 2.15 $\pm$ 1.24 years. 9 of the patients were siblings and 22 of them had positive family

history. Parental consanguinity rate was % 87.1. 8 patients were MPS IIIA, 26 were MPS IIIB, 3 were MPS IIIC, and 2 were MPS IIID. All of them had psychomotor retardation on admission. The other complaints were; not being able to speak in 8 patients, epilepsy in 6 patients, coarse face in 5 patients, hepatosplenomegaly in 2 patients, and recurrent hernias in 2 patients. Also, 34 patients had recurrent upper respiratory infections, 15 patients had cardiac manifestations, 3 had hearing impairment, and 3 had dysostosis multiplex. None of them had ocular involvement. 2 of them have died due to aspiration pneumonia. The mean current age of the rest of the patients was 8.43 ± 4.97 years. Molecular analyses were performed in 30 patients. 10 patients had an unidentified mutation, but described as highly damaging with *in silico* programs. Among these 7 were on *NAGLU* gene, 2 were on *SGSH* gene, and 1 was on *HGSNAT* gene. **Conclusion:** Mucopolysaccharidosis type III is a devastating neurodegenerative lysosomal storage disorder. There are no disease-specific treatments. However, intrathecal enzyme replacement therapies are under investigation. Thus, it is important to understand the natural history of this disease and identify the interventions needed to help these patients.

658 - Analysis of Heparan Sulfate and Non-Reducing Ends in Mucopolysaccharidosis Type I

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Mucopolysaccharidosis type I (MPS-I) is a lysosomal storage disorder caused by deficiency of alpha-L-iduronidase, resulting in impaired degradation of dermatan and heparan sulfate and their accumulation in tissues. Heparan and dermatan sulfate are degraded in lysosomes by specific enzymes through a sequential process starting at the non-reducing end (NRE) of the molecule. The heparan sulfate non-reducing end (NRE) specific for MPS I is a disaccharide composed of iduronic acid and N-sulfoglucosamine referred to as I0S0. In patients with the different forms of MPS I, ranging from the severe neuronopathic Hurler Syndrome to the attenuated Hurler-Scheie and Scheie Syndromes, enzyme replacement therapy (laronidase) and hematopoietic stem cell transplant early in life (only for Hurler syndrome) slow disease progression and improve some clinical symptoms. Here, we evaluate the clinical utility of total heparan sulfate and MPS I specific heparan sulfate NRE using the LC-MS/MS based Sensi-Pro® assay in a cohort of patients with MPS I. At diagnosis, the highest values for the ratio I0S0: HS (>0.06) were seen in patients with Hurler syndrome, while the lowest (0.02) was observed in one patient with Scheie syndrome, the least severe phenotype. Patients with Hurler-Scheie had intermediate values of the ratio (0.02-0.06). The concentration of total GAGs and total heparan sulfate, although elevated in all patients, could not discriminate the three sub-groups. Enzyme replacement therapy (ERT) and/or bone marrow transplant decreased the ratio I0S0: HS and correlated with slowing of symptom progression. Non-compliance with ERT regimen in one Hurler-Scheie patient resulted in increased I0S0: HS ratio. Our preliminary results suggest that the ratio I0S0/total heparan sulfate (I0S0: HS) is a good predictor of disease severity and that disease specific NREs may be used as biochemical markers to monitor treatment compliance and clinical progression in MPS I.

659 - Pentosan Polysulfate for the Mucopolysaccharidosis and Other Lysosomal Storage Diseases

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The mucopolysaccharidoses (MPS) comprise a group of 11 lysosomal storage disorders (LSDs) due to deficiencies of enzymes involved in glycosaminoglycan (GAG) metabolism. Our laboratory has specifically focused on understanding the skeletal biology in these diseases, leading to a series of papers documenting the involvement of the toll-like receptor 4 (TLR4) inflammatory pathways. This led to the evaluation of an FDA/EMA-approved drug, pentosan polysulfate (PPS), in two animal models of MPS, rats with MPS VI and dogs with MPS I. Weekly or biweekly subcutaneous injection of PPS into these animals reduced inflammation, improved certain pathological and/or clinical findings, and led to a surprising reduction of GAG storage in tissues and urine. The mechanism(s) responsible for PPS-mediated GAG reduction in MPS are under investigation, but hypotheses include an inhibitory effect on GAG synthesis (i.e., substrate reduction), and/or a stabilizing effect on lysosomal integrity and function. The findings in MPS animals led to two small "proof-of-concept" clinical trials in adult MPS I and MPS II patients, confirming the reduction in inflammation and/or GAG storage. We have also recently completed studies in a mouse model of MPS IIIA, indicating a reduction in systemic and neurological inflammation, and improvement in several other neuropathological findings. Based on these findings we propose that PPS may provide broad benefit across all MPS types, and perhaps other LSDs as well. With regard to this latter point, recent data has shown a reduction of inflammation in cells from patients

with Fabry and Gaucher diseases, indicating that the effects of PPS may not be limited to MPS.

660 - A New Recombinant Enzyme for Morquio A Enzyme Replacement Therapy: N-Acetylgalactosamine-6-Sulfate Sulfatase (Galns) Produced in a Glycoengineered Escherichia coli Strain

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Morquio A disease (Mucopolysaccharidosis IV A, MPS IVA) is a lysosomal storage disease (LSD) produced by mutations in the gene encoding for the lysosomal enzyme N-acetylgalactosamine-6-sulfate sulfatase (GALNS, EC 3.1.6.4). The enzyme replacement therapy (ERT) for MPS IVA was recently approved, which is based in the infusion of the recombinant enzyme Elosulfasa Alfa, produced in Chinese Hamster Ovary (CHO) cells. A growing number of studies have shown the possibility to produce active and therapeutic forms of lysosomal proteins in microorganisms. Although *Escherichia coli* is one of the most used host for the production of recombinant proteins, this expression systems does not perform some the posttranslational modifications observed in mammalian proteins, such as N-glycosylations. In this study, we used a glycoengineered *E. coli* strain to produce an N-glycosylated recombinant GALNS. The N-glycosylated recombinant GALNS (glycoGALNS) was characterized by its pH stability, kinetic parameters, cell uptake, intracellular traffic, and in vitro reduction of keratan sulfate (KS), and mouse biodistribution. The glycoGALNS showed higher stability at low pH values than the non-glycosylated version of the protein. Furthermore, the addition of N-glycosylations also improved the affinity for the artificial substrate. Cell uptake assays, using Morquio A fibroblasts, showed that the enzyme was taken up reaching intracellular enzyme activity levels similar to those observed in fibroblast from healthy individuals, in a process mediated by mannose receptors. Intracellular traffic assay, using HEK293 cells and an AlexaFluor 568 stained enzyme, showed that this enzyme was targeted to the lysosome. It is noteworthy that glycoGALNS allowed the reduction of KS in Morquio A fibroblasts. Finally, the biodistribution assay in C57BL/6 wild type mice showed that this recombinant GALNS was mainly distributed to kidney after 24 hours posttreatment. In summary, these results showed the potential of glycoGALNS towards the development of a new enzyme replacement therapy for Morquio A disease.

661 - Phenotypic Characterization and Neuroimaging Findings in a Case Series of Three Patients With Mucopolysaccharidosis VI (Maroteaux-Lamy Syndrome) in Ireland

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Objective: Mucopolysaccharidosis (MPS) type VI (Maroteaux-Lamy Syndrome) is a rare autosomal recessive disorder caused by a deficiency of arylsulfatase B. The resultant accumulation of glycosaminoglycans, specifically dermatan sulfate, causes progressive multi-organ involvement that can lead to various progressive symptoms. Clinical features comprise, e.g, coarse facies, skeletal dysplasia, hepatosplenomegaly, hearing loss, cardiac and lung disease. Neurological symptoms may include ventriculomegaly and cervical cord compression. Enzyme replacement therapy (ERT) with recombinant galsulfase is currently the mainstay of treatment along with multidisciplinary medical care, but acts in stabilization rather than cure of the condition. Early treatment and diagnosis has been associated with a better outcome in these patients. **Methods:** We here evaluate our cohort of MPSVI patients in Ireland, including clinical course, disease progression and response to treatment. **Results:** There was significant variation in the initial presentation of MPS VI in the Irish cohort of three male patients. All developed hearing loss, respiratory dysfunction, cardiac pathology, visual symptoms and a variable degree of developmental delay / motor impairment. All patients were commenced on ERT, although with differing degrees of responsiveness. One adult case had extensive signal abnormality on brain MRI and narrowing of the foramen magnum. Two children underwent brain MRI scans at 14 months and at 5 years of age, respectively. Both show extensive abnormal signal in the deep white matter. Enlargement of the perivascular spaces as seen in many forms of MPS, was seen alongside the corpus callosum in the younger boy and in the thalami in the 5-year-old. Follow-up scans on one child showed complete resolution of the white matter abnormality and partial resolution of the thalamic abnormality after commencing treatment with ERT. **Conclusion:** Early diagnosis is important to tailor treatment and surveillance, including ERT. We demonstrate a complete resolution of the white matter abnormality and partial resolution of the thalamic abnormality after commencing treatment in one child with MPS VI; this improvement has been maintained on subsequent brain MRI scans over 8 years. Acknowledgement: We wish to acknowledge the valuable support of staff at NCIMD TSCUH as well as in the local services

and to express our thanks to all those involved in patient care. We thank BioMarin for financial support.

662 - Presenting Signs and Symptoms of MPS: Results of a Systematic Literature Analysis

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Background: Mucopolysaccharidosis (MPS) disorders are rare lysosomal storage disorders whose subtle and non-specific early signs and symptoms present a diagnostic challenge for physicians who rarely encounter these patients in daily practice. The purpose of this systematic literature review was to identify presenting signs and symptoms of MPS disorders and to characterize reported diagnostic delays. **Methods:** The literature search was conducted in MEDLINE and Embase on June 27th, 2016, and yielded 1466 unique publications. Inclusion criteria specified that publications must contain a clinical description of an MPS patient at presentation or during the course of diagnosis. Therapeutic, biochemical, and molecular assay based publications were excluded. Following application of inclusion/exclusion criteria, 194 publications were found suitable for extraction. Publications reporting multiple MPS types were extracted into MPS type-specific records. For each MPS type, data on presenting signs and symptoms, delayed diagnosis, and/or misdiagnosis were extracted and analyzed. **Results:** Of the 194 publications included, 13% reported observational studies; the remainder included case reports, case series, surveys, guidelines, and reviews. The MPS type-specific sample size was less than 10 in 42% of studies. Reported delays in diagnosis ranged from <1 year (21% of studies) to greater than 10 years (19.4% of studies); 37% of studies reported delays of 1-4 years. The most frequently reported misdiagnoses were juvenile arthritis, rheumatoid arthritis, bilateral Perthes disease, spondyloepiphyseal dysplasia, and rheumatic fever. Facial coarsening and hepatomegaly were the only presenting signs consistently reported across all MPS types. Hearing loss, recurrent ear, nose, and throat infections, and joint involvement were also commonly reported across most MPS types. **Conclusion:** This literature review highlights key early signs and symptoms, and clusters thereof, as well as common diagnostic pitfalls. This information can be used to facilitate earlier referral, diagnosis, and treatment of MPS, ultimately leading to improved patient outcomes.

663 - Cellular Localization of Iduronate-2-Sulfatase, Proteome Isolation, and Identification of Changes in the Proteomic Profiling in a Murine Model of Hunter Syndrome

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Iduronate-2-sulfatase (IDS) is a lysosomal enzyme that participates in the metabolism of heparan and dermatan sulfate. Mutations on IDS gene produce mucopolysaccharidosis II (MPSII). This pathology is characterized by intracellular accumulation of both substrates causing severe damage to the central nervous system and other tissues. In this study, we used a neurochemistry and proteomic approach to identify the brain distribution of IDS, as well as its interacting proteins to get insights into the physiological role of this protein in brain. The IDS brain distribution was evaluated by immunodetection methods. Brain IDS proteome was isolated by using a recombinant IDS produced in *Pichia pastoris*, which was purified by anion exchange and molecular exclusion chromatography. The isolation of IDS-interacting partners was carried out with IDS-sepharose affinity chromatography**.** BN-PAGE was implemented for the identification of mouse brain protein complex from wild-type and IDS-KO. Extracted peptides and protein complex from affinity chromatography and BN-PAGE were analyzed by HPLC-ESI-MS2. The interaction of IDS with seven proteins was evaluated by immunocapture. IDS immunoreactivity was detected in different regions with a robust staining throughout the entire brain and, within the cytosol of neurons

and astrocyte bodies in the grey matter. We identified 217 partners proteins of IDS, from which 50 were identified as Hubs. The interactions with some of these proteins were confirmed by BN-PAGE and CoIP. In addition, the comparative analysis of protein complex from wild-type and IDS-KO showed changes in molecular functions such as a decrease in antioxidant response, structural molecules activity, receptor binding, protein transport and an increase in catalytic activity. In the protein complexes from Hunter murine model, we identified hubs like molecular chaperons, proteins implicated in conformational changes in ribosome, nucleosome synthesis, actin filaments and chemoattractant for inflammation process. These results for the first time highlight the presence of the enzyme under physiological conditions in neurons and astrocytes, and constitute experimental evidence of IDS-interacting proteins (mainly involved in metabolic process, axogenesis, exocytosis, vesicle-mediated transport and neuron projection development). The proteins identified in this study under both, physiological and pathological conditions would provide potential therapeutic targets.

664 - Improvement of the Quality of Life With Use of Enzyme Replacement Therapy in a Wheelchair Patient With Morquio A Syndrome

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Goals: To introduce objective improvement (need for emergency attendance and adapted 6 minutes walking test—6MWT) and subjective (patient perception about quality of life) after 1 year of the use of enzymatic infusion in a patient with Mucopolysaccharidosis (MPS) Type IVA. **Methods:** Clinical follow-up and patient interview. The patient started the infusions on 03/30/2015, and before the start of the infusions we performed the adapted 6MWT (crawling test). We performed the test after 6 months and after 1 year of enzyme replacement therapy (ERT). In addition, we performed patient interviews, clinical follow-up and evaluation of medical consultations in an emergency room. **Results:** After 1 year of ERT, the patient presented an increase in the modified 6MWT (crawling test)—initially 20 meters and 40 meters after 1 year—and, mainly, according to the patient's own report, an important improvement in the quality of life. As a marker of improvement, we found that after initiation of the infusions the patient did not need emergency services anymore, due to the symptoms of dyspnea, dizziness and arthralgia founded prior to ERT. **Conclusion:** The impairment of the patient's mobility, associated with the difficulty of dislocation to a larger medical center, made it impossible to perform complementary exams at baseline and during treatment. However, we consider that the adapted 6MWT, the evaluation of morbidity reduction and the subjective improvement attested by patient interview, made it clear that the ERT performed an established benefit for the patient with late diagnosis and handicapped.

665 - Trajectory of Patients With Mucopolysaccharidosis, "From Symptom to Diagnosis"

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Introduction: Mucopolysaccharidosis (MPS) is a group of diseases of low prevalence that is characterized by the deficiency of enzymes involved in the degradation of glycosaminoglycans (GAGs) with the progressive lysosomal accumulation of these macro molecules leading to multi-organ dysfunction. Its inheritance is mostly autosomal recessive. Its diagnosis is clinical, radiological and biochemical. **Objective**: To describe the trajectory performed by patients with Mucopolysaccharidosis until the diagnosis of their disease. **Materials and methods**: Retrospective descriptive study of patients with a diagnosis of MPS in the period January 1999 to April 2017. Inclusion criteria: patients diagnosed with MPS by enzymatic dosing in leukocytes. **Variables:** Diagnostic age, clinical characteristics, consanguinity, and diagnostic trajectory.

Results: N = 11.5 MPS I patients, 4 MPS VI patients, 1 MPS IV-A patient and 1 MPS III-A patient. Age at diagnosis: MPS between 11 and 17 months, MPS VI between 19 months and 10 years, and between 19 months and 10 years. Age of diagnosis of 5 patients with MPS I range 11 to 17 months, of 4 patients MPS VI range: 19 months and 10 years. There was 1 patient with MPS IV-A and 1 patient with MPS III-A diagnosed at 4 years. Of the patients born before 2014 (6/11), the diagnosis was made before 17 months of life in 1 patient; of those born after 2014 (5/11), all were diagnosed before 17 months of age. Background of inbreeding in 3 patients with MPS VI. Most frequent signs / symptoms: bone involvement and coarse facies. Multiple consultations by specialists prior to diagnosis in 9/11 patients. 6/11 patients were referred by Traumatology and Orthopedics. **Conclusions**: Early diagnostic identification is fundamental for the timing and therapeutic strategy (enzymatic replacement and bone marrow transplantation). The younger age at diagnosis of children born after 2014 could be due to greater knowledge and dissemination of these diseases, early clinical suspicion, and greater availability / accessibility of specific complementary tests

666 - Mutation Prediction Analysis Across the MPS Genes

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Mucopolysaccharidoses (MPS) are rare monogenic disorders caused by genetic alterations in enzymes involved in degradation of glycosaminoglycans. With molecular diagnostic advance, the number of new variants of uncertain significance (VUS) described in these genes increases daily. Diverse programs exist for pathogenicity evaluation. However, discordant results are frequently found. Therefore, the purpose of this study was to compare different prediction software in the evaluation of missense alterations of genes associated with MPS. **Methods:** Mutations from all MPS genes were obtained from gnomAD browser. They were assessed with Polyphen2, MutPred, SIFT, PROVEAN, SNPs&GO, PhD-SNP, PANTHER and M-CAP. Pathogenicity status of heterozygous mutations was determined by consensus of at least 6 programs (75%). To further analyze sensibility and specificity, programs were run with a curated list of *IDUA* mutations and their respective pathogenicity, based on HGMD and literature. **Results:** 3095 mutations were analyzed. An average 33% discordance rate between predictors was observed, ranging from 15.8% (*HYAL*) to 44.4% (*IDS*). For *GLB1*, *GUSB*, *IDS*, and *SGSH*, the amount of discordant results was greater than amount classified either as pathogenic or benign. Consensus in prediction outcomes significantly differed between genes ($P < .001$). Consensus of pathogenicity was achieved in 12.8% to 37.5% of mutations (*HGSNAT* and *GALNS*, respectively). Consensus for classification as benign was obtained in most genes, ranging from 30% to 50% (*GLB1* and *HYAL*, respectively). Panther and M-CAP did not evaluate 23.7% and 2.6% of all variations, respectively. *IDUA* mutations included 93 pathogenic and 7 nonpathogenic mutations. M-CAP resulted in maximum sensitivity. However, it did not evaluate 6 mutations and displayed 0% specificity. On the other end of the spectrum, SNPs&GO presented maximum specificity with low sensitivity (0.495). MutPred, Polyphen2, PROVEAN, PhD-SNP, PANTHER and SIFT achieved sensitivity/specificity of: 0.817/0.714, 0.903/0.429, 0.796/0.571, 0.753/0.714, 0.611/0.714, and 0.742/0.714. **Conclusion:** In the MPS related genes, performance of prediction pathogenicity software varied significantly. For *IDUA*, MutPred is one of the most suitable tools, as presented both high specificity and sensitivity. This highlights the need for evaluation of predictors' performance in each gene, in each situation, and the use of other parameters for assessment of pathogenicity.

667 - Molecular Characterization of MPS IVA Patients in Andean Region of Colombia

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Introduction: Mucopolysaccharidoses (MPS) are a group of inherited metabolic lysosomal storage disorders a subgroup of this is Morquio disease an autosomal recessive condition. Which overall incidence is 0.68 per 100 000 live births. In Colombia, studies suggest that MPS IVA is likely the highest prevalence worldwide. **Materials and Methods:** [HMPL1] Sixteen families and nineteen patients from a different region of the country were tested for mutation identification, the sequence was compared to the GALNS reference sequence NM_000512.4, and gene variants were reported. Bioinformatics analysis was done by SWISS-MODEL, the mutant proteins were generated by homology from the wild-type GALNS 4FDI template obtained from PDB database and visualization was performed using Swiss-PdbViewer. The predictive analysis was run in PolyPhen-2 software (Polymorphism Phenotyping v2) and SIFTS human protein v1.03 software. **Results:** 79% of the cohort was homozygous and 21% were compound heterozygous. The mutation c.901G>T was the most frequent mutation with 74% of the alleles 10,5% followed by mutation c.1156C>T. In addition, 1 novel mutation was described in c.214T>A predictive analysis identify it as pathogenic variant. **Conclusion:** This study revealing the mutation spectrum of MPS IVA in the Colombian population. The mutation spectrum data for MPS IVA disorder in Colombian population are not yet complete characterized. The high prevalence of c.901G>T mutation suggest it as a founder effect cause disease in this particular region. In addition, this spectrum data will be useful in the provision of better genetic counseling, and prenatal diagnosis.

668 - Hurler Syndrome, Clinical Characterization of a New Molecular Variant Identified in a Colombian Patient

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Objective: Mucopolysaccharidosis type 1 is one of the most frequent lysosomal diseases, Hurler syndrome is the most severe form of these. Due to the similarity with other MPS types, it is necessary to perform several biochemical and genetic tests to get the best accurate diagnosis. This study aims to describe the clinical, biochemical and molecular diagnosis of a new variant mutation. **Clinical case:** the patient is a 1-year-old Colombian male, infant born at term, black ethnicity, nonconsanguineous parents, pregnancy and delivery uncomplicated. At birth, he was hospitalized for hypoglycemia, he has history of laryngomalacia and adenoid hypertrophy, patent ductus arteriosus, and several respiratory infections since he was 3 months, also swallowing difficulties and no motor or language delays. Physical examination showed coarse facial features, a prominent broad forehead, mild corneal clouding, wide palpebral fissures, epicanthal fold, exophthalmos, flat nasal bridge, wheezing at auscultation, umbilical hernia, spinal

gibbus deformity, claw left hand, and articular stiffness in hands and toes. **Results:** We identified a case of a Colombian male infant with non-enzymatic activity, IDUA sequencing identified a new homozygous missense mutation c.1045G>T, causing a deleterious amino acid change p.ASP349Tyr. Data bases Polyphen-2, SIFT, and Mutation Taster all predicts this variant to be deleterious. **Conclusion:** Clinical, biochemical, and molecular characterization are essential for elaboration and correct diagnosis, clinical management, correlation genotype-phenotype, and better genetic counseling.

669- Alpha-Mannosidosis in a Patient With Mild Phenotype and Nonsense Mutation

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Introduction: Alpha-mannosidosis is a rare autosomal recessive lysosomal disease. The phenotype is classified as type I, severe form, with recurrent infections and early demise, and type II, mild form, with longer survival and slower progression. So far, more than a hundred mutations have been described, many of them private. **Objective:** To report the clinical profile of a patient diagnosed with alpha-mannosidosis and identification of a nonsense mutation. Patient and method: male patient, 16 y, child of consanguineous parents, first cousins, born by forceps delivery, full term, presented prolonged neonatal jaundice due to ABO incompatibility and mother IIG, IP, IA. Patient presents a history of delayed neuropsychomotor development, congenital cataract, and hepatosplenomegaly. At physical examination, bilateral convergent strabismus, coarse facies, macrocrania, cognitive impairment, pyramidal signs, gait ataxia. Presented a slow progressive neurological worsening of gait and cognition since 15 y, however, visceral improvement due to regression of hepatosplenomegaly. Skeletal X-ray showed multiple dysostosis, and brain MRI with cortical atrophy. He performed two oligosaccharide profiles in the urine, resulting in a normal sample and another sample with altered oligosaccharide and normal sialosaccharide bands. We continued the investigation with alpha fucosidase and beta-mannosidase enzymatic activity in leukocytes with normal results and alpha-mannosidase activity undetectable. Sequencing of the MAN2B1 gene showed variant exon 22 in homozygosity, p.Ser899X. In literature, this mutation has already been described, but without the phenotypic description of the patient. The patient's parents have the same mutation in heterozygosity. **Conclusion:** In the literature, to date, there is no correlation between the genotype and the phenotype in alpha-mannosidosis. The patient presents a mild phenotype, with a slow progression, classified as type II, although the enzymatic activity is undetectable, and has a nonsense mutation in Homozygosity.

670 - Molecular Genetics Profile of Mucopolysaccharidoses in the State of Santa Catarina, Brazil

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Objective: To report the molecular profile of patients with MPS in the State of Santa Catarina. **Methods:** A cross-sectional study was conducted from 1998 to 2016, which collected medical records from MPS patients. **Results:** 35 patients with mucopolysaccharidoses were included: 12 with MPSIV A, 12 with MPS I (9 Hurler, 2 Hurler Scheie, 1 Scheie), 8 MPS III (6 IIIB and 2 IIIC), and 3 MPS II (2 non-neuronopathic 1 neuronopathic). Thirteen patients with MPS underwent molecular genetics analyses. The genotypes were: MPS IVA patient 1 p.Arg94Leu /p.Arg94Leu; patient 2 p.Gly116Ser/p.Gly116Ser; patient 3 and patient 4 (sisters) p.Arg94Leu/ p.Arg94Leu; patient 5 p.Arg386Cys/unknown mutation. MPS I patient 1 c.1205G> A / c.1047C>; patient 2 and 3 c.208C> T / c.1205G; patient 4 c.1205G> A / c.1205G> A. MPS III: patient 1 IIIC p.Leu55Ter / c.1464 + 1G> A; Patient 1 IIIB p.Leu296 fs / unknown mutation. MPS II with IDS/IDS2 common inversion; patient 2 p.E344; patient 3 intron 8 deletion including pseudogene. **Conclusion:** In Santa Catarina, there is a high relative prevalence of Morquio A syndrome and the finding of identical mutations in unrelated cases, in our sample, can be possibly explained by founder effect. Molecular genetics analyses have limited utility for the screening of MPS due to extreme genetic heterogeneity. However, genotypic characterization is instrumental for the phenotype prediction, identification of carriers, and prenatal diagnosis.

671 - Effect of Losartan on Cardiovascular Disease in MPS I Mice

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Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder caused by deficiency of alpha-L-iduronidase. Aortic dilatation and heart enlargement are common features in MPS I patients. Alterations of TGF-β pathway have been related to cardiovascular diseases and it may be associated to MPS I heart dysfunction. Based on this, we verified the effects of blocking

the TGF-β pathway with losartan on cardiovascular disease in a murine model of MPS I. MPS I mice were treated with losartan at 2 months of age (0.6 g/L in drinking water; n = 11) or propranolol (0.5 g/L; n = 5). Wild-type (WT) and untreated MPS I mice were used as controls (n = 12 each). All animals underwent ultrasound examination at 6 months of age before euthanasia. Echocardiography analyses were performed to determine the left ventricular (LV) dimensions and cardiac function by shortening fraction (LVSF). Pulmonary vascular resistance (PVR) was obtained as the ratio between the acceleration and ejection times (AT/ET) at the pulmonary valve. Aortic root diameter was determined by M-mode, and in situ using a digital caliper immediately after euthanasia. Cardiovascular tissues were embedded in paraffin. Sections were stained with H-E & Alcian Blue to determined valve thickness or Verhoef Van Gieson (VVG) to analyze elastin breaks in the aorta. Aortic root diameter was increased 67% in MPS I mice compared to WT (1.07 mm ± 0.13 vs 1.79 mm ± 0.25; $P < .01$). Losartan decreased 25% the aortic diameter compared to MPS I (1.34 mm ± 0.15; $P < .01$). Echo analysis showed that losartan also improved the LVSF and the AT/ET ratio. MPS I mice presented reduced contractility (36.7% ± 5.1 in WT vs 27.6% ± 3.6, $P < .05$) while the heart function in treated mice with losartan was similar to WT (34.8% ± 5.7). Losartan treatment also prevented enlargement of the LV. MPS I mice presented increased heart valve thickening ($P < .01$) and elastin breaks per mm compared to normal mice ($P < .01$). No effect was observed on the valve thickness, but losartan normalized the elastin breaks. Mice treated with propranolol improved cardiac function and LV dimensions but had no effect on the aorta. We suggest that losartan is a potential new therapy for cardiovascular disease in MPS I. Propranolol only improved cardiac function, which suggests that heart dysfunction may be independent from TGF-β signaling. Losartan may target the underlying pathophysiology in MPS possibly by antagonism of TGF-β or other pathways which will be investigated.

672 - Follow-Up of Patients With Mucopolysaccharisosis Type II and IV With Enzyme Replacement Therapy. A Chilean Referral Center Experience

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Introduction: Mucopolysaccharidoses (MPSs) are a group of inherited lysosomal storage disorders characterized by deficiencies in specific enzymes involved in the catabolism of glycosaminoglycans (GAGs). These deficiencies cause excessive metabolites to accumulate in multiple organs. Mucopolysaccharidosis type II (MPSII) is an X-linked recessive genetic disorder, caused by deficiency of iduronate-2-sulfatase enzyme (IDS). Mucopolysaccharidosis IVA (MPS IVA; Morquio A) is caused by deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS), with an autosomal recessive trait. Enzyme replacement therapy (ERT) is available for both of these disorders (MPSII Idursulfase, MPS IVA elosulfase alfa). ERT improves respiratory impairment, functional capacity and joint mobility, reduces organomegalies and coarse facial features and stabilizes cardiac manifestations. Aim: To analyze clinical form of presentation, enzymatic defect and response to ERT in a cohort of MPSII and MPS IVA Chilean patients. **Methods:** A descriptive-comparative study of 20 patients with confirmed diagnosis of MPS (9 MPSII and 11 MPS IVA) describing age at diagnosis, tolerability and response to ERT. Results: 9 MPSII patients (1female), aged 3-28 years old. Median age at diagnosis: 36 months. 8/9 are receiving ERT (time 8-38 months). 1 patient retired due to adverse event associated to ERT. Median age at ERT initiation 9 years. Cardiac disease 6/9 pre-ERT. Organomegaly 6/9. Impaired PSG 5/9. Response to therapy: improvement of OSA: 1/5, improvement of left ventricular hypertrophy: 2/4, improvement in organomegaly 2/5. 11 MPS IVA patients (5 female), aged 3-50 years old. Age at diagnosis from 6 months to 33 years (median 57.5 months). 9/11 received ERT (time 3-26 months). Median age at ERT initiation 14.5 years. 2 patients have a recent diagnosis and therefore, have not initiated ERT. All patients present short stature, kyphoscoliosis, genu valgum, laxity of joints and hypoacusia. Cardiac disease 5/11.Impaired PSG 5/11. Spinal cord compression 4/11. 3/9 patients in ERT presented adverse effects, 2 suspended therapy because of severe adverse reaction. **Conclusions:** Almost all of our patients are receiving ERT. We found some degree of improvement in cardiac disease, respiratory function and organomegaly in MPSII patients. Due to short time of follow up, we could not evaluate ERT effects in MPS IVA. 3/18 patients suspended therapy because of severe adverse effects.

673 - Correlation of Ventriculomegaly, Size of Foramen Magnum and Jugular Foramina, and Cerebral Venous Outflow in Mucopolysaccharidoses

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Objective: In MPS patients, one hypothesis for communicating hydrocephalus is the reduced venous outflow through bone dysostosis of the skull base and chronic dural venous hypertension with impaired CSF absorption, however it has never been

proven. The goal of this study was to determine a correlation between jugular foramina and foramen magnun narrowing, ventriculomegaly and decreased cerebral venous outflow. **Methodology:** Forty-three MPS patients (12 MPS I, 15 MPS II, 5 MPS III, 9 MPS IV A and 2 MPS VI) performed brain MRI. The mean age of the patients was 13.7 years (age range 0.9 -36 years). Twenty-five patients were male (58.1%). Severe forms of the disease were found in 13 patients (30.2%). Macrocephaly was present in 32.6% of the patients. Ventriculomegaly was defined by an Evans' index greater than 0.3, a width of the III ventricle greater than 10 mm and a callosal angle less than 90°. The surface area of the foramen magnum and the bilateral jugular foramina was measured using a region of interest surface measurement tool and was dichotomised using median values. Intracranial and extracranial venous mean flow (mL/min) were obtained by using phase-contrast MRI and the adopted cut-off values were: straight sinus < 85, sagittal sinus < 235, right and left jugular veins < 200. This study (13-0252) was approved by the Scientific Committee and the Research Ethics Committee of Hospital de Clínicas de Porto Alegre. **Results:** The three indices of ventriculomegaly did not correlate with the skull base foramina measurements. Ventriculomegaly, especially the third ventricle width, was associated with reduced venous flow in the sagittal sinus and in the right jugular vein. Also, the decreased venous flow in the right jugular vein was associated with the narrowing of the right jugular foramen and with an increased aqueductal CSF flow. **Conclusions:** Based on our results, communicating hydrocephalus in MPS patients seems to be not only related to the obstruction of CSF reabsorption, but it is likely that cortical venous system hypertension due to reduced venous blood outflow through the narrowed right jugular foramen may play a role in the genesis of ventricular dilatation.

674 - Mucopolysaccharidosis Type VI (MPS VI) and Molecular Analysis: A Review of Published Classified Variants in the *ARSB* Gene

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Background: MPS VI (Maroteaux-Lamy syndrome) is an autosomal recessive lysosomal storage disorder caused by mutations in the *ARSB* gene that result in deficient activity of the lysosomal catabolic enzyme N-acetylgalactosamine-4-sulfatase (arylsulfatase B, ASB). Deficient ASB activity causes lysosomal storage, and elevated urinary excretion, of the glycosaminoglycan enzyme substrates dermatan sulfate and chondroitin-4-sulfate. MPS VI is diagnosed through clinical findings and deficient ASB enzyme activity with normal activity of control enzymes; detection of pathogenic variants in each *ARSB* allele can independently confirm the diagnosis and facilitate genetic counseling. A previous report on a cohort of 105 individuals diagnosed with MPS VI documented that most *ARSB* variants are rare or private. Since 2007, with the increasing use of molecular testing, many new *ARSB* variants have been published. **Methods:** To uniformly summarize all *ARSB* variants, we collected and analyzed from the literature and public databases 822 reports of 193 distinct variants in the *ARSB* gene from individuals diagnosed with MPS VI. **Results**: In agreement with previous reports, most variants are missense (60%; 115 of 193); next most common are deletions (18%; 34 of 193), followed by nonsense (12%; 23 of 193), and splice site/intronic variants (6%; 11 of 193). Many reported *ARSB* variants are rare, with 33% (64 of 193) reported only once. Zygosity of individuals with MPS VI (n = 411) distributed as: 51% homozygous (209 of 411) and 42% heterozygous (173 of 411); in 7% of cases only one allele was identified (29 of 411). Of the 193 unique *ARSB* variants summarized here, only 18% (34 of 193) are recorded in public databases (Clinvar, EmvClass, Invitae, ARUP) in association with supporting evidence/clinical significance. **Conclusions:** This analysis illustrates the heterogeneity of alleles linked to MPS VI and lack of representation of otherwise characterized pathogenic *ARSB* alleles in publicly available variant databases. We emphasize the importance of maintaining high clinical suspicion during MPS VI diagnosis and confirming diagnosis via ASB enzyme testing as many *ARSB* alleles may have yet to be formally classified as MPS VI-associated. Timely submission and classification of *ARSB* variants in public databases in association with biochemical and clinical data will help improve a timely diagnosis of MPS VI.

675 - Protocol Proposal for Mucopolysaccharidosis (MPS) Risk-Population Screening (MPSRPS) to the Ecuadorian Public Ministry of Health to be Included in the Ecuadorian Health System

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Some rare diseases, such as mucopolysaccharidosis (MPS), if they are detected early through access to early screening, this may allow to improve the quality of life of the patient and their family. This allows for them to receive the appropriate medical treatment, if there is access to it, which improves the illness' prognosis. Ecuador has little development in the management of rare diseases such as MPS at the early detection level as well

as its proper treatment. The country offers, since the end of 2011, to the Ecuadorian public a newborn screening called "*Con Pie Derecho*" (On the Right Foot) Our investigation is aimed to propose a protocol for the Ecuadorian population, from the necessary laboratory tests for screening and diagnosis up until the adequate offer within the first level of attention in the National Health System. In this sense, our investigation work is to deliver a protocol for our health system and includes a protocol proposal for laboratory test for screening and diagnosis with adequate and opportune access for the at-risk population. **Objective:** Propose a protocol to the Ecuadorian Ministry of Health to be included in the Ecuadorian Health System. **Discussion:** The clinical validity of the most frequently used laboratory tests indicates the qualitative and quantitative determination due to turbidimetry of glycosaminoglycan (GAGs) which are low sensitivity, on the other hand, the DMMB spectrophotometric values are highly sensitive and specific when it comes to suggest it as a reference test for our protocol proposal. The determination in the blood or plasma analysis of the enzyme activities with mass spectrometry of the deficient enzyme has high sensibility and specificity in MPS but low access and high costs for the Ecuadorian Health system which therefore for this reason will not be included in our proposal. MPSRPS protocol is indispensable to be delivered and socialized to the Ecuadorian Ministry of Health so that there are policies within the first level physicians and which includes in the Ecuadorian Health System services to be adequately accessed in the population at risk. In its second phase of this investigation we need to verify the validity of the laboratory tests with the corresponding equipment and reagents according to its costs. Therefore, a guide can be created for the adequate management of patients from its first level of attention for its later corresponding referral.

676 - Hematopoietic Stem Cell Transplantation for Patients With Mucopolysaccharidosis II

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Background: There is limited information regarding the long-term outcomes of hematopoietic stem cell transplantation (HSCT) for Mucopolysaccharidosis II (MPS II). In this study, clinical, biochemical, and radiological findings were assessed in patients who underwent HSCT and/or enzyme replacement therapy (ERT). **Subjects:** Demographic data for 146 HSCT patients were collected from 27 new cases and 119 published cases and were compared with 51 ERT and 15 untreated cases. Glycosaminoglycan (GAG) levels were analyzed by liquid chromatography tandem mass spectrometry in blood samples from HSCT, ERT, and untreated patients as well as age-matched controls. Long-term MRI findings were investigated in 13 treated patients (6 ERT and 7 HSCT). **Results:** Mean age at HSCT was 5.5 years (2 to 21.4 years) in new cases and 5.5 years (10 months to 19.8 years) in published cases. None of the 27 new cases died as a direct result of the HSCT procedure. Graft-versus-host disease occurred in 8 (9%) out of 85 published cases, and 9 (8%) cases died due to transplant-associated complications. Most HSCT patients showed greater improvement in somatic features, joint movements, and ADL compared to ERT patients. GAG levels in blood were significantly reduced by ERT and levels were even lower after HSCT. HSCT patients showed either improvement or no progression of abnormal findings in brain MRI while abnormal findings became more extensive after ERT. **Conclusion:** HSCT seems to be more effective than ERT for MPS II in a wide range of disease manifestations and could be considered as a treatment option for this condition.

T) Lysosomal Disorders: Sphingolipidoses (677 to 736)

677 - Identification of Lysosomal and Extralysosomal Globotriaosylceramide (Gb3) Accumulation in Endomyocardial Biopsies Before the Occurrence of Typical Pathological Changes of Fabry Disease

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Background: Early initiation of enzyme replacement therapy (ERT) could be effective in stabilizing the progression of Fabry disease (FD), and thus potentially preventing irreversible organ damage. Certain Type-2 cardiac FD treatment guidelines suggest performing an endomyocardial biopsy to confirm typical FD histopathological changes as a prerequisite for the initiation of ERT. However, the sensitivity of routine histological

examinations for FD has neither been discussed nor investigated before. **Objectives:** To evaluate the sensitivity of routine histological examinations for endomyocardial biopsies of FD patients with Immunofluorescence (IF) staining. **Methods:** IF staining of Globotriaosylceramide (Gb3) and lysosomal-associated membrane protein 1 (LAMP-1) was performed on the endomyocardial biopsies of the patients who were suspected of Fabry cardiomyopathy, yet had negative or only slight Gb3 accumulation determined by routine histological examinations (Hematoxylin and Eosin [H&E] staining, toluidine blue staining, and electron microscopy examination). **Results:** The IF staining results revealed that all patients had abundant Gb3 accumulation in their cardiomyocytes, while extralysosomal Gb3 accumulation were found in some patients. **Conclusion:** Current routine histopathological examinations for FD cardiac biopsies mainly focus on the existence of Gb3 inclusion bodies. However, before the formation of Gb3 inclusion bodies, significant Gb3 had already accumulated in the cardiac tissues. Moreover, the presence of significant extralysosomal Gb3 suggested the irreversible damages of cardiomyocytes might have occurred. We propose that Gb3 IF staining to be performed as a re-evaluation method when no typical FD pathological findings are observed in the biopsies of patients who are highly suspected to have Fabry cardiomyopathy.

678 - BIG Project (Bone Involvement in Gaucher Disease): Screening for Gaucher Disease With Focus in Bone Affection

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Type I Gaucher disease (EG1) is characterized by hematological, visceral and skeletal signs and symptoms. Around 80% to 95% of patients suffer from bone clinical signs or imaging skeletal problems. The clinical suspicion generally is because of hepato/splenomegaly or cytopenia, however some of the patients present in the first physician visit with bone symptoms. The objective is to present a project for medical education and screening for Gaucher disease with focus in bone affection. A diagnosis algorithm was developed based on bone problems. The screening will be done by assaying glucocerebrosidase in dried blood filter paper, and pathological ones will be confirmed in leukocytes in the following patients: 1) below 18 years old with osteomyelitis, recurrent fractures without diagnosis, Perthes disease, recurrent bone crisis or avascular necrosis (AN), 2) between 18 and 50 years old with recurrent fractures without diagnosis, recurrent bone crisis, hip arthroplasty or avascular necrosis (AN), 3) below 50 years old with reduced bone mineral density (Z score < -2) and bone pain without diagnosis with abnormal ferritin and platelet counts. This algorithm will be implemented along with an educational program about early diagnosis and bone affection in Gaucher disease in several centers from Argentina. It is important to improve the dissemination of knowledge among orthopedists, pediatricians, endocrinologists, rheumatologists, and biochemists, in order to improve early detection and diagnosis of Gaucher patients in order to have an early access to specific treatment.

679 - Moving Forward Toward Personalized Medicine in Rare Diseases: Examples From Lysosomal Disorders

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Understanding phenotypic variability in rare genetic diseases, such as Gaucher disease (GD), is challenging because it is hard to recruit large cohorts of patients with different symptoms to perform association studies. To overcome this problem, we chemically induced GD in 15 inbred mouse strains because their SNPs profile are known, followed by GWAS. GD-induced strains mimicked the divergent phenotypes observed in patients, which range from neuropathic disease with short lifespans to others with no evident CNS involvement and longer survival times. GWA analysis identified a small collection of candidate loci underlying the variable strain phenotypes, which allowed us to successfully predict the severity of the disease in other strains upon GD induction and to identify a novel therapy for the neuropathic forms of GD. By the end of the talk, I will discuss other approaches that we are currently following to uncover modifier genes and therapeutically relevant pathways including exome sequencing in twins presenting with different disease severity and multi-omics in fibroblasts derived from patients with other rare lysosomal diseases.

680 - Cause of Genetic Variations in Severe Clinical Phenotypes of Female Fabry Disease

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Clinical spectrum of female Fabry disease shows wide range of clinical pictures. Generally, female clinical phenotype is caused by the degree of skewed X percentage of α-Gal A gene/ percentage of residual α-Gal enzyme activities. However, there are severe clinical phenotypes of female Fabry disease, almost equivalent to those of classical type of male Fabry disease. These severe clinical phenotypes are caused by main three factors: 1) The X screwed degree of methylation of alfa-Gal A gene in female- a case with complete methylated allele in alfa-Gal A gene. 2) Cases associated with Turner syndrome, XO/Mosaic show severe clinical phenotypes. 3) Compound heterozygote/ homozygote states in two alleles of alfa-Gal A gene. Present report concerns unique severe clinical phenotype, a 37-year-old female Fabry disease associated with mental retardation and coarse face. Mild cardiomegaly was observed with mild proteinuria. Electron microscopic examination of skin demonstrated massive lamellar inclusion bodies in various skin cells. Chromosomal analysis showed chromosome 10q26 micro deletion demonstrated by CGH array which leads to mental retardation. Furthermore, plasma lyso Gb3 was markedly increased, more than 10 times of controls and this patient also excreted large amount of urinary Gb3 and Gb2 which showed more than classical male patient, her gene mutation was identified in exon 1, c36C>A. Furthermore, this patient showed complete methylation of alfa-Gal A gene in CpG island at exon 1 which leads to zero activity of alfa-Gal in leukocytes. This unique female is first case with complete methylated allele of alfa-Gal A gene associated with chromosome 10q26 deletion syndrome. Female Fabry disease associated with Turner syndrome, compound/homozygote state of female gene mutations or methylation occurred in non- mutated allele of alfa-Gal gene which caused complete inactivation of alfa-Gal enzyme activity, sometimes showed serious clinical course such as cardiac failure and CNS problems, and also may not response well to enzyme replacement therapy because of higher titer of serum antibody formation against enzyme protein. In practical point of view, it is important to take an account these clinical conditions in severe clinical phenotype of female Fabry disease.

681 - Report of a Novel Mutation in the Human *GLA* gene (Fabry disease) in the Same Family With 3 Female Generations Affected

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Lysosomal diseases originate in synthesis or function disorders of a lysosomal acid hydrolases. Fabry is an X linked hereditary defect of glycosphingolipid storage caused by mutations in the gene encoding the lysosomal acid hydrolase alfa-galactosidase A (GLA, alfa-galA). To date, over 400 mutations causing amino acid substitutions have been described. Some of these mutations are not related to the classical Fabry phenotype especially in women. We identified by molecular study 3 female patients belong three different generations in the same family at Cauca Department (Southwest from Colombia) with high values of Lyso-Gb3 and the heterozygous mutation (c.1124G>p.G375A). The clinical symptoms include chronic renal failure (index case of 54 years old, grandmother), hypohidrosis, microalbuminuria, acroparesthesias, cornea verticillata (index case of 35 years old, mother) and a positive microalbuminuria test (index case of 3 years old, grand-daughter). We conclude the three patients are suffering from Fabry disease, due to a new mutation in the *GLA* gene, recently classified as pathogenic.

682 - Cardiac Manifestations in Niemann-Pick Type C Disease With Mutations in the *NPC1* Gene: Case Report

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The present study is the first report of Niemann-Pick type C with mutations in the *NPC1* gene with cardiac manifestation. We present a male patient, from a nonconsanguineous family with diagnosis of Niemann-Pick type C at 7 years old. At 13 years old, a discrete cardiomegaly was identified in a routine echocardiogram, with no prejudice in left ventricular function. A discrete left ventricular hypertrophy was identify in follow up. A mild diastolic dysfunction was identify in 2016. At 16 years old, a new echocardiogram was performed showing a mild left ventricular concentric remodeling with an interventricular septal hypertrophy. Left ventricle mass of 115 g ($Z > 2.0$) and in wall thickness of 0.42 ($Z > 2.0$). Niemann-Pick disease type C is an autosomal recessive disease due to mutation in the *NPC1* and *NPC2* genes leading to alterations in trafficking of endocytosed cholesterol and storage of glycosphingolipids in tissues. Cardiac involvement is a common in others lysosomal storage diseases, but, till data, none cardiac involvement was describe in Niemann-Pick type C patients. This case report provides important new information about cardiac involvement in a patient with Niemann-Pick type C and advance the understanding of this disease.

683 - Evaluation of Endothelial Function With Reactive Hyperemia-Digital Peripheral Arterial Tonometry as a Non-Invasive Biomarker to Reflect Vascular Pathology in Patients With Fabry Disease

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Introduction: Fabry disease is one of the sphingolipidoses due to a defective alpha-galactosidase A encoded by the *GLA* gene on X-chromosome. A deficiency of this enzyme results in accumulation of globotriaosylceramide (GL-3) in systemic tissues including kidneys, heart, and the blood vessel system. Progressive kidney dysfunction has been known as one of the major causes of death in patients with Fabry disease. Microalbuminuria is considered one of the first signs of kidney dysfunction although irreversible changes leading to deterioration of kidney function may precede. Noninvasive biomarkers to diagnose early renal pathologic changes to prevent further deterioration are urgently needed. Endothelial dysfunction is thought to precede the development of microalbuminuria. An inverse relationship between microalbuminuria and brachial artery flow mediated vasodilation, which reflects nitric oxide (NO) dependent endothelial function, has been reported. The NO dependent vasodilation is known to be down regulated in Fabry disease. Objectives: This study evaluated reactive hyperemia index (RHI), which is a measure for endothelial function, based on reactive hyperemia-digital peripheral arterial tonometry (RH-PAT) with the EndoPAT 2000^{R}system (Itamar Medical Ltd, Caesarea, Israel) and compared to urine microalbumin concentrations in young male patients with Fabry disease to evaluate if endothelial dysfunction precedes abnormal microalbuminuria. **Methods:** Fourteen young male patients (age range: 6-20 years) with Fabry disease participated in the study. Endothelial function was evaluated by RH-PAT with the EndoPAT2000^{R}system. RHI < 1.67 was used as the cut-off for abnormal endothelial function. Microalbumin in first void urine specimens was also measured. Results: The study identified endothelial dysfunction in all subjects, however, no subjects showed abnormal microalbuminuria. **Discussion:** The evaluation of endothelial function with RH-PAT may diagnose early renal involvement prior to microalbuminuria and provide a rational as to when enzyme replacement therapy should be initiated to prevent irreversible progressive renal damage and renal failure.

684 - A Rare Form of GM1-Gangliosidosis: The Late Infantile Variant

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Background: GM1 gangliosidosis is a sphingolipid metabolism disorder due to lysosomal acid β-galactosidase deficiency. As the mean age of the onset of clinical signs and severity varies according to the severity of the enzyme deficiency, three different clinical phenotypes, including early infantile (type 1), late infantile (type 2), and adult forms, have been identified. Specific oligosaccharide pattern in urine and decreased β-galactosidase enzyme activity in white blood cells are important for diagnosis. There is no currently available effective therapy. This case is reported because of its rarity and its novel mutation. **Case Report:** A 19-month-old female case presented with the complaint of inability to walk. She was born full-term, as 3260 g from a twin pregnancy of a 35-year-old mother. Her twin sibling was lost in utero. Her neurological control was normal up to the age of 8 months when she started to lose her neurological skills such as head control and sitting. Physical examination revealed neuromotor retardation, mild hypotonia but no hepatosplenomegaly. X-rays graphics revealed mild dysostosis in the thoracolumbar region and cranial MRI showed bilateral thalamic hyperintensities and reduced myelination. Urine oligosaccharides were elevated and beta-galactosidase enzyme level was decreased [7 nmol/mg/hr (100-400 nmol/h)]. A diagnosis of GM-1 gangliosidosis was made. Compound heterozygous p.R201C (c.601C>T) / p.Q567* (c.1699C>T) mutation was detected at the analysis of the *GLB1* gene. p.R201C (c.601C>T) mutation was previously identified and reported to be associated with the disease, while p.Q567* (c.1699C>T) mutation was not previously identified, and according to the analyses performed with Mutation Taster program and because it creates an early stop codon, it is considered as a most likely disease-causing mutation. **Conclusion:** Cranial MRI evaluation of cases with neurological disability might help diagnose metabolic diseases.

685 - Evaluation of Enzyme Replacement Therapy for Bone Lesions of Gaucher Disease

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Introduction: Gaucher disease (GD) is an inherited metabolic disorder which glucocerebroside accumulates in the liver, spleen, bone marrow, because the β-glucocerebrosidase activity is genetically deficient or decreased. We evaluated bone lesions of GD and examined the effectiveness of enzyme replacement therapy (ERT). S**ubjects and Methods:** We studied bone lesions of 7 patients with GD (5 patients with type I and 2 patients with type III) to examine by X-ray, MRI, and dual-energy X-ray absorptiometry (DXA). Bone metabolism markers were analyzed for 3 patients with type I GD who had undergone ERT for more than 10 years. **Results:** Bone lesions include bone pain (n = 5), bone crisis (n = 2), hip joint and femoral bone deformity (n = 3), femoral head necrosis (n = 3), Erlenmeyer flask deformation (n = 3), scoliosis (n = 3), bone fracture (n = 2), and a decrease in bone density (n = 6). Three patients with type I GD who had received ERT for more than 10 years recognized lower bone mass (n = 3) and left femoral head necrosis (n = 1) showed with MRI before the initiation of ERT, but the bone mineral density was maintained mildly (82% of YAM) without deterioration. The osteogenic markers were almost normal and there was no decrease in osteogenesis. The bone resorption markers showed an increase of TRACP-5b levels (n = 3) and enhanced bone resorption. When bisphosphonate (BP, risedronate) was used in combination with ERT, TRACP-5b levels decreased and inhibition of bone resorption was observed (n = 3). **Discussion:** Bone lesions of GD are

frequently observed and one of the factors that causes a decrease in QOL. It was considered that exacerbation of bone lesions was suppressed by ERT with Imiglucerase did not show deterioration of bone lesions. In addition, suppression of bone resorption was suppressed by using BP in combination with ERT. It was reported that BP inhibits osteoclastic bone resorption, suggesting the possibility of improving skeletal manifestations of GD by appropriately combining BP with ERT. It was considered that exacerbation of skeletal disease was suppressed by Imiglucerase as ERT because it did not show deterioration of bone lesions. **Conclusion:** ERT for GD suppressed the progression of skeletal manifestations and further suggested the possibility of improving the bone disease by appropriately using BP in combination with ERT.

686 - Physician Awareness of Gaucher Disease in Indonesia

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Gaucher disease is the most common lysosomal storage disease. However, awareness of this disease in developing countries, such as Indonesia, is still low, while treatment options are also limited. There should be more than 4000 cases in Indonesia, considering Indonesia's population of 250 million. **Aim:** To demonstrate a series of Gaucher disease cases in Indonesia, focusing on the struggle to reach diagnosis and the clinical outcomes. **Methods:** We performed retrospective study of Gaucher disease cases diagnosed between 2012 and 2017. We recorded clinical presentations, biochemical data, and outcomes of these patients. **Results:** The first Gaucher disease patient was diagnosed in 2012 and thus raising awareness of its presence in Indonesia. During a period of 6 years, there were five new cases of Gaucher disease. Mostly are male (4/5), diagnosed between the age of 3 months and 3 years. Two patients resided in Jakarta (the capital city), two patients came from neighboring provinces (West Java and Banten), and one came from another island (Jambi). All patients presented with bicytopenia and hepatosplenomegaly. However, only two of them were referred from hematologist, while the rest were consulted by immunologist and gastrohepatologist. They had been misdiagnosed at least once before reaching the correct diagnosis. Initially, they were misdiagnosed as malignancy, cytomegalovirus infection, Tangier's disease, or glycogen storage disease. Time from initial presentation to diagnosis was 3 - 53 months, but time needed to reach the diagnosis of Gaucher disease in our hospital was 1 to 3 months. All patients underwent bone marrow aspirations which revealed Gaucher cells and diagnosis was confirmed by enzymatic test in Taiwan. Four patients died due to numerous causes, including infection and bleeding, and only one patient survived until now. The last three patients were included in the International Charitable Access Program for enzyme donation. However, two patients died before the enzyme was available in Indonesia. The surviving patient will receive enzyme replacement therapy in May 2017. **Conclusions:** Awareness of Gaucher disease in Indonesia has improved in recent years. To raise awareness among the pediatricians, we have given lectures in national meetings for physicians from various subspecialties, especially hematologist and gastrohepatologist.

687 - Assessing the Effectiveness of Miglustat in Niemann-Pick Disease Type C

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Objective: Niemann-Pick disease type C (NP-C) is a rare, autosomal recessive, neurodegenerative disease associated with a wide variety of progressive neurological manifestations, visceral symptoms, and early mortality. Miglustat is the only approved disease-specific therapy for NP-C, indicated for the treatment of progressive neurological manifestations in both adults and children. Since approval in 2009 there has been a vast growth in clinical experience with Miglustat, and its effectiveness has been assessed using a wide range of measures. **Methods:** Comprehensive review of published data from studies of cellular neuropathological markers and structural neurological indices in the brain, clinical impairment/disability, specific clinical neurological manifestations, and patient survival. **Results:** Cranial diffusion tensor imaging and magnetic resonance spectroscopy studies have shown reduced levels of choline (a neurodegeneration marker), and choline/*N*-acetyl aspartate ratio (indicating increased neuronal viability) in the brain during up to 5 years of Miglustat therapy. A 2-year immunoassay study showed significant reductions in CSF-calbindin during treatment, indicating reduced cerebellar Purkinje cell loss. Magnetic resonance imaging studies have demonstrated a protective effect of Miglustat on cerebellar and subcortical structure that correlated with clinical symptom severity. Numerous cohort studies assessing core neurological manifestations (impaired ambulation, manipulation, speech, swallowing, other) using NP-C disability scales indicate neurological stabilization over 2-8 years, with a trend for greater benefits in patients with older (non-infantile) age at neurological onset. A randomized controlled trial and several cohort studies have reported improvements or stabilization of saccadic eye movements during 1-5 years of therapy. Swallowing was also shown to improve/remain stable during the randomized trial (up to 2 years), as well as in long-term observational cohorts (up to 6 years). A meta-analysis of dysphagia—a potent risk factor for aspiration pneumonia and premature death in NP-C—demonstrated a survival benefit with Miglustat due to improved/

stabilized swallowing function. **Conclusions:** The effects of Miglustat on neurological NP-C manifestations has been assessed using a range of approaches, with benefits ranging from cellular changes in the brain through to visible clinical improvements and increased lifespan.

688 - A Randomized Controlled Trial of Two Low-Dose Agalsidase Beta Regimens in Male Pediatric Patients With Fabry disease: GL-3 Clearance From Kidney Cells

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Fabry disease is a multisystem, X-linked disorder caused by deficient α-galactosidase A activity. Progressive accumulation of globotriaosylceramide (GL-3) starts early in life in multiple tissues, including the kidney. This is a 5-year study of the efficacy of agalsidase beta low-dose regimens (0.5 mg/kg 2-weekly [0.5q2w, n = 16] or 1 mg/kg 4-weekly [1q4w, n = 15]) in pediatric male patients without clinically evident vital-organ involvement (NCT00701415). Exploratory outcomes included renal function (microalbuminuria, proteinuria, glomerular filtration rate [GFR, iohexol elimination]), retinal imaging (dilatation, tortuosity), angiokeratomas, plasma/urine biomarkers, and optional renal biopsies at baseline and year 5. Data are presented on 7/31 patients who had paired kidney biopsies. Median age of these 7 patients at baseline was 15 (range 10–17) years. Semi quantitative light microscopy GL-3 scoring showed complete GL-3 clearance in 6/8 kidney cell types assessed at year 5. Clearance was incomplete in non-capillary smooth muscle cells (2/7 patients) and podocytes (4/7 patients). Absolute podocyte GL-3 inclusions volume (quantitative electron microscopy) decreased in 4 patients (3 on 0.5q2w, 1 on 1q4w) and increased in 3 (1 on 0.5q2w, 2 on 1q4w). These changes did not correlate with peak anti-α-galactosidase antibody titers (median 400, range: 0-12,800), kidney function, or podocyte injury. Fabry arteriopathy severity increased in 6/7 patients. No significant development of interstitial fibrosis or glomerulosclerosis was observed. In all 7 patients, plasma and urine GL-3 levels rapidly normalized, and lyso-GL-3 levels reduced to near normal, with fluctuations after year 2. Renal function remained normal. All had retinal vessel involvement at baseline with no substantial changes at year 5. Angiokeratomas worsened in 5/7 patients. Estimated GFR values using the CKD-EPI formula in patients ≥18 years were distinctly higher than values measured by the Bedside Schwartz formula in patients <18 years. In conclusion, despite normal renal function, renal histological and retinal vessel abnormalities were present at baseline. Low-dose agalsidase beta regimens appeared sufficient to clear GL-3 from 6/8 renal cell types examined, but with variable reductions of podocyte GL-3 in both low-dose regimens. These observations suggest that podocyte GL-3 clearance could be agalsidase beta dose dependent. **Funding** (study, abstract): Sanofi Genzyme.

689 - Final Efficacy and Safety Results From a Phase 2 Clinical Trial After 8 Years of Treatment With Oral Eliglustat in Treatment-Naïve Adults With Gaucher Disease Type 1

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Eliglustat is an oral substrate reduction therapy approved as first-line treatment for adults with Gaucher disease type 1

(GD1) with poor, intermediate, or extensive CYP2D6-metabolizer phenotypes (>90% of patients). We report final long-term efficacy and safety results of a Phase 2 trial (NCT00358150, Sanofi Genzyme) after 8 years of eliglustat treatment in previously untreated patients. Adult GD1 patients who had splenomegaly with thrombocytopenia and/or anemia received 50 or 100 mg eliglustat tartrate (equivalent to 42 or 84 mg eliglustat) twice daily, dosed by plasma trough levels. Efficacy outcomes included changes in hemoglobin, platelets, spleen and liver volumes, disease-related biomarkers, skeletal manifestations, and achievement of therapeutic goals for anemia, thrombocytopenia, splenomegaly, and hepatomegaly (Pastores. *Semin Hematol.* 2004; Lukina. *Blood.* 2010). Of 26 enrolled patients, 19 completed the trial and 7 withdrew: 2 due to asymptomatic nonsustained ventricular tachycardia detected during 36-hour ECG safety monitoring on Day 1 (plasma drug levels were undetectable); 1 after 1 year due to progression of a bone lesion retrospectively identified at baseline; 1 chose to withdraw after 2 years; and 3 due to pregnancy. After 8 years of eliglustat, mean $\pm$ SD hemoglobin and platelet count increased by 2.1 $\pm$ 1.7 g/dL (from 11.3 $\pm$ 1.6 to 13.4 $\pm$ 1.3 g/dL) and 110% (from 67.5 $\pm$ 21.1 to 130.7 $\pm$ 59.8 $\times 10^9$/L), respectively. Mean spleen and liver volumes decreased by 68% (from 17.3 $\pm$ 10.4 to 5.1 $\pm$ 3.5 multiples of normal [MN]) and 31% (from 1.6 $\pm$ 0.5 to 1.1 $\pm$ 0.3 MN), respectively. All patients met $\geq$3 of 4 long-term therapeutic goals (spleen, 100% of patients; liver, 100%; hemoglobin, 93%; platelets, 53%) by 8 years. Median chitotriosidase levels decreased by 84%, CCL-18 by 82%, and glucosylsphingosine by 88%; plasma glucosylceramide normalized. Total lumbar spine mean Z-score increased by 0.88 (from 1.27 $\pm$ 1.02 to 0.39 $\pm$ 1.13) and mean T-score by 0.95 (from 1.64 $\pm$ 1.07 to -0.69 $\pm$ 1.31). Eliglustat was well-tolerated. All quality of life measures (SF-36, Gaucher disease severity score [DS3], Fatigue Severity Score) improved over time. No new safety concerns emerged. Most (98%) adverse events were mild or moderate and 94% were considered unrelated to treatment. In summary, clinically meaningful improvements in hematologic, visceral, biomarker and bone parameters continued or were maintained in previously untreated GD1 patients treated with eliglustat for 8 years.

690 - Liver-Based Expression of the Human Alpha-Galactosidase A Gene in a Murine Fabry Model Results in Continuous Therapeutic Levels of Enzyme Activity and Effective Substrate Reduction

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Fabry disease (FD) is an X-linked lysosomal storage disease caused by mutations in the *GLA* gene encoding alpha galactosidase A (α-GalA). FD is characterized by progressive systemic accumulation of α-GalA's substrates, globotriaosylceramide (Gb3) and lyso-Gb3, leading to renal, cardiac and cerebrovascular disease, leading to premature demise. The mutation and any residual α-GalA enzyme level determine whether the disease presents as classic FD or as later onset phenotypes. FD is treated by enzyme replacement therapy (ERT) by lifelong biweekly infusions, with variable results. Thus, an improved, more effective, and long-lasting treatment is needed. Two AAV-mediated, liver-targeted gene therapies were evaluated in a mouse model (GLAKO) lacking α-GalA activity, resulting in high levels of Gb3/lyso-Gb3 in plasma and tissues. The first strategy employs an episomal AAV vector encoding human *GLA* cDNA (h*GLA*) driven by a liver-specific promoter. Vector administration led to supraphysiological (up to 50-fold of WT) plasma α-GalA levels at day 14 sustained continuously for up to 6 months. At study end, dose-dependent α-GalA activity was increased in liver, heart, kidney and spleen with a corresponding reduction of Gb3/lyso-Gb3 to near normal levels. The second strategy (currently in clinical studies for Hemophilia B and Mucopolysaccharidoses) uses Zinc-Finger Nuclease (ZFN)-mediated genome editing to permanently integrate a corrective h*GLA* gene in the *Albumin* locus in liver cells. This strategy exploits the high transcriptional activity of this locus to create stably modified hepatocytes with potentially life-long transgene expression. Administration of 3 AAV vectors (2x ZFN vectors, 1x h*GLA* cDNA vector) achieved supraphysiological levels of up to 50-fold of WT of α-GalA activity in plasma (sustained for duration of the 2 month study) and high activity levels in liver, heart, kidney and spleen. Gb3 in these tissues averaged <10% of that measured in untreated mice. Importantly, appropriate glycosylation of the α-GalA enzyme was confirmed to ensure efficient lysosomal uptake in target tissues. Thus "proof-of-concept" for use of AAV-mediated targeting of hepatocytes to express therapeutic levels of human α-GalA has been demonstrated. A marked reduction of pathological accumulated Gb3/lyso Gb3 in key tissue sites was found, further supporting our liver-based AAV approaches as potential therapies for FD.

691 - Case Report: Niemann Pick C 1 Mutations: Exon 20 (c.3019CG) and Exon 22 (c.3249_3250delGT)

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Introduction: Niemann-Pick C disease (NP-C) is a rare autosomal recessive disorder with an incidence of 1/150,000 live births characterized by neurovisceral atypical lysosomal lipid storage (OMIM: 257220). In the brain, the stored lipids are gangliosides. NP is divided into four main types based on genetic cause and the signs and symptoms: type A, type B, type C and type D. The two most common mutations in the *NPC1* are: p. I1061 T and p. P1007A. **Objective:** To present the genotype–phenotype correlation in the heterozygous patient for 3249_3250del GT; to describe this mutation with clinic presentation in NPC1 (late infantile period). **Case**

Summary: A 10-year-old male with history of loss of acquired abilities at 5 years, followed by ocular abnormalities and hearing loss who complains of cognitive impairment, dysarthria, delay in speech, ataxia, slow movements while walking. Clinical assessment of the disease progression can be evaluated by video record. Due to suspicious of NP-C with a late infantile clinical onset the following diagnostic test were done: NP C Suspicion index (NP-C SI) was $\geq$70, should be considered as possibly having NP-C and should undergo further molecular test. The liso -SM-509:2,1 ng/ml (reference:<0,9 ng/ml). The patient is heterozygote for: c.3019C>G (p. Pro1007ALA) [exon 20] and c.3249_3250delGT (p. Phe1084Leufs*12) [exon 22]. He is receiving Miglustat as treatment with clinical improvement: ataxia, walking movement. **Conclusion:** To our knowledge this is the first time the mutation on exon 22 c.3249_3250delGT (pathogenic variant Type 1) is presented, although the exon 20, c.3019C>G mutation was described by Greer 1991 (most recurrent *NPC1* mutation in Europe, is the prototype of a Biochemical variant mutation). Due to the variability of clinic and genetic presentation it is necessary to determine strategy to identify affected patients among suspected cases with infantile cognitive impairment and neurological symptoms. The identification of two pathogenic NP C gene mutations should be considered sufficient for at initial diagnosis in few day, in the juvenile patient. Further data are required to correlate for the new NPC1 gene mutations variant with evolution, treat responses and other aspects.

692 - GALAC Deficiency in Pediatric and Juvenile Patient (Krabbe disease)

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Introduction: Krabbe disease (KD) or globoid leukodystrophy (LCG) is a sphingolipidosis (storage diseases) with autosomal recessive inheritance (OMIM 245200), incidence 1/100,000. KD affects peripheral and central nervous systems. The gene *GALC*, in Chromosome 14q31.3 encodes the β galactocerebrosidase with the progressive accumulation of the sphingolipid metabolite galactosylsphingosine (psychosine). There are neonatal/infantile form (psychomotor regression, developmental delay before few months). The juvenile/adult form is characterized by weakness, vison loss, intellectual regression and psychiatrics symptoms. **Objective**: To describe two patients with Krabbe Disease, infantile and adult form with the aberrant sphingolipid metabolism. **Cases Summary**: The child patient (KD A) with neonatal form presented tonic—clonic seizures, and psychomotor regression, irritability, spasticity, cry frequently, feeding difficulties, hypertonicity, optic atrophy. He is 4 years old, and is in Stage II—III, he has no contact with his surroundings. The juvenile patient (KD B) is clinically normal until 16 years old, after he presents psychiatric symptoms (psychosis), and intellectual regression, walked slowly, and generalized neurologic deterioration and Hemiparesis. He is in Stage II and is 30 years old. The Neurophysiologic studies are abnormal: peripheral neuropathy (Sensory and Motor conduction velocities are low), the brain images (MRI) are: in KD A, demyelination evidence on the brain (parieto occipital region) and cerebellum. In KD B: demyelination evidence on the brain (frontal, parieto occipital region, thalami, caudate, corona radiata, central semiovale and brain stem). The testing of GALC enzyme activity (both patients) was deficient (0%), in leukocytes isolated from heparinized blood and Arylsulfatase was Normal in both patients. It was not possible to establish the molecular diagnosis in both patients. The treatment of both individuals, with infantile and adult Krabbe disease who are diagnosed in stage II is limited to supportive and palliative care. **Conclusion**: The identification of newborns with the potential to develop Krabbe disease by newborn screening facilitates the initiation of treatment before neurologic damage has occurred. The genetic counseling is very important, and the carrier detection by molecular genetic testing is possible if the pathogenic variants have been identified.

693 - A Randomized Controlled Trial of Two Low-Dose Agalsidase Beta Regimens in Male Pediatric Patients With Fabry disease: GL-3 Clearance From Superficial Skin Capillary Endothelium

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Fabry disease is an X-linked disorder caused by *GLA* mutations that reduce α-galactosidase A activity. Progressive lysosomal accumulation of globotriaosylceramide (GL-3) starts early in life and occurs in a variety of cell types, including capillary endothelial cells, renal, cardiac and nerve cells. This study (NCT00701415) evaluated the efficacy of two agalsidase beta low-dose regimens (0.5 mg/kg 2-weekly [0.5q2w, n = 16] or 1 mg/kg 4-weekly [1q4w, n = 15]) administered for 5 years in pediatric male patients without clinically evident vital-organ involvement. The primary outcome was GL-3 clearance from the superficial skin capillary endothelium (SSCE). Skin biopsies (baseline, years 1, 3, and 5) were available for 29/31 boys (including 2 who received dose increases to 1 mg/kg q2w [labeled dose] for clinical deterioration; excluding 2 drop-outs). The patients' median age was 13 (range 5-18) years; 11 had a truncating *GLA* mutation (0.5q2w: 3; 1q4w: 8) and 18 a non-truncating mutation. No patient had severe SSCE GL-3 accumulation (score 3) at any time point. Of the 20 out of 29 patients with a non-0 SSCE GL-3 score at baseline, 13 (65%) reached and maintained 0-scores on low-dose agalsidase beta (0.5q2w: 7; 1q4w: 6). Shifts from baseline non-0 to 0-scores for SSCE GL-3 were statistically significant at each time point in the overall study population and both treatment groups, except at year 5 in the 1q4w group. 6 of the 7 patients with baseline 0-score maintained 0-scores (0.5q2w: 2, 1q4w: 4). The shift from non-0 to 0-score was also significant for GL-3 in deep vessel endothelial cells. Patients with the highest peak anti-α-galactosidase IgG titers tended to show less robust SSCE GL-3 clearance. Two patients with dose increases to 1 mg/kg q2w showed complete, sustained SSCE GL-3 clearance, despite one of them having the highest peak IgG titer (102400). In summary, low-dose agalsidase beta showed variable clearance of SSCE GL-3, compared to those seen with agalsidase beta 1 mg/kg q2w (Germain et al. 2007, Wraith et al. 2008), suggesting possibly a dose-response effect for agalsidase beta. Funding (study and abstract): Sanofi Genzyme.

694 - Long Term Effects of Enzyme Replacement Therapy in an Algerian Cohort of Type 3 Gaucher Patients

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Type 3 Gaucher disease is very rare and it is characterized by a slowly progressive brain involvement, in addition to severe disease of the other organs of the body. Enzyme replacement therapy (ERT) has been demonstrated to be effective in non-neuropathic Gaucher disease, but long-term results in patients with GD3 are still limited. **Patients and methods:** All patients affected by GD3, treated with ERT, and followed-up in our Pediatric unit of University hospital in Algiers were included. Data on clinical conditions, laboratory values, neurological and ophthalmologic examinations, radiological and electrophysiological features were collected. **Results:** Ten (10) patients (6 females, 4 males) homozygous L444P/L444P, mean age 6.6 years (range 8 months-16 years). They received ERT infusions from 3 months to 10 years. Hematological parameters and organomegaly improved/normalized in 7/10 patients. During the follow-up, three (3) patients showed severe progressive skeletal deformities (kyphoscoliosis). 7/10 developed pulmonary involvement and one of them died with severe pneumoniae. 6/10 developed horizontal gaze palsy and strabismus, two had seizures. Lymphadenopathy in the abdomen was observed in 9/10 patients and hepatic gaucheroma in one patient. 2/6 did not show any neurological symptom after 2 and 8years of treatment respectively. **Conclusions:** ERT improved the systemic manifestations in patients with GD3, but was not able to counteract the progression of neurological, pulmonary and skeletal symptoms in the long term. An additional treatment will be necessary to improve the outcome of type III GD.

695 - Cognitive Impairments and Subjective Cognitive Complaints in Fabry Disease

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The X-linked Fabry disease is a lysosomal storage disease with progressive organ affections, including cerebral vascular disease. Many patients complain of cognitive disturbance and have a general tendency of non-compliance to treatment and follow-up. Our hypothesis was that Fabry patients have cognitive deficits to a degree that interferes with their ability to perform complex aspects of activities of daily living. Patients (n = 41, 71% females, mean age 47 years, range 20-75) were recruited from the Danish national Fabry cohort. Time from onset of symptoms were from 0-69 yrs (mean 28), alpha-GAL activity was 11.8 nmol/h/mg protein (range 0-33, reference range: 20-65), and MSSI Fabry score was 23.3 ranging from 1 to 42. Memory was assessed by Selective Reminding Test and the Rey Complex Figure Test. Psychomotor speed/attention was assessed by Trail Making Test A & B and Symbol Digit Modalities Test (SDMT). Executive functions were assessed with the Stroop test (100 items), and verbal fluency tests. Cognitive complaints were assessed with Perceived Deficits Questionnaire (PDQ), a self-report measure with 20

questions to be scored in four categories, 0 = never to 4 = almost always. Total score ranges from 0 to 80. Perceived cognitive deficits vary among healthy persons and in this study more than two standard deviations (PDQ score >40) greater than previously found in healthy persons was considered abnormal. According to an a priori definition of cognitive impairment, 12 patients (29.3%) were cognitively impaired (all of them were impaired on ≥ four cognitive tests). The results demonstrated that psychomotor speed and attention had the highest frequency of impairment, as assessed by Stroop test, SDMT and Trail Making Test B. The patients were generally not impaired on memory tests. In general, disease related variables did not have a significant impact on the categorization of patients as being cognitively impaired or non-impaired. The levels of subjective cognitive complaints were generally very low in studied patients. Patients with cognitive deficits did not have significantly more complaints of cognitive dysfunction than healthy controls, Thus, about 1/3 of patients with Fabry disease have cognitive test-performances in a range where it may have a significant impact on everyday functioning and absence of subjective cognitive complaints does not exclude the presence of cognitive problems.

696 - Lysosomal-nucleus players in Fabry disease cultured fibroblasts

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Introduction: Fabry disease (FD) is one of the lysosomal storage diseases with an X-linked recessive inheritance. A mutation in *GLA* gene causes α-galactosidase A (α-Gal A) deficiency, which leads to an abnormal accumulation of globotriaosylceramide (Gb3) in the lysosomes. The lysosomal homeostasis is required for vital processes and is regulated by a lysosomal-nucleus signaling. The mammalian Target Of Rapamycin (mTOR) is the protein complex of the lysosomal nutrient sensing machinery, which can phosphorylate the cytoplasmic transcriptional factor EB (TFEB) when activated. When the TFEB is phosphorylated in the cytoplasm, it cannot translocate to the nucleus, where controls its network genes that will regulate autophagy, biogenesis and other lysosomal functions. Thus, mTOR and TFEB are the two main players of the machinery that regulates the autophagy and lysosomal biogenesis. AIMS: In this work, we aimed to compare mTOR and TFEB localization in fibroblasts from a FD patient and from a healthy donor. **Methods:** Male human skin fibroblasts from FD patient and control individuals were cultured with DMEM 15% Fetal Bovine Serum, 1% Penicillin/Streptomycin and 1% L-glutamine to confluence, fixed on a glass slide and labelled with anti-Phospho-mTOR (1:50) and anti-TFEB (1:50). Images were acquired in a laser scanning confocal microscopy for localization. Anti-Phospho-mTOR (1:50) was also used to quantify the p-mTOR protein by flow cytometry. **Results:** Both p-mTOR and TFEB were localized in the cytoplasm of fibroblasts from Fabry patients and control individuals, showing no difference in localization. Also, equal amounts of p-mTOR were detected when we compared both cells types by flow cytometry. **Conclusion:** When comparing mTOR and TFEB localization, no differences were observed between fibroblasts from FD patient and from healthy donor. The presence of TFEB at the cytoplasm infers that it is phosphorylated and the similar amount of p-mTOR means that there is not enough stimulus to trigger the regulation of the lysosomal homeostasis, suggesting that in FD patients the lysosomal-nucleus signaling is not compromised. **Financial Support:** AFIP, CNPq, and CAPES.

697 - Once Versus Twice-Daily Dosing of Eliglustat in Patients With Gaucher disease Type 1: The Phase 3, Randomized, Double-Blind EDGE Trial

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Eliglustat is a first-line oral therapy for adults with Gaucher disease type 1 (GD1) and compatible CYP2D6 phenotypes (>90% of patients). The randomized, double-blind EDGE trial (Sanofi Genzyme NCT01074944) evaluated once-daily (QD) dosing compared with the approved twice-daily (BID) regimen of eliglustat in previously treated or untreated adults with GD1 who attained clinical stability on BID dosing during a 6 to 18 months lead-in period (LIP). To be randomized to the 1-year primary analysis period (PAP), patients had to attain prespecified therapeutic goals for hemoglobin, platelets, spleen and liver volume, and bone on a stable dose of 50 or 100 mg BID, and have peak plasma eliglustat <50 ng/mL in the LIP. To meet the PAP primary endpoint, per-protocol patients had to maintain stability relative to PAP baseline in 5 measures (no decrease in hemoglobin >1.5 g/dL; no decrease in platelet count >25%; no increase in spleen or liver volume >25% or >20%, respectively; no symptomatic bone disease), and meet tolerability requirements including maintaining plasma eliglustat <150 ng/mL. Non-inferiority of QD vs BID dosing

(primary endpoint) was determined by a difference of <−15% in the lower bound of the 95% confidence interval (CI) of the difference between percent stable on QD vs BID. All patients, including non-randomized patients, could continue in an open-label extension. Of 170 enrolled patients, 87% had prior enzyme replacement therapy (ERT); 157 (92%) completed the LIP; 131 met all 5 therapeutic goals and other randomization criteria and entered the PAP. After 1 year, QD dosing did not meet the criteria to be declared non-inferior to BID dosing in tolerability and clinical stability. The lower bound of the 95% CI difference between percent stable on QD vs BID (80% vs 83%) was -17.7%. Randomized patients (QD and BID) maintained mean values for hematologic and visceral measures within therapeutic goal thresholds established for patients on ERT (Pastores et al. *Semin Hematol* 2004) during the PAP and extension. Eliglustat was well-tolerated in this long-term trial encompassing 566 patient-years of eliglustat exposure, with 4 withdrawals (2%) for related adverse events (AE), and similar AE profiles for QD vs BID. The dose regimen specified in the drug label is confirmed by this study. Although QD dosing did not meet non-inferiority criteria, overall clinical stability and safety for both QD and BID dosing was consistent with other clinical trials of eliglustat.

698 - Guidelines for the Management of Patients With Acid Sphingomyelinase Deficiency

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Objectives: To develop consensus recommendations for the assessment and effective disease management across the spectrum of phenotypes of acid sphingomyelinase deficiency (ASMD). **Methods:** Evidence-informed consensus process was employed by an international group of experts in various research and clinical fields related to ASMD. This process involved the review of published literature and personal clinical experience. **Results:** In the absence of an approved disease-modifying therapy, ASMD management consists of multi-organ symptom management. The appropriate evaluations and subsequent management are determined by the patient's disease phenotype. For patients with chronic forms of ASMD, it is recommended that regular assessments are performed to evaluate for liver, spleen, cardiovascular, and pulmonary disease, as well as the extent of neurological, bone, and hematologic involvement. Lifestyle modifications that may lessen the impact of symptoms are also recommended. Since the most severe, infantile neurovisceral form of ASMD is uniformly fatal by 3 years of age, in these patients, assessments should be made at the time of diagnosis, but not on an ongoing basis. Family counseling is advised to determine the individual approach needed to ensure patient quality of life. All patients with ASMD and their parents should receive genetic counseling. Additionally, siblings of patients with ASMD should be evaluated for ASMD. **Conclusions:** ASMD is a multiorgan system disorder requiring a multidisciplinary clinical team. Current recommendations for disease management include symptom management. With future therapies, disease management goals should aim to avoid splenectomy and other disease complications, and to improve liver function and respiratory status, with the ultimate goal of reducing serious morbidity and mortality. Supported by Sanofi Genzyme.

699 - Safety and Efficacy of Intrathecal Delivery of Recombinant Human Arylsulfatase A Produced Using a Revised Manufacturing Process in Children With Late-Infantile Metachromatic Leukodystrophy

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Metachromatic leukodystrophy (MLD) is an autosomal recessive disorder characterized by deficient activity of arylsulfatase A (ASA), resulting in sulfatide build-up in CNS and PNS cells. In late-infantile (LI)-MLD, neurodegenerative symptoms develop rapidly before premature death. This phase 1/2 multicenter, open-label, non-randomized study investigated the safety of intrathecally delivered recombinant human (rh) ASA in children with LI-MLD (NCT01510028). In cohort 4 (C4) patients received 100 mg rhASA every other week (EOW) produced using a revised manufacturing process (process B)

to improve process efficiency and robustness. Results from C4 were compared with the previously reported initial 40-wk study in which cohorts C1, C2 and C3 received rhASA EOW at 10, 30 and 100 mg, respectively (process A). Eligibility criteria for all four cohorts was confirmed MLD diagnosis, first symptoms <30 mths and ambulatory (able to walk forward 10 steps with one hand held). In C1-C3 patients were aged <12 years and in C4 <8 years. In C4, Gross Motor Function Measure-88 (GMFM-88) was ≥40 at screening and ≥35 at baseline. Mean ± SD enrollment age was 48.5 ± 24.22 mths in C4 compared with 31.5 ± 11.50, 47.3 ± 20.23, and 52.2 ± 31.17 mths in C1, C2, and C3, respectively (n = 6 per cohort). Overall, ≥1 treatment emergent adverse event (TEAE) occurred in 24 patients (100%; 474 events), ≥1 rhASA-related TEAE occurred in 13 patients (54.2%; 50 events) and ≥1 serious TEAE occurred in 14 patients (58.3%; 44 events). There were no deaths or discontinuations due to study drug or device. Decline in motor function (LS mean ± SE change [baseline to wk 40] in GMFM-88 total scores) were –18.1 ± 9.14% and –19.5 ± 8.54% in C4 and C3 (100 mg doses) vs –31.9 ± 8.76% and –29.0 ± 8.58% in C1 and C2. Changes in N-acetylaspartate/creatine (NAA/Cr) metabolite ratios were evaluated by MRS (baseline to wk 40). In right parieto-occipital white matter, LS mean change was –0.1 ± 0.07 (n = 3) and 0.1 ± 0.05 (n = 5) in C4 and C3 vs –0.4 ± 0.08 (n = 2) and −0.2 ± 0.08 (n = 2) in C1 and C2. A trend towards stabilization of MRI-MLD severity score in C4 was seen, as described previously in C3. CSF sulfatides fell to the normal range by wk 28 in C3 and C4. In conclusion, intrathecal rhASA produced using process B was generally safe and well tolerated. The 100 mg cohorts had a lesser fall in motor function and positive effects in lowering CSF sulfatides and MRS (NAA/Cr) than the 10 or 30 mg cohorts. Shire funded this study and medical writing support.

700 - Disease Burden in 220 Latin American Female Patients With Fabry disease: A Fabry Registry Analysis

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Fabry disease is an X-linked, lysosomal storage disorder with remarkable genetic variability. Clinical expression in female patients is heterogeneous (from asymptomatic to severe presentations as seen in classic males) and is dependent on multiple factors including the type of mutation and the X-chromosome inactivation profile. We assessed the burden of Fabry disease in 220 Latin American female patients enrolled in the Fabry Registry (NCT00196742). Natural history data from females never treated with enzyme replacement therapy (ERT−) and females eventually treated with agalsidase beta (ERT+) were compared. Natural history data were obtained from 110 ERT- (median age 33 [range 1.9-83.6] years) and 110 ERT+ females (median age 46.7 [range 14.5-76.7] years) from Brazil (68, 78, respectively), Chile (21, 18), Argentina (14, 14), and Colombia (7, 0). Onset of first Fabry symptoms (any) occurred at median age 15.3 (ERT−) and 14.4 years (ERT+), and diagnosis at age 27.2 (ERT−) and 40.9 years (ERT+). ERT+ patients started ERT at a median age of 43.7 (range 12.9-73.7) years. Percentages of females reporting specific Fabry symptoms (% [median age at first report, years]) for ERT- and ERT+, respectively, were: abdominal pain: 23.2 [17.3], 45.8 [30.2], $P = .01$; diarrhea: 18.9 [21.2], 43.1 [25.7]; abnormal sweating: 68.7 [24.4], 89.1 [32.9]; peripheral pain: 69.6 [23.5], 96.7 [26.3]; acute pain crisis: 20.2 [20.4], 32.3 [31.4]; angiokeratoma: 21.1 [30.3], 32.2 [43.5]. Available data on symptom history (absence/presence) was more limited in the ERT+ group which hampers interpretation. There were no notable differences between the ERT- and ERT+ groups in the percentages of females with renal dysfunction or cardiac pathology (normal median values [averaged and most recent assessments]). Severe clinical events in ERT- and ERT+ groups included: cardiac: 0.9%, 4.5%, respectively; dialysis/transplant: 3.6%, 0.9%; stroke 0.9%, 2.7%; TIA 3.6%, 8.2%. In conclusion, female Fabry patients often experience symptom onset during adolescence but are at risk of delayed diagnosis well into adulthood. Renal and cardiac disease burden in ERT− and ERT+ females does not appear to be substantially different. In general, not all female patients are submitted to profound multidisciplinary clinical investigations as reflected by the limited data. All female patients should be regularly monitored and treatment guidelines should be adhered to. Sponsor Fabry Registry and abstract: Sanofi Genzyme.

701 - Healthcare Resource Utilization in Gaucher Disease in Israel

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Gaucher disease (GD) is a rare autosomal recessive disorder caused by a mutation in the glucocerebrosidase gene. Relatively little is known about healthcare resource utilization (HCRU) for orphan diseases such as GD. The purpose of this study was to assess the burden to the healthcare system of a GD

population over time using retrospective data from Clalit Health Services (Clalit), the largest of four managed care organizations in Israel. A retrospective cohort study was conducted to assess HCRU for registered Clalit patients in the following groups: patients with GD versus matched controls from the general population; patients with GD with and without bone events at baseline; patients with GD with and without hematological abnormalities or organomegaly at baseline. Patients with continuous enrollment in Clalit 1 year pre- and post-baseline (1/1/2008) were examined and followed for up to 8 years. The study included 2610 Clalit members: 435 patients (3276 person-years [PY]) with GD and 2175 controls (16 240 PY) without GD. Over an 8-year period, patients with GD had significantly higher HCRU per PY than those without GD, including increases relative to their matched controls of 23.9% for community visits, 12.3% for specialist visits, 37.9% for ambulatory admissions, 57.3% for imaging use, and 25.5% for hospital admissions (all $P < .05$). Within the GD population, 123 patients (897 PY) with a bone event at baseline had higher HCRU rates than those without (n = 269, 2049 PY), with increases of 41.6% for community visits, 24.1% for specialist visits, 39.0% for ambulatory admissions, and 51.5% for imaging use (all $P < .05$). GD patients with hematological abnormalities or organomegaly (n = 330, 2491 PY) at baseline had higher HCRU rates for ambulatory admissions (80.6%), imaging use (67.1%), and number of hospital admissions (200.3%; all $P < .001$) than those without these baseline symptoms, but similar rates for community and specialist visits and length of hospital stay. Overall, rates of HCRU were greater for patients with GD compared to the general population. High HCRU rates were seen for imaging use and ambulatory admissions among GD patients with a bone event, hematological abnormalities, or organomegaly at baseline, which are consistent with the monitoring requirements of the symptoms of GD, anemia, and thrombocytopenia. Further studies should examine outcomes related to the management of GD in at-risk populations.

702 - Bone Pathology in Type 1 Gaucher Disease: Results From a Systematic Literature Review

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The occurrence of bone pathology remains a major clinical issue in patients with type 1 Gaucher disease (GD1). However, the relationship between bone disease and indirect markers of GD1 severity, and the role of bone pathology in overall disease burden, are unclear. A systematic review of the literature was undertaken to identify all publications on GD1 reporting on: (1) the relationship between indirect disease markers and bone pathology; (2) disease epidemiology and bone involvement; (3) the humanistic burden associated with bone disease; and (4) economic evaluations of treatments in relation to improving bone pathology. The searches identified 36 publications of interest: 24 papers reported results on the relationship between indirect disease markers and bone pathology; 4 reported on epidemiology; 8 on the humanistic burden; and 1 on economic evaluation. The occurrence of bone disease in GD1 was found to be related to specific variants in a number of genes, including glucocerebrosidase 1 (GBA1), bone morphogenetic protein 4 (BMP4), cytochrome P450 family 1 subfamily A member 1 (CYP1A1), interleukin 1-alpha (IL-1a), interleukin 1-beta (IL-1b), and interleukin 1 receptor antagonist (IL1RN). Several features of bone disease, including avascular necrosis (AVN) and glycolipid deposits in bone marrow, were found to be related to elevated chitotriosidase levels. Factors associated with an increased risk of developing bone disease in GD1 were older age at diagnosis, increased ferritin concentration, and decreased bone marrow fat fraction. Data on the prevalence of bone disease in GD1 were only available for AVN, with a reported incidence of 0.228 per 100 000 person-years in untreated individuals, versus 0.138 per 100 000 person-years in patients receiving enzyme replacement therapy (ERT). There were limited data on the economic burden of bone disease in GD1, but 1 study suggested higher annual healthcare costs relative to GD1 patients without bone disease. GD-related bone disease is associated with specific indirect markers of disease activity, and several of its features were found to be related to increased levels of chitotriosidase. Although there are limited data on the impact of treatment on bone disease, findings suggest that ERT is associated with a lower incidence of AVN.

703 - Evaluation of the Sleep Quality and Chronotype in Fabry Disease Patients

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Fabry disease is a lysosomal storage disease characterized by a mutation in the *GLA* gene, located on X chromosome, whose main consequence is the enzymatic deficiency of alpha-galactosidase A and accumulation of globotriaosylceramide (Gb3). The storage of Gb3 is progressive and affects several tissues, especially the smooth muscle. In addition to the symptoms commonly reported by individuals with the disease, such as acroparesthesia and angiokeratomas, excessive daytime sleepiness is also often described by the patients. Alterations in sleep parameters have already been described in individuals with Mucopolysaccharidosis type III, another type of LSD, which suggests alteration of biological rhythmicity, since the propensity to sleep presents a strong circadian component, synchronized with the light/dark cycle. Therefore, the purpose of this study is to describe quality of sleep and the chronotype of individuals with Fabry Disease. To achieve this goal, 17

volunteers (7 men and 10 women)—median age of 43 years (17-62)—diagnosed by biochemical or molecular analysis with Fabry Disease regularly attended at the Center for Reference of Inborn Errors of Metabolism at UNIFESP were matched by sex and age with 16 healthy controls (6 men and 10 women), with a median age of 42.5 years (18-59), totaling 33 individuals. The volunteers answered three questionnaires: Epworth Sleepiness Scale, Morningness–eveningness questionnaire and Pittsburgh Sleep Quality Index. Among Fabry Disease patients, 52.9% presented a positive result for ESS, which corroborates previous data in the literature. Chronotype analysis showed that the control group presented an expected distribution of the profiles, replicating what occurs in the general population, where the most extreme chronotypes are less frequent and the intermediaries are more frequent. The Fabry group presented a different distribution, in which 53% of the subjects presented de morningness profile (71.4% males) against 18.8% in the control group (33.3% males). This result may be related to a different rhythm profile for individuals with Fabry Disease. Most individuals in both groups reported poor sleep quality (50% control group and 58.8% Fabry group). These results confirm data described in the literature and highlight some rhythmic differences in individuals with Fabry Disease. Further studies, using markers of biological rhythmicity, may be performed to add new information in these important biological aspects.

704 - Enzymatic Studies of Gaucher Disease in Colombia, A Compilation of the Results of Seventeen-Years of High-Risk Screening

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Introduction: Gaucher disease is included within the genetic alterations that compromise the metabolism of the sphingolipids. This disease is categorized as a lysosomal storage disorder caused by Beta-glucosidase deficiency (D-glucosylacylsphingosine glucohydrolase, EC 3.2.1.45), which is inherited in autosomal recessive form (1q2.1). The clinical presentation shows a multisystemic compromise that includes, among other manifestations, hepatosplenomegaly, anemia, thrombocytopenia and bone lesions with varying degrees of severity. Therefore, enzymatic evaluation studies are of great importance, when they are used as a mechanism to confirm pathology. In Colombia, high-risk studies of sphingolipid metabolism disorders have shown that Gaucher disease is one of the most frequent lysosomal diseases, and in concordance with global case reports, more than 95% of the cases detected correspond to the non-neuronopathic form. **Aim:** Here we present enzymatic studies for Beta-glucosidase isolated from total leukocytes, in a Colombian population with high-risk for Gaucher disease (n = 2073 patients), Period: 2000 to 2016. **Methodology:** Beta-glucosidase enzymatic activity was measured in 800 control individuals (Age: 2 months - 95 years-old) and 2073 patients showing clinical features compatible with Gaucher disease (Age: 1 month - 80 years-old). Enzyme assays were performed using the artificial substrate, 4MU-b-glucopyranoside in the presence or absence of Conduritol Beta-Epoxide (CBE). The enzyme used as a control was beta-galactosidase (Activity Units: nmol/mg protein /hour). **Results and conclusion**: Enzymatic activities from control individuals ranged from 5.0 to 16.5 nmol/mg protein/hour, whereas enzymatic activities from patients exhibiting symptoms of Gaucher disease were 0.0-3.21 nmol/mg protein/hour. The total number of patients confirmed with enzymatic deficiency was 196 (9.5%) in a range of age from 2 months to 72 years-old (32% $\leq$ 10 years-old), 108 women (55%) and 88 men were affected (45%). Our results show an important frequency of the Gaucher disease in Colombia compared with other sphingolipidosis, however a late diagnosis is observed. We suggested using CBE as an inhibitor to avoid the interference of enzymatic isoforms, which degrade the artificial substrate, although they are not related to the disease.

705 - Renal Involvement in Fabry Disease

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Introduction: Fabry disease is an X'R disease that is caused by mutations in the gene encoding the α-galactosidase enzyme. The main clinical findings are cutaneous angiokeratomas, acroparesthesias, neurological findings, hypertrophic cardiomyopathy, corneal verticiliata and kidney involvement. In Fabry's disease, both glomerular and tubular dysfunction develop due to GB3 deposition in renal cells. **Method**: 16 Fabry patients and 21 healthy people as control group were included into the study. BUN, creatinine, 24-hour urine protein, 24-hour urine microalbumin, spot urine protein and microalbumin levels were studied in patient and control group. Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) and N-acetyl-β-D-glucosaminidase (NAG) were evaluated to assess tubulopatyhy and acute renal damage. NGAL, KIM-1 and NAGase values of the patients were compared with the control group. **Results**: The Fabry group consisted of 16 patients, 8 males and 8 females. Eight of the patients were taking enzyme replacement therapy. The control group consisted of 21 people, 9 females and 12 males. None of the patients had elevations in BUN, creatinine levels. When compared the NGAL, KIM-1, NAG levels with the control group, no significant relationship were found ($P \leq .05$ significant). But spot urine protein, microlbumin and 24 h urine protein and microalbumin were significantly high in the Fabry group ($P \leq .05$). **Discussion:** In this study, it was considered that there was no increase in NGAL, KIM-1, NAG, because the disease

relies on a very chronic pathogenesis from the acute process. It was considered that more patients should be studied in order to assess the efficacy of renal injury in Fabry patients.

706 - Niemann-Pick C (NPC) Through Parent's Eyes. Not Just a Case Report

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NPC is a progressive lysosomal storage disease associated with mutations in the *NPC1* and the *NPC2* genes. It is supposed to affect approximately1:150,000 people. Treatment became available in the last few years. Thanks to the generosity of her parents who share the lived experience in order to prevent their suffering from recurring in other families, we report the life story of ESM, a girl with NPC diagnosis. ESM was born on 1988. Just a few weeks before her 16th birthday she was discovered to have Niemann-Pick type C as the cause of her progressive deterioration. Up to 8 years of age she was a "normal" girl who interacted with peers, played, and practiced swimming, dancing, cycling and skating. Due to learning difficulties, poor attention span at school and writing worsening was referred to a psychologist. After a few months of therapy, the doctor evaluated that her problems were not psychological, so she was referred to a neurologist. ESM went through many medical studies and was diagnosed with ATAXIA of unknown etiology. She received no specific treatment. The symptoms progressed. Fine motor area and in the ocular movements began. She developed loss of the stability and balance, successively appeared difficulties in the speech, inconveniences in the swallowing of liquids, convulsive episodes and increasingly severe mandibular rigidity. As the new symptoms appeared, motor and balance were diminishing. Her menarche was at age 13, and always with irregular periods, her urine alternates between clear and odorless and cloudy with very strong odor. At that point, she was under vitamin E, high transaminases were detected and as there was no improvement, vitamin E was suspended. A new series of medical test studies did not give diagnostic confirmation. After almost 8 years of unsuccessful searches a skin biopsy was compatible with NPC, the genetic study confirmed the diagnosis. At the age of 16 she presented paralysis of the vertical and horizontal gaze, severe dysarthria, dystonic postures in both hands, severe postural instability, nasogastric tube for feeding. She assisted to rehabilitation treatments aiming to keep her occupied and happy. Her family and health caregivers tried at all times to improve her quality of life but unfortunately could not stop her progressive deterioration. We consider that the oral, write, photographic and videos registries of ESM's life will help physicians to improve early detection, understanding and treatment of NPC patients and families.

707 - Niemann-Pick Disease Type B: A Case Series of Brazilian Patients

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Objective: To describe a series of Brazilian patients with Niemann-Pick disease type B (NPD-B) characterizing the clinical features and to determine how genotype influences phenotype. **Methods:** A retrospective study of clinical records of patients with Niemann-Pick disease type B admitted at the Clinical Hospital of Ribeirão Preto, University of São Paulo - Department of Inborn Errors of Metabolism. Serial clinical examination, laboratory tests, radiologic studies were evaluated. Genotyping of the specific mutations in the acid sphingomyelinase (SMPD1) gene was performed. Genotype-phenotype correlations are presented. **Results:** The sample consisted of 6 patients aged 8 to 21 years and no family consanguinity was reported. The diagnosis was suspected based on clinical findings suggestive of (NPD-B). The coexistent conditions were: hepatosplenomegaly, elevation of transaminases and abnormal pulmonary function. Dyslipidemia and thrombocytopenia were detected in half of the patients. Analysis of plasma specimens revealed acid sphingomyelinase deficiency ranged from 0,1 to 142 *pmol/spot*20 h (mean level: 29 *pmol/spot*20 h) and markedly increased levels of chitotriosidase that varied from 763 to 7154 nmol/h/ml (mean level: 2338 nmol/h/ml). The Lysosphingomyelin-509 (Lyso SM 509) ranged from 2,5 to 10ng/ml (mean level 4,2ng/ml). The molecular analysis found mutations in the *SMPD1* gene in all patients. **Conclusion:** This study documents the multisystemic character and the clinic heterogeneity in the type B Niemann-Pick disease, which is mainly characterized by hepatosplenomegaly, liver dysfunction, atherogenic lipid profile and gradual deterioration of pulmonary function. The majority of patients with NPD-B have no neurologic abnormalities. To confirm the diagnosis, it is required to determine the genotyping of the acid sphingomyelinase gene. Some potential biomarkers, such as chitotriosidase may help to evaluate the disease activity and to monitor patient treatment responses. The Lyso SM 509, a new sensitive and specific biomarker reflects the burden of the disease and it can be used for the easy diagnosis and monitoring progression.

708 - LysoGb3 in Argentinean Fabry patients

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Fabry disease (FD) is an X-linked lysosomal storage disorder caused by *GLA* mutations with an unknown incidence in Argentina. The α-galactosidase (α-Gal) deficiency results in the

progressive accumulation of lysosomal substrates: leading to the storage of sphingolipids such as globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3) in lysosomes of all cells in the body. Despite the pattern of inheritance, this disease may present a wide spectrum of clinical manifestations in both men and women. More than 800 mutations and some polymorphisms were detected in the GLA gene and, because of the disease heterogeneity, a useful biomarker is essential not only for treatment monitoring but also as a diagnostic tool when a new mutation or a variant of uncertain significance mutation is detected. **Aim**: To evaluate the LysoGb3 level in 46 Argentinean Fabry patients (FP) at the diagnosis. **Material and methods**: Since we validate the method for the quantification of Lyso Gb3 in 2016 we have the opportunity to measure plasma Lyso Gb3 from 46 FP by UPLC- tandem mass spectrometry analysis: 29 females and 17 males. Genotyping was performed in all of them by sequencing and deletion/duplication analysis. **Results**: 40 out of 46(14 men and 26 women) FP showed elevated Lyso Gb3 level and 6 patients had the biomarker within normal limits, (men cut off< 1.0 nM and women cut off< 1.2 nM). The mean level for men was 61.51 nM(Min: 0.40 nM, Max: 138.9 nM) and for women 4.25 (Min: 0.30nM, Max: 21.20 nM). The most frequent mutation found was p.L415P, described as responsible of the classic phenotype of the disease. All FP with this mutation had high levels of LysoGb3. In 5 patients, 3 women and 2 men, all of them from the same family, an unclassified variant was detected that has not been described in FP: p.N34D (c.100A>G). The level of Lyso Gb3 founded in this family was as high as in patients with classic mutations. The mutations of the patients who showed LysoGb3 within normal limits were p. R301Q and p.R363 H, described as responsible of late-onset phenotype. **Conclusion:** The Argentinean FP reported is this study with classic phenotype has LysoGb3 levels out of normal limits. This biomarker helped us to classify a new mutation that was not described previously. We want to bring out the value of this biomarker to monitor the evolution of the disease and eventually the response to the treatment provide.

709 - Novel Mutations in a Case of a Peruvian Girl With Metachromatic Leukodystrophy

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We present the case of female patient from Piura (Northern region of Peru) with clinical and molecular diagnosis of metachromatic leukodystrophy. Infantile form of these autosomal recessive diseases is characterized by onset before 30 months of age, motor and mental deterioration, white matter lesions, and neuropathic changes in electromyography. The injury is caused by arylsulfatase (ARSA) deficiency leading for sulfatides deposit. Our patient had normal development before 1 year and 6 months when she began to regress. At the age of 2 years and 3 months she was evaluated by a geneticist. Her growth percentiles were normal except the cephalic perimeter (p2). She has diminished muscle tone, clonus, dystonia and increased osteotendinous reflexes. Her karyotype study had normal result (46, XX). MRI revealed increased white matter's signal. Her electromyographic study showed peripheral neuropathy. The ARSA activity was low by enzymatic study. WES (Whole exome sequencing) result was clinically relevant with variants with significant phenotype overlapping with this case: previously unreported heterozygous variant in the *ARSA* gene, c.1130_1132del (p.Phe377del), confirmed by Sanger sequencing and detected in the mother. It is a variant of uncertain significance (class 3) according to the recommendations of the Centogene and ACMG. Also, another unreported variant was found in heterozygosity: c.190T>G (p.Phe64Val), detected in the father. It is also a variant of uncertain significance (class 3). We are postulating these novel mutations as the cause for metachromatic leukodystrophy.

710 - Clinical, Biochemical, and Molecular Characterization of Patients With Gaucher Disease From Pará

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Gaucher disease (GD) is an autosomal recessive disease caused by β-glucosidase deficiency by mutations in the *GBA* gene, which encodes the enzyme. Chitotriosidase is a human chitinase secreted by active macrophages and used as a biomarker in the monitor of DG, the most common deposit lysosomal disease. The objective of this work was to genotype the *GBA* and *CHIT1* genes in patients with GD from Pará State. Mutations in the *GBA* and *CHIT1* genes were identified by PCR followed by direct sequencing. Three new previously described mutations in the *GBA*: S gene (-24) G (g.1872A> G), L (-25) S (g.1870T> C) and H419Y (g.6874C> T) and 15 mutations previously reported in other studies. The N370 S and RecNciI mutations were the most frequent, respectively, and then I489 T, the G377 S mutation was not found in our study. The genotyping of the *CHIT1* gene revealed 3 (23%) patients homozygous for the allele with 24 bp, 3 (23%) heterozygous and 7 (54%) dominated homozygotes for the allele. In this way, it was possible to conclude that it is important to identify the spectra of mutations in the *GBA* gene to better understand the molecular basis of the disease. In relation to the *CHIT1* gene, it is important to use other biochemical markers to monitor the disease, where the use of chitotriosidase is limited.

711 - Fabry Disease: A Way for Improving the Detection of Heterozygous Females in Dried Blood Spots and Analysis of Four New Genetic Variants Identified

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Fabry disease (FD) is an X-linked lysosomal disorder caused by mutations in the GLA gene, encoding the alpha-galactosidase A enzyme (AGAL). Heterozygous females (HF) for FD show a wide range of AGAL activity in vitro, from very low to normal values regardless of the type of sample analyzed: dried blood spots (DBS), plasma, leukocytes or fibroblasts. For this reason, the Gold Standard method for detection/diagnosis in women is GLA gene sequencing with large deletion/duplication analysis, an expensive and time-consuming method. HF may be asymptomatic although is likely that show some manifestations of disease, even these could be similar to those observed in men with classic FD. Detection is therefore essential for an early diagnosis and treatment, and for proper genetic counseling. **Aim**: to improve detection of HF for FD through enzyme activity assays in DBS and analysis of the new genetic GLA variants found. **Materials and Methods**: The study was conducted in 220 women with clinical suspicion and/or positive family history for FD. AGAL and Beta-Galactosidase (BGAL) enzyme activity were measured in DBS (fluorometric method with deproteinization), and BGAL/AGAL activity ratio were calculated in all women. BGAL is the routine enzyme run in our lab as a control to assess the quality of DBS samples. GLA gene analysis (sequencing + MLPA) was carried out in all women. Bioinformatic and familial pedigree analysis were performed for new variants. **Results**: By genetic test 65/220 women were detected as HF. Although only 22/65 (34%) HF showed low DBS AGAL activity, 58/65 (89%) had an increased BGAL–AGAL ratio (cutoff: 8.25, ROC curve AUC = 0,962, positive predictive value: 87%, negative predictive value: 95%). BGAL/AGAL ratio was normal in 146/155 of control group. Twenty different mutations in GLA gene were identified, where 4 were classified as variants of unknown significance because were previously not reported (N34D, A156_A160del, c.369+17T>C, c.640-1G>C). **Conclusions**: BGAL–AGAL ratio in DBS reduced significantly false negatives, improving the detection of HF in 164%. This allowed detect 89% of HF for FD versus 34% detected by AGAL activity in DBS, without any extra work. Although more studied are needed to measure the impact of this new variants found into the protein, in a first approach, N34D, A156_A160del and c.640-1G>C seem to be disease causing variants, whereas are unclear the effect that c.369+17T>C could have on protein structure.

712 - Statistical Analysis of the Biochemical and Genetic Data in the Largest Global Fabry Cohort Reported to Present

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Fabry disease is an X-linked inherited lysosomal storage disease characterized by a deficient alpha-galactosidase caused by mutations in the *GLA* gene. Fabry diagnosis is performed in a high-throughput stepwise manner: (a.) males- enzymatic activity, lyso-Gb3 quantification, followed by *GLA* gene sequencing and, (b.) females- *GLA* gene sequencing followed by lyso-Gb3. In the development and validation of the enzymatic assay we observed that alpha-galactosidase determination in DBS alone is insufficient for a precise diagnosis of Fabry disease, both in females (ppv 78%) and males (ppv 94.2%) due to multiple variables: leukocyte count for different individuals; hematocrit level; sample handling, lyonization effect in females. To eliminate the differences between samples several optimizations were introduced: chemical blank for each sample, a standard curve measured in the presence of blood extract and the ratio alpha-galactosidase to another lysosomal enzyme (beta-glucuronidase). Lyso-Gb3 was measured using mass spectrometry (LC/MRM-MS) from DBS extract. Mild mutations or late onset patients present levels of lyso-Gb3 in normal range (21.59% of all Fabry male cases). However, by combining the data from three different biochemical parameters (Lyso-Gb3, alpha-galactosidase and ratio alpha-galactosidase / beta-glucuronidase) we can distinguish between the cohorts of normal controls, mild (or late onset) Fabry cases and affected Fabry cases. The biochemical diagnosis was confirmed in all cases by genetic analysis. We report here the screening of over 72 000 individuals that led to the identification of over 3000 affected individuals and over 900 carriers. We sequenced over 71 000 alleles, resulting in the identification of 480 unique pathogenic variants (40% of which never published before).

713 - Glucosylsphingosine (lyso-Gb1) Plays a Central Role in the Diagnosis and Correct Assessment of Disease Severity in Gaucher patients

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Gaucher disease (GD) is an autosomal recessive, rare genetic disorder characterized by the deposition of glucocerebroside in cells of the macrophage-monocyte system. Although the disease is getting more apparent based on the available enzyme replacement treatment, the rate of proper early diagnosis of GD patients is still low. Standardized, simple and highly reproducible workflows for the diagnosis are crucial. We have developed and systematically validated a high-throughput workflow for the simple testing of GD patients: beta-glucocerebrosidase enzymatic activity, glycosylsphingosine (lyso-Gb1) quantification in DBS followed by *GBA* gene sequencing from the sample blood sample. Here, we report data from a screening study that compassed 4 years a screening of over 2,700 individuals that led to the identification of 873 Gaucher individuals (563 affected Gaucher patients and 310 carriers). Determination of lyso-Gb1 is performed by LC/MRM-MS. The lyso-Gb1 levels found

Gaucher patients were clinically divided in: very mild (12ng/ml - 25.0 ng/mL), mild (25.1 - 50 ng/mL), moderate (50.1 - 200 ng/mL) and severe (>200 ng/mL). Lyso-Gb1 was proven to have a sensitivity as well as specificity of 100%. We sequenced 2,257 alleles and identified 199 unique pathogenic GBA variants. From thee unique variants, 35% were not reported in literature up to now. The clinical severity of the mutations and their location can be correlated with the lyso-Gb1 concentration in the homozygous cases, e.g. c.1295G>T is correlated with mild lyso-Gb1 values while c.1060G>A and c.518C>A with extremely high lyso-Gb1 values (> 600 ng/mL). The most common mutations were c.1226A>G (30.1%) and c.1448T>C (24.7%). Lyso-Gb1 for c.1226A>G can vary from very mild to moderate. c.1448T>C is a severe mutation correlating to a massive increase of lyso-Gb1 up to 1,250 ng/mL. Lyso-Gb1 concentrations in blood can be used for the easy and early diagnosis of Gaucher patients and for treatment monitoring.

714 - The Increased Glucosylsphingosine Level in Patients With Parkinson's Disease With GBA Mutations

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Introduction: Gaucher disease (GD) is one of the most common lysosomal storage disorders, caused by mutation in glucocerebrosidase gene (*GBA*). The result of deficient enzyme activity is glucocerebroside and its derivative—glucosylsphingosine (GlcSph) accumulation in cells and certain organs. Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder. Recently, mutations in the *GBA* gene have been identified as the most common known genetic risk factor for the development of PD. Association studies have demonstrated a higher incidence of *GBA* mutations in PD patients of different ethnic origins including Russian, particularly those with early onset of the disease. The aim of our study was to estimate GlcSph level in dried blood spots (DBS) in sporadic PD (sPD) patients, in GBA-PD patients and in healthy controls. **Materials and methods:** GlcSph level DBS from 17 patients (group GBA-PD) with PD with GBA mutations - 8 (N370 S, L444P) and polymorphic variants - 9 (E326 K, T369 M), 66 patients with PD with the absence of mutations in the *GBA* gene ($_S$PD) and 42 healthy controls was estimated by LS-MS/MS method. Comparisons of medians were made using Mann-Whitney test. The level of significance was set at $P < .05$. Statistical analysis was carried out using SPSS 12.0. **Results:** We found increased GlcSph level in the group of GBA-PD (median, min-max: 1.05 ng/mL (0.59-2.01) in comparison with $_S$PD (median, min-max: 0.87 ng/mL (0.50-1.42), and the control group (median, min-max: 0,80 ng/ml (0.45-1.75) ($P = .001$, $P = .004$, respectively). The level GlcSph in the group of SPD did not differ from the control ($P = .616$). Moreover GBA-PD patients with *GBA* mutations showed a significant increase in GlcSph level (median, min-max: 1,25 ng/ml (0.76 -2.01) compared to control ($P = .005$), but not GBA-PD with *GBA* polymorphic variants (median, min-max 0.95 ng/mL (0.59-1.52) ($P = .106$). **Conclusion:** The obtained data show that in the development of PD the accumulation of substrate occurs in heterozygous carriers of *GBA* mutations. Our results support the notion that substrate reduction therapy could be discussed for the treatment of GBA-PD.

715 - The Clinical and Demographic Features of 23 Niemann-Pick Type A/B Patients From South and Southeast Parts of Turkey

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Background: Niemann-Pick disease (NP) is a progressive lysosomal storage disease due to sphingomyelinase deficiency. Accumulation of sphingomyelin within the monocyte-macrophage systems causes multisystemic involvement which mimics other diseases. Although NP is classified into 2 types; A: rapidly progressive early-onset neurovisceral form and B: late-onset milder form with relatively good prognosis, phenotypic variability is high. As the current treatment is symptomatic and ERT trials going on, natural history will serve as a guide. Here we report the clinical-molecular data of 23 Turkish patients. **Methods:** The medical records of a single center with 23 Niemann-Pick A/B patients (19 children, 4 adults) were evaluated retrospectively. **Results:** The mean age of onset of symptoms was 9.25 months (1-264). The first complaints were; hepatosplenomegaly (HSM): 100%, liver dysfunction: 52%, neurological troubles: 39.1%, hematological problems: 34.8%, infections: 30.4%, failure to thrive: 26%, respiratory problems: 17.4%, short stature: 13%, and eye involvement: 4.3%. 5 Patients had spontaneously resolved cholestasis in newborn period. Before diagnosis, 7 patients had bone marrow aspiration, 3 had liver biopsy, splenectomy was performed to 20 patients, and 65.2% of patients had a different diagnosis and admitted at least 2 centers. On admission, all patients had HSM, 70% liver dysfunction and bicytopenia, 43.5% dyslipidemia and neurological defects, 26.5% respiratory problems and eye involvement. The median time between the first symptom-the specific diagnosis was 8.5 months. Genetic analysis revealed 9 different mutations in 18 patients, among them 3 were new. One of the new mutations was detected both in infant and adult onset forms. At last visit after a follow-up period of 2-156 months, 4 children died and 6 patients could not be seen again. Cardiac manifestations were present in 2 patients and 6 patients had neurological deterioration. **Conclusions:** According to the historical

classification 9 patients were A, 9 were B and the remaining 5 were intermediate types. Although this report documents the most common signs and symptoms as HSM, liver dysfunction, bicytopenia and neurological problems, a phenotypic variability with a lower ratio of eye involvement and a higher ratio of dyslipidemia is found. Classifying the intermediate form will be important in the future for deciding about the treatments like ERT. Our report adds 3 new mutations to the literature.

716 - Niemann Pick Disease Type B: Identification of a New Mutation

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Introduction: Niemann-Pick type B lysosomal disease is caused by the alteration in the sphingomyelinase phosphodiesterase acid 1 encoded by *SMPD1*, which converts sphingomyelin to ceramide, which allows the accumulation in tissues causing cell death. The clinical course is very heterogeneous. **Objective**: Describe a case of Niemann-Pick type B disease, and report a new mutation. **Materials and methods**: We reviewed the clinical history of a patient with Niemann Pick criteria were quantified of the enzymatic activity of acid sphingomyelinase and the concentration of lyso-SM-509 biomarker in dry blood sample. Sequencing of the *SMPD1* gene was performed by PCR in centogene. **Results**: She is 8 years old and her parents are consanguineous. She had abdominal enlargement from 6 months of age and a failure to thrive. Her abdominal ultrasound at 9 months of age documented hepatosplenomegaly, associated with elevated transaminases and hyperlipidemia. The liver biopsy revealed altered architecture suggestive of glycogenosis, although a lysosomal disease was not discarded. The patient showed mild delay of neurodevelopment. Clinically was considered glycogenosis type 3. After that, she had recurrent respiratory disease. At 4 years, she went hospitalized for a diffuse interstitial lung disease and hypoxemia. The diagnosis of glycogenosis was reevaluated, and it is suspected Niemann Pick disease. The concentration of lyso- SM-509 biomarker which was found increased and the decrease on sphingomyelinase acid; both indicators of Niemann-Pick type A/B disease. Gene sequencing of SMPD1 identified a homozygous mutation, in exon 4 c.1328G>C (p.Arg443Pro), has not been previously described. **Conclusion**: The c.1328G>C mutation is in the catalytic metal phosphate domain of the protein; this could explain the failure of protein function and clinical manifestations.

717 - Determination of Lysosphingolipids by LC-MS/MS in Lysosomal Storage Diseases

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Study's objective: To analyze plasma Lysosphingolipids (LysoSLs) by LC-MS/MS in lysosomal storage diseases (LSD). **Methods:** Lysosphingomyelin (LysoSM), lysosphingomyelin-509 (LysoSM-509), hexosylsphingosine (HexSph), and globotriaosylsphingosine (LysoGb3) were simultaneously analyzed by LC-MS/MS method. We analyzed 75 plasma samples from 14 patients with molecularly proven Niemann Pick C (NPC), 4 patients with acid sphingomyelinase deficiency (ASMD), 2 females with Fabry disease (FD), 5 with Gaucher disease (GD), 5 with GM1gangliosidosis, 3 with Lysosomal Acid Lipase Deficiency (LALD). **Results:** As expected the levels of LysoSM and LysoSM-509 (and also of oxysterols) were elevated in NPC patients ($P < .001$). Stratifying NPC patients into 3 groups according to their neurological phenotype, we found that LysoSM and Lyso-509 levels were higher in early-infantile [LysoSM = 30.8 (nv<23.5) and Lyso-509 = 60.7 (nv<10 nmol/L), respectively], intermediate in late-infantile (22.6 and 34.6 nmol/L), and lower in the juvenile form (14.3 and 21.2 nmol/L). Among LSDs, the highest levels of LysoSM (85 nmol/L) and LysoSM-509 (293 nmol/L) were recorded in ASMD. Noteworthy, higher values of LysoSM (1140 *vs* 412 nmol/L; $P < .01$) and Lyso509 (305 *vs* 104 nmol/L; $P < .01$) were observed in ASMD patients with chronic neurovisceral phenotype when compared to those with the visceral one. Interestingly, a 14-year-old patient with a mild visceral phenotype (and with elevated oxysterols in only one out of 4 determinations), showed the lowest levels of LysoSM (46 nmol/L) and Lyso509 (16 nmol/L), paralleling the clinical picture. LysoGb3 and HexSph were elevated in female FD ($P < .001$) as well as in GD and KD ($P < .001$), respectively. LysoSM was slightly increased in juvenile GM1 ($P < .001$) but not in late infantile GM1. No LysoSLs abnormalities were recorded in LALD. **Conclusions**: These preliminary results confirm that analysis of LysoSLs represents useful and reliable biomarker in several LSDs. LysoGb3 and HexSph allow the diagnosis of FD and GD, whereas LysoSM and Lyso-509 of ASMD and NPC, respectively. Noteworthy, LysoSM and Lyso-509 correlate with the clinical phenotype and disease severity in ASMD and in NPC.

718 - Determination of Oxysterol for NPC Screening in Patients With Cholestasis Syndrome

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Introduction: Niemann-Pick disease type C (NPC) is an inherited autosomal recessive disorder in the group of lysosomal storage diseases. The earliest clinical manifestation of NPC, regardless of the clinical form of the disease is cholestasis syndrome, but can manifest isolated splenomegaly or hepatosplenomegaly. Manifestation in the neonatal period is noted in 45%-65% of cases. In most cases, signs of cholestasis in this disease spontaneously resolved by 6-8 months of age. Analysis of oxysterol cholestane-3β-5α-6β-triol (C-triol) is a sensitive screening test in the diagnosis of NPC. Aim of our study was to estimate efficiency to determine the level of C-triol in children with cholestasis for NPC screening. **Materials and methods:** Biochemical analysis of the C-triol was carried out in plasma by mass spectrometric detection (HPLC-MS/MS). Molecular-genetic analysis of all the exons and nearest intronic regions of *NPC1* and *NPC2* genes were made by direct sequencing by ABI PRISM 3500xL bioanalyzer. Target panel of 47 genes responsible for liver damage and cholestasis sequencing by using NGS technology on PGM Ion Torrent. DNA probes were prepared with designed Ampliseq set of primers. **Results:** The study included 108 patients (aged 2 weeks to 7 years). The group#1 had cholestasis at the time of the survey (n = 90) and group#2 had cholestasis in anamnesis (n = 28). High level of C-Triol (normal range <45 ng/mL) was found in 6 patients from group#1 (range 45-57.8 ng/mL) and 1 patient from group#2 (97.6 ng/mL). All 7 patients underwent a complete analysis of the genes *NPC1*, *NPC2*. Two mutations in compound-heterozygous state (c.2090T>C (p.Val697Ala)/c.3591+1G>A) in gene *NPC1* have been found in the only 1 patient from group#2. Thus, in patients with cholestasis in anamnesis we didn't find false-positive results. For 6 patients from group#1 was used NGS-panel. For 3 patients established the following diagnosis: 1-fructose-1,6 bisphosphatase deficiency (n = 1), Alagile syndrome (n = 1), familial intrahepatic cholestasis type 1 (n = 1). **Conclusion:** Despite the fact that the determination of C-triol is high sensitive test for NPC screening, the specificity of this test is not absolute and in some cases of inherited diseases with cholestasis syndrome oxysterol levels could be above the normal range.

719 - Acid Ceramidase: One Gene, One Enzyme, and Multiple Phenotypes

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Acid ceramidase (AC) is the lysosomal enzyme required to hydrolyze ceramide into sphingosine and free fatty acids. Mutations in the acid ceramidase gene (*ASAH1*) result in two rare, recessively inherited disorders, Farber Disease (FD; OMIM #228000) and Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy (SMA-PME; OMIM #159950). The *ASAH1* cDNA and gene have been isolated, and recombinant human AC (rhAC) has been produced and characterized from Chinese hamster ovary cells. Proof-of-concept enzyme replacement therapy (ERT) studies also have been carried out in AC deficient mice and FD cells, and "first-in-man" clinical trials in FD patients are currently been planned. Due its central role regulating ceramide and other sphingolipid metabolism, including sphingosine and sphingosine-1-phosphate, AC is a key rheostat controlling the balance between cell death and survival, and abnormal AC expression has been described in numerous other diseases, including cancer, age-related dementia, diabetes, blindness, and lung diseases such as COPD. Of particular note, AC deficiency occurs in respiratory epithelial cells of patients and mice with Cystic Fibrosis (CF), and inhalation of rhAC into CF mice reduces inflammation and prevents or reverses acute infection with *Pseudomonas aeruginosa*. We have also shown that rhAC can be used to enhance the outcome of *in vitro* fertilization procedures by promoting the survival of unfertilized oocytes and preimplanation embryos. It also enhances the survival of primary chondrocytes and induces chondrogenesis of mesenchymal stem cells, and can be used to improve the outcome of cell-based cartilage repair procedures as well. This presentation will provide an overview of AC biology and its involvement in various diseases.

720 - A cross-sectional natural history study design to address the lack of clinical data on acid ceramidase deficiency presenting as Farber disease

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Introduction: Acid ceramidase deficiency (Farber disease; Farber lipogranulomatosis) is caused by mutations in both alleles of the *ASAH1* gene, resulting in the deficiency of acid ceramidase and accumulation of the pro-apoptotic and pro-inflammatory sphingolipid, ceramide. A cohort of 45 patients (some of whom are since deceased) from 30 countries on 6 continents has been identified since 2014, with clinical presentations indicating a much broader phenotypic spectrum and potentially higher incidence than previously thought. **Objectives and methods:** Our goal was to develop a natural history study structure and design to enable systematic collection of information on living patients who have or have not undergone hematopoietic stem cell transplantation (HSCT), as well as on deceased patients through medical records. The study also functions to evaluate the applicability of clinical assessment tools used for other diseases with similar symptoms, as well as those developed specifically for Farber disease. Pre-existing clinical assessment tools include (among others) the Childhood

Health Assessment Questionnaire (CHAQ), and Wong-Baker Faces Pain Rating Scale, 6-minute walk test, pulmonary function testing, and joint range of motion measurements. We developed a unique patient reported outcome measure related to Farber disease symptoms including physical impairment, pain, and emotional distress, and a method for the assessment of change in size of subcutaneous nodules. **Conclusion:** Consideration of the broad spectrum of ages and heterogeneous phenotypes in a small population of patients available to participate in a study of the natural history of acid ceramidase deficiency led to the design of a cross-sectional cohort study with retrospective and prospective components. By including deceased patients and patients having undergone HSCT in the study design, the amount of data that can contribute to the understanding of this very rare disease is substantially increased. Furthermore, the specific prospective assessments will allow us to follow disease progression over time, and to evaluate the degree to which the assessment tools and techniques may be appropriate for registering change in certain symptoms over time. Enzyvant is developing enzyme replacement therapy for Farber disease, and it is our hope that the data collected will contribute to the selection of methods for measuring therapeutic efficacy in any future clinical trials.

721 - A Qualitative Research Study Documenting the Clinical Impact of Symptoms in a Diverse Population of Patients and Caregivers With Acid Ceramidase Deficiency (Farber Disease)

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Introduction: Acid ceramidase deficiency (Farber disease; Farber lipogranulomatosis) is considered an ultra-rare disease. Since 2014, Enzyvant has identified a cohort of 45 patients from 30 countries on 6 continents, with a much broader phenotypic spectrum and demonstrating a potentially higher incidence than previously thought. The cardinal symptoms of acid ceramidase deficiency are: arthritis, subcutaneous nodules, and hoarseness. Farber disease is most often misdiagnosed as Juvenile Idiopathic Arthritis. **Objectives:** Conduct a qualitative research study to understand the symptoms and impact of Farber disease from the perspective of patients and caregivers by conducting one-on-one interviews for concept elicitation, content validity, and patient interpretation of newly developed patient-reported outcome (PRO) tools. **Methods:** This study consisted of semi-structured qualitative interviews to understand Farber disease symptoms and the evaluation of new PRO measures to capture the impact of symptoms on patients including: subcutaneous nodules, voice, overall pain, arthritis, ability to move joints, ability to perform daily tasks, and level of fatigue. Participants were asked to rate the symptoms' impact on a scale of 0-10, with 0 meaning no impact and 10 meaning greatest impact. **Results:** Eight interviews were conducted: 2 adult patients (attenuated phenotype); 1 caregiver of deceased child (severe phenotype); 1 transplanted adult patient (moderate phenotype); 1 caregiver of transplanted pediatric patient (moderate phenotype); 1 caregiver of a pediatric patient (moderate phenotype); and 1 caregiver/patient dyad of a non-transplanted pediatric patient (moderate phenotype). Interviews revealed the symptoms of acid ceramidase deficiency have measurable clinical impact across a broad spectrum of phenotypes and symptom severity. The symptoms with the highest-rated impact were the ability to move joints, average impact rating of 6.6 (range 1-10); the ability to perform daily activities and subcutaneous nodules, both rated 5.9 (range 1-10 for daily activities and 3-9 for nodules); voice impact was rated 5 (range of 3-9). **Conclusion:** The information gathered provides a better understanding of methods useful for measuring symptom impact in Farber disease, potentially in the context of future therapeutic trials, and may allow better discussion of symptom impact between physicians, patients and caregivers.

722 - Niemann Pick Type C Diagnostic Methods and Survey: National Intervention-Free INSPECT Registration Study Protocol Presentation

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Objective: Niemann-Pick Type C (NPC) is a rare neurovisceral lysosomal storage disorder that leads to progressive neurodegeneration and premature death. Clinical findings, age at onset and severity of the disease are heterogeneous. In this study, we aimed to increase the NPC awareness in Turkey, to collect data about the natural course of the disease, to use effective diagnostic methods and to test the validity of the NPC

suspicion index in terms of national patients' profile. **Methods:** INSPECT is a multicenter, national, intervention-free epidemiologic registry study. Patients with definite and suspicious diagnosis of NPC were recorded. Twenty-seven centers including pediatric neurology, metabolism, gastroenterology, endocrinology and psychiatry departments were participated. **Results:** Seventy-six patients with definite diagnosis of NPC and 75 patients with suspicious diagnosis; total 151 patients were recorded between July 2012 - December 2016. The mean ages of diagnosis were 8.6 years, 24% were under the age of 4 years, 56% between 4 and 16 years, 20% were over the age of 16. Ninety-two percent of the patients had mutations in the NPC1 gene and 8% of the NPC2 gene. According to suspicion index, prolonged neonatal jaundice or cholestasis, splenomegaly, vertical supranuclear gaze paralysis, visceral and psychiatric combination were detected with a higher rate in the patients with a certain diagnosis of NPC compared to suspected cases ($P < .05$). Miglustat usage rate was 65% in patients with NPC during registration. **Conclusion:** Systemic symptoms were most prevalent in early childhood-onset patients, as well as in adolescent/adult-onset patients. Neurological findings profiles were consistent with previous publications. In this study, we will evaluate the sensitivity of suspicion index, data for diagnosis and treatment, analyzes for genotype/phenotype correlation in molecular diagnosed patients.

723 - Fabry Disease's Clinical Picture in Brazilian Dialysis Centers

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Fabry disease (FD) is an X-linked disease with an estimated incidence of 1:40 000–170 000 and is usually marked by chronic pain, angiokeratomas, hypohidrosis, heat/cold intolerance, corneal abnormalities, renal injury, stroke, and cardiac complications. Complications may become life-threatening, including renal failure. Our objective was to verify the prevalence of clinical signals/symptoms and of some comorbidities in FD patients. A cross-sectional study, approved by the Research Ethics Committee of Campos Medical School, was performed in Brazilian dialysis centers from July 2013 to July 2015. Each patient signed a consent form. The sample consisted of FD-suspected patients, placed into two groups: positive FD patients (FD+), (diagnosed by biochemical and/or molecular analysis) and negative FD patients (FD−), (excluded by biochemical and/or molecular analysis). The sample was selected by a database called DataGenno Interactive Research®: first, a clinical questionnaire was created based on FD signals/symptoms and comorbidities; the obtained data were entered in the database in order to be sorted into the groups above. The variables were: cornea verticillata, abdominal pain after food ingestion, angiokeratoma, hearing problems, decreased/absent sweating, numbness sensation, pain crises, burning sensation, exercise intolerance, cold/heat intolerance, recurrent fever, cerebrovascular disease, palpitation/precordialgia, obesity, diabetes mellitus (DM), and high blood pressure (HBP). Renal failure was not considered because it was present in all patients. A total of 9081 patients were select from 35,201 patients from dialysis centers; 130 were FD+ and 8951, FD-. The average age was 43.93 ($\pm$16.53) and 45.86 ($\pm$14.07) for FD+ and FD−, respectively. Females were more prevalent than males in FD+ comparing to FD− ($P < .001$). More prevalent signs and symptoms in FD+ were: palpitation/precordialgia (39.23%) followed by decreased/absent sweating, numbness sensation, cold/heat intolerance (34.61% each), pain crises (33.84%), cerebrovascular diseases (27.69%) and burning sensation (21.53%), among others. Angiokeratoma was present in less percentage (12.03%) and the comparison between FD+ and FD− patients was significant ($P < .001$). HBP occurred in 37.69%, DM in 8.46% and obesity in 2.30% of FD+, percentages significantly lower than in FD−. FD is clinically heterogeneous and probably under diagnosed; Datagenno may be a valuable tool to improve FD diagnosis

724 - Natural History of Early-Infantile Krabbe Disease

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Background: Krabbe disease is a rare neurodegenerative disease caused by a deficiency in the lysosomal enzyme, β-galactocerebrosidase, resulting in demyelination of the central and peripheral nervous systems. If left without treatment, it results in progressive neurodegeneration with reduced quality of life and early death. Krabbe disease is classified into subtypes based on age of onset, with early-infantile (EI) onset representing the most severe and most rapidly progressing form. **Objective:** The purpose of this prospective study was to describe in a standardized manner the natural progression of EI Krabbe disease in a very large cohort of patients. **Methods:** Patients with EI Krabbe disease referred to the NDRD Program were consented to participate in the study. Data sources included diagnostic testing, parent questionnaires, and standardized multidisciplinary neurodevelopmental evaluations assessing for adaptive behavior, cognitive function, motor function, and speech/language skills. A number of patients were evaluated annually in order to monitor disease

progression. Only pretreatment evaluations were included for patients who received hematopoietic stem cell transplantation (HSCT). **Results:** We evaluated 71 children with EI Krabbe disease. Median age of symptom onset was 4 months; median time to diagnosis after symptoms was 3 months. Initial symptoms included irritability (48%), feeding difficulties (31%), spasticity (31%), and developmental delay (29%). By 6 months, 100% of patients presented with appendicular spasticity. By 10 months, 100% of patients had axial hypotonia. Other symptoms included vision and hearing impairment, orthopedic complications, loss of head control, and dysautonomia. Results of nerve conduction studies showed that 100% of patients developed peripheral neuropathy by 6 months of age. Median galactocerebrosidase enzyme activity for 49 patients was 0.05 nmol/h/mg protein (range, 0-0.3 nmol/h/mg protein). For the 26 patients followed longitudinally, survival rate to 2 years was 56%. **Conclusions:** To our knowledge, this is the largest study depicting the natural history of EI Krabbe. It clearly delineates the variability of disease progression during the first 2 years of life. With recent advances in newborn screening and promising therapeutic interventions, developing a greater understanding of disease progression in EI Krabbe will be critical for deciding whether a patient should be treated with HSCT or any other novel therapies.

725 - Correlation of Lyso-GB3 Level and *GLA* Mutations in Patients From Screening Cohort Among Dialysis Centers in Russian Federation

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Background: Fabry disease is a rare inherited disorder that affects mainly males with mutation in the *GLA* gene. It causes accumulating of Lyso-Gb3 and reducing of α-GalA activity. **Methods:** In all adult patients with suspected Fabry disease from dialysis centers the alpha-galactosidase A activity was estimated in dry blood spots by MS-MS method. In cases when the alpha-galactosidase activity was lowered the measurement of Lyso-GB3 marker by UPLC-MS-MS method and molecular genetic testing of all coding and flanking intronic regions by Sanger method were carried out. **Results:** In 39 examined patients, the alpha-galactosidase A activity was decreased and 36 of them have showed higher Lyso-Gb3 levels. Sequencing of 39 patients revealed 3 males with slightly reduced alpha-galactosidase activity and *c.376A>G* and *c.427G>A* mutations, both of these mutations are considered as nucleotide replacements with conflictive relevance. Among 36 patients with mutations in the *GLA* gene in 18 persons (50%) 16 novel mutations were found and confirmed by in silico analysis. Novel mutation *c.895G>A* (*p.D299 H*) was revealed twice in unrelated families. **Conclusion:** Measurement of Lyso-Gb3 helps to confirm the diagnosis in patients with questionable pathogenicity of revealed nucleotide replacements. High Lyso-Gb3 level correlate with most severe clinical performance of Fabry disease, with early onset of chronic kidney disease and with mutations of class one of pathogenicity leading to frameshift, splice and stop variants.

726 - Acute Neuronopathic Gaucher Disease (Gaucher type 2) in Two Turkish Infants

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Objective: Acute neuronopathic Gaucher disease (Gaucher type 2) is present in early infancy with brainstem dysfunction (horizontal gaze paralysis, dysphagia), visceromegaly, neck retroflexion, spasticity, growth retardation, cachexia. Neurodegeneration is rapid and very few patients can survive to two years of age. **Case report 1:** A 15-month-old male patient was brought in with the complaint of throwing his head backwards. Complaints of the patient whose development was normal previously started when she was 5 months old. First degree consanguinity was reported and death of a sibling at three months of age due to jaundice and hepatosplenomegaly. Physical examination showed head retroflexion, hepatosplenomegaly, spasticity in hands and feet, and flexor contracture in both hips. The ß-glucocerebrosidase activity was 0.3 μmol/L/h (> 3.2) lower and molecular genetic analysis revealed a pN370 S in one allele and deletion (p403 H, L444P, A456P, V460 V) of 55 bases in exon 9 in the other allele. He died when he was 16-month-old. **Case report 2:** A 3-month-old male patient was brought up with a complaint of restlessness, crying, vomiting, throwing back the head. It was learned that these complaints had begun from the birth. From the second month, it was reported that there was additional weight loss. There was no consanguinity marriage between the parents. Physical examination showed head retroflexion, hepatosplenomegaly, increase in muscle tone, and developmental retardation. Eye examination, brain MRI and brain diffusion MRI were normal. The ß-glucocerebrosidase activity was 0.3 μmol/L/h (> 3.2) lower, chitotyrosidase activity was higher and molecular genetic analysis is under investigation. He died when he was 5-months-old. **Conclusion:** Neck retroflexion and hepatosplenomegaly were the important clues in neurodegenerative patients to suspect "Gaucher Type 2" in the differential diagnosis.

727 - Limited Benefits of Presymptomatic Cord Blood Transplantation in Neurovisceral Acid Sphingomyelinase Deficiency (ASMD) Intermediate Type

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Introduction and objectives: Niemann-Pick disease (NPD) is a lysosomal storage disease caused by deficiency of acid sphingomyelinase (ASM). Neurovisceral NPD type A is an early onset severe and fatal neurodegenerative disease. Type B is the visceral (hepatosplenomegaly, interstitial lung disease) non-neurologic form. Intermediate phenotypes have also been described. There is currently no therapy for the neurological impairment; some nonspecific options are available to improve the visceral signs: splenectomy, liver transplantation, whole-lung lavage. **Method:** We report the case of two siblings affected by the intermediate type of ASM deficiency: the elder brother had developed neurodevelopmental delay, and had died from severe visceral complications at the age of 3. The younger brother had cord blood transplantation at the age of 5 months while he had a normal development and showed a moderate hepatomegaly. **Results:** The transplanted patient showed a neurodevelopmental delay with severe cerebellar ataxia at 8 years old. Conversely, visceral involvement was minimal, with moderate hepatomegaly and no splenomegaly or interstitial lung disease. **Conclusions:** The transplant prevented visceral progression and early death, but it could only delay neurocognitive deterioration.

728 - Skeletal Manifestations in Algerian Children With Gaucher disease

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Enzyme replacement therapy (ERT) is effective for the treatment of the systemic manifestations of Gaucher disease (GD) and can have a significant impact on skeletal manifestations. Bone disease in children is a major cause for concern as it puts them at risk of developing irreversible and debilitating bone complications and interferes with normal growth and achievement of optimal bone mass during a critical period of growth. **Objectives**: We report the clinical features of patients Gaucher disease type 1 and 3 and also the effects of enzyme replacement therapy (ERT) on bone manifestations. **Methods and material:** Twenty (20) patients are included in this study. The data were collected from clinical features, results of laboratory and mutation analysis. To evaluate bone involvement, radiography, dual energy X-ray absorptiometry (DEXA), and magnetic resonance imaging were performed. **Results:** Twenty (20) patients, ten (10) girls and ten (10) boys, mean age for GD1 12,6 (6-19 years), and for GD3 7,6 (15 month-17years). Ten (10) GD3, Nine (9) GD1, and one (1) GD2. Most of them had « Erlenmeyer flask deformity », followed by osteopenia or osteoporosis, avascular necrosis in one GD1 and fracture in one GD3. Kyphoscoliosis is frequent. Three (3) patients had bone pain. None of them had bone crisis. Mean BMD was −2.1(−4.8/+0.1 ZS) before ERT, then +0.37(−2.5/+1ZS) after ERT. Two (2) patients with splenectomy had more severe bone disease (severe kyphosis and fracture). **Conclusion:** Affecting both the marrow and mineral compartments, GD-related bone disease is the most significant cause of morbidity and long-term disability for patients. In some patients, bone manifestations persist or worsen despite enzyme therapy.

729 - Natural History of Metachromatic leukodystrophy

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Metachromatic leukodystrophy (MLD) is a rare demyelinating disorder caused by deficient activity of arylsulfatase A, a lysosomal enzyme involved in the degradation of 3'-O-sulphogalactosylceramide (sulfatide). MLD is divided into subtypes based on age of onset, with each subtype having a variant disease course. The purpose of this prospective study was to investigate disease progression in each subtype in order to identify early clinical symptoms. 104 MLD patients with late-infantile (LI), early-juvenile (EJ), late juvenile (LJ), or adult onset were evaluated from 2000 to 2017. Assessments included physical exams, neurodevelopment evaluations (cognitive, motor, expressive/receptive language, adaptive behavior), neuroimaging and neurophysiologic tests. LI onset was most prevalent (60.57%), followed by EJ (21.15%), LJ (13.46%), and adult onset (4.81%). Median age of onset in each cohort was 16, 50.5, 76, and 168 months, respectively, with a median delay of 11, 16, 23, and 108 months between onset and diagnosis. Initial LI symptoms involved delayed achievement of GM milestones (46.03%), abnormal gait (28.57%), and GM regression (20.60%). Similar initial symptoms were seen for EJ patients, although some cases involved impairment of fine motor skills and language acquisition. The majority of individuals with LJ onset displayed cognitive deficiencies, with 50% of children initially presenting with memory/attention/learning difficulties and changes in personality (21.43%). In the adult group, the onset had greater variability and entailed changes in personality and psychiatric disturbances. Abnormal NCV studies were present for 96% of LI patients, with the one normal study in an asymptomatic 8-month-old. Mixed results were seen in EJ and LJ NCVs; only 25% of adult NCVs were

abnormal. Periventricular T_2 white matter hyperintensities were the most common MRI finding in all cohorts, although cerebral hyperintensities became more common as age of onset increased. This is the largest longitudinal study of MLD performed at the same site using a standardized evaluation. The results show the predominantly early involvement of peripheral nerves affecting motor skills, with a predominance of cognitive and behavioral symptoms emerging as patients get older. Further evaluation of the neurophysiological studies and brain MRIs will be important for understanding the impact of these abnormalities on disease progression.

730 - Natural history of late-infantile Krabbe disease

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Background: Krabbe Disease (KD) is a rare neurodegenerative disorder caused by a deficiency in the lysosomal enzyme galactocerebrosidase. Unlike early-infantile KD, late-infantile (LI) may present with a more variable disease course. Because of the slower progression, late onset patients are thought to benefit from transplantation to a greater extent than early onset patients. However, there are currently no studies on the variability of disease progression in the later onset phenotypes. **Objective:** The purpose of this study was to characterize the natural progression of LIKD in a large cohort of patients evaluated via a standardized protocol. **Methods:** 29 patients (22 boys, 7 girls) with LIKD were prospectively evaluated from 2000 to 2017. Assessments included neurodevelopment evaluations (cognitive, motor, expressive/receptive language, adaptive behavior), neuroradiologic and neurophysiologic tests, and cerebrospinal fluid protein analysis. **Results:** Median age at symptom onset was 12 months, with a median delay of 5 months between onset of symptoms and diagnosis. Two groups were identified with initial symptoms before and after 18 months of life. Twenty children had symptoms between 6-18 months of life. The most common initial symptoms for patients <18 months were irritability and developmental delay, and for patients with onset >18 months, it was abnormal gait. For both groups, the most common MRI abnormality was increased T2 signaling in the periventricular white matter. In 17 of 20 (85%) patients, nerve conduction velocity results were abnormal. The 3 patients with normal studies had disease onset after 18 months, one of whom was asymptomatic. Abnormal CSF levels were obtained for 11 of 13 symptomatic children with available data. Median CSF protein level for the 11 abnormal studies was 142 mg/dL (range 83-214). The 2 asymptomatic children with available CSF data had protein levels within normal limits. **Conclusion:** Although conventionally LIKD has encompassed patients with symptom onset between 6 and 48 months of age, the results of the current study suggest two distinct LI trajectories, with onset either between 6 and 18 months or onset between 19 and 48 months. The older group had less peripheral nerve involvement and is, therefore, more likely to benefit from transplantation. Ultimately, the proposed classifications will allow physicians to more precisely predict treatment outcomes.

731 - Nieman-Pick C1 Disease: A Novel Mutation in an Algerian Child

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Niemann-Pick disease type C is a neurodegenerative disorder caused by mutations in NPC1 and NPC2 genes. NP-C disease is very heterogeneous in its presentation. It has a variable age of onset, and a range of visceral, neurological, and psychiatric clinical features can arise at different disease stages and progress at different rates. **Case report:** To report a novel mutation of Nieman Pick C1 disease in an Algerian girl. Ilham a 3-year-old female, has ataxia since the age of 2 years, with splenomegaly and hypotonia. Gaucher disease diagnosis has been suspected. Betaglucosidase activity is in normal range. Diagnostic of Nieman-Pick C disease is suggested. The concentration of the biomarker lyso-SM-509 was pathologically increased 3.2 ng/mL (reference: $\leq$ 0.9 ng/mL) and the concentration of the biomarker lyso-SM-465, measured as an internal control, was normal (17.2 ng/mL, reference: <46.3 ng/mL). These results are suggestive of Niemann-Pick disease type C1/C2. The sequencing of the *NPC1* and *NPC2* genes detected a previously unreported homozygous variant in intron 21 of the *NPC1* gene, c.3245+1G>T. This substitution is located in the donor splice site of intron 21, and a skip of exon 21 is very likely. To date, this variant is not described in the Exome Aggregation Consortium, Exome Sequencing Project, or the 1000 Genomes Browser.

732 - Improvements in Brain Development Following Hematopoietic Stem-Cell Transplantation in Krabbe disease

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Background: Krabbe disease is a rare but severe neurodegenerative disorder mainly affecting infants. It is characterized by deficiency of galactocerebrosidase, resulting in abnormal myelination in the brain and peripheral nerves. Neurological symptoms progress quickly and often lead to death within 2 years. Currently, hematopoietic stem cell transplantation (HSCT) is the only treatment available that can alter disease progression in asymptomatic or minimally symptomatic patients. **Objective:** While the benefits of transplantation have been shown using behavioral exams, this study seeks to directly investigate

the longitudinal changes in cerebral myelination using brain MRI and propose a more sensitive tool that identifies disease before symptoms are evident. It also aims to measure the effects of HSCT on changes in myelin integrity. **Method:** We longitudinally scanned 55 Krabbe patients with early infantile onset, of which 14 were treated with HSCT after their first MRI scan. The scans from these 55 patients were compared to those of 255 similarly aged controls. Diffusion tensor imaging (DTI) was used to assess white matter (WM) integrity of the brain. The fractional anisotropy (FA) has been shown to measure the organization of the corticospinal tracts, which are responsible for relaying action potentials from the motor cortex to the spinal cord. Lower than normal FA values indicate disorganization of myelination around the tracts. **Results:** Patients that were not treated with HSCT present with lower than normal FA and showed some increase before decreasing significantly within two years. Patients treated with HSCT generally showed normal development of the corticospinal tracts, albeit in the lower part of the normal range. The FA values are consistent with the observed motor function. **Conclusion:** FA measurements of the brain show compromised WM integrity in early infantile Krabbe patients who are not treated with HSCT. In contrast, patients who are treated with HSCT early in life show an increase in myelination of the corticospinal tract similar to, albeit lower than normal controls. This study supports using DTI as a tool to measure WM integrity and effects of treatment. Thus, this newly developed tool can be used as a marker of disease progression in neonates diagnosed through statewide newborn screening programs.

733 - Expression of Acid Sphingomyelinase in Human Saliva and its Diagnostic Importance in Niemann-Pick Disease Type b

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Introduction: Niemann-Pick type B (NP-B) is an autosomal recessive lysosomal storage disorder caused by a deficiency of acid sphingomyelinase (ASM) coded by *SMPD1* gene. NP-B is a multisystem disease with progressive hepatosplenomegaly and gradual deterioration of pulmonary function; most type B patients have little or no neurologic involvement and survive into adulthood. Saliva is a biofluid used to analyze the health/ disease condition of an individual. It constitutes a diagnostic means in oral disorders, such as periodontal diseases, oral and breast cancers, Sjogren's Syndrome and the neuronal Ceroid Lipofuscinoses in our Center. Diagnostic assays for this enzyme were developed using fibroblasts, leukocytes, plasma and dry blood spots, but there are no expression studies in saliva in the literature. **Aim:** To determine if ASM is expressed in saliva, to establish the population's reference range of the enzyme activities in that fluid, and to validate the method in NP-B patient. **Materials and Methods:** We standardized a fluorometric method to determine ASM activity in human saliva of control subjects and in one NP-B patient. **Results:** ASM activity was detected in all saliva samples. The range of ASM in saliva for 15 control subjects was 8.8- 70.4, with an average of 31 nmol/17 h/ mg of protein. Values in plasma were significantly lower, a 0.23-7.8 range, with an average of 1.7 nmol/17 h/ mg of protein. There is no correlation between saliva and plasma samples ($R^2 = 0.001$). ASM was markedly deficient in the saliva activity of 0.6 nmol/17 h/ mg of protein as well as in the leukocyte pellet (0.125 nmol/h/mg protein) and the plasma (0.09 nmol/17 h) of one NP-B proband. **Conclusion:** This finding indicate that the saliva could be an alternative biofluid to plasma and to leucocytes to measure ASM activity, representing a non-invasive, easy-collection diagnostic means, which would allow the identification and characterization of these entities in our medium.

734 - Psychosine, A Marker of Krabbe Phenotype and Treatment

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Newborn screening (NBS) for Krabbe disease, a rare neurodegenerative disorder caused by deficient galactocerebrosidase (GALC) enzyme activity, has recently been implemented in a number of US states. However, the spectrum of phenotypic manifestations associated with deficient GALC activity complicates the management of screen-positive newborns and underscores the need to identify clinically relevant biomarkers. Earlier studies with a small number of patients identified psychosine, a substrate of the GALC enzyme, as a potential biomarker for Krabbe disease. In this study, we provide, for the first time, longitudinal data on dried blood spot (DBS) psychosine concentrations in different Krabbe disease phenotypes for both untreated patients and those treated with hematopoietic stem cell transplantation (HSCT). From 2010 to 2016, DBS samples were prospectively collected from 69 patients with a confirmed diagnosis of KD, four sibling carriers, and two asymptomatic NBS-positive patients during clinical evaluations at the Program for the Study of Neurodevelopment in Rare Disorders (NDRD). Based on the age at which symptoms appeared, patients with KD were classified as having EIKD (0-

6 months at onset), LIKD (6-48 months at onset), or juvenile-onset KD (4-18 years at onset). These samples were tested for psychosine levels and plotted by age, subgrouping by disease onset and transplant status. **Results:** Substantially elevated DBS psychosine concentration during the newborn period was found to be a highly specific marker for infantile Krabbe disease. This finding supports the use of DBS psychosine concentration as a second-tier NBS test to aid in the identification of patients who require urgent evaluation for HSCT. In addition, longitudinal assessments showed that both natural disease progression and treatment with HSCT were associated with decreases in DBS psychosine concentrations. **Conclusions:** Based on these findings we provide recommendations for the interpretation of psychosine concentrations in DBS specimens collected during the first year of life. Future studies should aim to better delineate the relationship between DBS psychosine concentration and disease onset in patients with later-onset forms of Krabbe disease.

735 - Next-Generation Versus Sanger Sequencing: Validation of NGS for Genotyping *CHIT1* Alterations in Gaucher Disease Patients

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Gaucher disease (GD) is an inborn error of metabolism of sphingolipids caused by the beta-glucocerebrosidase deficiency which leads to substrate accumulation within the lysosomes of reticuloendothelial cells. GD can be classified in type I—a mild form, mainly presenting visceral enlargement; and types II and III—presenting acute and chronic neurological impairment, respectively. The specific treatment options for GD are enzyme replacement or substrate reduction therapy. To monitor the treatment efficacy in patients, several biomarkers can be measured. Chitotriosidase (chito) is a macrophage-produced enzyme encoded by the *CHIT1* gene. It can be increased in up to 1000-fold the normal level in untreated GD patients, decreasing over treatment. There are eight *CHIT1* polymorphisms known to impair chito activity reported at the Human Gene Mutation Database (HGMD). Thus, it is important to stratify the patients according to *CHIT1* genotype for a better treatment follow-up. The aim of the study was to validate a next generation sequencing (NGS) method for *CHIT1* analysis in the Reference Center for GD at the Hospital de Clínicas de Porto Alegre. DNA samples from 21 GD patients were amplified by multiplex PCR using an Ion Ampliseq Panel™ and sequenced by Ion Torrent PGM machine. The results were analyzed using Ion Reporter v.5.2 software and compared to results obtained from conventional PCR followed by Sanger sequencing using ABI 3500 Genetic Analyzer. The variants found were c.304G>A (p.G102 S), classified as pathogenic; c.1325C>G (p.A442G) and c.1049_1072dup24—both likely benign. The alleles frequencies found within the sample population by Sanger sequencing were 0.309, 0.095, and 0.142; by NGS were 0.309, 0.119 and 0.142, and in the 1000genomes database are 0.290, 0.109 and 0.289, respectively. The NGS results had a 95% consistency rate when comparing to Sanger sequencing (20 out of 21). In the one discordant case, NGS was able to detect all three variants in heterozygosis against two (p.G102 S and dup24) found by Sanger. It may be explained due to NGS greater sensitivity to detect low frequency variants (<10%). Although a few discrepancies might occur, NGS showed to be a reliable and promising high throughput method for genotyping *CHIT1*.

736 - Tay-Sachs Disease, p.Arg137* and p.Arg178His Variants

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Objectives: Report a case of Tay-Sachs disease (TSD) with p.Arg137* and p.Arg178His variants at HEXA gene. **Methods:** Medical record review, literature review. **Results:** C.S.H.L., male, 5 years, non-consanguineous parents, maternal grandmother had progressive supranuclear palsy. He was born at hospital, by cesarean delivery, full term, with a weight of 3410 g, Apgar 8/9. He started to support his neck at 6 months, walked at 14 months and spoke some words, however after 19 months presented speech and motor development regression. At physical examination, at 3 years, were observed hyperreflexia, superior limbs in pronation, gait only with support, normal skull circumference, no dysmorphisms. Complementary tests indicated an increase in liver enzymes, lactic dehydrogenase and alpha amylase, electroencephalogram with slow waves in paroxysms, brain magnetic resonance imaging showing discrete ectasia of the lateral ventricles, a questionable reduction in the thickness of the white matter, which presented hypersignal in T2 flair and could correspond to periventricular leukomalacia and myelinization delay of the peritrigonal white matter, in addition, discrete reduction of the thickness of the middle third of the corpus callosum and elevation of myoinositol peak in the white matter regions; levels of HEX A and total hexosaminidase below the reference values and concomitant Exome analysis evidenced two pathogenic variants in

heterozygosis in the HEXA gene (p.Arg137* and p.Arg178His), the last one, previously related to B1 variant of TSD, more common in Portuguese descendants. **Conclusions:** The TSD has three clinical forms, it is probable that the case in question fits into the subacute or juvenile classification, where there is apparent normality at birth, but between two and five years of age the clinical manifestations begin, among them the regression of the development, especially in speech and vital capacity and decline in cognition. Is also characteristic of the heterozygote pattern of the mutated gene, and the residual HEX A enzyme activity is low. Because of its autosomal recessive nature, there is a 25% risk of this mutation affecting future offspring, so genetic counseling is essential. Therapy is aimed at supporting, nourishing and preventing complications of vital systems, patients need to be followed up with multidisciplinary team in order to stimulate physical and intellectual development, delaying loss of skills already acquired.

U) 21. Lysosomal Disorders: Others (737 to 777)

737 - Molecular Insight, Identification of Novel Genotype, Establishment of Genotype-Phenotype Correlation, and Preparation of Mutational Dataset for Metachromatic Leukodystrophy of Indian Ethnic Population

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Objectives: Metachromatic Leukodystrophy (MLD) comes under the umbrella of lysosomal storage disorders with the prevalence of 0.0025% worldwide. It is a rare autosomal recessive disorder caused by the deficient arylsulfatase-A enzyme activity and sulfatide accumulation. It involves progressive Neurodegeneration in the central and peripheral nervous systems due to mutation in ARSA gene. Objectives of the study was to identify the spectrum of pathogenic mutations in Indian patients and to establish a genotype phenotype correlation. **Design & Methods:** A total of 32 enzyme deficient subjects were recruited in the study. DNA analysis of 26 subjects was performed. Prenatal analysis of 2 subjects was also done to rule out pathogenic mutations. **Results:** Mutations were identified in 22 subjects. Patients groups were divided in Late Infantile, Early Juvenile, Juvenile and Adult onset categories. Two novel pathogenic mutations c1112C>T, with splice site change & c325G>C with single base exchange, two insertions c1158_1159 INS T & c.752_753 INS T, one deletion c818_818 DEL C were found. Mutation c1172C>G was found in almost 17% subjects, mutations 919G>A & 733G>A were found in almost 9% subjects. Mutation 883G>A & 325G>C were found in compound heterozygous state with mutation c.752_753insT & 733G>A respectively. Rests of the mutations c731G>A, c937C>T, c302G>A, c251G>A, c911C>T, 979+1G>A were found scattered in different subjects. Two prenatal analyses were also done in 20th week of the pregnancy with CVS-DNA, out of which in one subject, mutation c1578C>G was found in homozygous state and mutation c937C>T was found in heterozygous state, respectively. A genotype–phenotype correlation was made. **Conclusions:** Since there is a paucity of data on MLD has been reported from India ethnicity and the prevalence and mutational spectrum in our ethnicity is still unknown & unclear, though our study is a step ahead to provide the useful information regarding the clinical & mutational parameters, when correlated with the diseased state

738 - Gastrointestinal Endoscopy in Disease Monitoring

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Objective: There are few reports of gastrointestinal (GI) endoscopy findings in lysosomal acid lipase deficiency (LAL-D) patients, and the objective of this work is to describe the findings of this exam in a family with LAL-D. **Method:** Retrospective study of medical records (Ethics Committee Approval CEP-UNIFESP #2007/11). **Case Reports:** Patient 1 is female, 36y, she is the mother of patients 2 and 3, and had elevated total cholesterol (T-Chol), low density lipoprotein cholesterol (LDL-C) and triglycerides (TRYG), low high-density lipoprotein cholesterol (HDL-C); liver biopsy revealed hepatocytes dilatation due to lipid accumulation, lipid filled histiocytes, broadening portal spaces suggesting lipid storage disease. GI endoscopy revealed normal esophagus, stomach and first portion of duodenal findings, and second portion with elevated and confluent lesions with yellow mucosa suggesting lipid storage. Patient 2 is male, 8 years 10 months by the time of GI endoscopic evaluation (current age 11 years), onset of symptoms at 3 years, presenting symptom was hepatosplenomegaly, height for age (HFA) Z-score of −2.52 and weight for age (WFA) Z-score of −1.69. He presented elevated T-Chol, LDL-C, and TRYG and low HDL-C. GI endoscopic findings were irregular nodular and yellow colored mucosa of first portion of duodenum and second portion with yellow mucosa with nodes larger than 5 mm reaching the jejunum. Patient 3 is male, younger brother of patient 2, asymptomatic, diagnosis at 6y due to familial screening, at the time of GI endoscopy he was 8 years 10 months (current age 9 years 7 months), HFA Z-score −0.29, WFA Z-score −0.37. He presented elevated T-Chol, LDL-C, and TRYG and low HDL-C. GI endoscopic findings were first portion of duodenum with normal findings and second portion

with yellow mucosa with nodules. All 3 patients had enlarged cytoplasm histiocytes infiltration of core villi on duodenum biopsy. **Conclusion:** Our study showed the variable severity of GI endoscopy findings that do not linear correlate to age or patient's symptoms. GI endoscopy present characteristic findings in LAL-D and should be part of the diagnostic work-up in patients with micro vesicular steatosis with unknown cause as it seems to be a helpful tool. We believe that we should raise awareness of LAL-D as a differential diagnosis whenever a professional who performs the GI endoscopy face a massive yellow mucosa, because it is a treatable disorder and the earlier the onset of treatment, the better the outcome of the patient.

739 - Genotype-Phenotype Parameters of Mucolipidosis of II and III A Types in Russian Patients

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Purpose: To present the results of analysis of genotype-phenotypic parameters with mucolipidosis of II and III A types. **Methods:** The activity of lysosomal enzymes in plasma was measured by the standard method with the use of chromogenic and fluorogenic substrates, followed by comparison with reference values. Genomic DNA of leukocytes was isolated using a set of Prep 100 reagents (DIAtom ™) according to the manufacturer's instructions. Amplification of all the exons of the *GNPTAB* gene was carried out by PCR followed by direct non-radioactive sequencing by Sanger. **Results:** 50 patients aged 1.5 to 10 years were examined. Clinical symptoms of the disease included Hurler-like phenotype, growth retardation, skeletal, cardiovascular and CNS damage. I-cell disease was characterized by the most severe course. The clinical diagnosis was confirmed by normal indices of GAG, high activity of lysosomal hydrolases in blood plasma, and detection of mutations in the *GNPTAB* gene. 35 probands were fully genotyped. In 8 patients only 8 mutant alleles were detected. In 7 patients, no mutations were found. Six new mutations in exons 1 (p.I31 N; p.Q36P), 10 (p.L398P), 11 (p.W446X), and 13 (p.S738X; c.2250delT) were found, among which mutation p.S738X was found to be frequent (21% of alleles). This mutation results in the formation of a stop codon in the middle of the 13th exon of the *GNPTAB* gene and is associated with a severe I-cell form of the disease. The presence of the second and more simple mutation (missense or microdeletion/duplication), which does not lead to the shift in the reading frame, contributes to the formation of mucolipidosis of III A type. Deletion c. 3503_3504delTC, leading to the shift in the reading frame, was the most frequent (31.4% of alleles). It was confirmed that a clinical picture of the I-cell disease develops depending on the nature of the second mutation (deletion in the homozygous state, mutation with a shift of the reading frame or nonsense mutation). The presence of the missense mutation is a reliable criterion for the formation of type III mucolipidosis. A similar situation is typical for the frequent mutation (p.R375X) found in Russian patients (18.6% alleles). **Conclusion:** Identification of mutations in the *GNPTAB* gene provides predictions of the severity of the disease course, and an understanding of the mechanisms of its development, which will contribute to the development of pathogenetic treatment methods.

740 - Characterization of Patients With Galactosialidosis and Sialidosis Type I

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Galactosialidosis (GS) is a lysosomal disorder characterized by a combined deficiency of glycoprotein-specific *N*-acetyl-α-neuraminidase and β-galactosidase, secondary to a defect in protective protein/cathepsin A (PPCA). Three subtypes of GS have been described: early infantile or severe neuropathic, late infantile or attenuated, and juvenile/adult or neuropathic. Sialidosis, also a lysosomal disorder, is characterized by a neuraminidase deficiency and is recognized in two forms: Type 1 (ST-1) usually presents in the second decade of life and can have neurological involvement; Type 2 is more severe with an earlier onset. Both GS and ST-1 are possible therapeutic indications for a novel recombinant human enzyme PPCA. **Objective:** To understand the clinical manifestations of GS and ST-1, and the patient experience with these diseases to inform selection or development of appropriate and meaningful clinical endpoints. **Methods:** A search of the GS and ST-1 literature was conducted in PubMed, and after exclusion of animal, biomedical and non-GS or ST-1-specific studies, relevant articles were reviewed. A clinician interview guide was developed based on key features of GS and ST-1 identified in the literature. An experienced qualitative researcher conducted in-depth, telephone interviews with expert clinicians. Thematic analysis of the verbatim transcripts was conducted, which led to development of a protocol and interview guides for patient and caregiver interviews. **Results:** Twenty-five articles, the majority (n = 22) being case studies, were reviewed and summarized. Five clinicians and one genetic counselor with experience diagnosing or managing patients with GS or ST-1 were interviewed from Italy (n = 2), USA, Brazil, Canada, and Saudi Arabia. Conceptual models were developed incorporating key signs, symptoms and impacts of the diseases based on the literature and interviews. The most frequently

reported signs/symptoms for GS were hepatosplenomegaly, growth disturbance, skeletal abnormalities, coarse facial features, cardiovascular problems, hernia, cherry red spots and corneal clouding. For ST-1, myoclonus, seizures, ataxia, and optical symptoms were most frequently reported. Patient interviews are currently ongoing. **Conclusion:** GS and ST-1 are rare, heterogeneous, and complex diseases. The evidence gathered from this research will be used to characterize disease burden and identify outcome measures and endpoints for future clinical studies of PPCA.

741 - Efficacy of Pegunigalsidase Alfa (PRX-102), A Chemically Modified Plant Cell Culture Expressed Human α-Galactosidase-A Enzyme, on Neuropathic Phenotypes in a Mouse Model of Fabry Disease

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Fabry disease (FD) is an X-linked glycosphingolipidosis caused by deficient activity of α-galactosidase A (α-gal A); it exhibits a variety of clinical manifestations, of which small-fiber neuropathy is a key feature of the disease. Enzyme replacement therapy (ERT) is currently the standard care for Fabry patients (agalsidase alfa and beta). Pegunigalsidase alfa, a novel PEGylated, chemically modified α-gal A, shows a superior stability and markedly increased half-life in the circulation and enhanced mouse tissues delivery compared to approved ERTs. **Objective:** The goal of this preclinical study was to compare the efficacy of pegunigalsidase alfa with approved ERTs at preventing or slowing the peripheral neuropathy in a mouse model of FD. **Methods:** 1 mg/kg pegunigalsidase alfa, agalsidase beta and agalsidase alfa at the approved clinical doses (1, 0.2 mg/kg, respectively) were injected intravenously to 2 months old Fabry mice every 2 weeks for a total of 6 injections. The therapeutic effects were evaluated 1 week after the last injection. Expression of macrophage marker, Iba1, in dorsal root ganglia (DRG) was assessed by immunohistochemistry. Thermal sensation was assessed by hot-plate test at 55°C. **Results:** Untreated Fabry mice had significantly increased number of Iba1 positive (+) cells in DRG compared to wild-type (WT) mice, suggesting association of inflammation with peripheral neuropathy. Compared to untreated Fabry controls, Fabry mice received 1mg/kg pegunigalsidase alfa had significantly decreased number of Iba1+ cells in DRG that was similar to WT levels. In contrast, the number of Iba1+ cells in Fabry mice received agalsidase alfa or beta was not different from untreated Fabry mice. Compared to WT, untreated Fabry mice exhibited a significantly delayed response to heat stimuli. The response time in Fabry mice that received pegunigalsidase alfa ($P < .01$) and agalsidase beta ($P < .02$) was significantly decreased compared to untreated Fabry mice while the response time in Fabry mice that received agalsidase alfa was not different compared to untreated Fabry mice. **Conclusions:** These data suggest that ERT initiated from asymptomatic stage may slow the onset and progression of small-fiber neuropathy. Restored Iba1 expression in Fabry mice treated with pegunigalsidase alfa, but not with currently approved ERTs, suggests that the novel chemically-modified enzyme may have advantages in the treatment of the Fabry small-fiber neuropathy.

742 - Cholestane-3β,5α,6β-triol and Chitotriosidase Combined Analysis: Potential Use for Niemann-Pick Type C Diagnosis and Screening

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Niemann-Pick type C (NPC) is a severe genetic disorder, mainly characterized by neurological dysfunction and liver damage. It is caused by mutations in the genes NPC1 or NPC2, leading to an abnormal accumulation of cholesterol within cellular lysosomes. Diagnosis is usually performed by the demonstration of cholesterol excess in fibroblasts (Filipin test) or by molecular analysis. However, considering the great benefits of the early treatment, new biomarkers able to screen NPC patients with high sensitivity and specificity have been stablished. With this purpose, we investigated the potential use of the combined analysis of cholestane-3 β,5 α,6 β-triol (triol) and the chitotriosidase (CT) activity for diagnosis, screening and monitoring of NPC patients. The levels of triol were investigated by LC/MS in plasma samples of 122 untreated individuals with clinical suspicion of NPC and also in 5 patients with previous diagnosis of NPC under treatment with miglustat. We detected 16 subjects with abnormal concentrations of triol (higher than 100 ng/mL), most of them also presented increased CT activity and 11 were confirmed as NPC by the Filipin test. Two patients of this group presented inconclusive results in the Filipin test, being one eventually diagnosed as NPC by molecular investigation and the other eventually diagnosed as Niemann-Pick type A or B (NPA/B) by the low acid sphingomyelinase (ASM) activity presented. Three patients with high triol concentrations had a negative result in the Filipin test, as well as low ASM activity, being diagnosed as NPA/B. On the other hand, triol concentrations were normal in NPC patients treated with miglustat, although CT activity in these individuals remained altered. In

the 106 patients with normal triol concentrations (lower than 100 ng/mL), most presented a normal activity of CT. No patient of this group had a positive Filipin test and the few patients with inconclusive Filipin test did not present pathogenic mutations in the NPC1 or NPC2 genes. In conclusion, our data demonstrated that the combined analysis of triol and CT is a promising approach not only to help the diagnosis and monitoring of NPC patients, but also to enable the screening of these patients before the disease worsening. **Financial Support:** CNPq, FAPERGS, FIPE-HCPA.

743 - Detection of Lysosomal Storage Diseases by a Multiplex Tandem Mass Spectrometry Method

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Lysosomal storage diseases (LSDs) comprises a heterogeneous group of more than 50 genetic disorders with a combined incidence of 1 per 7700 live births. They are characterized by progressive accumulation of macromolecular substrates within lysosomes, leading to cellular dysfunction, which may affect both somatic organs and the central nervous system. Since substrate accumulation and its tissue distribution can be variable even among patients with identical genotypes, the symptoms of LSDs are not always recognized early in life. In this context, interest in screening methods for LSDs has increased in the last years, especially because early diagnosis is essential to prevent the complications of these diseases, and tandem mass spectrometry (MS/MS) has been appointed as the most appropriated methodology for this purpose. So, in this work we aimed to evaluate the performance of MS/MS for detection of six LSDs (Niemann-Pick A/B, Krabbe, Gaucher, Fabry, Pompe, and MPS-I diseases). Standard curves and quality control dried blood spots were assayed to evaluate the precision, linearity and accuracy of the method. Samples from 150 controls, grouped according to age, and from 59 patients with previous diagnosis of LSDs were subjected to the measurement of acid sphingomyelinase, galactocerebrosidase, β-glucocerebrosidase, α-galactosidase, α-glucosidase, and α-L-iduronidase. The results obtained from calibration curves demonstrated good linearity and accuracy and the intra- and interassay precisions varied from 1.17% to 11.60% and 5.39% to 31.24%, respectively. For most enzymes, the activities in controls were higher in newborns compared to children and adults. As expected, all affected patients were well discriminated, presenting activities significantly lower compared to all control subjects. In conclusion, our results showed that MS/MS is a promising methodology to screen LSDs, being extremely useful for guiding other biochemical or molecular investigations necessary to conclude the diagnosis. **Financial Support:** CNPq, FAPERGS, FIPE/HCPA.

744 - Evolving Observational Registries: An Evidence Based Approach to Understanding Data Availability in the Sanofi Genzyme Rare Disease Registries

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The Sanofi Genzyme-sponsored Rare Disease Registries (Gaucher [ClinicalTrials.gov NCT00358943], Fabry [ClinicalTrials.gov NCT00196742], Pompe [ClinicalTrials.gov NCT00231400], and MPS I [ClinicalTrials.gov NCT00144794]) represent the largest global, observational databases for these lysosomal storage diseases. Since the inception of the first Registry in 1991, Registry data have been utilized to advance disease understanding, ensure access to therapies, and optimize patient outcomes. Operational support provided to Registry sites ensures operational success with compliance and data integrity. To further guide the evolution of the Registries in a systematic, evidence-based manner, a project was undertaken to identify barriers to data entry and to understand the quantity of data available for analysis. On-line surveys focused on data entry barriers were distributed to all active Registry site users; 151 surveys were completed (19%) within 4 weeks. Survey results informed the development of a qualitative interview guide. Interviews were conducted with 27 Registry participants from 12 countries. To evaluate the quantity of data available for analysis, a cross-Registry subset of 97 parameters was evaluated along the stepwise progression of data classified as "entered" to "usable." Data entry barriers identified include the absence of data when assessments are not performed, limited time and resources to enter data, challenges obtaining medical records, and difficulties matching data from medical records to the Case Report Forms. The percentage of patients with usable data for any one of the 97 parameters ranged from 2% to 93%. A decline in quantity from data classified as "entered" to "usable" was observed for both untreated and treated patients; however, treated patients had a consistently larger amount of usable data. These results illustrate the determinants of data in the Registries and provide a roadmap to inform changes in what and how data are collected. These data will complement continual efforts aimed at evolving the Registries so they continue to meet current and future scientific research needs.

745 - Natural History of Two Adult LALD Siblings: 25 Years of Follow-Up

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Background: Lysosomal acid lipase deficiency (LALD) is an autosomal recessive storage disease caused by *LIPA* gene mutations. Later LALD presentation is commonly seen with unexplained persistent elevations in aminotransferase levels, high LDL and low HDL cholesterol levels, fatty liver, and hepatomegaly. Information about long term follow-up of these patients is needed to improve knowledge of the natural history. **Cases report:** We report the cases of two LALD adult siblings of non-consanguineous parents (homozygous mutation c. 894 G>A). The index case, a 31 years-old male, had a first record of palpable liver in the first year of life. At 6 years dyslipidemia, persistently increased liver enzymes and hepatomegaly were diagnosed. Liver biopsy revealed microvesicular steatosis and periportal fibrosis. The second case, a 30-year-old female was diagnosed with dyslipidemia at 4 years-old (screened after index case). She had hepatomegaly, elevated transaminases, microvesicular steatosis, and periportal fibrosis on biopsy at 10 years old. Storage diseases were excluded and genetic study for familial hypercholesterolemia was negative in both. They were followed up by dyslipidemia and steatohepatitis under low-fat diet, the younger under lipid-lowering therapies too. Both had normal school and professional evolution. The genetic diagnosis was made at 30 and 29 years old respectively in the screening of LALD. Liver biopsy 20 years later shows microvesicular steatosis (NASH-CRN score 7.1c in both) and the same degree of fibrosis (Ishak score 2). Fibroscan shows 8.6 KPa and 10.3 KPa respectively (median elastography). Mild splenomegaly is now observed in the male. Atherosclerotic evaluation shows 25%-30% stenosis at the beginning of the right subclavian artery and a higher coronary calcium score (54) in the first case. **Discussion:** LAL-D predominantly presents in pediatric patients. Both cases fit the classic profile with low HDL and high LDL cholesterol with persistent elevation in aminotransferase levels, hepatomegaly, microvesicular steatosis and periportal fibrosis. After 20 years similar involvement on liver biopsy was observed, although the first case mainly on diet) has now mild splenomegaly, and different atherosclerotic repercussion. The availability of enzyme replacement therapy warrants greater awareness of this disorder as well better knowledge of natural evolution to evaluate results

746 - Follow-Up Results of Fabry Disease Patients in Relation With Risk Perception

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Introduction: Fabry disease (FD) is a rare X-linked lysosomal storage disease (LSD). It results from mutations in the GLA and more than 400 mutations have been identified in this gene. Diagnosis of FD is quite difficult; however, follow-up is even more difficult in our region. This paper was prepared to give the follow-up results of FD patients in relation with perceived risk. **Methodology:** The clinical data of 23 Fabry patients (7 males, 16 females) from 7 families from Kırıkkale Pediatric Metabolic Disease Department were given. Beck Depression Inventory (BDI), and Sjoberg Risk Perception Scale (SRPS) were used for individual depression and risk perception at the time of the diagnosis. Risk perception was assessed with a scale from 1 to 7, where "1 is not at all serious and 7 is very serious." **Results:** Between the years 2015-2017, 825 patients were investigated with a suspection of FD and 23 had the diagnosis. Diagnoses were verified by enzyme and genetic mutation analyses. The FD patients had a mean age of 38.7 (14-62) years. The most common mutations were p.A143 T (c.427G>A) (7 patients) and p.D313Y (c.937G>T) (7 patients), p.T385A(c.1153A>G) mutation was found in 1 patient. Enzyme levels were significantly higher in patients who had p.D313Y mutation. Ten patients had neurological 4 had cardiac and 4 had renal problems. 2 patients were going under hemodialysis with terminal renal failure. According to Turkish Ministry of Health's regulations 9 patients were started to receive enzyme replacement therapy (ERT). After 1 year, ERT in 4 patients the lyso Gb3 levels were found to be normal. BDI scores and risk perception of our patients were 13.1 ± 7.4 and 3.2 ± 2.5, respectively. 13 (57%) FD patients (3/9 from the ERT receiving group) stop coming for controls without giving any reason. There was no relation with depression scores and stopping follow-up. However, risk perception levels were lower in that group. A woman who stopped receiving therapy at the 4-month ERT, attended to the hospital with stroke. **Conclusion:** As individuals usually believe them to be at lower risk for the outcomes of the disease, they might minimize the threat's severity and stop the follow-ups and treatment easily.

747 - A Possible Link Between Urinary Gb3, Lipid Peroxidation, and Nitrosative Stress in Fabry Patients Before and During Long-Term ERT

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Fabry disease (FD) is caused by deficient activity of the lysosomal enzyme α-galactosidase A. Its substrates, mainly globotriaosylceramide (Gb3), accumulate and seem to induce the pathophysiological findings of FD. Elucidating the underlying mechanisms in FD pathophysiology is essential to the development of additional therapeutic strategies, once the available one, enzyme replacement therapy (ERT), is not completely efficient on preventing disease progress. Based on previous findings of higher lipid peroxidation during short-term ERT, the already described relationship between nitrosative stress and vasculopathy, and the induction of pathophysiology mechanisms by Gb3, the objective of this study was to investigate lipid peroxidation, nitrosative stress and Gb3 in Fabry patients at diagnosis and to compare it with patients during long-term ERT and controls. For that, we investigated 58 Fabry patients (23 male and 35 female) subdivided into two groups (at diagnosis and during long-term ERT) and compared them to healthy individuals. Fabry patients at diagnosis presented higher lipid peroxidation levels (malondialdehyde - MDA), nitric oxide ($NO^{\cdot}$) equivalents and urinary Gb3. MDA and $NO^{\cdot}$ equivalents remained higher in patients during long-term ERT, whereas Gb3 levels were lower than at diagnosis but still higher than controls. These data demonstrated that lipid peroxidation and excessive $NO^{\cdot}$ production occur in Fabry patients before and after long-term ERT, probably as a consequence of Gb3 accumulation, providing targets to future therapy approaches using antioxidants in combination with ERT in FD. **Financial support:** CNPq, CAPES, FIPE-HCPA

748 - Lyso-Gb3 Induces Oxidative DNA Damage in Cultured Kidney Cells

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Fabry disease (FD) is a lysosomal disorder caused by mutations leading to a deficient activity of α-galactosidase A with progressive and systemic accumulation of its substrates. Although FD is considered a systemic disorder, renal complications are major causes of morbidity in Fabry patients. Once DNA damage has been associated with disease progression in other chronic diseases and was recently found in high levels in Fabry patients, the main objective of this study was to verify the effects of a FD accumulated substrate on DNA damage in kidney cells. To reproduce pathological conditions, it was tested three concentrations found in Fabry patients (10, 50 and 100 nM) of the latest described biomarker for FD—globotriaosylsphingosine (lyso-Gb3)—in a cultured renal lineage—human embryonic kidney cells (HEK-293 T)—that has been used as a model of epithelial kidney cell-line in renal physiology studies. In all tested concentrations, lyso-Gb3 induced DNA damage (assessed by alkaline comet assay). To investigate a possible oxidative origin in purines and pyrimidines, the comet assay with endonucleases was also performed and again all lyso-Gb3 concentrations induced significant DNA damage when compared to negative control. To the best of our knowledge, this is the first study focusing on these effects of lyso-Gb3 and provides new information that could be useful to studies looking for new therapeutic strategies to slow renal disease progression in FD. **Financial support:** CNPq, CAPES, FIPE-HCPA.

749 - ".Omics" to improve the efficiency of lysosomal disease diagnosis

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The wide phenotypic variability inherent in lysosomal diseases presents challenges for accurate and timely diagnosis. Diagnostic confusion can also arise from interpretation of the degree of residual enzyme activity and is impractical when the disease is not due to an enzyme deficiency per se. We have profited from advances in mass spectrometry and developed two ".omics" platforms to improve the efficiency of lysosomal disease diagnosis. Employing urine chemistry, as a reflection of multisystemic disease, all ten mucopolysaccharidosis subtypes could be identified from a low molecular weight glycosaminoglycan fingerprint. Ranging in size from mono- to pentasaccharides, with up to two sulfates, these fragments have terminal residues complicit with the enzyme deficiency. From a total of 286 blinded urine samples, 93 were identified as specific mucopolysaccharidosis subtypes with the remainder as controls; this method has now become our urine mucopolysaccharidosis screening test replacing high resolution electrophoresis. Surrogate markers of disease burden, these oligosaccharides are also informative for biochemically monitoring patients in receipt of corrective therapies including bone marrow transplant, enzyme replacement and

a gene therapy clinical trial, in which the requisite oligosaccharides decreased, informing on efficacy. A second lipidomics platform provides for the sphingolipidoses following a single-phase lipid extraction of just 0.01 mL of plasma. From a total of 3000 samples, including 16 related inherited metabolic disorders, all 20 Gaucher disease patients were correctly identified. For biochemical monitoring patients in receipt of therapy, some patients returned near normal concentrations (<10 pmol/mL) whereas others were as high as 600 pmol/mL, suggesting that disease burden remains high in some patients. Patients with classical Fabry disease were identified with elevated globotriaosylsphingosine and concentrations remained unchanged in patients receiving replacement therapies. With the addition of sulfatide, confirmation of metachromatic leukodystrophy was afforded with elevated plasma 18:0-sulphatide concentrations, and lyso-sphingomyelin-509 allowed identification of Niemann-Pick C. The opportunity to profile multiple analytes in one assay not only improves diagnostic efficiency but likely provides a clearer picture of disease burden, facilitating both prediction of clinical course and longitudinal biochemical monitoring.

750 - A Pilot Test Evaluating DBS Chitotriosidase in Mexican Patients With Nephropatic Cystinosis

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Introduction: Nephropatic cistinosis (NC) is a rare lysosomal disease due to absence of cystine transporter protein and causes an abnormal intralysosomal of cystine. Diagnosis is confirmed by observation of cystine crystals in cornea, by elevated cystine levels in leucocytes or by presence of mutations at the *CTNS* gene. Chitotriosidase is a human chitinase produced by leucocytes and it is elevated in more than 40 conditions including lysosomal disorders. It is used as a screening biomarker and for monitoring therapeutic goals in Gaucher disease. **Objective:** To evaluate chitotriosidase levels in DBS and presence/absence of 24-pb duplication polymorphism at the *CHIT* gene in treated Mexican patients with NC and to correlate with adherence treatment. **Methods:** DBS chitotriosidase activity was measured in 15 Mexican patients with NC using the 4-MU-triacetylchitotrioside (Chamoles et al, 2002), DNA was extracted by Single Lysis Salting Out (Shaik et al, 2016), PCR amplification of a fragment of the *CHIT* gene was done to determine the presence/absence of 24-pb duplication polymorphism. **Results:** Chitotriosidase levels were normalized by 24-pb dup (5 of 15 were heterozygous and none was homozygous for 24-pb dup). In 9 of 15 patients, chitotriosidase were elevated compared with normal individuals. 12 of 15 patients were under treatment (11 with Cystagon and 1 with Procysbi), all of the 3 patients with no treatment showed elevated chitotriosidase; adherence treatment was observed in 9 of the 12 treated patients and only in 3 of them Chit was elevated, in the remaining 6 Chit was normal. Measures are being performed every 6 months for 2 years. **Conclusions:** This preliminary study indicates that chitotriosidase activity is a practical and useful marker to measure treatment adherence in patients with Nephropatic Cystinosis.

751 - CentoMD®: Genetic Variants—Related Biomarker Knowledge Database

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Clinical symptoms in lysosomal storage diseases (LSDs) are caused by the deficiency of specific enzymes function and resultant substrate accumulation in the lysosomes. Several biomarkers are already in use as indicators of the presence and monitoring of LSDs: lyso-Gb3 (Gb3) in Fabry disease (FD), Lyso-Gb1 (Gb1) in Gaucher disease (GD) and NP509 in Niemann–Pick (NP) disease. CentoMD® is a browser-based tool that enables access to a high-quality repository of genetic, biochemical and human phenotype ontology (HPO)-based clinical information. All patients provided informed consent before inclusion in the DB. We measured Gb3, Gb1 and NP509 in the DBS samples obtained from 5,603 patients (56.7 females, 39.1 males, 3.3% unknown) undergoing biochemical and genetic testing for verification of FD (71.7%), GD (15.8%) or NP (12.5%). The pathological cutoff for biomarker measurements was set to 1.8 ng/ml for Gb3, to 4.8 ng/ml for Gb1; and to 0.9ng/ml for NP509. Biomarker levels were correlated with clinical severity of the individual patients. The patient cohort (>128 000 genetically screened individuals in CentoMD® v3.3) covers >110 countries worldwide. 85% of patients with metabolic disorders are associated with biomarker data. We have confirmed the presence of the corresponding LSD in 73.1% (75.1% FD, 14% GD, 10.9% (NP) of the screened individuals and the carrier status in 26.9% (62.4% FD, 20.7% GD, 16.9% NP). The observed age at diagnosis varies from 11.9+/13.7 in NP cases, to 22.7 $\pm$ 19.6 in GD and 41.2 $\pm$ 19.9 in FD (39.9 $\pm$ 18.1 in males, 42.7 $\pm$ 21.0 in females). The most of the diagnosed LSD cases are originating from Europe (49.8%), followed by Latin America (36.2%), Middle East (9.5%) and Africa (2.4%). The level of biomarkers at time of diagnosis were 3.5+/−2.5 ng/mL for NP509, 299.8 $\pm$ 259.5 ng/mL for Gb1 and 3.9 $\pm$ 10.3 ng/mL in FD-females, and 19.8 ng/mL $\pm$ 32.9 ng/mL in FD-males. We have identified in total 945 unique genetic variants (44.4% are novel), associated with epidemiological information, clinical symptomatology and biomarker levels in CentoMD® (40.4% in FD, 34.8% in GD, and 55.1% in NP). In order to generate reliable predictions of genetic changes in severity and progression of LSDs, we investigated the relationship between the detected genetic variants, biomarker levels and clinical

phenotype. CentoMD® brings exceptional quality, unique and valuable information on genetic variants spectrum in different ethnical populations.

752 - Wolman Disease, Atypical Presentation Without Marked Hepatomegaly or Hypertransaminasemia

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Lysosomal acid lipase deficiency (LALD) is a rare autosomal recessive lysosomal storage disease caused by mutations in the *LIPA* gene. Patients presenting in infancy have the most rapidly progressive disease, so called Wolman disease, developing signs and symptoms in the first weeks of life and rarely surviving beyond 6 months of age without treatment. The patient, a full-term male newborn with normal birth weight (2880 g) and a history of consanguinity (parents are first-degree cousins), started vomiting and presenting abdominal distension in the first days of life, reason why he was admitted to hospital at 15 days of life. He continued with vomiting, diarrhea, failure to thrive, malabsorption, and developed marked splenomegaly. At 2 months of age he was transferred to our hospital. On physical examination, both weight (3840 g) and length (53 cm) were below the third percentile. He was pale, and abdominal distension with splenomegaly and cachexia were evident. A subtle hepatomegaly about 1-2 cm below the costal margin was present at that moment. Laboratory test showed anemia (Hb: 8.1 g/dL). AST and GGT were slightly elevated [AST: 90 U/L (normal range: 7-80); GGT: 108 U/L (normal range 10-35)], and ALT levels were normal [ALT: 35 U/L (normal range 5-47)]. LDL cholesterol value was normal (LDL: 113 mg/dL), whereas HDL cholesterol was low (12 mg/dL) and triglycerides were elevated (221 mg/dL). Although no marked hepatomegaly or hypertransaminasemia was present, clinical data led to the suspicion of Wolman disease. Blood film examination showed vacuolated lymphocytes (63% of lymphocytes). Acid lipase enzyme activity was null in dried blood sample, and genetic test showed novel mutation in homozygous, located in intron 6 canonical splicing donor. The following laboratory work showed an increase in the transaminases (AST: 188 U/L, ALT: 202 U/L, and GGT: 299 U/L), and hepatomegaly became more evident (about 4 cm below the costal margin) during the following weeks. Conclusions: In an infant with compatible physical examination (malnutrition, abdominal distension) and symptoms (vomiting, diarrhea, malabsorption, and failure to thrive), LALD should be investigated, although not marked hypertransaminasemia or hepatomegaly is present at the moment, as they may develop later. As in other metabolic diseases, in Wolman disease blood film examination for vacuolated lymphocytes can give a cheap, rapid and minimally invasive clue to the diagnosis.

753 - Lysosomal Acid Lipase Deficiency (LAL D): Clinical and Morphological Progression in Children and Young Patients

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LAL D is a rare hereditary disease characterized by a progressive course. Objective: to study clinical, laboratory and morphological manifestations in patients with LAL D. Methods: We examined 14 patients aged 2.5 to 21 years (avg. 8.8 $\pm$ 1.5 years) with enzymatic and genetically confirmed diagnosis of LAL D. Puncture biopsy of the liver and a morphological examination of the specimen were performed to all patients. Results: At the time of the study, the liver was enlarged in 13 children for 2-8 cm (avg. 4.4 $\pm$ 0.6) from under the edge of the costal arch, the spleen in 5 children was palpated below the left costal arch for 1-2 cm. The cytolysis syndrome was confirmed in all patients: AST—105.2 $\pm$ 16.1, ALT—132.2 $\pm$ 20.6 U/l. Hypercholesterolemia 7.4 $\pm$ 0.4 mmol/L and an increase in LDL-C—5.3 $\pm$ 0.4 mmol/L were detected in all children, hypertriglyceridemia in 5 patients (2.6 $\pm$ 0.3 mmol/L). The index of histological activity was 4.5 $\pm$ 0.5 points (Knodell). There were no signs of inflammation in the liver biopsy in 4 patients. Phenomenon of periportal hepatitis of minimal degree of histological activity was registered in 4, low degree—in 6 children. Attention was drawn to the presence of increased hepatocytes in 5 patients. Hepatocytes had a light vacuolized cytoplasm (micro vesicular or mixed steatosis) in all observations. Two children had binuclear hepatocytes. In biopsies of two more patients, the nuclei were shifted to the periphery. During the Schick reaction in 7 patients, the accumulation of the Schick-positive substance in hepatocytes was recorded, which was washed out by amylase control in 3 of them. In all patients, morphological picture was characterized by fibrosis of portal tracts, port-portal septums were detected in 12, perihepatocellular fibrosis in 6 children. The Desmet sclerosis index was 1 point confirmed in 4 (age of children 6.0 $\pm$ 2.4 years), 2 points in 7 (age 7.4 $\pm$ 1.1 years), 3 points for 3 patients (avg. age 7.5 $\pm$ 1.9 years). Thus, patients with LAL D are characterized by the presence of hepatomegaly, cytolysis syndrome, hypercholesterolemia and dyslipidemia due to an increase in LDL-C. Morphological signs of LAL D could be nonspecific. However, the overwhelming majority is determined by vacuolization of the cytoplasm, micro/mixed steatosis, minimal inflammatory

changes and the formation of liver fibrosis progressing with age as the duration of the disease increases.

754 - Analytical Scoring Tool Based on Enzyme Activity to Guide the Diagnosis of Pompe Disease Suspected Patients

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Pompe disease is a storage disorder with a partial or complete deficiency of acid α- glucosidase (GAA). The identification of acarbose as one of the effective inhibitors of isoenzyme maltase glucoamylase (MGA) derived from neutrophils permits the assessment of GAA activity in blood samples, including DBS (dried blood spots). Three enzyme reactions were performed by fluorometric enzymatic assay: 1- GAA, was measured at pH 4.0 in the presence of inhibitor acarbose; 2- total GAA (MGA+GAA) measured at pH 4.0 without acarbose; and 3-Neutral α glucosidase activity (NAG), measured at pH 7.3. The tGAA reflects the combined activity of the isoenzyme MGA and GAA and was measured to calculate the percentage of tGAA that was inhibited by acarbose by using the formula (tGAA - GAA) / tGAA. This study uses a unique scoring system based on GAA, NAG, and tGAA to guide the diagnosis of patients. The scoring system is composed of 10 classifiers, several of which were either taken or adapted from Chien et al., 2008 www.pediatrics.org/cgi/doi/10.1542/peds.2007-2222 Out of these 10 classifiers, 9 of them are binary with values 0 or 1 and the remaining is a floating-point classifier that ranges between −1 and 1. Overall, a given patients score can range between −1 to 10 and effectively incorporates tGAA, NAA, GAA and percent inhibition into the decision process. To get a glimpse of this scoring system's diagnostic ratio we have tested the algorithm on 35 patients, 28 of whom were healthy. The mean of the healthy population was 0.75 with maximum value, minimum value, +2SD and -2SD intervals being 1.76, −0.05, 1.67, and −0.16, respectively. The mean of the affected patients was 6.9 with maximum value, minimum value, +2SD and −2SD intervals being 10, 3.76, 12 (capped at 10), and 1.867. Based on these values a threshold with a score of 1.8 yields excellent diagnostic odds ratio, classifying all patients correctly. **In conclusion:** Our scoring system provides separation between healthy and affected patients which cannot be explained by GAA, tGAA, NAG or inhibition percent alone. Additionally, this system provides basis for: - development for more sophisticated algorithms that incorporates regression analysis; - fine-tuning of the threshold using ROC curves based on dynamic patient databases; and - in cases where diagnostic metrics show bimodal/trimodal distribution, extension of classifiers to incorporate ethnic background to increase reliability.

755 - β-Mannosidosis: Case Report of the First Two Slovak Patients

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Background: β-mannosidosis is a very rare panethnic autosomal recessive disorder. It results from the deficiency in the function of the lysosomal enzyme β-mannosidase involved in the glycoprotein catabolism. The result is an excessive accumulation of oligosaccharides in lysosomes with its high excretion in the urine. The age of onset varies from infantile to early adulthood. Clinical picture is extremely variable with developmental delay, behavioral disturbance, susceptibility to respiratory infections and hearing loss as mostly common symptoms. Seizures, facial dysmorphia, skeletal deformation, hypotonia and skin involvement are reported for a minority of the cases. **Case Report:** We present 2 siblings of consanguineous Roma parents (their fathers are cousins). Both girls 7 and 4 years old presented with identical clinical picture: short stature (< 3 percentile), mental retardation, hypotonia, hyperactivity, autistic features, slight facial dysmorphia (coarse features), slight barrel- shaped chest, hypacusis, and ethnically atypical fair hair. Both are from uncomplicated pregnancies, with the evident developmental delay from the end of first year, without verbal communication till now. The younger girl overcame meningococcal sepsis at 13 months of age. The diagnosis of β-mannosidosis was established by pathological urine excretion of oligosaccharides with dominant excessive excretion of disaccharide Man(β1→4) GlcNAc and subsequently markedly low activity of leukocyte β-mannosidase −0.8 and 0.9 nmol/h/mg of protein (normal range 65-180). Mutation analysis revealed homozygosity for c.2158-2A>G in introne 15 in the *MANBA* gene. **Conclusion:** Clinical heterogeneity and absence of typical symptoms of lysosomal storage disease make the clinical diagnose difficult. Urinary oligosaccharides examination with special β-mannosidosis detection is a cheap and reliable laboratory screening method. Presented cases are from the second Roma origin family from 17 reported families. This fact indicates the possible higher incidence in Roma ethnicity.

756 - Screening Tests for Pompe Disease in 6522 Samples From Colombian Patients, the Need of Confirmation

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Pompe disease is a hereditary disease of the glycogen metabolism. The mutations are transmitted in an autosomal recessive way and affect the molecular structure of lysosomal enzyme α-Glucosidase (EC 3.2.1.20/3). This alteration leads to progressive storage of glycogen in muscle cells lysosome and alteration of other cellular mechanisms generating a broad phenotypic clinical expression including proximal muscle weakness, restrictive respiratory insufficiency, severe hypotony, hyperckemia, macroglosya, and cardiomyopathy between others. Enzymatic analysis techniques for screening and diagnosis have been developed for this specific metabolic disease. The first ones have been developed in solid phase samples (DBS), allowing then to carry out extensive high-risk population screenings. The latter ones have been developed in isolation of total leukocytes, as a confirmation technique for altered cases in the screening tests. Since 2005, screening studies in high-risk populations have been carried out in Colombia, using the DBS technique because of its easy handling, collection and shipping characteristics, achieving a wide coverage above the country. The results of ten years (2006-2015) of screening in high risk population with suggestive findings of Pompe disease submitted to analysis in our center are presented here. A total of 6522 samples on filter paper were analyzed in that period. The age range was 2 months to 95 years. The methodology included an end-point enzymatic assay, using 4MU-alpha-D-glucopyranoside (4MU) as an artificial substrate in the presence of acarbose as an inhibitor of non-disease-related enzyme isoforms. 90 samples with alterations in the enzymatic activity were detected with this screening. Subsequently, these 90 individuals were carried out to a later confirmation using an end-point enzymatic assay with 4MU in acarbose presence, in isolated leukocytes; obtaining enzymatic alteration in 40 individuals, thus confirming the pathology in them. 50 samples (0.72%) show results comparable to normal population values, considering then these individuals, as false positives of DBS screening technique. This finding constrains the confirmation by using complementary tests in all cases and in some of them even to evaluate the enzymatic activity using specific natural substrate (Glycogen) for this entity.

757 - Selective Screening of 32 940 Colombian Patients for the Detection of Lysosomal Metabolic Disorders: Memories of 22 Years of Research (1995-2016)

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Introduction: Disorders of lysosomal metabolism (LDs) involve a group of more than 50 hereditary alterations, in which enzymes belonging to the glycosaminoglycans, oligosaccharides and sphingolipids catabolic pathways are affected. LDs cause chronic deposition of macromolecules in the lysosomal environment, resulting in a multisystemic compromise that includes organomegaly, connective-tissue disorders, dysfunction of the central nervous system, and so on. A mass screening protocol for metabolic disorders is not available in Colombia, especially for lysosomal disorders, because they are not considered as a priority in the public health system, due to their low frequency. Evaluation is usually made under clinical criteria that may lead to the underdiagnoses of many of these pathologies. **Aim:** To present the findings on high-risk screening for alterations of lysosomal metabolism in Colombia from 22 years, showing a contrast between the first decade of classical methods using liquid samples (cell isolation, plasma or serum, and urine samples), and the last period using solid phase samples (dry blood collected on filter paper, DBS), which have facilitated the diagnostic process by increasing the number of patients under study. **Methodology:** A total of 32 940 individuals with suspicion of lysosomal disease (LD), referred from 1995 to 2016, of whom 3834 (11.6%) were studied with classic procedures using liquid samples (urine/serum/whole blood) and 29 106 (88.4%) who were referred by GSSPF since 2005. **Results / Discussion:** Confirmatory tests showed 652 (2%) patients affected by some LD. Diseases detected: Fabry (n = 47), Fucosidosis (n = 2), Gaucher (n = 196), Gangliosidosis-GM1 (n = 21), Krabbe (n = 1), Metachromatic leukodystrophy (n = 12), Mucolipidosis (n = 8), Mucopolysaccharidosis (tipo-I(n = 27)), tipo-II(n = 43), tipo-III(n = 14), tipo-IVA(n = 192), tipo-IVB(n = 2), tipo-VI(n = 40), tipo-VII(n = 1)), Pompe(n = 40), Sandoff(n = 2), Sialidosis(n = 1) and Tay Sachs(n = 3). **Conclusion:** Studies in DBS allowed the coverage of screening of these disorders to be extended and indirectly support the diagnosis of other LDs. However, it is important to consider that these are screening tests, whose positive cases must be confirmed with reference methodologies or cellular extracts.

758 - Characterization of Plasma Lipoprotein Particles in Spanish Patients With Lysosomal Acid Lipase Deficiency

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Objective: Lysosomal acid lipase deficiency (LALD) is a rare lysosomal storage disease characterized by progressive liver disease and dyslipidemia. Clinically, LAL-D is classified into two major phenotypes: infantile-onset Wolman disease (WD) and later-onset cholesteryl ester storage disease (CESD). LALD typically presents with high serum levels of LDL-C, low serum levels of HDL-C and, in some cases, elevated triglycerides. The reduction in HDL-C levels has been linked, until now in vitro, to reduced formation of mature HDL. The objective of this study was to characterize plasma lipoprotein particles in the full clinical spectrum of LALD. **Methods**: Twelve LALD patients were included: one infant (WD) and eleven CESD patients (two 10-year-old children and three adults treated solely with statins ± ezetimibe, two 11-year-old children and one adult not treated with lipid-lowering treatment and three adults with liver transplants). Plasma samples were analyzed using a novel advanced lipoprotein test based on 2D diffusion-ordered ^{1}H-NMR spectroscopy (Liposcale®). Lipoprotein particle concentrations, lipid load and sizes were measured. Reference values were calculated from 2,300 healthy adults. **Results:** The children presented with very similar lipoprotein profiles: high concentrations of IDL-C (17.6 mg/dL, NV:<11), LDL-C (167.8 mg/dL, NV:<130) and LDL-P (1248 nmol/L, NV:<900) and low HDL-C concentrations (38.3 mg/dL, NV:>40). However, the infant presented with a very different profile than the children, with normal levels of LDL-C and LDL-P, significantly higher levels of IDL-C (52.9 mg/dL), IDL-TG, VLDL-TG, LDL-TG, and notably lower levels of HDL-C (11.6 mg/dL). In the adults on statins, VLDL-C and/or IDL-C were elevated, whereas LDL-C was only elevated in one patient (193.8 mg/dL). Three of four adult patients had very low concentrations of HDL-C (36.2 mg/dL). LDL-P was high in one adult. HDL particle diameter was small in two children and one adult. The transplanted adults' lipoprotein profiles had shifted to a healthy state. **Conclusions:** LALD patients have varied plasma lipoprotein profiles with a highly atherogenic pattern despite lipid-lowering therapy, especially in WD and in children. This pattern is characterized by high levels of ApoB-containing lipoproteins, including IDL. Low HDL-C concentration is the most common finding in LAL-D. In few cases, mature HDL particle levels are low. Liver transplantation seems to control dyslipidemia.

759 - CLN8 Deficiency Impairs Dendritic Development in Hippocampal Neuronal Model

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Introduction: CLN8-disease belongs to the group of Neuronal Ceroid Lipofuscinoses, neurodegenerative disorders characterized by the abnormal lysosomal accumulation of lipofuscin-like compounds. It is caused by a deficiency of CLN8p, an Endoplasmic Reticulum resident protein. It was related with the synthesis, transport and detection of lipids; however, its role remains unknown. We aim to study the effect of *CLN8* expression levels on the morphology of neurons. **Methods**: Embryonic hippocampal rat neurons of 2 and 9 d.i.v. were transiently transfected with pYFP (control) alone or co-transfected with pCLN8wt (overexpression) or pshCLN8 (silencing). 2 d.i.v. neurons were marked with anti-Tau and anti-Tubulin by immunostaining for axonal length measure. 9 d.i.v. neurons were used for dendritic branching study by Sholl analysis. Images were taken in an epifluorescence microscope and analyzed with ImageJ-Fiji software. One-way ANOVA test was used to evaluate axonal length, and two-way ANOVA test with repeated measures was used for dendritic evaluation. **Results**: 1) 2 d.i.v. neurons did not show meaningful differences regarding axonal length among treatments ($P > .05$). 2) 9 d.i.v. neurons did reveal significant variations in dendritic ramification measured. Dendritic development in pshCLN8-treated cells was diminished compared with control cells ($P < .0001$). Interestingly, neurons overexpressing *CLN8* showed values between the other two conditions. **Discussion**: CLN8p deficiency affects dendritic development, but not the axonal length. Our previous results showed a tendency of *CLN8* to alter lysosomal distribution in the cell body. An appropriate lysosomal function and distribution are required for a correct dendritic development. Moreover, it was shown that changes in the morphology of dendrites are related with some neurodegenerative disorders. Now we propose that both overexpression and silencing of *CLN8* affect the development of dendrites possibly through altering lysosomal dynamics. These morphological changes may be part of the pathophysiology of CLN8, as was shown for some other neurodegenerative diseases.

760 - Lysosomal Acid Lipase Deficiency (LAL D): Clinical and Morphological Progression in Children and Young Patients

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LAL D is a rare hereditary disease characterized by a progressive course. Objective: to study clinical, laboratory and morphological

manifestations in patients with LAL D. Methods: We examined 14 patients aged 2.5 to 21 years (avg. 8.8 ± 1.5 years) with enzymatic and genetically confirmed diagnosis of LAL D. Puncture biopsy of the liver and a morphological examination of the specimen were performed to all patients. Results: At the time of the study, the liver was enlarged in 13 children for 2-8 cm (avg. 4.4 ± 0.6) from under the edge of the costal arch, the spleen in 5 children was palpated below the left costal arch for 1-2 cm. The cytolysis syndrome was confirmed in all patients: AST—105.2 ± 16.1, ALT—132.2 ± 20.6 U/L. Hypercholesterolemia 7.4 ± 0.4 mmol/l and an increase in LDL-C—5.3 ± 0.4 mmol/L were detected in all children, hypertriglyceridemia in 5 patients (2.6 ± 0.3 mmol/L). The index of histological activity was 4.5 ± 0.5 points (Knodell). There were no signs of inflammation in the liver biopsy in 4 patients. Phenomenon of periportal hepatitis of minimal degree of histological activity was registered in 4, low degree—in 6 children. Attention was drawn to the presence of increased hepatocytes in 5 patients. Hepatocytes had a light vacuolized cytoplasm (micro vesicular or mixed steatosis) in all observations. Two children had binuclear hepatocytes. In biopsies of two more patients, the nuclei were shifted to the periphery. During the Schick reaction in 7 patients, the accumulation of the Schick-positive substance in hepatocytes was recorded, which was washed out by amylase control in 3 of them. In all patients, morphological picture was characterized by fibrosis of portal tracts, port-portal septums were detected in 12, perihepatocellular fibrosis in 6 children. The Desmet sclerosis index was 1 point confirmed in 4 (age of children 6.0 ± 2.4 years), 2 points in 7 (age 7.4 ± 1.1 years), 3 points for 3 patients (avg. age 7.5 ± 1.9 years). Thus, patients with LAL D are characterized by the presence of hepatomegaly, cytolysis syndrome, hypercholesterolemia, and dyslipidemia due to an increase in LDL-C. Morphological signs of LAL D could be nonspecific. However, the overwhelming majority is determined by vacuolization of the cytoplasm, micro/mixed steatosis, minimal inflammatory changes and the formation of liver fibrosis progressing with age as the duration of the disease increases.

761 - Profile of the Brazilian NPC-C Patients: Preliminary Phenotypic and Genotypic Data

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Introduction: Niemann-Pick disease type C (NPD-C, or NPC) is an autosomal recessive inborn of cholesterol trafficking, with an estimated incidence around 1:150 000. The progressive storage of unesterified cholesterol inside the lysosomes, together with secondary pathogenic mechanisms still not well understood, leads to progressive visceral and neurological manifestations, with reduced life expectancy. **Objective:** The purpose of this study was to evaluate a Brazilian cohort of NPD-C patients, regarding selected characteristics, including genotype. **Methods:** Retrospective study based in chart reviews of Brazilian NPD-C patients treated or not with miglustat. The variables ascertained were: date of birth, age at diagnosis, age at onset of neurological manifestations, age at death, genotype information. The information related to miglustat treatment was recorded. **Results and Conclusions:** Fifty-four patients were included, with a mean age of 15.4 years, being 28 female and 26 male (52%/48%). Of the 54 patients, 27 were diagnosed before the age of 6 years, 4 between 6 and 11 years, 7 between 12 and 18 years and 16 after 18 years. The majority of patients (30/54, or 59%) presented the first neurological manifestations before 6 years of age. A significant number of patients (33/54) received treatment with miglustat, with a mean treatment duration of 3.09 ± 2.36 years. Regarding genotype information, from the 54 patients included, we were able to obtain genetic data from 48 of them, in whom we sequenced the 25 exons of NPC1 and the 5 exons of NPC2 genes, and performed MLPA studies in some. In 33, we were able to find 2 pathogenic mutations, all in the NPC1 gene. Considering 66 alleles (33 patients), we found 14 alleles with nonsense/frameshift mutations and 52 alleles with missense mutations. In 10, we found just one pathogenic variation, while in 5 no pathogenic variation was found. Although these data should be further evaluated in more detail, they provide an insight on the profile of Brazilian NPD-C patients.

762 - Neuronal Ceroid Lipofuscinosis: Two Families, Two Forms, Two New Mutations

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Neuronal ceroid lipofuscinosis (NCL) is a lysosomal storage disease characterized by neurodegeneration, seizure and visual loss. It is most commonly seen neurodegenerative disease group in childhood. The patients regarding two different NCL forms will be presented with clinic, laboratory, imaging and genetic analysis results. A 3-year old male patient born as a result of consanguineous marriage forwarded to our hospital with regression in speech commenced in the 18th month and loss of motor acquisitions, seizures, and ataxia. In his physical examination, it was determined that he lost his speaking ability, had walking ability and had low response to external stimuli. In the cranial MRI scan, cerebral atrophy was established. Palmitoyl-proteinthioesterase (PPT) enzyme activity was 0 nmol/spot 45*h (N:0.25-2.5) and in PPT1 gene analysis, p.H187Y change—undefined previously—was determined as homozygous. A 5-year-old male patient born from a consanguineous marriage forwarded to us with regression in speech at the age of 2.5 years, seizures beginning at the age of 3 years and imbalance in walking added while he was 4 years. On his physical examination, it was established that there was no walking and speech ability, no response to external stimuli, there were involuntary movements on hands. Cerebral and cerebellar atrophy were identified in the cranial MRI scan. In the *MFD8* gene analysis concerning the form—late infantile neuronal ceroid lipofuscinosis—known to be common in Turkish society was established as previously undefined Q365X homozygous mutation. In the family screening, the same homozygous mutation was identified in the MFDS8 gene analysis from the 3-year-old sibling with no complaints except for being not able to talk. NCL is one of the diseases that should be considered in the definitive diagnosis of a patient applying to hospital with neurodegeneration.

763 - Glycogen Storage Disease Type 9 is the Most Common Among Cohort of Russian Pediatric Patients With Glycogenosis

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Background: Glycogen storage disease (GSD) is a group of inherited diseases characterized by disorder of carbohydrate metabolism and manifest with hepatomegaly, hypoglycemia, hyperlactatemia, hyperlipidemia and hyperuricemia. GSD, type 9 is the pathology which is genetically poorly surveyed earlier. Most likely it is bound to the big extent of 4 genes (*PHKA2, PHKA1, PHKB and PHKG2)* mutations in which lead to development of this disease. Also, it is bound with mainly smooth state of the disease in comparison with other GSD types. **Methods:** We examined 98 patients with a clinical phenotype of different GSD. The coding and adjacent intronic genes region *G6PC, SLC37A4, GAA, AGL, GBE1, PYGM, PYGL, PFKM, PHKA2, PHKB, PHKG2, PHKA1, PGAM2, LDHA, ALDOA, ENO3, PGM1, GYG1, GYS2, GYS1, LDHB,* and *LAMP2* were examined by NGS in all patients. **Results**: In 89 patients, the diagnosis was verified by methods of laboratory diagnostics. Among them 27 patients (27.6%) were diagnosed with GSD, type 9. In all 4 genes mutations in which cause GSD, type 9 infrequent nucleotide replacements were found. The greatest number of patients was with mutations in the *PHKA2* gene—22 (82%), 4 patients (14%) with mutations in the *PHKG2* gene, and by one patient (4%) with mutations in *PHKB* and *PHKA1* genes. At the same time, 7 novel mutations in *PHKA2* gene, 2 mutations in *PHKB* gene, 2 mutations in *PHKG2* gene, and 1 mutation in *PHKA1* gene were revealed. Interestingly that the patient with unique nucleotide replacement *c.897T>G* in *PHKA2* gene has two pathogenic nucleotide replacements *c.784T>G* and *c.857C>T* in *GNPTG* gene which can lead to development of mucolipidosis, type 3. At the same time, according to a clinical picture the patient had characteristics of both pathologies. **Discussion**: The applied technology made it possible to determine that GSD, type 9 is the most frequent among cohort of Russian pediatric patients with GSD and that mutation *c.884G>A* in the *PHKA2* gene is most typical for patients with GSD, type 9a

764 - DNA MFSD8/CLN7 Variants of Late Infantile (LI) Neuronal Ceroid Lipofuscinosis in Argentina

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The neuronal ceroid lipofuscinoses (NCLs) are characterized by retinal damage and brain pathology, loss of neurons, neuroinflammation and autofluorescent lipofuscin-like lipopigments. NCLs are caused by mutations in at least 13 different genes. *MFSD8/CLN7* underlaying the autosomal recessive CLN7 disease (OMIM#610951) is a childhood neurodegenerative disorder caused by the defective lysosomal membrane protein MFSD8/CLN7. Pathological DNA variants may remain elusive to genomic screening in rare recessive conditions. Our

aim is to describe *MFSD8/CLN7* DNA variants in Argentina in individuals #1, #2, and #3, males with LI onset ages, refractory seizures 3.6 to 10 years, psychomotor regression, visual failure, ataxia or other movement abnormalities absent, and positive electron microscopy (EM). Screening for DNA variants were either by NGS with NCL panel or PCR/Sanger. #1: onset 4 years, 8 years old, EM curvilinear profiles (CL); NCL-panel result, MFSD8/CLN7 nonsense variant c.103C>T: p.Arg35* hom.; confirmation by Sanger, segregating in the mother. #2 and #3: EM fingerprint-like profiles (FP), diseases CLN1/CLN2/CLN3 excluded; genes *CLN5*, *CN6*, *MFSD8/CLN7* and *CLN8* screened by PCR/ Sanger. #2, onset age 3.6y, death 15y, *MFSD8/CLN7* intron 2c.63- 4delC het., segregating from the mother, being absent in 200 control chromosomes of Italian and Norafrican population. It was previously identified as heterozygous in a Polish affected individual. #3, onset age 1.6 years, 20 years old (last seen with 11 years), MFSD8/CLN7 nonsense variant c.1444C>T: p.Arg482* het. described before in one French subject. All the 3 patients showed late infantile onset. Interestingly #1(CLN7-disease index subject) of Spanish-Guarany ancestors, with variant p.Arg35* hom. shows positive EM for CL profiles, while individuals #2 of Antic Yugoslavian-Ukranian-German ancestors, and #3 of Spanish-Italian ancestors, differed in the EM with FP-like profiles seen in the second biopsy, and variants intron 2-c.63-4delC het. and c.1444C>T: p.Arg482* het. both of uncertain pathological significance, the NGS panel/exomic/genomic sequencing is pending. This study underscores the importance of the phenotype study, and highlights the impact of NGS on the genetic characterization of rare recessive conditions. A mosaicism of DNA variants in the brain could be hypothesized, and genomic DNA variants search in cerebrospinal fluid could be informative on the elusive genetic background of some NCLs.

765 - Toward Small-Molecule Therapies for a Juvenile Form of Batten Disease

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The *ATP13A2/PARK9/CLN12* gene encodes a lysosomal P5-type ATPase and several mutations in this gene are known to cause an autosomal recessive form of early-onset Parkinsonism, called Kufor-Rakeb syndrome (KRS). Recently, different mutations in this same gene were identified to lead to neuronal ceroid lipofuscinosis (NCL, also called Batten disease), in members of a Belgian family, and to spastic paraplegia 78 in 5 patients from 3 unrelated families. The link between these neurodegenerative diseases is further supported by studies in animal models, but the molecular mechanism(s) underlying the connection between ATP13A2 deficiency and disease pathogenesis is not understood. Nevertheless, all this indicates that ATP13A2 plays a crucial role in neuronal cells and that its deficiency leads to neurodegeneration. In order to elucidate the molecular mechanisms involved in the pathogenesis, we recently initiated functional studies of the ATP13A2 protein, mainly in the context of the juvenile form of NCL (JNCL), which is characterized by the accumulation of autofluorescent storage material in lysosomes. Taking advantage of the fact that ATP13A2 is highly conserved from yeast to humans, we have developed disease models for the Batten disease and Kufor-Rakeb syndrome variant of ATP13A2 in budding yeast. The results obtained so far with these models point to an implication of ATP13A2 in heavy metal resistance, with potentially different pathways involved for different types of metal. In parallel, we are developing a knock-out model for ATP13A2 in zebrafish which we seek to use for further validation of the phenotypes observed in the yeast model. Additionally, we have developed a screening strategy to exploit the combination of both models for a more rapid identification of bioactive compounds with therapeutic potential as orphan drug candidates for JNCL.

766 - Screening for Farber Disease Using a C26Cer Specific Biomarker in Dry Blood Tests (DBS) Followed by *ASAH1* Gene Sequencing

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Farber disease (FD) is a rare autosomal recessive disease caused by mutations in acid ceramidase gene (*ASAH1*). FD is characterized by low ceramidase activity, resulting in accumulation of fatty substances, mainly ceramides. At clinical level, Farber disease is manifesting through hallmark symptoms such as: periarticular nodules, lipogranulomas, swollen and painful joints and a hoarse voice or a weak cry; in addition to these, also hepatosplenomegaly, rapid neurological deterioration or developmental delay are reported. Seven different Farber types were described, with phenotypes varying from mild cases with a longer life expectancy to very severe cases, where the patients do not survive past their first year of life. The diagnostic aspects of FD are poorly developed also due to the rarity of the disease and the significant variability of the clinical symptoms. In the present study, the screening for ceramides and related molecules was performed in Farber affected patients (n = 10), carriers (n = 11) and control individuals (n = 192). This study has the highest number of enrolled Farber patients and carriers reported to present. Liquid chromatography multiple reaction mass spectrometry (LC/MRM-MS) studies revealed that the ceramide C26:0 (C26Cer) and especially its cis-isoform (cis-C26Cer) is a highly sensitive and specific biomarker for FD ($P < .0001$), with pathological values in a range of 39.2-150.0 nmol/L blood (normal range 13.6-23.4 nmol/L blood, N = 192, healthy individuals). We characterized the 2 isoforms of the C26 ceramide by ion mobility—high resolution mass spectrometry (IM-QToF). The new biomarker can be determined directly in the dried blood

spot extract with low sample consumption, easy sample preparation, high reproducibility and it presents the possibility of being used in high throughput screenings

767 - The Mutation p.D313Y is Associated With Organ Manifestation in Fabry Disease

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Fabry disease (FD) is a multisystem lysosomal storage disorder caused by mutations in the *GLA* gene. The clinical significance of the mutation p.D313Y has been debated. We performed a retrospective chart analysis of clinical (neurological, cardiac, renal, ophthalmological), genetic, and biochemical (lyso-globotriaosylsphingosine, lyso-Gb3; enzyme activity) data in all our patients with the mutation p.D313Y. Seventeen patients from 7 families (12 female, 5 male; age range 4-51 years) were included. Typical symptoms and organ manifestations compatible with FD were identified in 13 patients. Cerebrovascular events occurred in 5 females. Pain or acroparaesthesia were reported by 8 patients. Ocular manifestations included cornea verticillata in one patient and mild retinal vascular tortuosity in 7 patients. Lyso-Gb3 was elevated in 2 females who suffered from cerebrovascular involvement. Classical cardiac, renal or skin manifestations could not be identified. The mutation p.D313Y in the *GLA* gene may lead to organ manifestations and elevation of the Fabry-specific biomarker lyso-Gb3. Neurological symptoms (stroke and pain) and ocular manifestations seem to be the leading findings. Annual routine visits are recommended for patients carrying the p.D313Y mutation. Enzyme replacement therapy should be considered in symptomatic patients.

768 - Lyso-SM-509 is a Highly Specific and Sensitive Biomarker for the Identification of Niemann-Pick Patients—A 30 Months Study

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Niemann-Pick Type C (NPC) disease is an autosomal recessive disease caused by mutations in NPC1 or NPC2 genes translated in defects of the lysosomes cholesterol transport system leading to abnormal accumulation of cholesterol and glycolipids in the lysosome. The severity of the NPC symptoms and the fact that treatment options are available makes NPC diagnosis of high most importance. We present data from a 30 months screening study in a global cohort of potential Niemann Pick patients. Diagnosis was performed in a stepwise manner, using lyso-SM-509 biomarker determination, followed by sequencing of NPC1/2 genes. The levels of lyso-SM-509 in blood and can be used for the easy diagnosis of NPC patients and for the monitoring of the disease progression. Determination of lyso-SM-509 is performed by LC/MRM-MS in plasma, serum, EDTA blood and dried blood spots (DBS). We screened over a world-wide cohort using lyso-SM-509 analysis in DBS and we identified 456 NPC individuals (284 affected and 172 carriers) and 233 NPA/B individuals (157 affected and 76 carriers). The diagnosis was confirmed by sequencing of the NPC1/NPC2 by single gene sequencing, NGS, or MLPA. In NPC1/2 sequencing negative patients with increased lyso-SM-509 concentrations the sequencing of sphingomyelinase (SMPD1) gene was done. Lyso-SM-509 has a sensitivity of 100% and specificity of 99.15% for NPC1/2. Most of the NPC cases were diagnosed in the age of 3 to 10 years (30.65%). We could identify over 6797 NPC1 alleles, 73 NPC2 alleles and 1641 SMPD1 alleles. From the 312 unique variants found (213 NPC1, 14 NPC2, and 85 SMPD1) only 142 were previously published (45%). Most NPC cases were linked with detailed clinical information; the most common symptoms were: hepatomegaly (63.6%), splenomegaly (55.8%), neurodevelopment delay (53%), ataxia (35.6%), psychopathology (34.6%), brain atrophy (30.4%), ophthalmoplegia (30.4%), spasticity (30%), dystonia (24.4%), and seizures (20.7%)

769 - A Novel Mutation in the *Neu1* Gene Causing Sialidosis Type1 in Two Saudi Patients

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Sialidosis (OMIM# 256550), an autosomal recessive lysosomal storage disease is caused by mutations in the *NEU1* gene encoding the enzyme neuraminidase. Sialidosis is classified into two types on the basis of phenotype and age of onset. Patients with Sialidosis type 1, present at 8-25 years of age with decreasing visual acuity, macular cherry red spots and or myoclonus. Myoclonus is usually an early feature of the disease and begins in the limbs. As the disease progresses they may develop seizures. We report two unrelated Saudi adolescents who presented with macular cherry red spots. They had

normal growth, intellect and no dysmorphic features. They had reduced visual acuity and were otherwise asymptomatic. Family history was unremarkable. Cultured fibroblast from one patient showed markedly reduced activity of neuraminidase 0.02 nmol/min/mg (reference: >0.10). However, molecular testing revealed a novel mutation in *Neu1* (c.451G>A; p.Val151Ile) which has not been previously reported as pathogenic.

770 - Study on the Use of Forced Oscillation Technique (FOT) in Patients With Lysosomal Storage Diseases

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Background: The loss of enzymatic activity in lysosomal storage diseases (LSD) results in progressive cellular accumulation of macromolecules. In these patients, respiratory dysfunction is usually the leading cause of morbidity and mortality. Thus, clinical assessments and objective measures of lung function, such as spirometry, are crucial for prompt diagnosis and management. The use of spirometry, however, is limited to patients who can perform forced expiration. The "forced oscillation technique" (FOT), on the other hand, is performed during tidal breathing; thus, it is amenable to most patients who fail spirometry test. This study describes the feasibility of using FOT in patients with LSD. **Methods:** This study was conducted at Tawam Hospital Outpatient Services (Al Ain, Abu Dhabi) between January 2016 and May 2017. During this period, 22 patients with confirmed LSD were enrolled in the study; their mean $\pm$ SD age was 15 $\pm$ 13 years. Two patients were excluded because of tracheostomy. Their airway resistance was assessed using FOT at 5 Hz (R5, in $cmH_2O.s.L^{-1}$), and the results were expressed as R5 z-scores. **Results:** Thirteen patients with mucopolysaccharidoses (MPS) were studied. The FOT study was successful only in two patients (MPS-I and MPS-IVa), despite several attempts. The R5 z-scores for these two patients were 4.3 and 5.4 (previously failed spirometry). The ten patients with MPS-III failed to do the test. A patient with Niemann-Pick disease type C had R5 z-score of 4.2, a patient with Mucolipidosis type III had R5 z-score of 2.4, a patient with Alpha-mannosidosis had R5 z-score of 2.4, and a patient with Gaucher disease type I had R5 z-score of 2.7. A patient with Fabry disease had R5 z-score of 0.68, Tay Sachs disease -1.0, and Gaucher type I 0.16. **Conclusions:** Most patients with MPS failed to comply with the FOT study maneuver. Nevertheless, this methodology uncovered a remarkably increased airway resistance in patients with LSD, indicating these patients had profound respiratory disease. Regular use of FOT in patients with LSD should be considered.

771 - Body Composition in Infantile Onset Pompe Disease

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Study's objective: To analyze body composition in Infantile Onset Pompe Disease (IOPD). **Methods:** The study included 8 patients with genetically confirmed IOPD (M/F ratio 1:1, age range 18 months-16 years): 5 with the classical phenotype (4/5 non-ambulatory all requiring ventilatory support) and with 3 non-classical IOPD (all ambulatory, 1/3 with ventilatory support). All pts were on ERT and on a high-protein diet. We recoded anthropometric measures (body weight, height, and BMI) and Bioelectrical Impedance Analysis (BIA) using a BIA 101 device (Akern, Italy). The following BIA measures were considered: Fat Free Mass (FFM), Fat Mass (FM), Phase Angle (PhA) and Total Body Water (TBW). **Results:** Patients were classified according to their BMI Z-score as severe underweight (<-3, 1 pt), moderate underweight (>-3 to ≤-2, 1 pt), mild underweight (>-2 to < -1, 2 pts), normal weight (≥ -1, 4 pts). Two children <5 years, lacking reference BIA parameter values, were excluded from evaluation. Among the remaining 6 pts, only one with non-classical IOPD without signs of muscle weakness showed normal anthropometric and BIA parameters. Normal BMI was recorded in 4/6 patients, 3 showing an elevated FM (2 non-ambulatory with classical-IOPD and 1 with non-classical IOPD with abnormal gait). Two patients with classical IOPD were underweight, both showing a reduced FM. PhA was below normal range in 4/6 patients, 1 with severe classical IOPD and 3 with non-classical phenotype. The %TBW was reduced in 3 patients, while in the remaining 3 patients the %TBW was above the normal range. **Conclusions** These preliminary data in IOPD patients, partially parallel those reported in late onset Pompe disease, showing a discrepancy between BMI and FM, highlighting that BMI alone may underestimate the increase in FM.

772 - Danon Disease in a Female Girl With a Severe Cardiomyopathy—A Case Report

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Danon disease, also known as X-linked vacuolar cardiomyopathy and myopathy, is caused by mutation in the gene encoding lysosome-associated membrane protein-2Danon disease is an X-linked dominant disorder predominantly affecting cardiac muscle. Multisystem involvement can be observed, leading to more severe clinical manifestations including skeletal muscle involvement, ocular manifestations and mental retardation, specially in male patients. It is caused by mutations in the gene

encoding lysosome-associated membrane protein-2 (*LAMP2*). **Objective:** To describe a case of Danon syndrome in a girl with a severe cardiomyopathy. **Methods:** Case Report. Patient A, 20 years old, female, only child of a young and non consanguineous couple. She had been asymptomatic for the last 18 years. A severe cardiomyopathy was diagnosed by the time she was evaluated for a surgery procedure. Echocardiography: laterobasal, middle and apical side wall hypertrophy. Diastolic dysfunction grade II. Wolf Parkinson White arrhythmia. Muscle enzymes were normal. A multigene testing panel for hypertrophic cardiomyopathy revealed a *LAMP2* mutation classified as very probably pathogenic variant - NP_002285.1: p.Asn242Thrfs*41. The mother (a presumed carrier of the mutant gene, that is under mutational analysis) and the father of the patient have no evidence of cardiomyopathy. **Discussion:** *LAMP2* mutations typically cause multisystem disease, but also can present as a primary cardiomyopathy. Some studies show that both male and female patients can manifest an equal prevalence of dilated and hypertrophic cardiomyopathy. Men are affected before the age of 20 years, whereas most affected women seem to develop cardiomyopathy in adulthood. Our patient probably developed the cardiomyopathy from infancy to adolescence period, which is not usual. Cardiomyopathy is a genetically heterogeneous heart disease with limited therapeutic options. A strict cardiologic follow-up is mandatory considering the risk of severe adverse events including sudden death, and the eventual requirement of a heart transplant. We reinforce the need to apply multigene testing panels for cardiomyopathy both in pediatric and in adult patients, due the potential benefits for further treatments and genetic counseling.

773 - Prenatal Hypertrophic Cardiomyopathy due to Danon Disease

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Background: Pediatric cardiomyopathy poses diagnostic challenge. Inborn errors of metabolism may explain hypertrophic forms (HCM) associated with impaired energy production or substrate storage. In rare prenatal-onset presentations, abnormal glycogen storage due to PRKAG2 mutations has been described. In some patients with HCM, Danon disease, a lysosomal glycogen storage disease due to X-linked lysosome-associated protein-2 (*LAMP-2*) mutations, may underlie. Clinical features include Wolff-Parkinson-White syndrome, skeletal myopathy, variable mental retardation and retinal disease. Most cases in males reveal at adolescence, whereas carrier mothers present approximately ten years later with more protracted course. Even if next generation screening has allowed identification of younger patients, no fetal or neonatal cases are reported. **Case report:** We describe the fifth child of French nonconsanguineous parents. At 32 weeks of GA, mother reported reduced fetal movements and echography revealed HCM. Birth was induced by cesarean section at 37 weeks of GA because of hydrops fetalis. The boy necessitated ventilation due to respiratory and cardiac distress. Marked hypotonia and rare spontaneous movements were noted with facial amimia, absent swallowing and bilateral microphthalmia. Severe HCM was confirmed and death occurred at day 12 due to cardiac failure. Histochemistry of cardiac muscle samples revealed vacuolation and glycogen deposition. Genetic testing using next generation sequencing gene panel, including LAMP2 and 51 other cardiovascular genes, found a hemizygous missense mutation in exon 3 (c.299c>T) predicted to truncate LAMP2 protein. **Discussion:** The fetal heart preferentially utilizes oxidation of pyruvate, provided by glycogenolysis, for energy supply. Antenatal onset of HCM may therefore involve glycogen storage. In Danon disease, HCM may be extreme, and explained the largest heart by weight ever reported in literature in an adolescent. The earliest reported symptom onset was at age 4 months in a male suffering from hypotonia and cardiac failure due to marked vacuolar myopathy and severe HCM. Antenatal onset of HCM in Danon disease is not surprising and mutational analysis of *LAMP2*, besides of *PRKAG2*, should be proposed in diagnostic work-up of fetal cardiomyopathy, in order to offer genetic counselling and treatment to families. **Conclusion:** Danon disease should be integrated in diagnostic work-up of fetal cardiomyopathy and non-immune hydrops fetalis.

774 - The Clinical Features and Diagnosis of Metachromatic Leukodystrophy: A Case Series of Turkish Pediatric Patients With Three Novel Mutations

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Objectives: Metachromatic leukodystrophy (MLD) is a rare autosomal recessively inherited lysosomal storage disease caused by lack of arylsulfatase A (ARSA), which is required for the hydrolysis of sulfated glycosphingolipids. Mutations of *ARSA* gene are responsible for this disorder. Enzyme deficiency results in excessive accumulation of sulfatide in myelin of the nervous system, bile ducts of the liver, and distal tubules of the kidney. Based on the age of the symptoms onset, MLD can be divided into three forms: late infantile, juvenile, and adult. **Methods:** In this study, we aimed to determine the epidemiological, clinical and biochemical profiles of 5 Turkish MLD patients. Diagnosis was confirmed with the analysis of

ARSA gene in all patients. **Results:** 4 of the patients were late infantile form and one was juvenile MLD. Delay of motor milestones, frequent falls and bulbar problems were the most common symptoms. Spasticity, decreased bulk of muscles and brisk deep tendon reflexes were the other findings of the patients. MRI of patients showed a leukodystrophic pattern with periventricular deep white matter involvement. All cases had significantly decreased leukocyte ARSA levels from 2 to 9 nmol/per mg of protein/hr (normal level >60 nmol/per mg of protein/h). With the analysis of the *ARSA* gene of our patients with different clinical variants of MLD, three novel mutations: c.473G>A(p.C158Y), c.893G>A(p.G298D), c.905G>A(p.C302Y) were identified. **Conclusion:** Our data once more showed that there are still several mutations to be discovered in *ARSA* gene. Molecular characterization of the disease is important for genotype–phenotype correlation, for understanding the molecular basis of the disease, for prenatal diagnosis and for developing new therapeutic strategies like chaperones.

775 - Oropharyngeal Dysphagia in Infantile-Onset Pompe Disease

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Background: Enzyme replacement therapy (ERT) has significantly prolonged lifespan of infantile-onset Pompe disease (IOPD), leading to the emergence of a new phenotype including oropharyngeal and swallowing disorders. **Method:** Oral feeding and swallowing capacities were investigated in 5 IOPD patients (2- to 7-year-old, treated by ERT), using clinical observation of repeated meals, speech therapist evaluation, videofluoroscopy swallow study (VFSS) and endoscopic nasopharyngeal evaluation. **Results:** All patients had facial muscle weakness. Macroglossia and tong weakness were also reported in 3 of 5. Articulation was disordered with reduced speech intelligibility and hypernasal resonance. Oral feeding difficulties were reported in all the patients with various degrees of severity, limited to difficulties to close the mouth during mastication for one patient, to mild ($n = 3$) to severe ($n = 1$) mastication disturbances with a high risk of aspiration for the others. VFSS demonstrated no abnormality in the pharyngeal and esophageal stages of swallowing, excepted for one patient presenting no muscle contraction and massive penetration and aspiration. The latter was the most severely affected patient by IOPD. **Conclusion:** Mastication and the oral stage of swallowing were defective in all the patients whereas impairment of the pharyngeal and esophageal stages was only present in the most severely affected patient, suggesting that degree of swallowing impairment was related with overall physical strength and function. Because of the predominantly involvement of the oral stage of swallowing, early treatment by a speech therapist may be recommended.

776 - Simple and Reliable Preparation of Internal Quality Control Material for the Confirmatory Diagnosis of Lysosomal Storage Disease

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Confirmatory diagnosis of lysosomal storage diseases (LSD) is performed by determination of lysosomal enzymatic activities in different biological fluids. Internal quality assurance system for lysosomal enzyme analysis is complex because commercial control samples for enzyme assays are not available. We developed and evaluated leukocytes homogenates as internal quality control (QC) material for their use in the confirmatory lysosomal enzymatic assays. **Methods:** Once leukocytes homogenates were obtained and isolated from whole blood collected in heparinized tubes, the pellet was split in two in order to count with two types of control material: a normal and inactivated QC material. Inactivated quality control (IQC) material was obtained by incubating the homogenate at 80° C for 2 hours and the other was kept intact. Both were store in aliquots at −80 C until the performed of enzyme assays. The usefulness for their application in the routine analysis of ten lysosomal enzymes was evaluated. Normal QC (NQC) was evaluated by the determination of day-to-day variance by repeated analysis of QC material sample in a period of 10 months. IQC was processed at each analytical run to ensure correct identification of enzymatic deficiencies in the assay. **Results:** The day-to-day coefficient of variation (CV) average of NQC for the evaluated enzymes was 13.9%. CVs range from 9.3% to 19.7%. For all enzymatic assays IQC had results close to blank. **Conclusion:** The use of QC material obtained from leukocyte homogenates is a useful alternative for the internal quality assurance system of any laboratory performing diagnostic tests for LSD to ensure their results and diagnosis.

777 - Nephrophatic Cystinosis: Report of Mexican Population

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Introduction: Cystinosis is a rare autosomal recessive metabolic disorder caused by a mutation in the *CTNS* gene (MIM 219800). Biochemically characterized by an anormally high intracellular content of free cystine in different organs due to a transport defect of cystine in lysosomes, cytinosine, cystine's transporting protein, causing an abnormal deposition of cystine crystals in lysosomes mainly in the kidneys, cornea, thyroid gland, pancreas, muscle, bone marrow, and nervous system.

Objective: Report the clinical characteristics of Mexican patients with nephrophatic cystinosis. **Material and Method:** Patients with diagnosed confirmed to nephropatic cystinosis 1976 to 2017. **Results:** 44 Mexican patients (17 females and 27 males) from 31 different families with diagnosis of nephrophatic cystinosis. All subjects had Fanconi's syndrome, 41 were diagnosed with infantile cystinosis, and three others with juvenile cystinosis. Start of symptoms was at 2.2 years, (8 months to 15 years), age to diagnose was 5.1 years, four patients were diagnosed after of kidney transplant, eighteen died, twenty four alive, and two lost. The symptoms; 39/44 polyuria and polidipsy, 31/44 dehydration, 31/44 vomiting and hyporexy, 36/44 failure to thrive, 29/44 rickets, 18/44 hypothyroidism, 35/44, 31/44 kidney terminal disease, 12/44 received kidney transplant, 35/44 showed cystine corneal crystals deposits, 19/44 had rickets, and 14/44 glucosuria. Only 21/44 had DNA study, 16 with severe phenotype, 3 moderate, and 2 very severe. 4/44 were diagnosed by cystine quantification in fibroblast cultures, four by renal biopsy, one by corneal and conjunctiva biopsy, one by bone marrow biopsy, 24/44 by an eye exam and the six others due to their sibling's similar medical history with the same diagnosis. **Conclusion**: Mexican patients had the similar symptoms described, delayed diagnoses, and high mortality is presented.

V) Lysosomal Disorders: Treatment, Enzyme Replacement Therapy (778 to 813)

778 - Anti-Agalsidase Antibodies are Associated With Severe Kidney Disease and Truncated Alpha-Galactosidase A in Fabry Disease

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Fabry disease (FD) is a progressive and multisystemic X-linked disease in which mutations in *GLA* gene lead to alpha-galactosidase A deficiency with subsequent increase of glycosphingolipids such as *Lyso*Gb3. Enzyme replacement therapy (ERT) with agalsidase alfa or agalsidase beta has been available since 2001 in Europe with the development of anti-agalsidase antibodies of unclear significance. We aimed to determine the clinical and biological impact of these antibodies. From December 2014, we have initiated the *French FAbry Biobank and RegistrY* (FFABRY) as a multicenter, independent, and collaborative clinical database associated with a biobank gathering biological samples from French patients with genetic and/or enzymatic diagnosis of FD, treated or not with ERT. Anti-agalsidase status for both ERT was prospectively assessed with a home-made ELISA. Plasma Lyso-Gb3 was measured with by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). Enzyme inhibition assays were performed in plasma and PBMCs. Among the 103 patients (53 males) included, 18 of the 45 (40.0%) males and 2 of the 25 (8.0%) females exposed to agalsidase were seropositive without difference between molecules. Antibody titers were correlated with enzymatic inhibition (r^2>0.77, $P < .0001$) and plasma *lyso*Gb3 in men ($r^2 = 0.52$; $P < .0001$). All IgG subclasses were observed but IgG4 reached the highest concentrations (0.05 to 1.45 mg/mL). Seropositivity, but not serum inhibition was associated with higher frequencies of renal transplantation or dialysis (hazard ratio 30.2; $P = .002$). Mutations leading to truncated alpha-galactosidase A were significantly associated with antibodies (relative risk 2.88; $P = .006$) in men and correlated with worse cardiac MSSI in women. Anti-agalsidase antibodies are associated with a more severe renal disease and higher plasma *lyso*Gb3. Systematic and independent screening for anti-agalsidase antibodies and associated mutations should be

performed in FD patients. A better understanding of the role of genotype in antibody development is needed. New therapeutical algorithm should already be defined: (i) increase the dose of ERT, (ii) associate or switch to chaperone therapy to hide epitopes, and/or (iii) use immunosuppressive therapies.

780 - Evaluation of a Case With Niemann-Pick Type C Under Miglustat Therapy

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Background: Niemann-Pick Type C (NPC) is an autosomal recessive neurovisceral lipid storage disorder that has genetic, biochemical and clinical heterogeneity. It is characterized by the accumulation of non-esterified cholesterol and glycolipids in lysosomes due to impaired intracellular transport of cholesterol. Miglustat is a glycosphingolipid synthesis inhibitor and is the first approved, disease-specific therapy for the treatment of NPC patients that disrupts the formation of lipids known as glycosphingolipid and thus prevents build-up of lipids in the brain. This report aims to evaluate the pre-treatment and post-treatment clinical and laboratory characteristics of a case who received treatment with Miglustat for 29 months. **Case Report:** The 45-month-old male patient was initially admitted to our outpatient clinic when he was 1-year-old with complaints of reduced movement, jaundice, swelling in the abdomen and difficulty in eye tracking. The family history revealed that the parents were cousins and that the elder brother was lost when he was 3.5 months old due to liver failure. The *NPC1* gene molecular analysis performed at the age of 16 months revealed a new homozygote mutation of p.W949G (c.2845T> G). Treatment with Miglustat at a dose of 100 mg/day was initiated. No drug-related side effects were observed. The dose was increased to 200 mg/day when the case was 40 months old. The clinical and laboratory characteristics at the initiation and completion of the treatment of the case, who was 45 months old at the final evaluation and who is still alive, are given in Table 1. **Conclusion:** Neurological findings moderately deteriorated and SDSs of height, weight, and head circumference worsened. On the other hand, no significant change was detected in laboratory evaluation. No drug-related side effects occurred. These findings indicate no clear benefit from a course of 29 months of Miglustat treatment in this case with NPC type 1.

781 - Early Hematopoietic Stem Cell Transplantation in a Patient With Severe Mucopolysaccharidosis II: A 7 Years Follow-Up

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Mucopolysaccharidosis type II (MPS II - Hunter syndrome) is an X-linked lysosomal storage disorder caused by a deficiency in the enzyme iduronate-2 sulfatase (I2 S), leading to the accumulation of the glycosaminoglycans, affecting multiple organs and systems. Enzyme replacement therapy does not cross the blood brain barrier, limiting results in neurological forms of the disease. Another option of treatment for severe MPS, hematopoietic stem cell transplantation (HSCT) has become the treatment of choice for the severe form of MPS type I, since it can preserve neurocognition when performed early in the course of the disease. To date, only few studies have examined the long-term outcomes of HSCT in patients with MPS II. We describe the seven-year follow-up of a prenatally diagnosed MPS II boy with positive family history of severe MPS form, submitted to HSCT with umbilical cord blood cells at 70 days of age. Engraftment after 30 days revealed mixed chimerism with 79% donor cells; after 7 years engraftment remains at 80%. I2 S activity 30 days post-transplant was low in plasma and normal in leukocytes and the same pattern is observed to date. At age 7 years growth charts are normal and he is very healthy, although mild signs of dysostosis multiplex are present, as well as hearing loss. The neuropsychological evaluation (Wechsler Intelligence Scale for Children—Fourth Edition—WISC-IV), disclosed an IQ of 47. Despite this low measured IQ, the patient continues to show improvements in cognitive, language and motor skills, being quite functional. We believe that HSCT is a therapeutic option for MPS II patients with the severe phenotype, as it could preserve neurocognition or even halt neurodegeneration, provided strict selection criteria are followed.

782 - Monitoring Physical Activity Using a Wearable Device in Pompe Disease

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Late-onset Pompe disease (LOPD) is a rare metabolic disease with an age of onset of $\geq$ 1 year. Patients experience a progressive decline in respiratory and skeletal muscle function which can lead to decreased mobility as well as respiratory weakness. Activity tracking devices offer a unique opportunity to monitor Pompe patients' mobility in real world settings. The Pompe Disease Symptom Scale (PDSS) is a newly developed instrument aimed at capturing the disease symptoms from patients' perspective. The scale captures breathing difficulties, fatigue and tiredness, muscle weakness and muscle ache, pain, and headache. Part of the ongoing validation

work for this instrument includes administering it to Pompe patients and exploring correlations between the PDSS scores and patient monitored activities using a commercially available activity tracker. The objectives are to 1) Evaluate the measurement properties of the PDSS; 2) Explore the relationship between patient reported symptom severity and device measured activity, which includes step count, distance, and elevation, using an activity tracker in a real-world setting. LOPD patients were given Fitbit One devices for 6-8 weeks for activity monitoring and separately engaged in observational data donation on the PatientsLikeMe website. PDSS scores were captured at baseline and exit; during the study patients also reported general and Pompe specific symptoms on the PatientsLikeMe portal. Daily activity, including steps, patient engagement, and peak 6-min walk intervals were measured during the study. Psychometric properties of the PDSS were tested to develop a preliminary score. The association between PDSS and device measured activity were examined. Median age of patients enrolled was 44 years, 83% (n = 29) female, with 94% diagnosed $\geq$ 18 years (n = 33). Median activity (step count) of the population was less than other publically available chronic disease populations ($\sim$3000 steps/day), activity patterns varied by age, age at onset, PDSS, and use of enzyme replacement treatment (ERT). Baseline PDSS was negatively correlated with total steps (r = -0.32) and peak 6-min walk test ($r = -0.52$). Members in the first quartile of the PDSS (least severe) reported nearly twice as much activity as members in all other quartiles. Using activity trackers as part of validation activities for newly developed PRO instruments offer novel insights from the real world on the interpretation of instrument scores.

783 - Pegunigalsidase Alfa for Treating Fabry disease—Immunogenicity and Pharmacokinetics Results From Phase 1/2 Studies

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Pegunigalsidase alfa (PRX-102) is a novel, PEGylated, chemically modified, α-galactosidase A enzyme replacement therapy for the treatment of Fabry disease (FD). Immune responses to therapeutic protein could potentially impact its efficacy and/or safety. In FD, an X-linked disorder caused by the loss of function of the lysosomal enzyme α-galactosidase-A, anti-drug antibody (ADA) formation toward the enzyme has been shown to occur in a high percentage of male patients, especially with nonsense mutations. **Objective**: To evaluate the safety, pharmacokinetics (PK), and efficacy parameters of FD patients treated with Pegunigalsidase alfa, in a phase 1/2 clinical study. **Methods**: A total of 16 FD patients participated in a Phase 1/2 study in which Pegunigalsidase alfa was administered IV every other week in three cohorts (cohort 1, 2 and 3 received 0.2, 1.0, and 2.0 mg/kg). Safety and efficacy parameters were evaluated in addition to PK and immunogenicity. **Results**: PK: Pegunigalsidase alfa has a favorable PK profile [maximum concentration (Cmax) and overall enzyme amount (AUC)] with higher amount of active enzyme available throughout the 2-week treatment intervals. Immunogenicity: Of the 16 patients who completed 1 year of treatment, three (3) male patients developed treatment induced IgG antibodies to pegunigalsidase alfa (ADA+); this comprised of two patients from Cohort 1 (0.2 mg/kg) and one from Cohort 2 (1 mg/kg). There were no ADA+ patients in Cohort 3 (2 mg/kg). All 3 ADA+ patients became negative following 1 year of treatment, consistent with reduced immunogenicity and induced tolerance during pegunigalsidase alfa treatment. Impact of ADA+ on PK: The 2 ADA+ patients in Cohort 1 exhibited a distinct and reversible effect on PK profile resulting in decreased Cmax and AUC at 3 and 6 months compared to Day 1. PK profile and parameters improved and returned to the baseline profile after 12 months of treatment, suggesting that the ADA impact on Pegunigalsidase alfa activity was transient and reversible. The ADA+ patient in Cohort 2 had low ADA titers and had stable PK parameters throughout the study. **Conclusions**: Pegunigalsidase alfa has an extended circulatory half-life and higher AUC together with a low immune response. The improved PK profiles of Pegunigalsidase alfa is associated with reduced immunogenicity that may reflect long term induction of tolerance in previously seroconverted patients and has the potential for clinical benefit in treating FD patients.

784 - Characterization of a Chemically Modified Plant Cell Culture Expressed Human α-Galactosidase-A Enzyme for Treatment of Fabry Disease

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Protalix, Carmiel, Israel

Fabry disease is an X-linked recessive disorder caused by the loss of function of the lysosomal enzyme α-Galactosidase-A. Pegunigalsidase alfa (PRX-102) is a novel enzyme for the therapy of Fabry disease expressed in a BY2 Tobacco cell culture. Pegunigalsidase alfa is chemically modified, resulting in a cross-linked homo-dimer. **Objective:** Characterization of the in vitro and in vivo properties of Pegunigalsidase alfa. **Results:** Chemical reaction parameters were optimized to select the prime PEG moiety not to interfere with enzymatic activity and to gain the maximal stabilization effect. The obtained molecule demonstrates preserved 3D structure, identical glycosylation profile, improved kinetic parameters and thermal stability. Pegunigalsidase alfa is taken up by primary human Fabry fibroblasts and is targeted to the lysosome. Pegunigalsidase alfa has a relatively simple glycosylation pattern, characteristic to plants, having mainly tri-mannose structures with the addition of either α (1–3)-linked fucose or β(1–2)-linked xylose, or both, in addition to various high mannose structures. Results show that Pegunigalsidase alfa has prolonged *in-vitro* stability in plasma and under lysosomal-like conditions Pegunigalsidase alfa maintains over 80% activity following 10 days of incubation. Pharmacokinetic profile of Pegunigalsidase alfa measured in male Fabry mice shows an increased $t_{1/2}$ of 581 minutes. **Conclusions:** Our data demonstrate that Pegunigalsidase alfa has superior stability and prolonged circulatory half-life. Therefore, Pegunigalsidase alfa is a promising alternative for treatment of Fabry disease.

785 – One-Year Follow-Up Safety and Efficacy of Fabry Disease Patients Treated by IV Administration of Pegunigalsidase Alfa—A Novel, PEGylated, and Cross-Linked Homodimer α-Galactosidase-A Enzyme

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Fabry Disease (FD) is an X-linked disorder caused by the loss of function of the lysosomal enzyme α-galactosidase-A. Pegunigalsidase alfa (PRX-102) is a novel, PEGylated chemically modified α-galactosidase A, which makes the enzyme a more stable homo-dimer, expressed in ProCellEx® system using plant cell line in suspension. The enzyme has enhanced pharmacokinetic properties including a half-life of approximately 80 hours, and substantially higher AUC (overall enzyme amount). The current report is a 1-year follow-up of FD patients treated with Pegunigalsidase alfa including subgroup analysis of a classic phenotypic presentation of the disease as part of two phase 1/2 clinical studies: PB-102-F01 and its extension study PB-102-F02. **Objective:** To evaluate the safety, pharmacokinetics and efficacy parameters on patients treated with Pegunigalsidase alfa administered IV every 2 weeks. **Methods:** Symptomatic FD naïve male and female patients (>18 y.o.) were recruited to the study. Pegunigalsidase alfa was administered IV every other week in three cohorts (cohort 1, 2 and 3 received 0.2, 1.0 and 2.0 mg/kg pegunigalsidase alfa, respectively). Primary outcome consists of safety; adverse events, clinical laboratory, physical examination and ECG. Secondary outcome includes pharmacokinetics, and exploratory efficacy parameters: plasma Gb3 and Lyso-Gb3; kidney function: eGFR and proteinuria; and BPI questionnaire to assess pain. Additional parameters were kidney Gb3 inclusion bodies in renal peritubular capillaries (PTC) (BLISS score; baseline and 6 months assessed by biopsies) cardiac MRI and MSSI (Mainz Severity Score Index). **Results:** After 1 year of treatment, classic FD patients presented a total reduction of Gb3 inclusions in PTCs of 84.1 ± 3.3% (mean ± SE) in all 3 doses; a mean reduction in plasma Gb3

and Lyso Gb3 of 33.3 ± 7.6% and 57.6 ± 6.8%, respectively, stability in annualized eGFR and cardiac MRI, and an improvement in MSSI and BPI. Pegunigalsidase alfa was well tolerated, with the majority (98%) of adverse events being mild and moderate. Only one of the patients experienced an event of hypersensitivity, and only 3 patients, (~ 19%) developed treatment induced antibodies. **Conclusions:** During 12 months of treatment Pegunigalsidase alfa demonstrated improved PK parameters, effectiveness in various disease endpoints and was well tolerated.

786 - ERT and intrathecal baclofen Therapy in Patient With Hunter syndrome

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Background: Enzyme replacement therapy (ERT) with Idursulfase has clinical benefits in Hunter syndrome, but has limited effect on musculoskeletal system. Many patients despite ERT suffer from progressive joint stiffness and contractures with profound loss of joint motion and spasticity with walking on their toes. Intrathecal baclofen therapy (IBT) is commonly used in childhood spasticity, so we used it together with ERT in our patient. **Method:** Patient was treated with intravenous Idursulfase from 3 years and at the age of 9 years on the basis of progressive loss of joint motion, we indicated baclofen test, which showed reduction of spasticity and improvement of his fine motor function. Then programmed baclofen pump was inserted used modified subfascial technique for pump insertion with pump catheter implanted intrathecally at L1-L2 interspace and patient was gradually set to current baclofen dose of 250 μg /day. **Results:** During the two further years of continuous ERT and IBT we observed significant reduction of spasticity with increase range of active and passive movements. Mainly it was facilitation in fine motor function, including dismounting of toys and grabbing objects, many of which patient was not able to execute properly before. We observed also better walking pattern but with persistence of previous stiff contractures on gastrocnemius muscle bilaterally, which can be explained by late beginning of IBD. **Conclusion:** 2-year experience on both ERT and IBT showed improvement of patient's quality of life with increased range of motion, improved walking pattern and better fine motor function. We consider it as feasible treatment of muscle spasticity in selected patients with Hunter syndrome.

787 - Long-Term Safety and Efficacy of Intracerebroventricular Enzyme Replacement Therapy With Cerliponase Alfa in Children With CLN2 Disease: Interim Results From an Ongoing Multicenter Extension Study

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Background: CLN2 disease, a rare, inherited, pediatric, neurodegenerative lysosomal storage disorder caused by TPP1 deficiency is characterized by seizures, ataxia, language and motor function loss, blindness, and early death. A phase 1/2 study (NCT01907087) demonstrated that intracerebroventricular (ICV) infusion of 300 mg Cerliponase alfa, a recombinant human TPP1 enzyme, every other week for 48 weeks was associated with attenuation of CLN2 disease progression. This extension study (NCT02485899) assesses the long-term safety and efficacy of ICV-administered Cerliponase alfa in children with CLN2 disease for up to 240 weeks. Design: Subjects who completed the phase 1/2 study enrolled in this open-label extension study, and continued receiving 300 mg Cerliponase alfa qow. Cumulative data from both studies were used to evaluate long-term safety (assessed by analysis of adverse events (AEs)) and efficacy (assessed by changes in motor and language (ML) functions using the CLN2 clinical rating scale). **Results:** 24 subjects were initially treated with Cerliponase alfa in the phase 1/2 study (9 male, 15 female, mean (SD) age 4.3 years (1.24)); 23 subjects enrolled in the extension study (74 to 124 weeks total exposure). All had AEs; most were Grade 1-2. Common AEs included pyrexia, hypersensitivity and convulsion. Nineteen (79%) subjects had at least one serious AE, which were mostly consistent with neurodegenerative disease in a pediatric population. Significant attenuation of the rate of decline in ML score (mean (95% CI): 0.32 (0.13, 0.52) points/ 48 weeks, $P < .0001$) was observed compared with a rate of decline of 2.0 points/48 weeks in untreated patients. The responder (<2 point loss) rate at 81 weeks (87%, $P = .0002$) was unchanged compared to that observed at 48 weeks, suggesting a persistent treatment effect. **Conclusions:** These data suggest that enzyme replacement therapy with ICV-administered Cerliponase alfa has an acceptable safety profile and a sustained effect over time.

788 - Antibody Formation to Enzyme Replacement Therapy in Classic Infantile Pompe Disease: Effects of Immunomodulation in Naive Patients

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Objective: To evaluate whether immunomodulation at the start of enzyme replacement therapy (ERT) prevents formation of anti-recombinant human acid alpha-glucosidase (rhGAA) antibodies in classic infantile Pompe patients. **Methods:** Patients were treated with 4 weekly doses of Rituximab, weekly administration of methotrexate, monthly intravenous immunoglobulins and alglucosidase alfa 40 mg/kg/week. Antibody titers were measured using ELISA and immunoprecipitation; also, their neutralizing effects were determined. Clinical efficacy was evaluated by (ventilator-free) survival, reduction in left ventricular mass index and improvement of motor function. **Results:** Three classic infantile patients, one Cross Reactive Immunological Material (CRIM) negative, and two CRIM positive patients participated. B cell depletion was obtained immediately after initiation of immunomodulation. B cell recovery was observed after 7 months. Despite immunomodulation antibodies against recombinant human alpha-glucosidase developed. Peak antibody titers were 6250: 200 000 and 800 000. Initially antibodies showed no neutralizing effects, but neutralizing effects were observed in one patient at study end. Patients survived ventilator-free, learned to walk, and showed reduction left ventricular mass index. The patient with the highest titer temporarily lost the ability to walk at study end. **Conclusions:** The current immunomodulation protocol prevented formation of rhGAA antibodies only during B cell depletion. After B cell recovery, high titers were found in 2 patients. The patient with the highest titer neutralizing effects increased over time, during which he showed a clinical decline in motor function.

789 - Successful Allogeneic Bone Marrow Transplantation From a Carrier Sibling for the Treatment of Alpha Mannosidosis

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Introduction: Alpha Mannosidosis is a storage disease caused by the mutation of *MAN2B1* gene resulting in the loss of lysosomal alpha mannosidase. Symptoms may include distinctive facial features, skeletal abnormalities, hearing loss, intellectual disability, and dysfunction of the immune system. Treatment options include bone marrow transplantation (BMT) and, possibly in the future, enzyme replacement therapy. We describe a case with a-mannosidase who underwent successfully HSCT from a carrier sibling. **Case:** A 5-year-old girl who had a sibling diagnosed with alpha Mannosidosis admitted to outpatient for screening. Her history revealed speech delay and trouble in forming sentences so she was having special education. Having chest deformity since birth, facial features which became distinct after age 3 and leukocyte enzyme level of 1.35 (231.4 $\pm$ 81) nm/g/h. She was diagnosed with alpha Mannosidosis. There are ongoing research studies for enzyme replacement therapy however; the only treatment method for today is BMT. Although no neurological or skeletal impairment is expected after; BMT stops the progression of the disease therefore it was decided to proceed with BMT. Having no matched unrelated donor, transplant was made from her full-match sister who was a carrier for the disease. Myeloablation was made with *Busulfan* and *Cyclophosphamide* and to prevent ¨graft versus host¨ disease, *Cyclosporine* and *Methotrexate* was initiated. Myeloid and thrombocyte engraftments were seen shortly and she was discharged with no major complication on day 37. She's currently on 13th month after BMT and enzyme level raised to 110 nm/g/h, same level as her carrier sibling. She did not have any other complications, moreover her intellectual ability augmented and hearing got better. She's still on special education due to articulation problems. **Results:** In Mannosidosis, the use of BMT should be decided by the patient's profit/loss assessment. BMT should be considered as an important treatment modality in some metabolic diseases commonly seen in our country which have progressive course and low treatment chances with current treatment modalities.

790 - A Rare Case of Fabry Cardiomyopathy Requiring Permanent Pacemaker and Implantable Cardioverter-Defibrillator Despite Enzyme Replacement Therapy

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A 58-year-old male farmer referred to our hospital due to ECG abnormality, dyspnea on exertion and cardiac enzyme elevation after diagnosis of scrub typhus at local clinic. A 12-leads electrocardiogram (ECG) revealed normal sinus rhythm with 1st degree atrioventricular block (AVB), complete right bundle branch block (RBBB) and pathologic Q wave in I, II, aVL, V5 and V6. Transthoracic echocardiogram showed diffuse severe concentric hypertrophy of the left ventricle (LV)

of an average ventricular wall thickness of 30 mm. A dynamic left ventricular outflow track obstruction LVOT) was observed during the Valsalva maneuver. Right ventricle (RV) was also hypertrophied (RV free wall thickness, 9 mm). Cardiac magnetic resonance imaging revealed diffuse delayed hyper-enhancement with various degrees of transmurality. More detailed familial history taking informed that his younger brother and older sister suffered from cardiac problem. His oldest sister died of sudden cardiac death at the age of 60. Genetic disorder was suspected and confirmed as Fabry disease (FD) via GLA gene sequencing. A missense mutation was identified at exon 6 within a *GLA* gene. The mutation is known to cause FD of a cardiac variant type by literature review. Additionally, 4 family members were confirmed to have FD with same mutation. Index patient initiated enzyme replacement therapy (ERT) with intravenous agalsidase-beta via outpatient department (OPD). Follow-up ECG showed first degree AVB and left bundle branch block (LBBB). He experienced two episodes of syncope and frequently complained of dizziness during OPD follow-up. ECG revealed complete AVB. Beta blocker was discontinued more than two weeks after that. However, complete AVB didn't improve. Permanent pacemaker (PPM) which has a function of implantable cardioverter-defibrillator (ICD) was implanted considering that he has high risk features of sudden cardiac death such as syncope, severe hypertrophy of LV septal wall and family history of sudden cardiac death. Peak pressure gradient of LVOT markedly decreased to 36 mmHg after PPM implantation from 63 mmHg before that. Symptoms of dyspnea and dizziness also markedly improved. Progression of Fabry cardiomyopathy can lead to severe conduction dysfunction such as complete AVB requiring PPM despite ERT. It suggests that treatment must be initiated early in the course of the disease to be optimally effective.

791 - Urinary Glycosaminoglycan Levels in a Mucopolysaccharidosis Type II Pediatric Population Aged ≤ 18 Months Receiving Idursulfase Therapy: Data From the Hunter Outcome Survey (HOS)

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Specific treatment for mucopolysaccharidosis type II (MPS II; Hunter syndrome) is available in the form of intravenous enzyme replacement therapy (ERT). Idursulfase (Shire, Lexington, MA, USA) stabilizes or improves many of the somatic features of MPS II in patients aged ≥ 5years; tolerability and initial efficacy outcomes in patients starting Idursulfase therapy aged 1.4–7.5 years have been shown to be similar to those of the first clinical studies but experience in patients aged ≤ 18 months remains limited. Our ability to diagnose MPS II early in life is improving, creating a need for increased understanding of the impact of early initiation of ERT. We analyzed data from patients enrolled in the HOS global, observational registry who started Idursulfase aged ≤ 18 months (n = 45 prospective patients; all male; June 2016). Patients were diagnosed at a median (P10, P90) age of 0.4 (0.0, 1.3) years; symptom onset was at age 0.4 (0.0, 1.1) years. Almost half had cognitive impairment (48.8%; 20/41). Patients had received Idursulfase for 53.3 (2.1, 91.9) months, starting at age 0.8 (0.2, 1.4) years; three individuals stopped ERT and never re-started. Sixteen patients started Idursulfase at < 6 months (median 0.3 [0.0, 0.4] years). Urinary glycosaminoglycan (uGAG) measurements (dimethylmethylene blue spectrophotometric assay, adjusted for urine creatinine; n = 33 patients) showed a trend toward a decrease from elevated baseline levels with Idursulfase therapy. Among those with data at both baseline and 1 year of Idursulfase (n = 11), uGAG levels changed by −61.6% (−81.8, 44.1%). Between baseline and year 2 (n = 9), the change was −73.5% (−87.7, 115.7%). As yet, patient numbers are low, but current findings indicate decreased uGAG levels following Idursulfase therapy in this group. Additional follow-up and analysis of a range of clinical outcomes will further increase our understanding of early treatment with Idursulfase. Previously presented at WORLD-Symposium 2017. **Funding information:** Shire sponsors HOS and funds medical writing support.

792 - COMET Methodology: Comparison of the Efficacy and Safety of the Enzyme Replacement Therapies, NeoGAA and Alglucosidase alfa, in Treatment-Naive Patients With Late-Onset Pompe Disease

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Pompe disease is caused by deficiency in the lysosomal enzyme acid alpha glucosidase (GAA), responsible for lysosomal glycogen breakdown. Consequently, glycogen accumulates in body tissues, particularly cardiac and skeletal muscles, leading to progressive muscle weakness and respiratory failure. Alglucosidase alfa, a recombinant human GAA (rhGAA), was the first treatment approved for Pompe disease. NeoGAA, an investigational second-generation rhGAA, was designed to enhance glycogen clearance and improve clinical efficacy. This abstract describes the COMparative Enzyme replacement Trial (COMET; NCT02782741) methodology, which is designed to evaluate the efficacy and safety of neoGAA vs alglucosidase alfa in treatment-naïve patients with late-onset Pompe disease (LOPD). COMET is a Phase 3, multicenter, multinational, randomized, double-blind trial in treatment-naïve LOPD patients, aged $\geq$3 years, who at baseline are ambulatory for 40 m (without stopping/using assistive device), are not on invasive ventilation, and have $\geq$30 to $\leq$85% upright predicted forced vital capacity [FVC]). To control the number of patients with high baseline FVC, $\leq$15% of those enrolled will have baseline FVC $\geq$80% predicted. The target enrolment is 96 patients (non-inferiority test: 80% power with assumed treatment difference 2.0% predicted FVC; superiority test: 85% power with 3.5% predicted FVC). Patients randomized 1:1, with stratification on baseline FVC, sex, age, and country (Japan/ex-Japan), receive 20 mg/kg neoGAA or alglucosidase alfa IV qow for 49 weeks. They then enter an open-label, long-term follow-up phase ($\leq$96 weeks) and receive 20 mg/kg neoGAA IV qow. The primary objective of the trial is to determine the efficacy of neoGAA on respiratory muscle strength, as measured by upright FVC % predicted. Other assessments include: functional endurance, inspiratory, expiratory, and extremity muscle strength, motor function, health-related quality of life, patient-reported outcomes, pharmacokinetics, and safety. The first patient enrolled in the trial in November 2016 and the estimated completion is October 2020. The results from the 49-week primary analysis period should identify whether there are any treatment outcome differences between neoGAA and alglucosidase alfa. This trial is sponsored by Sanofi Genzyme.

793 - Efficacy and Safety of Migalastat, an Oral Pharmacological Chaperone for Fabry Disease: Renal Findings From Two Randomized Phase 3 Studies (FACETS and ATTRACT)

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Fabry disease is an X-linked disorder of lysosomal α-galactosidase A (α-Gal A) deficiency, leading to substrate accumulation, multiorgan disease, and progressive decline in renal function. Accumulation of globotriaosylceramide (GL-3) in the kidney is a known consequence of Fabry disease. We examined whether administration of Migalastat, an oral pharmacological chaperone that induces proper folding of specific mutant forms of α-Gal A and increases trafficking to lysosomes, helps stabilize renal function in patients with Fabry disease. Two randomized phase 3 studies were conducted for Migalastat 150 mg every other day. FACETS (011, NCT00925301) was a 24-month trial, including a 6-month double-blind, placebo-controlled period, in 67 enzyme replacement therapy (ERT)–naive patients. ATTRACT (012, NCT01218659) was an active-controlled, 18-month trial in 57 ERT-experienced patients with a 12-month open-label extension (OLE). Efficacy analyses focused on patients with *amenable* mutations (FACETS, n = 50; ATTRACT, n = 53). A post hoc analysis examined the eGFR annualized rate of change from baseline in the range of 60 to <90 or >90 mL/min/1.73 m^2; the range of 30 to <60 mL/min/1.73 m^2 was not included due to the low number of patients in this group. In FACETS, analyses revealed statistically significant reductions in kidney interstitial capillary GL-3 from months 0 to 6 (placebo-controlled; $P = .008$) and months 6 to 12 ($P = .014$). Over 24 months, the annualized rate of change in $eGFR_{CKD\text{-}EPI}$ $\pm$ SD with Migalastat was -0.3 ± 4.2 mL/min/1.73 m^2. The annualized rate of change $\pm$ SD for eGFR in the range of 60 to <90 mL/min/1.73 m^2 (-0.8 ± 4.16) was comparable to the rate in the range of $\geq$90 mL/min/1.73 m^2 (0.2 ± 4.11). During the 18-month controlled period of ATTRACT, the annualized means $\pm$ SD for eGFR for Migalastat (-0.40 ± 4.3) and ERT (-1.03 ± 7.4) were comparable. The annualized rates of change in eGFR $\pm$ SD in the range of 60 to <90 mL/min/1.73 m^2 for Migalastat and ERT were -0.2 ± 5.32 and -7.3 ± 15.90, respectively; the annualized rates in the range of $\geq$90 mL/min/1.73 m^2 were -2.0 ± 2.15 and -2.1 ± 5.30. Predefined renal events occurred in 24% and 33% of patients on Migalastat and ERT, respectively. During the 12-month OLE, eGFR remained stable. In FACETS and ATTRACT, renal function remained stable with Migalastat across patient subgroups. Migalastat has promise as a first-in-class oral chaperone for patients with Fabry disease with amenable mutations. This is an encore abstract.

794 - Long-Term Migalastat Treatment Stabilizes Renal Function in Patients With Fabry Disease: Results From a Phase 3 Clinical Study (AT1001-041)

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Progressive nephropathy is a hallmark of Fabry disease, a rare X-linked disorder of lysosomal α-galactosidase A (α-gal A) deficiency that results from lysosomal deposition of globotriaosylceramide and can lead to the complications of end-stage renal disease, dialysis, and renal transplantation. Progressive impairment of renal function has also been shown to be a major risk factor for cardiac events and premature death. Stabilizing or slowing renal decline is an important treatment goal in Fabry disease. Oral Migalastat (approved in the EU in 2016; in clinical development in the US) was designed to chaperone specific *amenable* mutant forms of α-gal A to lysosomes where the enzyme catabolizes accumulated disease-causing substrates. The long-term effects of Migalastat on GFR in enzyme replacement therapy (ERT)-naive patients with Fabry disease from the phase 3 extension study AT1001-041 (NCT01458119) were investigated. Patients completing clinical studies with Migalastat were eligible to enroll in the extension study and receive open-label Migalastat 150 mg every other day. Annualized rate of change in $eGFR_{CKD\text{-}EPI}$ was calculated using simple linear regression. One hundred twenty-seven patients with Fabry disease were enrolled in two phase 3 studies. All 54 ERT–naive patients completing the phase 3 study enrolled in the extension study and received open-label Migalastat for a mean of 3.4 years (range 1.5-4.9 years). Patients (male and female) receiving Migalastat had a mean annualized rate of change for $eGFR_{CKD\text{-}EPI}$ of −0.7 mL/min/1.73 m^2 (95% CI—1.83, 0.46) from baseline to month 48. In female patients, the corresponding mean annualized rate of change during this period was −0.49 (95% CI—2.0, 1.1). In male patients, the mean annualized rate of change was −1.06 (95% CI—2.8, 0.70). These average annualized changes in eGFR with Migalastat represent clinically relevant stabilization of eGFR compared with the annualized declines of −2.6 and −3.0 mL/min/1.73 m^2 reported in the literature for 2 large cohorts of untreated male and female patients with Fabry disease. Migalastat was generally safe and well tolerated, with no AE trends attributable to Migalastat. The most common AE reported was mild-to-moderate headache. Few serious AEs were considered related to Migalastat. In patients with Fabry disease and amenable mutations, long-term Migalastat treatment for up to 4.9 years resulted in clinically meaningful stabilization of renal function. This is an encore abstract.

795 - In Vitro Stopcodon Readthrough of Alfa-Galactosidase and Alfa-Glucosidase Premature Termination Codons Using Gentamicin, Geneticin, and Ataluren: Therapeutic Potential for Fabry and Pompe Diseases

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The objective of this study was to investigate in vitro stopcodon readthrough effect of Gentamicin, Geneticin and Ataluren (PTC124) for the treatment of Fabry and Pompe disease caused by Premature Termination Codons (PTCs). The fibroblast cultures of two Fabry patients bearing the p.R220X and p.R227X mutations, respectively, and one Pompe patient bearing p.E888X were treated for 48 h with Gentamicin (250-3000 μg/ml), Geneticin (125-1000 μg/ml) and Ataluren (2.5-20 μM) in Dulbecco's Modified Eagle Medium (DMEM). The best results were obtained for p.R227X mutation with G418, reaching a 30% and 20% recovery of alfa-galactosidase activity at 125 and 250 μg/ml, respectively while gentamicin at 250 and 500 μg/ml resulted in 10% and 22% recovery, respectively. Furthermore, Ataluren resulted in a 26% recovery at 5 μM for p.R227X. The synthesis of full-length alfa-galactosidase enzyme was demonstrated by western blotting technique upon stopcodon readthrough by Gentamicin, Geneticin and Ataluren. On the other hand, p.R220X mutation did not respond to treatment with Gentamicin, Geneticin and Ataluren possibly because of nonsense mediated decay (NMD), which might impair the readthrough response if a PTC-bearing mRNA is an NMD target. Generally, a purine in the 4th nucleotide position of the termination signal was reported to result in more efficient termination than a pyrimidine, which might explain the difference in response to readthrough treatment between p.R227X and p.R220X mutations. In our study, both p.R227X and p.R220X mutations are of UGA type where the first PTC is followed by A (Adenine) while the second PTC is followed by C (Cytosine). Treatment of the fibroblast culture of Pompe patient bearing p.E888X mutation resulted in 2.5% recovery of alfa-glucosidase activity at 750 μg/mL Gentamicin while Geneticin at 125 μg/mL and Ataluren at 5 μM resulted in 12.5% and 4% recovery of alfa-glucosidase activity, respectively. The synthesis of full-length alfa-glucosidase enzyme was demonstrated by western blotting technique upon

stopcodon readthrough by Gentamicin, Geneticin and Ataluren. In summary, this is a proof-of-principle study demonstrating the potential of stopcodon readthrough as a therapeutic strategy for the treatment of Fabry and Pompe diseases caused by PTCs. Measurement of storage materials in lysosomes would also be essential to determine if the level of readthrough is sufficient to restore phenotype to normal or to near to normal.

796 - Response of Patients With Fabry Disease With the Amenable *GLA* Mutation p.N215 S to Treatment With Migalastat

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Fabry disease is an X-linked disorder of lysosomal α-galactosidase A (α-Gal A) deficiency, leading to substrate accumulation and multiorgan disease. Migalastat, an oral pharmacological chaperone, binds to *amenable* forms of α-Gal A to restore lysosomal trafficking and cellular activity. ATTRACT (Study 012; NCT01218659) is a phase 3, randomized, open-label study comparing Migalastat and enzyme replacement therapy (ERT) in patients previously treated with ERT. Patients were randomized 1.5:1 to continue ERT or receive 150 mg oral Migalastat every other day for 18 months with an option to enter an open-label extension with Migalastat. The study randomized 60 patients with amenable and non-amenable mutations; 57/60 patients received treatment; 53/57 treated patients had amenable mutations. A retrospective subanalysis investigated treatment response at month 18 in patients with p.N215 S, a prevalent mutation associated with the non-classic phenotype. This subanalysis consisted of 8 patients (5 males) in the Migalastat group and 3 patients (1 male) in the ERT group. The average (± 95% CI) age for these patients was 56 (± 7.93) years. In all patients with p.N215 S, baseline left ventricular mass index (LVMi) was 103 ± 26 g/m^2; $eGFR_{CKD\text{-}EPI}$ was 87.0 ± 13 mL/min/1.73 m^2. Seven of eight Migalastat patients and 1/3 ERT patients had LVH at baseline. For the Migalastat group, mean (± 95% CI) change from baseline to month 18 was: $eGFR_{CKD\text{-}EPI}$ 0.50 (± 2.55); $mGFR_{iohexol}$ −1.03 (± 3.64); and LVMi −4.80 (± 4.10). Seventy-five percent of patients treated with Migalastat achieved a decrease in LVMi at month 18. Plasma globotriaosylsphingosine (lyso-Gb_3) remained stable after the switch from ERT to Migalastat in these patients. The mean (± 95% CI) change from baseline to month 18 for lyso-Gb_3 was 0.05 (± 0.79). For the ERT group, mean (± 95% CI) change from baseline to month 18 was: $eGFR_{CKD\text{-}EPI}$ −0.77 (± 1.30); $mGFR_{iohexol}$ −4.47 (± 3.57); plasma lyso-Gb_3 0.63 (± 1.34); and LVMi 2.47 (± 19.83). Overall, in patients with p.N215 S, there was a consistent decrease in LVMi following 18 months of treatment with Migalastat. This is an encore abstract.

797 - Effects of Treatment With Migalastat on the Combined Endpoint of Kidney Globotriaosylcermide Accumulation and Diarrhea in Patients With Fabry disease: Results From the Phase 3 FACETS Study

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Fabry disease is an X-linked disorder of lysosomal α-galactosidase A (α-Gal A) deficiency, leading to substrate accumulation and multiorgan disease. Patients with Fabry disease report gastrointestinal symptoms that significantly impact their quality of life. Migalastat, an oral pharmacological chaperone, binds to *amenable* forms of α-Gal A to restore lysosomal trafficking and cellular activity. FACETS is a phase 3, double-blind, placebo-controlled study to evaluate Migalastat in patients with Fabry disease with amenable mutations. The study randomized 60 patients; 57/60 patients received treatment; 53/57 treated patients had amenable mutations. A retrospective, unpublished analysis using Xu's Statistic, designed to evaluate if treatment has an effect on multiple outcomes simultaneously relative to control, assessed changes in kidney interstitial capillary globotriaosylceramide (KIC GL-3) combined with the Gastrointestinal Symptom Rating Scale diarrhea subdomain (GSRS-D). Logistic regression also assessed the correlation between changes in KIC GL-3 and GSRS-D, with a response in KIC GL-3 defined as a reduction of >0.1 inclusions per capillary (above background staining) and response in GSRS-D defined as a reduction >0.33 (above MCID). Eighty-three percent (15/18) of patients with amenable

mutations treated with Migalastat demonstrated a response in KIC GL-3 and/or GSRS-D when either or both were elevated at baseline, compared with 33% (5/15) treated with placebo. Xu's Statistic revealed a significant difference between treatments in the average change from baseline to month 6 for KIC GL-3 ($P = .021$; 1-sided), GSRS-D ($P = .029$; 1-sided), and the combined endpoint of KIC GL-3 and GSRS-D ($P = .009$; 1-sided). Patients with a reduction in KIC GL-3 were 4.3 to 5.6 times more likely to show improvement in GSRS-D than patients who did not have a reduction in KIC GL-3. Overall, reductions in KIC GL-3 are associated with improvements in diarrhea, and Migalastat simultaneously reduces disease substrate and improves gastrointestinal symptoms in patients with Fabry disease with amenable mutations. This is an encore abstract.

798 - Taliglucerase Alfa: Safety and Efficacy Across 6 Clinical Studies in Children and Adults With Gaucher Disease

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Taliglucerase alfa is an enzyme replacement therapy (ERT) approved for treatment of adult and pediatric patients with Type 1 Gaucher disease (GD) in several countries and the first plant cell–expressed recombinant therapeutic protein approved by the US FDA for humans. We report the findings across 6 key Taliglucerase alfa clinical studies. A total of 33 adult treatment-naïve patients were randomized to Taliglucerase alfa 30 U/kg or 60 U/kg in a 9-month, multicenter, randomized, double-blind, parallel-group, dose-comparison pivotal trial, after which eligible patients continued into 2 consecutive extension trials; 17 treatment-naïve adult patients completed 5 total years of treatment with Taliglucerase alfa. In the only ERT trial focused on exclusively pediatric patients with GD, 11 treatment-naïve children were randomized to Taliglucerase alfa 30 U/kg or 60 U/kg in a 12-month, multicenter, double-blind study; 9 completed 3 total years of treatment in a dedicated pediatric extension study. The effect of switching patients from Imiglucerase to Taliglucerase alfa was also investigated in a separate 9-month trial that included 5 children and 26 adults; 2 children completed a total of 2.75 years and 10 adults completed a total of 3 years of Taliglucerase alfa treatment in the extension studies. All trials evaluated safety and spleen volume, liver volume, platelet count, hemoglobin concentration, and biomarkers as measures of efficacy. Detailed results from baseline through the end of these studies will be presented. Taliglucerase alfa was well tolerated, and adverse events were generally mild/moderate in severity and transient. Treatment with Taliglucerase alfa resulted in improvements (treatment-naïve patients) or stability (patients switched from Imiglucerase) in visceral, hematologic, and biomarker parameters. Together, these comprehensive data support the treatment of adult and pediatric patients with GD who are naïve to ERT or who have previously been treated with Imiglucerase.

799 - Long-Term Safety and Efficacy of Olipudase Alfa in Adults With Acid Sphingomyelinase Deficiency (ASMD)

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Objective: To assess the long-term safety and efficacy of olipudase alfa, an investigational enzyme replacement therapy in development for the treatment of nonneurological manifestations of acid sphingomyelinase deficiency (ASMD). **Methods:** Five adults with chronic visceral ASMD (Niemann-Pick type B) were enrolled in a 26-week Phase 1b trial of olipudase alfa (NCT01722526). At the end of the 26-week trial period, all five patients enrolled in an ongoing 5-year extension study (NCT02004704). Here, we present data for cumulative 30 months (2.5 years) of treatment. **Results:** Olipudase alfa continues to show a favorable safety profile, with no deaths, serious adverse events (AEs), or AEs that led to discontinuation reported through 30 months of treatment. None of the 5 patients developed antibodies to olipudase alfa. Pre-infusion plasma levels of ceramide, a sphingomyelin catabolite, remained normal over time with minor individual fluctuations (especially within 72 hours of infusion). Baseline levels in dried blood spots of lyso-sphingomyelin were 5-fold above the upper limit of normal. Lyso-sphingomyelin showed a steady decrease with treatment, with near-normal mean levels attained at 18 months (-70.7% reduction; $P = .0005$) and sustained through 30 months. Mean spleen volume was 12.8 MN at baseline, and decreased to 6.7 MN at 30 months ($P = .005$), corresponding to a 47.3% decrease from baseline ($P < .0001$). Mean liver volume decreased from 1.7 MN at baseline to 1.07 MN at 30 months ($P = .02$), a 35.6% decrease ($P = .006$). At baseline, all patients showed evidence of infiltrative lung disease, which continued to improve through 30 months of treatment. Lung HRCT showed clearance of reticulonodular densities after 30 months ($p = 0.0061$). At baseline, patients had a pro-atherogenic lipid profile with increased mean triglyceride, total cholesterol, and LDL-C levels, and decreased mean HDL-C levels. By 30 months, triglycerides decreased by 42.99% ($P = .02$), total cholesterol by 12.7% ($P = .04$), LDL-C by 22.8% ($P = .007$), and HDL-C increased by 137.6% ($P = .01$). Mean levels

of chitotriosidase decreased by 72.3%, from 735 nmol/h/mL at baseline to 221.0 nmol/h/mL at 30 months ($P = .0007$). **Conclusions:** These data on 30 months of olipudase alfa treatment show a sustained safety profile and continued improvements in multiple clinically relevant parameters. Lyso-sphingomyelin may constitute a reliable biomarker for treatment monitoring and disease burden. Funded by Sanofi Genzyme.

800 - Left Ventricular Hypertrophy and Decreased Renal Function at Baseline Predict Worse Cardiovascular and Renal Outcomes in Patients in the FOS Registry

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Fabry disease is an X-linked genetic multisystem disorder. Cardiac and renal involvements are among the most common and detrimental. Heterogeneity in response to enzyme replacement therapy (ERT) suggests that the optimal timing of initiation is not always achieved. Longitudinal data over 15 years in the Fabry Outcome Survey (FOS) facilitates the exploration of long-term outcomes. This analysis focused on data from patients enrolled in FOS who received Agalsidase alfa ERT and who also had available treatment start date, age at diagnosis and values for left ventricular mass index (LVMI) or estimated glomerular filtration rate (eGFR) at ERT initiation. Renal outcomes included dialysis, transplantation, and renal failure. Cardiovascular outcomes included myocardial infarction, left ventricular hypertrophy (LVH), and heart failure. Patients with reported events before ERT initiation were excluded. Time to first renal or cardiovascular event was compared from ERT initiation up to 120 months of treatment in subjects with increased LVMI (>48 g/m$^{2.7}$ for females, >50 g/m$^{2.7}$ for males) vs. normal LVMI, and in subjects with reduced eGFR (<90 mL/min/m^2) vs. normal eGFR. Kaplan-Meier curves were compared using a log rank test. Cox regression with age at diagnosis as a covariate was used to estimate hazard ratios (HR) between the groups. Evaluation of the effect of LVMI at baseline included 650 and 697 patients for time to first cardiovascular and renal event, respectively. Evaluation of the effect of eGFR at baseline included 1084 and 1147 patients for time to first cardiovascular and renal event, respectively. Patients with LVH demonstrated higher probability of renal (HR = 2.1; 95% confidence interval [CI] 1.57:2.81; log-rank p<0.0001) and cardiovascular events (HR = 2.1; 95% CI 1.61:2.73; log-rank p<0.0001) over the follow-up period. Similarly, patients with reduced renal function demonstrated higher probability of renal (HR = 2.5; 95% CI 2.02:3.12; log-rank p<0.0001) and cardiovascular events (HR = 1.9; 95% CI 1.54:2.25; log-rank p<0.0001) over the follow-up period. Similar statistically significant effects were observed in males and females separately, for both cardiovascular and renal outcomes, with both baselines LVH and decreased renal function. The data show an important interaction between cardiac and renal involvement in Fabry disease. This observation indirectly supports the timely diagnosis and initiation of ERT to prevent negative renal and cardiovascular outcomes.

801 - Analysis of Renal and Cardiac Outcomes in male Fabry Outcome Survey Participants Starting Agalsidase alfa Enzyme Replacement Therapy Before and After 18 Years of Age

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Patients with Fabry disease may have better outcomes when enzyme replacement therapy (ERT) is started early in life. Data from the Fabry Outcome Survey (FOS; data extracted January 2017) were analyzed for males with childhood symptom onset who started Agalsidase alfa ERT at ≤18 years old (child treated males, CTM; n = 141), >18 and ≤30 years old (young adult treated males, YTM; n = 220), and >30 years old (adult treated males, ATM; n = 483). The following renal and cardiac outcomes were analyzed up to 10 years after ERT initiation: estimated glomerular filtration rate (eGFR), proteinuria, and left ventricular mass indexed to height (LVMI). Mean age (SD) at symptom onset was similar for the 3 groups: 7.4 (3.7) years for CTM, 8.7 (4.4) years for YTM, and 8.9 (3.7) years for ATM. Mean age (SD) at diagnosis was 10.4 (4.7) years for CTM, 18.6 (6.5) years for YTM, and 35.8 (15.9) years for ATM, and mean (SD) age at ERT initiation was 13.0 (3.8) years, 24.0 (3.5) years, and 44.9 (10.4) years, respectively. Mean (SD) FOS-Mainz Severity Score Index at baseline was 10.0 (7.3) for CTM, 13.6 (8.5) for YTM, and 19.9 (11.6) for ATM. Mean (SD) eGFR at baseline was 113.5 (27.5) mL/min/1.73m^2 for CTM, 120.3 (20.7) mL/min/1.73m^2 for YTM, and 89.7 (27.7)

mL/min/1.73m^2 for ATM, and the median (IQR) of the overall mean change per year over 10 years was +1.8 (−3.4;+8.1) mL/min/1.73m^2, −1.0 (−4.1;+1.5) mL/min/1.73m^2, and −1.9 (−4.9;+0.2) mL/min/1.73m^2, respectively. Median (IQR) proteinuria at baseline was 83.6 (27.0;142.4) mg/day for CTM, 140.0 (60.0;230.0) mg/day for YTM, and 337.3 (130.0;815.0) mg/day for ATM and the median (IQR) of the overall mean change per year over 10 years was +5.0 (−1.2;+16.8) mg/day, +0.5 (−5.2;+20.1) mg/day, and +5.4 (−18.9;+61.2) mg/day, respectively. Mean (SD) LVMI at baseline was 37.4 (8.2) g/m$^{2.7}$ for CTM, 40.6 (10.2) g/m$^{2.7}$ for YTM, and 58.2 (18.4) g/m$^{2.7}$ for ATM, and the median (IQR) of the overall mean change per year over 10 years was −2.3 (−4.9;0.9) g/m$^{2.7}$, −0.2 (−2.9;1.5) g/m$^{2.7}$, and −0.6 (−4.3;3.0) g/m$^{2.7}$, respectively. These data show that initiating ERT in childhood, before manifestation of severe symptoms, may attenuate progressive Fabry-related renal and cardiac disease, and that initiating ERT in adulthood, when renal and cardiac involvement is already apparent, may stabilize eGFR and LVMI progression. The results from this analysis suggest that greater clinical benefits may be obtained the earlier ERT is started.

802 - Profile of Patients With Mucopolysaccharidosis Type II Without Cognitive Impairment Who Started Idursulfase Treatment Aged >20 Years: Data on late Treatment Start From The Hunter Outcome Survey (HOS)

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Mucopolysaccharidosis type II (MPS II; Hunter syndrome) is a progressive X-linked disease caused by deficient activity of iduronate-2-sulfatase (I2 S). The so-called attenuated (slowly progressive) form has minimal CNS involvement; however, extra-neurologic manifestations can be as significant as in the severe (early progressive) form. Enzyme replacement therapy with recombinant I2 S (Idursulfase; Shire, Lexington, MA, USA) was available in 2006 in the USA, 2007 in the EU, and subsequently in other countries globally. Herein, we describe patients with MPS II without cognitive impairment from the Hunter Outcome Survey (HOS; a global observational registry) who were aged >20 years at treatment initiation. Exclusion of patients with cognitive impairment was based on the answer to the yes/no question from the database: "Cognitive impairment?". As of March 2016, 59/947 patients followed prospectively in the registry met these criteria. Patients were stratified by age at treatment start: >20 to <25 years (n = 21), ≥25 to <30 years (n = 18), ≥30 to <35 years (n = 7), and ≥35 years (n = 13). Retrospective analyses of median ages at symptom onset in years (P10; P90) were: 3.3 (0.8; 8.0), 3.5 (0.2; 7.5), 4.8 (4.1; 18.2), and 4.0 (1.0; 44.3), respectively. Overall, the systems most commonly affected (between birth and last visit) were musculoskeletal (59/59), abdominal/gastrointestinal (57/59), and ear (56/58). Median ages at diagnosis were: 7.2 (0.3; 19.0), 4.6 (2.9; 26.0), 18.8 (4.5; 32.6) and 7.5 (4.0; 19.0). Median ages at treatment initiation were: 22.5 (20.6; 24.8), 27.7 (25.2; 29.2), 31.9 (30.4; 34.2), and 39.3 (36.7; 47.8). Reasons for delaying treatment initiation after diagnosis are unknown. Apart from drug availability, factors may include mild clinical symptoms, physician/patient decision or perception of inefficacy after a certain age, balancing infusion regimens with daily life, or onset or worsening of symptoms. Further analysis is required to delineate these barriers and raise awareness of the importance of early diagnosis and timely treatment initiation. Previously presented at WORLDSymposium 2017. **Funding information:** Shire sponsors HOS and funds medical writing support.

803 - Nurse Led Administration of an Intracerebroventricular (ICV) Medication

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Background: ICV administration via an indwelling device enables medications to bypass the blood brain barrier, allowing high doses of drug to enter the central nervous system. This route is a well-established, and well-tolerated, method of drug delivery for pediatric and adult patients, including treatment of meningitis, intractable pain and cancer. With advances in medicine, ICV drug delivery is increasing in frequency and being introduced to treat a number of pediatric conditions. Children with CLN2 (Late Infantile Batten Disease) now have the opportunity to receive enzyme replacement therapy, via the ICV route, ensuring it bypasses the blood–brain barrier to target the affected area. The literature suggests this procedure is currently only undertaken by medical staff, but with increasing numbers of treatments and patients, physicians are placed under greater pressure. There is no clear evidence to suggest nurses could not safely perform the procedure. **Aims:** To establish a nurse led service administering medication via an ICV device in a ward environment. **Method:** An assessment was carried out, analyzing the risks and benefits of nurses accessing the ICV device and administering the enzyme via this route. We worked in partnership with the medical staff already performing this procedure to produce clinical practice guidelines, an operational policy, and a training package. This ensures staff competency and patient safety. Following establishment of the nurse led

service; the data were reviewed and compared with statistics in the current literature. We analyzed the device related complications occurring in >38 infusions on patients aged between 4 and 15, carried out in a ward setting. **Results:** Clinical practice guidelines are now in place. A training package, including theory and practical competencies has been established. This includes a register of staff certified to administer the enzyme via the ICV route. The CLN2 patients in our hospital have received a total of >38 infusions over >7 months. Two device related complications have occurred. One resulted in an unavoidable infusion cancellation. In both events, the issue was escalated appropriately and patient safety was maintained. **Conclusion:** There appears to be no clear indication of increased complications associated with nurses accessing ICV devices and administering medication via the ICV route when compared to current literature.

804 - Toward Establishment of a Minimal Clinically Important Difference in the Treatment of Alpha-Mannosidosis: First Results From Velmanase alfa (Human Recombinant Alpha-Mannosidase) Development Program

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Velmanase alfa (human recombinant alpha-mannosidase) is the first enzyme replacement therapy in development for the treatment of alpha-mannosidosis (AM). All 33 patients treated during clinical development were evaluated in a long-term follow-up (rhLAMAN10) study with a mean (SD) exposure of 29.3 (15.2) months; 10 of the 33 patients were exposed to an initial placebo period of 12 months before switching to active treatment. AM is a multisystem lysosomal storage disorder, presenting with heterogeneous severity, and large variability in terms of age of onset and manifestations. In this context, a single outcome measure does not provide the adequate sensitivity to quantify clinically meaningful treatment effect in a comprehensive way. The aggregation of multiple disease-specific domains has been proposed to overcome this issue, generating a clinically relevant overall treatment response. End points measured during the study were grouped into 3 domains defined as pharmacodynamics (reduction of serum oligosaccharides), functional (Three Minutes Stair Climb Test (3MSCT), Six Minute's Walk Test (6MWT), and Forced Vital Capacity (FVC) % predicted), and quality of life (Childhood Health Assessment Questionnaire (CHAQ) Disability Index (DI) and CHAQ pain). Responders were evaluated based on Minimal Clinically Important Difference (MCID) thresholds established in proxy-diseases. Adopted MCID were oligosaccharides below 4 μmol/L, increase of 7 steps/minute for 3MSCT (proxy: MPS IV A), increase of 30 meters for 6MWT (proxy: Pompe disease), increase of 10% for FVC (proxy: Pompe disease), decrease of 0.13 for CHAQ DI (proxy: Juvenile Arthritis) and of 8.2% for CHAQ pain (as in pediatric rheumatology). Patients were considered responders in one domain if the MCID was achieved in at least one parameter of the domain; responders to treatment were defined as patients with response in at least 2 domains. Based on these criteria, response to treatment was observed in 88% (29/33) of patients treated with Velmanase alfa; response during placebo phase was reported in 33% (3/10). Response in all 3 domains was achieved in 45% of treated patients versus 0% during placebo. This analysis represents the first attempt to establish an AM-specific disease score based on MCIDs for key outcome measures. The above reported results provide evidence of the clinical benefit of Velmanase alfa therapy in AM patients, highlighting its disease-modifying effect.

805 - Impact of Anti-Drug Antibodies (ADA) on Safety and Efficacy of Velmanase Alfa (Human Recombinant Alpha-Mannosidase) Long-Term Enzyme Replacement Therapy in Patients With Alpha-Mannosidosis

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Patient immune response to a therapeutic protein (either pre-existing or developed during treatment) can significantly

impact its safety and efficacy profile. Velmanase alfa is an enzyme replacement therapy in development for the treatment of alpha-Mannosidosis, an ultra-rare lysosomal storage disorder. In the clinical studies and after-trial treatment programs, 33 patients have been treated with a mean (SD) total exposure of 29.3 (15.2) months. Patients were tested for anti-drug antibodies (ADA) before and during treatment and defined ADA positive (ADA+) if at least one titer was above the lower limit of detection (1.4 U/mL). Global therapeutic response (GTR) was evaluated as improvements exceeding the Minimal Clinically Important Difference in at least one end point of at least 2 of the following domains: pharmacodynamics (serum oligosaccharides), functional (3 Minutes Stair Climbing Test, 6 Minutes Walking Test, Forced Vital Capacity), and quality of life (Childhood Health Assessment Questionnaire's Disability Index and VAS pain). In total, 8 patients were ADA+ before (^{pre}ADA+) and 3 developed ADA during treatment only (^{tx}ADA+). Infusion related reactions occurred in 2 of the 3 ^{tx}ADA+ patients (and none of the ^{pre}ADA+), and all resolved without sequelae or therapy discontinuation. One patient dropped out of phase II study after IRR events, but he was subsequently enrolled in the phase III trial; he is still receiving infusions of Velmanase alfa. One serious adverse event (acute renal failure) occurred in a ^{pre}ADA+ patient and was considered "possibly related," although additional potential causal factors were also present (i.e., high dose NSAIDs and dehydration). The event was moderate in intensity and the patient recovered without sequelae. GTR under treatment was achieved in 85.7% (6/7) of the ^{pre}ADA+ and 66.7% (2/3) of the ^{tx}ADA+ patients, in comparison with the 91.8% (21/23) of the ADA negative subjects. Thus, pre-existing ADA do not seem to affect therapeutic response to velmanase alfa. Similarly, the development of ADA during treatment was not associated with significant adverse reactions and 2 out of 3 ^{tx}ADA+ patients maintained a sustained positive clinical response. In conclusion, based on the currently available data, ADA do not appear to be a major concern for the clinical use of Velmanase alfa.

806 - Improvements in Endurance, Serum Immunoglobulin G Levels, and Quality of Life in Alpha-Mannosidosis Patients Switching From Placebo to Velmanase Alfa Long-Term Enzyme Replacement Therapy

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Velmanase alfa is the first human recombinant form of alpha-mannosidase in development as long-term enzyme replacement therapy for alpha-mannosidosis. Relevant information on efficacy of investigational drugs can be obtained by analyzing the placebo patients after switch to active treatment at the end of development trials. In the 12-month, phase III, rhLAMAN-05 trial, 10 patients (5 adults and 5 pediatrics) were allocated to placebo. At trial conclusion, all patients switched to Velmanase alfa in compassionate use or trial extensions, and 12-18 months data after the start of active treatment were collected in a comprehensive evaluation visit (last observation; LO). Results are presented as mean absolute values (SD) or mean relative changes (SD) versus baseline after 12 months of placebo ($n = 10$) and at LO during active treatment ($n = 9$). Endurance was measured by the Three Minutes Stair Climb Test (3MSCT) and the Six Minute Walk Test (6MWT), with average baseline performances of 55.5 (16.0) steps/minute and 465.7 (140.5) meters, respectively. During the 12 months of placebo treatment, patients decreased by −3.6% (13.5) and −0.8% (10.8) in the 3MSCT and 6MWT, respectively. At LO, the 3MSCT improved by 9.0% (25.1) and the 6MWT by 2.2% (13.1). Improvements were consistently observed in both pediatric and adult patients. Recurrent infections and immunological impairment are key features of the disease, especially in pediatric patients. Serum immunoglobulin class G (IgG) levels were 7.27 g/L (1.64) at baseline. During the placebo period levels remained stable compared to baseline (+1.0% (16.9)), while a marked increase was observed following Velmanase alfa treatment by +37.3% (16.1) vs baseline at LO. Patient's quality of life was assessed via the Childhood Health Assessment Questionnaire (CHAQ) that allows the evaluation of both patient disability and discomfort (pain). The disability index (DI) was equal to 1.56 (0.67) at baseline, increased (worsened) to 1.71 (0.50) after 12 months placebo treatment, and improved (decreased) to 1.43 (0.50) at LO; pain worsened during the placebo phase from 0.42 (0.59) to 0.52 (0.66) and improved to 0.36 (0.51) at LO. Results of the comparison between the placebo phase and the treatment with Velmanase alfa in the same patients provide evidence of a beneficial effects of the enzyme replacement on serum IgG levels and on an array of clinically-relevant domains, including mobility, endurance, pain, and quality of life.

807 - Impact of Elosulfase Alfa Treatment on Patient-Reported Outcomes in Morquio A Syndrome: Results From the First Year of an English Managed Access Agreement

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Background: Morquio A syndrome is an ultra-rare, inherited, multi-systemic disease which, if untreated, results in impaired functioning, mobility, and quality of life (QoL), and early death. Enzyme replacement therapy (ERT) with Elosulfase alfa is the only approved treatment. In England, access to Elosulfase alfa is granted to all patients on a conditional basis through a managed access agreement (MAA). Patients must fulfill four out of five response criteria to continue receiving treatment; one of the five criteria covers patient-reported outcomes (PROs). PROs support continuing treatment if stabilization or improvement are reported in two of the following three domains: QoL, depression, and pain. PROs for the first year of the program are reported herein. **Methods:** QoL was monitored using either the EQ-5D-5 L tool or the caregiver assistance domain of the MPS Health Assessment Questionnaire (MPS HAQ). The Beck Depression Inventory (BDI; only applicable for patients $\geq$13 years) was used to assess depression. Pain was measured using either the Adolescent and Pediatric Pain Tool (APPT) or the Brief Pain Inventory (BPI), depending on patient age. Thresholds for clinically meaningful changes vs. inherent assessment variability were established post-hoc and agreed upon by all MAA stakeholders. **Results:** As of March 2017, one year of data had been collected for 35 patients. Ten patients entered the program treatment-naïve, the remainder came from the clinical trial program (mean years on ERT = 6.08 [SD 1.36]; n = 25). Based on the EQ-5D-5 L tool, QoL remained stable in 67% of patients, improved in 21%, and deteriorated in 12% (mean change = 0.04 [SD 0.27], n = 33). Based on the caregiver burden domain of the MPS HAQ, QoL was stable in 91%, improved in 6%, and deteriorated in 3% (mean change = −0.86 [SD 9.10], n = 35). Depression remained stable in 89% and deteriorated in 11% (mean change = −1.44 [SD 4.72], n = 18). Pain severity decreased in 63%, remained stable in 34%, and worsened in 3% (APPT: mean change = −2.48 [SD 1.96], n = 25; BPI: mean change = −0.03 [SD 1.49], n = 10). Overall, PROs provided evidence supporting continued treatment for 33 of 35 patients. **Conclusions:** Based on PROs, the majority of patients met or exceeded the necessary level of treatment benefit established by the MAA stakeholders. Assessment of multiple domains was a critical component of the program due to patient heterogeneity and the importance of individualized patient management in Morquio A syndrome.

808 - Enhanced PK Profile of Pegunigalsidase Alfa Supports Once-Monthly Dosing of 2 mg/kg of Pegunigalsidase Alfa (PRX-102) for the Treatment of Fabry Disease

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Pegunigalsidase alfa (PRX-102), a novel PEGylated Enzyme Replacement Therapy (ERT) for Fabry disease (FD) was administrated IV every other week (EOW) to FD patients as part of a Phase 1/2 study, in three dose cohorts (0.2, 1.0, and 2.0 mg/kg). Pegunigalsidase alfa was found to be safe and well tolerated and demonstrated effectiveness in various disease parameters. The pharmacokinetic (PK) parameters of pegunigalsidase alfa are markedly improved compared to the two currently marketed ERT drugs for FD. The half-life of pegunigalsidase alfa is $\sim$ 80 hours vs. $\sim$2 hours of Agalsidase alfa and Agalsidase beta with C_{max} of the 2mg/kg cohort $\sim$10-fold higher than those published for Agalsidase beta. **Objective**: To evaluate a PK projection for 4 weeks infusion interval with Pegunigalsidase alfa based on Pegunigalsidase alfa 2 mg/kg EOW PK data. **Methods:** PK projection modelling: PK parameters of the 2mg/kg cohort administration (dosed EOW; phase 1/2 study) were projected (Phoenix WinNonlin Software) to estimate Pegunigalsidase alfa PK profile on weeks 3 and 4 after infusion once given every 4 weeks (E4 W). The weekly AUC and average concentration (C_{ave}) was projected and compared to Agalsidase beta published data dosed EOW (Agalsidase beta USPI). **Results:** PK modelling shows that over a 4-week time frame Pegunigalsidase alfa is estimated to have a greater AUC ($\sim$45 fold) after a single infusion of 2 mg/kg compared to EOW infusions of Agalsidase beta. The weekly projected C_{ave} indicates that there should be expected measurable levels of Pegunigalsidase alfa at the 3 rd and 4th week after an infusion in the same order of magnitude as Agalsidase beta in the 1st week

after infusion providing enzyme coverage throughout the 4 weeks intervals. **Conclusions:** The unique characteristics of Pegunigalsidase alfa together with the results of the PK projection modelling and the safety and efficacy results from phase 1/2 all serve as the rationale for initiating a phase 3 study that will assess the safety, efficacy and PK of 2 mg/kg Pegunigalsidase alfa administered IV E4 W in FD patients. Treating patients every 4 weeks is expected to improve the quality of life and treatment compliance, and has the potential to be efficacious, safe, with reduced immunogenicity. Additionally, it may address the clinical needs of early or younger diagnosed patients. The planned phase 3 study may offer health care providers and patients with FD alternative treatment options.

809 - Biochemical Correction Associated With Improved Muscle Function in a Phase I/II Clinical Trial of Clenbuterol in Pompe Disease Patients Stably Treated With ERT

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Objective: To investigate whether Clenbuterol therapy could increase cation-independent mannose 6-phosphate receptor (CI-MPR)-mediated uptake of recombinant human acid-α-glucosidase (rhGAA) during enzyme replacement therapy (ERT) in Pompe disease. The skeletal muscle response to ERT in Pompe disease is limited due to poor delivery of ERT and low CI-MPR expression in skeletal muscle. We have previously demonstrated the benefit of increased CI-MPR-mediated uptake of rhGAA ERT in mice with Pompe disease following addition of Clenbuterol therapy. **Methods:** In this 52-week Phase I/II double-blind, randomized, placebo-controlled study we have investigated the use of Clenbuterol in patients with late-onset Pompe disease (LOPD) stably treated with ERT. Inclusion criteria included LOPD pts who were treated with ERT for more than 1 year at the standard dose, and exclusion criteria included nonstandard ERT dosing, or a contraindication to Clenbuterol administration. The efficacy of Clenbuterol treatment during ERT in patients with LOPD was evaluated with muscle and pulmonary function testing as the primary end points. **Results:** One significant adverse event unrelated to Clenbuterol occurred, as well as transient minor adverse events and mild elevation of creatine kinase associated with Clenbuterol administration. From an efficacy perspective, at Week 52 the 6-minute walk test increased 40 meters (from 350 m $\pm$ 130 m to 390 m $\pm$ 120 m, $P = .08$) and 11% with regard to predicted performance ($P = .03$). The predicted maximum inspiratory pressure (MIP) increased 224% (from 21 $\pm$ 73 to 68 $\pm$ 30; $P = .02$) for the Clenbuterol treated group, demonstrating increased respiratory muscle strength. Exploratory endpoints improved including the QMFT and the GSGC. No significant changes were demonstrated in the placebo-exposed group. Biopsy of the vastus lateralis demonstrated 50% lower glycogen content and improved histology at Week 52. RNA-Seq from muscle samples revealed 853 genes with significantly altered expression ($P < .05$) in association with improved biochemical correction. Among these genes, the reactome for collagen formation was increased and 17 collagen genes were upregulated. The reactome for the respiratory electron transport chain trended downward, and 4 complex IV and one complex I subunit genes were downregulated. **Conclusion:** This study revealed initial safety and efficacy for adjunctive Clenbuterol therapy in patients with LOPD who are stably treated with ERT.

810-The Nurse's Role in the Multidisciplinary Management of Patients on Enzyme Replacement Therapy

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Enzyme replacement therapy (ERT) is a mode of therapy that provides an artificially manufactured enzyme to replace the deficient enzyme in Mucopolysaccharidoses (MPS) types I, II, and VI, Fabry-, Pompe- and Gaucher- diseases. It has been approved and funded in many countries for over a decade. ERT has been approved in Australia for these disorders for approximately 10 years and is funded by the Australian Federal Government under a special scheme: the Life Saving Drugs Program (LSDP). In order to access treatment, applications are submitted to the LSDP based on strict clinical, laboratory and diagnostic (e.g., mutation analysis) criteria. Continuation of the treatment is dependent on clinical and laboratory outcome measures presented to LSDP yearly. Patients require annual assessments by a large number of subspecialists. ERT is time consuming; it requires the patient and family to attend the hospital regularly for intravenous infusions and for specialists' appointments to monitor treatment effectiveness. In 2015, 11 patients were treated with ERT at the Royal Children's Hospital in Melbourne: seven with MPS type I, II, and VI, two with Pompe disease, one with Fabry disease and one with Gaucher disease. In 2017, ERT is administered to 16 patients: 12 with MPS I, II, VI, two with Pompe disease, one with Fabry disease and one with Gaucher disease. A patient on ERT treatment may need to come to hospital weekly or fortnightly for IV treatment and have 10-48 sub-speciality face-to-face encounters (April 2016- April 2017 data). The Nurse acts as a liaison between the family, the clinical teams, community health services and LSDP. Within the hospital environment the Nurse coordinates multidisciplinary care including sub specialties, the admissions manager, Day Medical Unit staff, Pharmacy, hospital school and the family to ensure seamless appointments and treatments. The Nurse builds a strong working relationship with the family by regular meetings and phone calls. The Nurse offers support in order to foster communication and compliance. Not least, the Nurse must consider the child's quality of

life and works with the family to facilitate the best outcome possible. Given the increase in the number of patients and predicted increase in the number of disorders for which ERT will be available, the nurse's role has become pivotal to maintaining the quality assurance of the treatment.

811 - ZFN-Mediated In Vivo Genome Editing Results in Therapeutic Levels of Alpha Galactosidase A and Effective Substrate Reduction in a Murine Model for Fabry Disease

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Fabry disease (FD) is an X-linked lysosomal storage disease caused by mutations in the *GLA* gene encoding alpha galactosidase A (α-GalA). FD is characterized by progressive systemic accumulation of α-GalA's substrates, globotriaosylceramide (Gb3) and lyso-Gb3, leading to renal, cardiac, and/or cerebrovascular disease and culminating in premature demise. The mutation and any residual α-GalA enzyme level determine whether the disease presents as classic early onset FD or as one of the later onset variants. FD is treated by enzyme replacement therapy (ERT) via lifelong biweekly infusions, which often create hardship for patients, and can yield variable results. Thus, an improved, more effective, and long-lasting treatment is needed. The presented strategy (currently in clinical studies for Hemophilia B and Mucopolysaccharidoses type I and II) uses Zinc-Finger Nuclease (ZFN)-mediated genome editing to permanently integrate a corrective h*GLA* gene in the *Albumin* locus in liver cells. This approach ensures long-term expression of the transgene by exploiting the high level transcriptional activity of the native *Albumin* enhancer/promoter in stably modified hepatocytes. This genome editing approach was tested in a mouse model (GLAKO) lacking α-GalA activity and containing high levels of Gb3/lyso-Gb3 in plasma and tissues. GLAKO mice were treated with a single injection of an AAV h*GLA* donor in the presence of *Albumin*-targeted AAV ZFNs under the control of a liver-specific promoter. Administration of the three AAV vectors achieved supraphysiological levels of up to 50× of WT of α-GalA activity in plasma (sustained for 2 month study duration) and high activity levels in liver, heart, kidney and spleen. Gb3 in these tissues averaged <10% of that measured in untreated GLAKO mice. Importantly, appropriate glycosylation of the α-GalA enzyme was confirmed to ensure efficient lysosomal uptake in target tissues. In follow-up studies, further improvements to the hGLA donor construct utilizing alternate signal peptides resulted in a-GalA activity levels of up to 250x of WT with near complete clearance of Gb3/Lyso-Gb3 storage material in target tissues. Thus "proof-of-concept" for use of AAV-mediated genome editing of hepatocytes to express therapeutic levels of human α-GalA has been demonstrated. A marked reduction of pathological accumulated Gb3/lyso-Gb3 in key tissue sites was found, further supporting our liver-based AAV approaches as potential therapies for FD.

812 - Liver-Based Expression of the Human Alpha-Galactosidase A Gene in a Murine Fabry Disease Model Results in Continuous Therapeutic Levels of Enzyme Activity and Effective Substrate Reduction

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Fabry disease (FD) is an X-linked lysosomal storage disease caused by mutations in the *GLA* gene encoding alpha galactosidase A (α-GalA), which acts on globotriaosylceramide (Gb3) and its deacylated version lyso-Gb3. The progressive systemic accumulation of these substrates leads to renal, cardiac, and/or cerebrovascular disease in adulthood and can result in premature demise. The mutation and any residual α-GalA enzyme level determine whether the disease presents as classic early onset FD or as one of the later onset phenotypes. FD is treated by enzyme replacement therapy (ERT), however the short half-life of the enzyme necessitates lifelong biweekly infusions, with variable results. An improved, more effective, and long-lasting treatment would benefit FD patients. An AAV-mediated, liver-targeted gene therapy was evaluated in a mouse model (GLAKO) that lacks α-GalA activity, resulting in high levels of Gb3/lyso-Gb3 in plasma and tissues. This strategy employs an episomal AAV vector encoding human *GLA* cDNA (h*GLA*) driven by a liver-specific promoter. Vector administration in GLAKO mice led to supraphysiological plasma α-GalA levels (up to 200-fold of WT at some doses) at day 14 sustained continuously for up to 2 months. At study end, dose-dependent α-GalA activity was increased in liver, heart, kidney, and spleen with a corresponding reduction of Gb3/lyso-Gb3 to near-normal levels. In a follow-up 6-month dose finding study in GLAKO mice, where six different doses of AAV h*GLA* cDNA were tested, it was found that expression of α-GalA (up to 30-fold of WT) was stable up to 6 months and well tolerated in these animals. Data for tissue uptake and Gb3/Lyso-Gb3 clearance at study end will be presented. Importantly, appropriate glycosylation of the α-GalA enzyme produced from liver cells was confirmed by in vitro and in vivo experiments to ensure efficient mannose-6-phosphate mediated lysosomal uptake in target tissues. Thus "proof-of-concept" for the use of AAV-

mediated targeting of hepatocytes to express therapeutic levels of human α-GalA has been demonstrated. A marked reduction of the pathological accumulated Gb3/lyso-Gb3 in key tissue sites was found, further supporting this liver-based AAV h*GLA* cDNA approach as a potential therapy for FD.

813 - Enzyme Replacement Therapy Infusion Related Reactions: A Single-Center review of Incidence and Management in Children With Lysosomal Storage Disorders

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Objectives: Infusion related reactions (IRRs) are well-recognized in lysosomal storage disorder (LSD) enzyme replacement therapies (ERTs), with mild reactions reported in 15%-50% of patients, and severe/anaphylactic reactions in 0.2-7.7%. We report a single-center practice review. **Methods:** Retrospective review of LSD patients receiving ERT over a 5-year period to determine IRR incidence. Acute assessment, investigation, and management compared to national anaphylaxis guidelines (Resuscitation Council UK; RCPCH). **Results:** At any given time, there were between 90 and 105 patients with LSDs receiving ERT. Eleven patients experienced significant IRRs (1 alglucosidase alfa, 2 idursulfase, 2 laronidase, 3 elosulfase, and 3 agalsidase beta). Diversity in reaction timing and nature was apparent. Clinical documentation varied. IRR ranged from hypersensitivity to anaphylaxis. Investigations obtained included tryptase, total IgE, and anti-ERT IgG antibodies. Acute management involved pausing or slowing infusions, administration of intravenous hydrocortisone and/or antihistamine, with small numbers receiving intramuscular adrenaline. Management for subsequent infusions included oral/intravenous steroids, antihistamine pre-medication and infusion rate modification. Individual regimes varied. **Conclusions:** ERT IRRs are unpredictable and necessitate structured treatment. A "Traffic Light" ERT reaction protocol has been developed to facilitate optimal assessment, investigation and management. The impact of the protocol will be reviewed, aiming to ensure consistent, effective management.

W) Glycosylation Disorders/CDG, Protein Modification Disorders (814 to 831)

814 - The Patient Diagnosed With Congenital Glycosylation Defect as a Refugee: Her Life is a Novel

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Introduction: It is harder to diagnose in recently defined types of hereditary metabolic diseases. It is intended to determine and resolve the problems by the experiences of a refugee. **Case:** An 8-month-old girl has applied for growth retardation. She was born mature with1500 g from consanguineous Syrian parents (third degree relatives). As she was six-months old, she was brought to İstanbul by her father employed as seasonal tourism worker. The neuro-motor retardation was realized during routine control in a local clinic and then the kid was directed to a pediatric neurology specialist. Subsequently, the kid was sent to our center for metabolic assessment. In the initial medical examination performed, decreased tonus and inverted nipples were observed and the patient was holding her head partially. The results of the previous blood tests were normal (carnitine profile, lactate, pyruvate, and blood amino acids). Transferrin isoelectric focusing applied to the patient with pre-diagnosis of Congenital Glycosylation Defects (CGD). CDG due to the significant cerebellar atrophy in magnetic resonance imaging of the brain and pathology was found. The blood sample taken from the patient has been sent to Belgium for advanced assessment and pathologic results have been confirmed accordingly. The family was moving constantly and it was finally contacted at İzmir. A group of volunteers providing assistance to refugees have supported the family failing to cover cargo expenses of the samples sent to Belgium. The molecular diagnosis possibility was also searched because she was the family's first child. A special genetic laboratory was found for assistance. After contact, the family returned to Aleppo-Syria and the geneticist in Gaziantep Public Hospital. The patient's blood was sent to a private genetic laboratory affiliated with the state hospital. The result consisted of a single line as: "*PMM2* gene p.V129G>A homozygote mutation, CDG type 1a". This result was obtained after 10 months following the application date by contacting with 4 laboratories in 4 cities located in 3 different countries. The patient was diagnosed with slight pericardial effusion in the last checkup when she was one-and-a-half year old, whereas the patient's family continues to migrate. **Conclusion:** Health-care services are a human right. International cooperation is required to provide solution in transition zones such as our country.

815 - A 20-Years Follow-Up of a Case of Congenital Disorder of Glycosylation Type 1b

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Congenital Disorder of Glycosylation (CDG) 1b is a rare metabolic disorder caused by phosphomannose isomerase activity and presents in childhood primarily with gastro-intestinal and hepatic symptoms. The condition responds to oral mannose treatment. We present a case of 20-year old female patient, homozygous for the D131 N mutation in the phosphomannose isomerase (PM1) gene that was in keeping with reduced

phosphomannomutase isomerase activity. Her Carbohydrate Deficient Transferrin was 16.8% (0-2.6). The diagnosis was made when she was 6-months of age after she presented with hypoglycemia during the episodes of diarrhea and vomiting and was associated with hyperinsulinism. She had hepatomegaly and gastrointestinal symptoms without neurological signs. Thrombotic tendency screen returned to normal within 10 days of commencing mannose and over the next five months her transferrin pattern also improved. There were no significant health problems throughout her childhood and teenage years. At the age of 20, her liver function tests were normal with normal clotting; Protein C was 99 μ/dL (70-140) and protein S 96 (53-123 μ/dL). Her glucose was 4.3 mmol/L after prolonged fasting of 59 hours. Due to poor compliance to mannose, the treatment was stopped and she has remained off mannose for three years now. She reports occasional loose stools that occur infrequently. Bilateral diffuse osteochondritis dissecans, significantly limiting her mobility, remained the main concern. She underwent two surgical procedures under general anesthetics. Six months later an USS of her knees showed osteophyte reoccurrence. She followed emergency regimen during peri-operative period to prevent hypoglycemia more than to treat it. In conclusion, CDG 1b is a treatable condition. Our patient seems to have grown out of the disease and is well despite no mannose administration. Enchondromatosis has been described in O-linked, but not in N-linked disorders of glycosylation. We report the first case of CDG1b with features similar to Ollier disease affecting her knees with no clear association between these two conditions.

816 - Improvement of Glycosylation Abnormalities in a Severely Affected Patient With SLC39A8 Deficiency and Mitochondrial Dysfunction

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The *SLC39A8* gene encodes a ubiquitously expressed, transmembrane manganese (Mn) and zinc (Zn) transporter which localizes to both cell and mitochondrial membranes. Mn and Zn are essential trace elements and cofactors for multiple biological processes including energy metabolism, neurodevelopment, blood clotting, immunological response, endocrine regulation, bone, and connective tissue growth. Due to the abundance of Mn- and Zn-dependent processes, SLC39A8 transporter deficiency has been associated with a severe multisystemic developmental disorder. Decreased serum Mn concentrations impair the function of β-galatosyltransferase that transfers UDP-galactose to N-acetylglucosamine of a glycan, resulting in reduced glycosylation and type II congenital disorders of glycosylation. Increasing the intracellular UDP-galactose pool by galactose and uridine supplementation completely restored galactosylation in a severely affected patient (Park et al 2015). Here, we describe a recently reported 13-year-old female with SLC39A8 deficiency, a primary disorder of Mn transport with secondary glycosylation abnormalities and mitochondrial dysfunction displaying profound psychomotor retardation, dystonia, seizures, failure to thrive, and bilateral basal ganglia hyperintensities on T2-weighted imaging and cerebral atrophy (Riley et al J Inherit Metab Dis. 2017). Initial supplementation with oral galactose and uridine, with subsequent addition of Mn led to significant improvement and transient normalization of the transferrin isoform pattern with clinically correlated reductions in dystonia, tremors, and improved attentiveness. It remains to be established whether introduction of these therapies early in the clinical course of the disease would have an ameliorating effect on disease progression.

817 - N-Acetylmannosamine (ManNAc) for the Treatment of GNE Myopathy: 18-Month Preliminary Results From a Phase 2 Open-Label Study

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Background: GNE myopathy is a rare, autosomal recessive, adult-onset myopathy caused by deficiency of the rate-limiting enzyme (GNE) of sialic acid biosynthesis. In GNE myopathy, progressive skeletal muscle atrophy decreases strength, function, and quality of life in patients. There is no approved therapy. *N*-acetyl-D-mannosamine (ManNAc), an uncharged monosaccharide and the first committed precursor in the sialic acid biosynthetic pathway, is an oral therapeutic candidate that prevents muscle weakness in mouse models of GNE myopathy. The Phase 1 trial showed that ManNAc is safe and increases sialic acid production in GNE myopathy patients. **Objective:** We present an interim analysis after 18 months of oral administration of ManNAc at doses of 6 grams twice daily in an ongoing open-label phase 2 study (NCT02346461) of ManNAc in GNE myopathy patients. **Methods:** Twelve patients were enrolled in two cohorts. Safety, pharmacokinetics, pharmacodynamics, as well as measures of strength, function and patient-reported outcomes were evaluated after administration of oral

ManNAc for 18 months. **Results**: Long-term administration of ManNAc appeared safe. Twice daily ManNAc dosing results in a sustained increase in levels of plasma free sialic acid, while ManNAc does not accumulate in plasma. Comparison of muscle biopsies taken at baseline and at 3 months showed improved membrane sialylation after administration of ManNAc. At 18-months we estimated a $\sim$45% slowing in the rate of decline, with a 96% probability that ManNAc slowed the rate of progression of disease. When subjects were asked about their ability to perform tasks or functions, the majority reported their functional ability to be the same or improved. **Conclusion:** These findings support further development of ManNAc as a therapy for GNE myopathy.

818 - CGD Type Ib Patient Diagnosed due to Hypoglycemia and Elevated Liver Function Tests

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Introduction: Congenital glycosylation defects (CDG) autosomal recessive disorders clinically characterized with growth retardation, hypotonia, and with multisystemic involvement. CDG type Ib is due to deficiency in phosphomannose isomerase which converts fructose-6-phosphate into mannose-6-phosphate. Patients usually present with hepatic or gastrointestinal symptoms lacking cranial involvement making their IQ completely normal. We present a case of CGD type Ib diagnosed in our clinic. **Case report:** A 10-month-old girl presents to emergency department with severe diarrhea, vomiting, and growth retardation. Her story reveals only preterm (32 weeks) birth and nothing significant. On physical exam her height, weight, and head circumference was below 3 rd percentile however her developmental steps were normal. She had her liver 2 cm and spleen 1.5 cm below mid-costal margin. On laboratory work up, her glucose level was low with 28 mg/dL and liver function tests were high with alanine aminotransferase (ALT) 244 IU/L and aspartate aminotransferase (AST) 274 IU/L. On follow up, her LFT's remained high and glucose level remained low; on further exam, her fasting insulin level was detected high with 11.7 mIU/L ($\geq$2) and diagnosed with hyperinsulinemic hypoglycemia and diazoxide was given as treatment. The patient then referred to our clinic for follow up. On first visit her laboratory revealed hypoglycemia and elevated LFTs. With all these finding, CDG type Ib was suspected and further evaluation was initiated. Protein C %27(%70-140), Protein S %59,5(%63.5-149), Antitrombin III %40 (%83–128) was detected low concurrent to our prediagnosis. Then transferrin isoelectric focusing testing was performed and type I pattern was seen which was typical for CDG type Ib. Her genetic testing later showed homozygous pM138 T mutation and her diagnosis was made definitively. Oral D-mannose treatment was started with 1.2 mg/kg/day q6 hours. She dramatically reacted to treatment: her diarrhea and hypoglycemia resolved, her liver and spleen decreased in size as well as her LFT's. Due to regulated blood sugar, diazoxide was stopped. **Result:** CDG type Ib is a newly defined disorder which dramatically benefits from D-mannose treatment. Children with GI symptoms, hyperinsulinemic hypoglycemia, palpable liver and spleen, growth retardation and elevated LFT's should be evaluated for the disease and thought in differential diagnosis.

819 - A 10-Year Research Program on Congenital Disorders of Glycosylation in Argentina

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Defects in the N-glycosylation pathway include more than 110 affected genes, and they are responsible for Congenital Disorders of Glycosylation (CDG) an inherited metabolic disease. Protein hypoglycosylation caused a clinical multisystem phenotype ranging from mild to severe that leads the diagnosis in a very challenging way. **The objective** was to communicate our 10-year CDG research program in Argentina and to present data to expand the molecular and clinical knowledge for CDG in Latin American countries. Here, we describe the current state of diagnostics together with our experience on potential pitfalls in the screening of CDG. **Methods:** Patients with clinical criteria for CDG referred by physician who presented ethical permissions have been studied. The clinical phenotype at neonatal period or during the first years of life showed psychomotor retardation, neurologic features, hematological and endocrine abnormalities. Serum samples and genomic DNA were obtained from normal controls and CDG suspected patients. Glycosylation in serum transferrin (Tf) were studied by isoelectric focusing (IEF), high performance liquid chromatography (HPLC), and/or capillary electrophoresis (CE). The genetic analysis was performed by Sanger sequencing or exome sequencing. **Results:** Testing of serum glycoproteins

from individuals presenting altered transferrin isoform type I pattern (8) or type II (2), showed a considerable decrease in the amount of transferrin isoforms terminating in sialic acid. We identified compound heterozygous mutations in four PMM2-CDG patients and homozygous mutations in two ALG2-CDG patients. The 30% of patients with altered Tf-IEF were observed as genetic defects different from CDG (*GALT*; *COLA2* and transferrin SNPs). **Summary:** patients with mutations affecting the N-linked glycosylation pathway are the most common CDG type with seventy distinct genes identified (Freeze et al., 2014). Here we report the first CDG program in Argentina. Mutations in the most frequent gene *PMM2* were detected by Sanger sequencing in all PMM2-CDG clinical suspected patients. We report the identification of only the second and third ALG2-CDG worldwide. The exome studies were made in collaboration with Dr. Hudson Freeze (ALG2-CDG) and Dr. Gert Matthijs (Bistué Millón et al. 2012). In this context, we organized two CDG Latin American Symposiums and the internships of Latin American professionals to increase CDG knowledge and detection in our Countries. www.cdgargentina.com.ar

820 - Exome Sequencing for the Clinical Diagnosis of Non-Progressive Hepatic Form of Glycogen Storage Disease IV

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Glycogen storage disease type IV-(GSDIV) is caused by homozygous or compound heterozygous mutation in the *GBE1* gene, which encodes the glycogen branching enzyme, on chromosome 3p12. GSDIV is a clinically heterogeneous disorder. The typical "classic" hepatic presentation is liver disease of childhood, progressing to lethal cirrhosis. The neuromuscular presentation of GSDIV is distinguished by age at onset into 4 groups: Perinatal, presenting the fetal akinesia deformation sequence and perinatal death; Congenital, with hypotonia, neuronal involvement, and death in early infancy; Childhood, with myopathy or cardiomyopathy; and Adult, with isolated myopathy or adult polyglucosan body disease. The enzyme deficiency results in tissue accumulation of abnormal glycogen with fewer branching points and longer outer branches, resembling an amylopectin-like structure, also known as polyglucosan. 2-year-old female patient with history of congenital hypothyroidism, global neuro-developmental delay, hypotonia, failure to grow, with metabolic studies: negative urine screening, negative amino acid chromatography, normal karyotype, cerebral magnetic resonance imaging showing mild atrophy cerebral, dysmorphic features without hepatic or cardiac involvement. Non-consanguinity is reported between parents, and no previously family history for this disease. Exome sequencing was performed on the Illumina platform, showing single nucleotide variant (G >A) on gene *GBE1*, located on chromosome-3 (rs80338672), with heterozygous inheritance mode, reported as missense, and previously associated with pathogenicity. Non-progressive hepatic form is less frequent, Burrow et al (2006) reported a 30-month-old girl with GSD4 who had stable congenital hypotonia with gross motor delay and severe fibro fatty replacement of the musculature, but no hepatic or cardiac involvement. It suggested that the unusually mild phenotype in this patient might be due to residual enzyme activity.

821 - Congenital Disorder of Glycosylation—Phosphomannomutase 2 Deficiency (CDG-PMM2/ CDG IA): Follow Up Findings in a 32-year-old Argentinian Patient

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CDG-pmm2/cdg1a is mainly characterized by a multisystemic age-related clinical manifestations including failure to thrive, hypotonia, developmental delay, strabismus, cerebellar hypoplasia, inverted nipples and fat pads. A 32-year-old female patient. She presented low APGAR score, hypotonia, feeding difficulties, low weight, strabismus, and fat pads noticed in the first months of life. After the first year, delayed developmental was evident and also ataxic gait, lower extremities hypotrophy, stroke like episodes (2) deep venous thrombosis, amenorrhea, cataracts, hypothyroidism, and supra renal insufficiency. MRI showed a progressive cerebellar atrophy. In the last year a thyroid neoplasia was detected and removed. Nowadays, she is a happy and friendly girl. The screening test of CDG was performed by Dr. Nestor Chamoles who suspected CDG1A, revealed a typical CDG1 pattern. Fibroblast test confirmed PPM deficiency, molecular study revealed homozygosity for p.R141 H mutation (Dr. Jaak Jaeken) Trough this presentation we aim to contribute to a better knowledge of this inherited error of metabolism, tending to increase the suspicion index and improve the follow up of CDG patients and families.

822 - PMM2-CDG and Sensorineural Hearing Loss

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A two-year-old boy was referred for evaluation of psychomotor disability, hypotonia, and sensorineural deafness since infancy. There was no hearing impairment in the family according to the parents. Physical examination showed a height of 80 cm (below 3 rd centile = 82 cm), weight of 8.2 kg (below 3 rd centile = 10.5 kg), and head circumference of 43 cm (below 3 rd centile = 47.3 cm). He showed facial dysmorphism (hypertelorism, small rounded nose, pronounced philtrum, thin upper lip, high palate, and micrognathia), a keel thorax, inverted nipples, abnormal fat distribution and mild hepatomegaly. Eyes were wide set with a shallow nasal bridge and synophrys. He had severe hypotoniaand hyporeflexia. He was not able to sit unsupported and was nonverbal. Blood investigation showed increased serum transaminases [AST: 1689 U/L, ALT: 1049 U/L(normal range: 0-40 U/L)] and gamma-GT: 29 U/L(normal:4-18 U/L). PT, aPTT, antithrombin, and factor XI were normal. Ultrasonographic examination showed minimally enlarged liver size with normal echogenicity. Echocardiography and ophthalmological evaluation were normal. Brain MRI showed cerebellar atrophy and widening of the posterior fossa and pericerebellar space. A type 1 pattern was found on serum transferrin isoelectrofocusing, and he was found to be homozygous for the known *PMM2* variant c.385G>A (p.Val129Met).The present patient had thus PMM2-CDG (phosphomannomutase 2 deficiency), the most common N-glycosylation defect. Two CDGs have been significantly associated with deafness namely RFT1-CDG (10 out of 13 patients) and ALG11-CDG (4/9). As to PMM2-CDG, we found 15 reports with altogether 20 patients showing sensorineural hearing loss/hearing impairment. At least 700 patients have been reported with PMM2-CDG. Although, calculation results in a 3% prevalence of hearing impairment in this CDG. On the other hand, hearing impairment is not rare in the general population. According to the WHO, hearing impairment in the European general population has a prevalence between 3.2 and 13% depending on the population considered. From these data, it seems that hearing impairment in PMM2-CDG is not a symptom but a coincidental finding.

823 - Toward a PMM2-CDG Therapy: Optimization Process of Potential Pharmacological Chaperones

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The functional characterization of Phosphomannomutase 2 (PMM2) disease-causing mutations has suggested that PMM2-CDG could be a conformational disease and that therapies addressed to improve the protein folding would be able to ameliorate clinical symptoms. From a 10 000 compound library screening, 8 possible pharmacological chaperones (PCs) were selected. The compound 1-(3-chlorophenyl)-3-3-bis(pyridine-2-yl) urea (compound VIII) stood out, based on its pharmacochemical properties, showing no inhibitory effect on PMM2 enzymatic activity and enhancing the activity and stability of a number of destabilizing PMM2 mutations. These results provided the first proof-of-concept of a possible treatment for PMM2-CDG and identified a promising chemical structure as a starting lead for the development of new therapeutic agents against this severe orphan disease. The aim of this work was setting-up an optimization process by methodological sequential rounds from a battery of chemical analogs of compound VIII in order to improve the physicochemical properties and cytotoxicity. **Methods:** Up to 795 chemical-analogs of compound VIII have been selected. These compounds have been evaluated by complementary approaches to determine their viability as potential PCs. First, pharmacochemical analysis of the compounds was performed by computational analysis using the SmartsFilter program. Afterwards, the in vitro effect on PMM2 enzymatic activity and stability was evaluated by IC_{50} and differential scanning fluorimetry assays using the recombinant PMM2 protein. Results: From 795 analogs, 165 passed every reactivity filter by the pharmacochemical analysis. Out of 165, 25 compounds were selected for further in vitro analysis. In this first selection, 7 of the 25 structure analogs have shown no concentration-dependent inhibitory effect on enzymatic activity, a mild stability improvement and no chemical reactivity. **Discussion:** This workflow for developing a potential therapy for PMM2-CDG has shown a dynamic progression from a promising PC to 7 new compounds that passed the first required critical selection points, allowing the process to move forward to the next screening levels in a cellular model which will provide new potential structures with pharmacological effects. PI16/00573; MINECO-FEDER. Fundación Isabel Gemio.

824 - Maternal Uniparental Isodisomy Causing a New Case of B4GALT1-CDG (CDG IId)

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Introduction: Till now only two cases of B4GALT1-CDG (CDGIId) have been reported in the literature. We describe a new case which presents a mutation in homozygosity not previously described, being also the first case of inheritance due to maternal uniparental isodisomy. **Clinical case:** A 4-month-old girl of Spanish origin presents with cervicoaxial hypotonia, peculiar phenotype and mild hepatosplenomegaly. Analytical data: CPK 1009 U / L, GOT 254 U / L, TSH 15.2 μUI / mL, free

T4 0.87 ng / dL, free T3 4.33 ng / dL, and a carbohydrate-deficient transferrin (CDT) of 34%. The serum sialotransferrin profile showed a significant elevation of asialo-, monosialo-, and disialo- transferrin isoforms. Cardiologic and ophthalmologic study, abdominal ultrasound, transfontanelar ultrasonography and skeletal series showed no alterations. TTPa elongation and high antithrombin levels are detected, with no clinical repercussions or need for treatment. The genetic study by exome sequencing identified a novel mutation in homozygous fashion in the gene B4GALT1. The mother bears the mutation in heterozygosis and the father is not a carrier. Further analysis by SNP array indicated a complete UPD of chromosome 9 and analysis of STR indicated a maternal isodisomy across chromosome 9. At present, at 2 years of age, psychomotor development is normal. She has a low-percentiles of the pondero-structural development, a hypothyroidism that is controlled with substitutive treatment, a myopathy with elevation of CPK and GOT without clinical repercussion and a mild hepatosplenomegaly. **Conclusion:** Our patient has a weight-loss delay and CPK levels much higher than those described in the other two B4GALT1-CDG patients reported. It will be necessary to know new cases of CDGIId in order to define or better establish its clinical spectrum but in this case is needed the evaluation of the consequence of the maternal isodisomy.

825 - Would Congenital Disorders of Glycosylation Incidence be Higher Than the Expected?

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Congenital disorders of glycosylation (CDGs) cause impaired synthesis of glycoconjugates and are genetically heterogeneous with pathogenic variants over one hundred genes that can cause it. ALG1 mutations cause rare autosomal recessive disorder, called ALG1-CDG. Main devastating features are neurodevelopmental retardation with multisystem involvement. But, it is difficult to diagnose when there are no stimulative findings. Here presented an ALG1-CDG case, who has unexplained seizures and neurodevelopmental delay. **Case:** A male patient at eight months old with neurodevelopmental delay and seizures. The patient was born with birth weight 3300 gr, after 40 weeks of unremarkable gestation. He had no head control until six months of age. His physical examination showed hypotonia. His eye and hearing examinations were normal. When he became eight months old, he had epileptic spasms. His electroencephalogram showed hypsarrhythmia. Blood gas, biochemical parameters, lactate, ammonia, tandem-mass spectroscopy, amino acids, very long chain fatty acids levels, urine organic acids, and amino acids levels were normal. There are no pathological signs on brain magnetic resonance imaging. Isoelectric focusing test showed type 1 pattern. The molecular analysis has been performed, and was found homozygous mutation on ALG1 gene [p.Gly145Asp(c.434>A)]. **Conclusion:** Our patient had non-specific clinical findings as neurodevelopmental delay and seizures. The patients, who have similar symptoms, apply to our clinics very often. Many times, etiology for those patients cannot be found. Normally, neurological involvements and/or dysmorphic features and at least other systemic involvements are expected for the ALG1-CDG patients, according to the literature. Therefore, it is difficult to think about this disease when a patient has only neurological involvement like our patient. But, based on the experience we had, we are evaluating this disease for such patients.

826 - Intellectual Disability With Autistic Features as Atypical Presentation of ALG8-CDG due to Novel Gene Variants Detected by Targeted Resequencing

Lorenzo Ferri[1], Beatrice Berti[1], Francesco Mari[1], Maria Alice Donati[1], Elena Procopio[1], Duccio Malesci[1], Rita Barone[2], Davide Mei[1], Elena Parrini[1], Renzo Guerrini[1], and Amelia Morrone[1]

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Background: ALG8-CDG (OMIM 608104) is caused by mutations in the *ALG8* gene which encodes ALG8 alpha-1,3-glucosyltransferase. ALG8-CDG is a congenital disorder of glycosylation (CDG) with dysmorphism (retrognathia, low-set ears, pes equinovarus), muscular hypotonia, hepatomegaly, coagulopathy, edema and ascites (including fetal hydrops), cardiorespiratory problems, protein-losing enteropathy, and cataracts. ALG8-CDG affects N-linked glycosylation and patients show abnormal profile of serum transferrin isoelectric focusing (TIEF). **Case Report:** A 11-year-old male patient born from non-consanguineous parents. He delayed early developmental milestones since the age of 8 months. He did not show failure to thrive. He exhibited facial dysmorphism (high-arched palate, micrognathia, saddle nose, and low set ears), inverted nipples, peculiar fat pads, overweight, mild scoliosis and mild hypotonia. During his life, he developed a severe cognitive impairment with a severe autistic spectrum disease with poor social interaction, motor and vocal stereotypies. TIEF showed an abnormal profile. **Methods:** Targeted next-generation sequencing (NGS) (exons + splice junctions) using HaloPlex enrichment method (Agilent Technologies) and Pair-End 2x150 bp on an Illumina MiSeq platform. Reads were mapped by BWA and variants called accordingly to GATK best practices. **Object:** To identify the genetic cause of CDG by performing a genetic differential diagnosis basing on targeted NGS of 85 CDG-related genes. **Results:** We identified three new rare variants in the patient's *ALG8* gene. A c.122G>A p.Arg41Gln variant was inherited from the patent's father. This variant

affects a highly conserved amino acid and is predicted to be deleterious by several bioinformatic tools. A c.445T>G p.Leu149Arg and a c.980C>G p.Thr327Arg variants were inherited both from the patent's mother. The p.Leu149Arg variant affects a highly conserved amino acid and is predicted to be deleterious while the p.Thr327Arg variant falls in a weakly conserved amino acid with conflicting bioinformatic predictions. Interestingly, the p.Leu149Arg variant affects an intramembrane portion of the ALG8 protein changing a non-polar (Leu) into polar (Arg) amino acid. **Conclusion:** Targeted NGS leads to *rapid* and cost-effective differential diagnosis among different CDG. We also expand the phenotype of ALG8-CDG to a severe intellectual disability with autistic features and inverted nipples.

827 - ALG13 Deficiency Causes X-Linked Mental Retardation and Congenital Disorder of Glycosylation Type I

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Background: ALG13 encodes together with ALG14 a UDP-GlcNAc transferase and is therefore essential in the pathway of N-linked glycosylation by catalyzing the second step in the endoplasmic reticulum. This UDP-GlcNAc transferase transfers the second N-acetylglucosamine to the dolichol linked oligosaccharide precursor. Disruptive variants in ALG13 cause a congenital disorder of glycosylation (CDG) presenting with X-linked mental retardation as main clinical phenotype. **Methods:** Two siblings were introduced presenting with mental retardation, dystrophy, retrognathia and muscular hypotonia in order to perform screening for CDG using high performance liquid chromatography and isoelectric focusing. To confirm our findings, we performed ESI-TOF MS. In order to identify the genetic cause, we used next generation sequencing, confirming our results with Sanger sequencing. **Results:** We identified a hemizygot variant c.1886 A>G (Y629C) in ALG 13 in both siblings. Their mother and grandmother are heterozygous for this variant. Both siblings revealed a positive screening for CDG with hypoglycosylation during high-performance liquid chromatography and isoelectric focusing. Mass spectrometry confirmed the missing of the second N-acetylglucosamine in the oligosaccharide chain of transferrin. **Discussion:** Deficiencies in ALG13 cause a congenital disorder of glycosylation, which might easily be overlooked by mainly presenting with an X-linked mental retardation. In order to ensure a more effective glycosylation a causal therapeutic approach is to correct hypoglycosylation, which is essential, since it would be one of few treatable X-linked mental retardations.

828 - ATP6AP2 Deficiency Causes a Congenital Disorder of glycosylation type II and Impaired Autophagy

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Background: ATP6AP2 deficiency impairs the correct assembly of a V-ATPase, which functions as a proton pump in different cell compartments. This V-ATPase is necessary to maintain a pH gradient in the Golgi apparatus as well as for correct autophagy. Congenital disorders of glycosylation (CDG) are a rapidly growing group of diseases caused not only by protein deficiencies directly involved in glycosylation, but also proteins concerning cellular homeostasis especially concerning the ER and Golgi apparatus. ATP6AP2 is therefore not only essential for autophagy but also in order to ensure an efficient glycosylation. **Methods:** To screen for congenital disorders of glycosylation high performance liquid chromatography (HPLC) and isoelectric focusing of transferrin was performed in two patients, with suspicion for CDG. Next generation sequencing was performed in order to identify the cause of an abnormal glycosylation pattern. Electron microscopy was used to analyze a liver biopsy as well as traditional histology. **Results:** A patient with mild hepatopathy and immunodeficiency due to hypogammaglobulinemia was introduced to us. His nephew was born after the diagnosis of this patient was known; due to prenatal diagnostics, it was already known at birth that he carries the same genetic variant. He developed liver failure at the age of 5 months, until today there are no signs for an immunodeficiency. Liver failure was due to a lipid accumulation in the lysosomes, which was seen by liver biopsy and electron microscopy. In the first weeks of life he presented with cutis laxa, his uncle shows increased joint laxity as well as cutis laxa. Both patients revealed hypoglycosylation concerning N-linked glycosylation in screening for CDG. We identified a hemizygote variant in ATP6AP2 (c. 212G>A (R71 H)) as genetic cause. **Discussion:** A deficiency in ATP6AP2 disturbs assembly of the V-ATPase necessary to create a pH gradient in the Golgi as well as causes a disturbed function of lysosomes and endosomes. A therapeutic approach is trying to correct glycosylation by supplementing substrates to support glycosylation and trying to retrieve lysosomal function by using metabolites to release lipid accumulation.

829 - Common Point of Congenital Disorders of Glycosylation Type Ia and Hereditary Fructose Intolerance

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Congenital disorders of glycosylation (CDG) are caused by defective glycosylation of proteins and lipids. Patients with CDG show a very broad spectrum of clinical manifestations. Isolectrofocusing of serum transferrin (IEF) is a screening method of choice for N-glycosylation disorders. Three patients with CDG 1a and one hereditary fructose intolerance were presented with different clinical features showing type 1 pattern characteristics. A 5-year-old boy presented with hypertrophic cardiomyopathy, growth retardation and elevated transaminase levels. On his physical examination, dysmorphic face, pectus carinatus deformity, inverted nipples, axial hypotonia and distal spasticity, 2/6 systolic murmur, and pubic fat pads were detected. Cranial MR revealed bilateral cerebellar atrophy. On abdominal ultrasound hepatosteatosis was detected. PMM2 gene analysis revealed homozygous mutations. Two siblings at the age of 2 years and 4 years complaint about motor mental retardation. Axial hypotonia, distal spasticity, inverted nipples were detected. Cranial MR revealed cerebellar hypoplasia in one of the sibling and corpus callosum agenesia in the other. In the *PMM2* gene analysis compound heterozygote mutations were detected. A 3.5-year-old female patient was evaluated for transaminases elevations, hypoalbuminemia, coagulopathy, metabolic acidosis. Physical examination revealed dysmorphic facial features, fat pads in the gluteal region and hepatosplenomegaly. Type 1 pattern was detected in IEF of serum transferrin. But forward genetic analysis revealed a homozygous mutation in the ALDOB gene and the patient was diagnosed as hereditary fructose intolerance. In the presence of unexplained neurological findings, cerebellar atrophy, abnormal liver function tests, coagulopathy, cardiomyopathy, growth retardation, skeletal involvement findings CDG should be kept in mind. IEF can be used as a screening method. It should not be forgotten that IEF may be false positivity in hereditary fructose intolerance.

830 - De Novo Variant Identified for WES in ALG13, First Report of CDG-1 s in a Colombian Patient

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Introduction: congenital disorders of glycosylation (CDG) are a group of inborn errors of metabolism caused by defects of glycan metabolism presenting with heterogeneous multisystemic clinical manifestations. To date, more than 60 different types of CDG have been reported. Type I (CDG-I) is caused by deficiencies in the genes involved in N-linked glycosylation. ALG13-CDG (CDG-IS) is X-linked infrequent form of these disorders, reported at first time in 2012. **Case report:** we present a case of a boy born for consanguineous parents, with early onset of hypotonia, developmental delay, intellectual disability, feeding problems, patent ductus arteriosus (PDA) and mitral insufficiency, without epilepsy and without a diagnosis despite an extensive genetic and metabolic evaluation (plasma and urine amino acids, urine organic acids, lactate, pyruvate, ammonia, creatine kinase). The classic CDG gene identification included sequential application of biochemical methods in blood samples and fibroblasts, however, is reported the normal glycosylation pattern in CDG-IS. Whole-exome sequencing (WES) was performed in this patient using an Illumina platform. A de novo variant of unknown clinical significance (VUS) in the X-linked gene ALG13 c.428C>T p.P143 L (NM_001099922) was identified; hemizygosity was confirmed by Sanger sequencing. Sequencing also showed that the mother was heterozygous for this change. This amino acid position is highly conserved across species and it's found in a presumed functional domain of this glycotransferase superfamily. During the clinical follow-up of the patient, his mother becomes pregnant, male sex of the gestation were confirmed, the ALG13 variant of his brother was searched and confirmed the carrier status, which voluntary pregnancy interruption (VPI) was performed. **Conclusion:** We report a patient with CDG-IS without epilepsy and microcephaly, both related with this form of CDG. This is the first reported Colombian case of this disorder with a de novo variant identified for Whole exome sequencing, this finding expands the phenotypic spectrum of CDG-I and broad the use of this powerful technology for the identification of disease-causing mutation in diseases with genetic and phenotypic heterogeneity like CDG.

831 - A Development Delay and Dysmorphic Case With *ALG13* Gene Pathogenic Mutation Identified by WES

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Glycosylation is an important and complex co- and posttranslational modification system involving some 1%-2% of all human genes. Congenital disorders of glycosylation (CDG) is a group of genetic diseases in which the synthesis of glycans and their union with glycoconjugates, proteins, and lipids is defective. This process occurs in cytosol, endoplasmic reticulum and the Golgi apparatus. Although, eight biochemical pathways are involved, the best characterized are that involved

in the N- and O-glycosylation of proteins. About 50 genetic defects have been described that affect N-glycosylation of proteins, either isolated or in combination with effects on O-glycosylation. Phosphomannomutase 2 (PMM2)-CDG of the N-glycosylation is the most common type of CDG. The *ALG13* gene defect on N-glycosylation is infrequent and normally, catalyzes the second step of lipid-linked oligosaccharide (LLO) biosynthesis. This defect has X-linked inheritance. Most CDG with N-glycosylation defect are multi-organ diseases with neurological involvement. N-glycosylation disorders are associated with sialic acid deficiency and are detected by serum transferrin isofocusing (IEF). Is our aim, to present a case of ALG 13 gene pathogenic mutation detected by WES, with clinic consistent with CDG and with normal serum transferrin IEF profile. **Clinical case:** 3 years of age male patient. Nonconsanguineous healthy parents. Three older healthy brothers. During pregnancy, increased nuchal translucency was detected. Born at term with normal anthropometry, Apgar test 8/9. Severely global delayed psychomotor development, hypotonia, failure to thrive, limited eyes contact, dysmorphic features, postnatal microcephaly, no epilepsy, abnormal brain MRI interpreted as gliosis. Normal metabolic studies as were the HCG and cytogenetic studies. WES detected novel pathogenic variant which was confirmed by Sanger sequencing in the patient and in his mother in *ALG13* gene: c.2458 G>A. **Conclusions:** we think our patient is affected of X-linked CDG disease: ALG13-CDG, although he has not abnormal serum IEF of transferrin. Our results implicate the potential of WES to unravel disease genes in complex pathway like CDG in newly diagnosed singleton families.

X) Neurotransmitter and Creatine Related Disorders (832 to 840)

832 - Neurometabolic Disorders in a Cohort of 221 Children Below Two Years of Age With Epilepsy: A Single-Center Experience in Saudi Arabia

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Introduction: Epilepsy is a common chronic disorder affecting children below 2 years of age. The contribution of metabolic disorders in the etiology of epilepsy in this age group is not well characterized. The objectives of this study were to identify the metabolic causes of epilepsy in the first 2 years of life and to describe their clinical, electroencephalographic, radiological, and molecular characteristics. **Methods:** A retrospective study was conducted in a tertiary center in Saudi Arabia between January 2010 and December 2011. All patients presented with epilepsy in the first 2 years of life were reviewed. We used The International League against Epilepsy definition of epilepsy and we excluded febrile seizures. **Results:** We identified 221 children under 2 years of age diagnosed with epilepsy. Metabolic disorders were the cause of epilepsy in 24 patients. The features of these 24 patients include consanguinity in 18 (75%), developmental delay in 13 (54%), generalized tonic-clonic seizures in 10 patients (42%), infantile spasms in 4 (17%), myoclonic in 7 (29%), and focal seizures in 3. Molecular diagnosis was confirmed in 17 children (71%) and enzyme deficiency in 7 (29%). The major diagnoses were nonketotic hyperglycinemia (n = 3), peroxisomal disorders (n = 3), neuronal ceroid lipofuscinosis (n = 2), Menkes disease (n = 2), mitochondrial disorder (n = 2), and biotinidase deficiency (n = 2). The remaining patients had lysosomal storage disease, fatty acid oxidation defects, aminoacidopathy, and organic aciduria. Seizure freedom was achieved in one-third of patients in our cohort. **Conclusion:** Different metabolic disorders contributed to the etiology of different types of epilepsy, especially infantile spasms and myoclonic seizures.

833 - Effect of Guanidinoacetate on Memory and Oxidative Status in Striatum

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Guanidinoacetate Methyltransferase (GAMT) deficiency is an autosomal recessive inherited disorder of the metabolism of creatine that leads to depleted levels of creatine and excessive concentrations of guanidinoacetate. Patients affected develop neurological symptoms during childhood, such as muscular hypotonia, involuntary extrapiramidal movements, convulsions, slurred speech, and even autism. Although the pathophysiology of GAMT deficiency is unclear, neurological dysfunction is commonly found in this disease and it has been mainly attributed to reduction of creatine or/and increase of guanidinoacetate (GAA) levels. This study's objective was to investigate the effects of an intrastriatal administration of GAA on non-aversive behavioral test and parameters of oxidative stress such as 2′7′dichlorofluorescein (DCF) oxidation and antioxidant enzyme activities. Sixty days Wistar rats received a single intrastriatal GAA administration or saline 48 hours after stereotactic surgery. Animals were subjected to behavioral or striatum biochemical tests 1 hour after the infusion. In the novel object recognition test, the time exploring the novel object increased in the control group ($P \leq .05$), while the same did not happened in treated animals. GAA significantly decreased the activity of superoxide dismutase (SOD) ($P \leq .01$) and catalase (CAT) ($P \leq .01$) as well as increased DCF oxidation ($P \leq .01$). A pretreatment with creatine for seven days prior to the surgery was able to prevent all alterations, except CAT activity. In conclusion, it is possible to presume that these biochemical alterations caused by high levels of GAA may contribute to neurological alterations found in

patients with GAMT deficiency. Furthermore, it is possible that creatine supplementation helps to prevent these features. Supported by CNPq.

834 - Creatine Synthesis Defects: Experience of 3 Indian Patients

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Background: Cerebral creatine deficiency in brain MR spectroscopy (^{1}H-MRS) is the characteristic hallmark of all Creatine Synthesis Defects (CSD). In India, many children affected with developmental delay, seizures, hypotonia and speech delay are routinely investigated by MRI. However, MRS is not performed routinely and hence these disorders may be missed. **Objective**: To evaluate clinical spectrum and outcome with creatine therapy in cases of CSD. **Methods:** This is a retrospective study of 3 patients detected by MRS with CSD- 2 with creatine transporter defect (CrTr) and 1 with GAMT deficiency. All 3 patients (all males) presented with global developmental delay, delayed speech, hypotonia, and episodes of seizure in patient with GAMT deficiency. Screening for creatine metabolites (creatine, guanidino aceto acetate, creatinine, and arginine) with liquid chromatography-tandem mass spectrometry was performed for all 3 patients in blood, urine, and plasma samples. Genetic studies were performed to confirm the diagnosis. **Result:** We analyzed GAA and Creatine levels in plasma and urine samples of these patients before and after commencing the therapy. Patient with GAMT deficiency was treated with low protein diet and oral creatine supplementation (400 mg/kg/day). This child also received anticonvulsants. He showed improvement in levels of Creatine in plasma as well as reduction of GAA in plasma and urine. Clinically, he showed improvement in tone, behavior, and no more seizures after treatment. There is improvement in cognition. One of the patients with CrTr was treated with Arg 200 mg/kg/day and Creatine 400 mg/kg/day. He showed mild improvement in blood and urinary creatine levels and improvement in tone, activity and some improvement in speech and cognition. Other patient with CrTr was treated with only Creatine initially with unsatisfactory response but later Glycine 150 mg/kg/day was added and following which he showed good recovery. There were no adverse effects seen with creatine supplementation and parental acceptance for therapy was good. **Conclusion:** All 3 of our patients were picked up by MRS and later confirmed by biochemical and genetic studies. All 3 patients responded favorably to treatment. 2 of them showed satisfactory improvement. Creatine therapy is affordable, tolerated well without any side effects and palpable improvements are seen within 3-6 months. We recommend inclusion of MRS while evaluating any child with neurodevelopmental problems.

835 - In Vitro Study of a New Pathogenic Variant of Human 6-Pyruvoyl-Tetrahydrobiopterin Synthase

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Objective: To present an in silico model of a new variant (c.331C>T or p.Ala111Ser) of human 6-pyruvoyl-tetrahydrobiopterin synthase (PTPS) that is the most frequent in Mexican patients with PTPS deficiency. **Methods**: For i*n silico* analyses, PTPS crystallographic structures deposited on the Protein Data Bank were used. For isolation of the *PTS* gene, mRNA was obtained from human embryo kidney cells (HEK293). *PTS* cDNA was obtained through reverse transcription. Amplification of PTS cDNA was performed by end-point PCR using specific oligonucleotides. **Results**: Mutagenesis *in silico* showed that replacing serine per alanine at 111 position, causes repulsive clashes besides, hydrophobic interactions between alanine and neighbor valines are lost. Wild type *PTS* gene coding sequence was successfully isolated from HEK293 cells and cloned in a bacterial vector with *NdeI* and *BamHI* restriction sites flanking the gene at 5' and 3' ends respectively. **Conclusion**: These advances represent the molecular basis for the further functional and structural characterization of p.Ala111Ser PTPS pathogenic variant, which will contribute to the study of genotype/phenotype relationship of patients with PTPS deficiency in Mexico, where this pathogenic variant seems to be the most frequent one.

836 - Diabetic Ketoacidosis in Vanishing White Matter

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Vanishing white matter (VWM) disease (OMIM #603896) is an autosomal recessive chronic and progressive leukodystrophy and is one of the most prevalent inherited childhood leukoencephalopathies. The symptoms of the disease can begin

shortly after birth or in adulthood. The phenotypic variation is very wide and the most common features include neurological deterioration, optic atrophy, seizures, coma, and death [3, 4, 5]. VWM disease is diagnosed on the basis of the clinical symptoms in combination with MRI results and molecular testing. Five Eukaryotic Initiation Factor "EIF" genes are known to be involved in VWM disease: EIF2B1, EIF2B2, EIF2B3, EIF2B4, and EIF2B. Hyperglycemia and diabetic ketoacidosis (DKA) have never been reported before in patients with VWM disease. We are reporting on a case with both VWM disease and DKA, and so to the best of our knowledge, this is the first case of a similar presentation. He was admitted at the age of 8 months through the emergency department with irritability and vomiting and was found to have a blood glucose level (25 mmol/L), HbA1c (11.4%), as well as ketosis (Ketones +3), metabolic acidosis (plasma bicarbonate 15 mEq/L), C-Peptide (22 pmol/L), Glutamate decarboxylase Abs (4 IU/mL), Islet Cells Abs (Negative; normal, Negative), and blood PH (7.1). The patient was readmitted again with intractable seizures and generalized tonic–clonic convulsions, and a CT scan of his brain showed diffuse confluent bilateral symmetrical low attenuation in white matter consistent with leukodystrophy, which was confirmed by an MRI of the brain revealing diffuse white matter disease. Five genes that associated with neonatal and infantile diabetic syndromes (KCNJ11, ABCC8, INS, GCK, and PDX1), and muscle biopsy for defects in mitochondrial respiratory chain complexes were all negative. Here was a history of abortion at 2 months of gestation and premature death of a baby boy at 6 months gestation. Further investigations including Whole Exome Sequencing (WES) revealed a homozygous likely pathogenic variant in EIF2B1 NM_001414: c.146T>G p.(Leu49Arg) in the proband; both parents were heterozygous. This is the first case of an association between VWM disease and DKA which yields additional observations regarding the role of EIF2B1 in glucose regulation and expands the phenotype of VWM disease. However, the relation between the eIF2B protein complex and glucose level is unclear and requires further research.

837 - CSF PLP Deficiency in Inborn Errors of Metabolism: Implications for Treatment

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Background: Pyridoxal 5'-phosphate (PLP) is an essential cofactor for >120 enzyme catalyzed reactions in the body, including neurotransmitter metabolism. A low concentration of PLP in the brain (reflected in a low CSF concentration) can lead to seizures and movement disorders. **Methods:** Data from 304 CSF samples collected from patients at our clinical center over an 18-month period was retrospectively reviewed. Information regarding CSF PLP concentration, diagnosis, symptomatology and medication was collected. CSF PLP was measured in samples protected from light using a commercially available kit (Chromsystems). CSF PLP concentrations were compared to age dependent reference ranges across four age groups as previously described. **Results:** The mean patient age was 5.3 years (range 0.01-18.1 years); 56.6% male. There was a significant ($P = .01$) negative correlation between age and CSF PLP concentration as previously described. 15 individuals had CSF PLP at the lower limit or below the reference range. The mean age of this group was significantly higher than the main cohort (10.9 years). 60% had seizures and were on anticonvulsants. One was receiving L-dopa. Diagnoses included primary inborn errors of metabolism (Non-ketotic Hyperglycinemia, Hyperprolinemic Type 1, Hyperphosphatasia with mental retardation syndrome type 3, Pyruvoyl-Tetrahydropterin Synthase Deficiency, Glucose transporter 1 Deficiency, Cobalamin C deficiency) and immunological disorders. One patient with B6 dependent epilepsy (Antiquitin deficiency) on pyridoxine treatment showed normal CSF PLP. **Conclusions:** This study has shown CSF PLP deficiency in several inborn errors of metabolism not previously described. These results have important treatment implications as seizure control may be improved with addition of Vitamin B6 as an adjuvant anticonvulsant.

838 - X-Linked Creatine Transporter (SLC6A8) Deficiency Syndrome: A Report on an Argentinian Family With a Variable Clinical Phenotype

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Cerebral creatine transporter deficiency, attributable to the deficiency in the *SLC6A8* gene, causes X-linked mental retardation, epilepsy, language delay, and autistic features. In contrast with creatine synthesis defects, the majority of patients with SLC6A8 deficiency do not respond to treatment. We describe an Argentinian family with this condition: the proband and his brother, with psychomotor delay and severe speech impairment. The family also includes their sister with psychomotor retardation, predominantly in language, and their mentally retarded mother. The proband brain MRI indicated hemispheric white matter abnormalities, while MR spectroscopy indicated markedly reduced creatine peak. The proband biochemical testing indicated increased urine creatine/creatinine ratio, with normal plasma creatine and guanidinoacetate.

His 17-year-old brother and 18-year-old sister had similar biochemical and clinical abnormalities. To confirm the diagnosis in the proband and his brother, sister and mother the SLC6A8 analysis by Multiplex ligation-dependent probe amplification (MLPA) showed an aberrant pattern, as a possible deletion in exon 3 of SLC6A8. To confirm the results the cDNA sequence analysis of the *SLC6A8* is in actual process in our Molecular Diagnostics Laboratory. This family illustrates the remarkable phenotypic variability in this condition. Investigation of creatine metabolism, brain MR spectroscopy are mandatory in patients with developmental delay of unknown etiology and molecular testing is useful to confirm the diagnosis.

839 - Tyrosine Hydroxylase Deficiency: A Report of Three Patients With a Severe Early Onset Form

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Tyrosine hydroxylase (TH) deficiency also called Dopa responsive dystonia is a rare autosomal recessive disorder resulting from cerebral catecholamine deficiency. Actually, Based on severity of symptoms and signs as well as responsiveness to levodopa therapy, there are three clinical phenotypes attributable to pathogenic variants in TH. **Aim:** We report three observations of children with a severe early onset encephalopathy caused by TH deficiency. **Case reports:** Two brothers and an unrelated boy present a few months after birth with a severe progressive encephalopathy, generalized dystonia, oculogyrosis, and signs of dysautonomy with hypersudation, thermal disruption and episodes of hypoglycemia. Hyperprolactinemia was found in all of patients. Electroencephalogram, electromyogram, brain MRI, evoked brain stem auditory and visual potentials were normal. Decreased cerebrospinal fluid concentrations of hemovigilance acid and 3-methoxy-4-hydroxyphenylethylene glycol, were found with low humanely/ hydoxy indolacetic acid ratio. L-Dopa treatment was initiated in the three patients with progressive dosages. After a short period of improvement severe dyskinesias were observed and the treatment was then discontinued and replaced with benzodiazepines. The two brothers died respectively at age six years and a half and four years and a half after inhalation pneumonia. The third patient is still alive with a severe encephalopathy. The molecular tests were made for the two brothers. A novel mutation was found but its pathogenicity is unknown, so prenatal diagnosis is still not possible for further pregnancies. For the third patient, the results are not yet available. **Conclusion:** Our patients had the TH-deficient progressive infantile encephalopathy form. These patients are usually very sensitive to L-Dopa thus the treatment is often discontinued because of intolerable dyskinesias. The molecular diagnosis is essential as it is the only method to perform prenatal diagnosis for this severe untractable encephalopathy.

840 - Monoamine Neurotransmitter Disorders in a Chilean Cohort of Infants and Children

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Introduction: The monoamine neurotransmitter disorder is a group of neurometabolic disorders caused by defects in the catecholaminergic pathway (adrenaline, noradrenaline, dopamine) and serotonin. Clinically, neurological symptoms of early onset like encephalopathy, dystonia and other movement and motor disturbances predominate. Clinical suspicion and cerebrospinal fluid (CSF) analysis are essential for diagnosis, which performed early allows an opportune therapeutic intervention. **Aim:** To analyze clinical form of presentation, enzymatic defect, and response to treatment in patients with monamine neurotransmitter disorders in infants and children. **Methods:** A descriptive prospective study of 9 children with characterization of early neurological symptoms; dystonia and associated symptomatology, analysis of neurotransmitters in CSF, and response to pharmacological treatment. **Results:** The most frequent clinical presentation was dystonia in all cases, with an average age of presentation at 23 months. 6/9 was generalized and 3/9 focal. 2/9 presented dystonic status. 6/9 patients were diagnosed before six years of age. Associated symptoms: 8/9 presented developmental delay, 5/9 pyramidal syndrome, 5/9 central hypotonia and tremor, 3/9 oculogyric crisis, 2/9 diaphoresis, 1/9 fever of central origin. Type of defect by CSF analysis: Tyrosine hydroxylase deficiency: 3/9 patients, GTP cyclohhydrolase-1 deficiency: 3/9, L-Dopa decarboxylase deficiency: 2/9. One case is still in analysis for dihydropteridine reductase/sepiapterine reductase deficiency. **Treatment:** All of our patients received treatment with L-Dopa: 4/9 with complete remission of dystonia, 5/9 with partial remission, and 3/9 presented adverse effects with conventional dosage of L-Dopa (dyskinesias/increased dystonia/agitation) in younger children. **Conclusions:** Dystonia is the most frequent neurological sign, associated with developmental delay in most cases. Onset of symptoms is early, beginning in infancy or early childhood in the majority. Our patients had different types

of enzymatic deficits. All of them responded to L-Dopa, highlighting the fact that younger patients present side effects with usual doses, which disappear with therapy at low doses. Early diagnosis of neurotransmitter diseases allows an early and timely treatment.

Y) Disorders of Vitamins, Cofactors, and Trace Elements (841 to 860)

841 - Novel Mutations of the *ATP7B* Gene in Czech Families With Wilson's Disease

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Study's objective: Wilson's disease (WD, MIM #277900) is an autosomal recessive genetic disorder of Copper metabolism caused by mutations in the *ATP7B* gene. The aim of our study was to analyze clinical presentations and diagnostic tests of pediatric patients with WD. **Methods:** We retrospectively analyzed the medical history of 35 patients (aged 17 months to 19 years) with confirmed diagnosis of WD treated at our institute from 2002 till March 2017. **Results:** The mean age onset of symptoms was 9.9 years of age. Hepatic presentations were the most common with either liver failure or more frequently increased transaminases. In 77.5% cases, ceruloplasmin serum concentrations were $\leq$0.2 g/L [median 0.16 (0.02; 0.28)]. In 72.5% patients, the basal urinary Copper excretion was $\geq$1.6 μmol/24 hours [median 2.3(0.82; 15.4)]. Mutation analysis was performed in all cases. The detection mutation ratio was 95.7%. We identified 2 novel *ATP7B* gene mutations [c.2732C>T (p.A911 V); c.2324C>T (p.A775 V)] and 17 known mutations. The most common mutation was c.3207C>A (p.H1069Q) (53.7%). **Conclusion:** Genetic testing is the most accurate and effective diagnostic method for early diagnosis.

842 - Skin Lesions in Cobalamin C Disease are Associated With Poor Metabolic Control and are Reversible

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An 8-year-old boy with Cobalamic C disease developed multiple skin ulcers. He was diagnosed with Cobalamic C disease by newborn screening and was treated with vitamin B12 injections and cystadane from beginning. However, family was poorly compliant with treatment. At time of this presentation, his homocysteine was 181.2 micromole/liter (normal <11). In addition, plasma methionine was very low and plasma methylmalonic acid was high. Home care nursing and school health services were involved to ensure compliance with treatment. Vitamin B12 injections were given by nurse and cystadane administration was supervised. Within a week, lesions started improving and healed by 6 weeks of supervised therapy. Metabolic parameters (homocysteine, methionine and methylmalonic acid) showed improving trend along with improvement in the lesions. Skin lesions in Cobalamin C disease has been rarely described. It was thought to be due to nutritional deficiencies associated with dietary restrictions or due to untreated metabolic condition. Biopsy of a new onset lesion of this patient showed acanthosis, spongiosis and perivascular lymphocytic and eosinophilic infiltrates. Our observation suggests that skin lesion in Cobalamin C disease is associated with poor metabolic control rather than nutritional deficiencies and probably a manifestation of microangiopathy characteristic of this disorder. Appearance of these lesions in a known patient suggests either poor compliance or inadequate dose and should be addressed promptly. Normal range in parenthesis

	Plasma Total Homocysteine (<11 μM/L)*	Plasma Methionine (7-47 μM/L)*	Plasma Methylmalonic Acid (<0.4 μM/L)*
At presentation	181.2	4.2	6.0
After 1 week	59.5	18.3	2.19
After 6 weeks	54.7	17.8	2.62
After 12 weeks	60.2	10.4	1.17

* normal range

843 - Challenges in Diagnosis of Wilson Disease in a Limited Resource Country

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Background: Wilson disease is a rare autosomal recessive condition caused by mutation in the *ATP7B* gene, with reduced excretion of Copper into bile, resulting accumulation of Copper in the liver, brain, kidneys, and eyes. The signs and symptoms of Wilson disease, such as hepatic dysfunctions, musculoskeletal manifestations, and neuropsychiatric features may difficult to distinguish from other diseases. **Objective:** To describe the challenges of the first diagnosed Wilson disease at Cipto Mangunkusumo Hospital. **Case:** A 13-year-old boy, born from non-consanguineous marriage, came with anemia one year ago. He was suspected with thalassemia, and then leukemia, but further diagnostic procedures showed negative results. He had a history of melena due to grade 3 esophageal varices and

underwent variceal ligation. Six months later he was then referred to Nutrition and Metabolic Diseases Clinic with suspicion towards inherited metabolic diseases due to involuntary movements of the limbs and dysarthria. On physical examination, patient was severely malnourished with skin pigmentation, Kayser-Fleischer rings on both eyes, and hepatosplenomegaly. Laboratory findings revealed anemia, thrombocytopenia, and slightly elevated liver transaminases, normal serum electrolytes, plasma glucose and renal function. Brain MRI T2-weighted images revealed high signal hyperintensities in bilateral basal ganglia. Wilson disease was one of our differential diagnosis, therefore, we performed blood and urine sample abroad. Results showed low serum ceruloplasmin and elevated urinary copper. Based on the above investigations, we diagnosed Wilson disease and he was then treated with D-penicillamine, zinc, and diazepam. **Conclusion**: A suspicion is required to diagnose Wilson disease especially in a child with liver disease and neurological manifestations. It is still challenging to diagnose Wilson disease due to the lack of awareness and limited diagnostic methods in our country.

844 - Biotidinase Deficiency: Novel Mutations in Algerian Patients

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Introduction: Biotidinase deficiency is an inherited autosomal recessive metabolic disease of biotin. The leading aspects of this disease are neurologic and cutaneous symptoms. The disorder can be treated with oral administration of Biotin. **Materials and Methods**: In the present study, we investigated four unrelated Algerian families including a total of five symptomatic. Genomic DNA was isolated from EDTA whole blood and strode at -20°C before analysis. **Results:** All patients have characteristic clinical features of this disease. Three patients have hearing loss and developmental delay. Age of diagnosis is between 3 weeks and 4 months. All families were consanguineous. All presented dermatologic and neurologic traits of the disorder. Five patients have a profound biotidinase deficiency and one child was characterized by a partial deficiency. Mutations analysis revealed three novel mutations: c.del631C and c.1557T>G within exon 4 and c.324-325insTA in exon 3. **Discussion:** The present study is the first reported molecular investigation of biotin deficiency in Algeria. Three novel mutations are isolated. The large number of homozygous mutation in this series is likely due to the high rate of consanguinity. Establishing the biotin deficiency mutation spectrum could help molecular diagnosis of this rare disease as well as genetic counseling, early management and follow-up. **Conclusion:** Since newborn screening is not available in Algeria, cascade and targeted screening in affected families would be helpful to identify at risk individuals.

845 - Mutations in *PROSC* Disrupt Cellular Pyridoxal Phosphate Homeostasis and Cause Vitamin B6-Dependent Epilepsy in Man

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Objectives: Determine the genetic basis of vitamin B_6-dependent epilepsy in a cohort of children with seizures in whom known causes of B_6-dependent epilepsy had been excluded and to understand the mechanism(s) involved. **Results:** A deficiency of PLP, the active form of vitamin B_6, can present as seizures and other symptoms which are treatable with PLP and/or pyridoxine. Whole exome sequencing of 2 children from a consanguineous family with pyridoxine-dependent epilepsy revealed a homozygous nonsense mutation, p.Ser78Ter, in proline synthetase co-transcribed homolog (bacterial) (*PROSC*). Segregation studies revealed a similarly affected brother was also homozygous for p.Ser78Ter. Subsequent sequencing of 29 unrelated individuals with pyridoxine-responsive epilepsy identified biallelic *PROSC* mutations in 4 additional children. Prior to this study the role of PROSC was unknown, although ubiquitous expression in human tissues and high levels of conservation throughout evolution suggest that PROSC has an important cellular function. Structural studies of yeast and *E.coli* homologs have shown that PLP binds to PROSC within the β-barrel domain of the protein without affecting quaternary structure suggesting that PROSC does not function as an enzyme. Complementation of a pyridoxine-sensitive *E.coli*

mutant lacking the PROSC homologue (ΔYggS) with human PROSC restored growth whilst PROSC bearing p.Leu175Pro, p.Arg241Gln and p.Ser78Ter did not. Pre-treatment cerebrospinal fluid samples showed low PLP concentrations and evidence of reduced PLP-dependent enzyme activity. However, cultured fibroblasts showed excessive PLP accumulation. A zebrafish with CRISPR induced PROSC null mutations, which affect the PLP-binding site, has been generated to enable further investigation of the role of PROSC in PLP intracellular homeostasis and to investigate the mechanisms underlying vitamin B_6-dependent epilepsy. **Conclusion:** Mutations in *PROSC* result in prenatal/neonatal seizures that respond to B_6 treatment. Our studies suggest PROSC is involved in intracellular homeostatic regulation of PLP, a highly reactive aldehyde, by supplying PLP to apoenzymes that require it as a cofactor while maintaining free PLP concentrations low enough to avoid unwanted reactions with other important cellular nucleophiles and phosphatase hydrolysis. The zebrafish loss-of-function mutant for *prosc* will be used to investigate the exact mechanism(s) involved, which remain an important as yet unresolved issue.

846 - Pyridox(am)ine 5'-Phosphate Oxidase Activity in Patients With Vitamin B6 Responsive Epilepsy: Findings and the Effect of the Common p.R116Q Variant Upon Enzymatic Activity

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Objective: Determination of PNPO activity in patients with variants identified in the *PNPO* gene, child hospital controls and those with other B_6-responsive seizure disorders. **Methods:** LC-MS/MS was used to quantify Pyridox(am)ine 5'-phosphate oxidase (PNPO) activity in dried blood spots (DBS) using a method that we have recently developed. **Results:** PNPO deficiency is an autosomal recessive disorder classically characterized by early onset epileptic encephalopathy responsive to high doses of vitamin B_6. Control PNPO activity ranges were established using DBS from 37 hospital controls (5 d-15 y) and 18 children (1 m-16 y) receiving B_6 supplementation for seizure control in whom PNPO deficiency had been excluded. These cohorts had PNPO activities of 10.0-95.0 pmol DBS^{-1} h^{-1} (mean = 41.7 pmol DBS^{-1} h^{-1}) and 23.0-85.9 pmol DBS^{-1} h^{-1} (mean = 56.0 pmol DBS^{-1} h^{-1}), respectively. No correlation of PNPO activity with age was found. Patients receiving B_6 supplementation had mildly increased PNPO activity compared to those not receiving supplements ($P < .05$). Patients with mutations in *PNPO* (n = 19) had PNPO activities significantly lower (nd: 4.6 pmol DBS^{-1} h^{-1}; mean = 1.1 pmol DBS^{-1} h^{-1}) ($P < .0001$) than those measured in control cohorts with all of the 14 potentially pathogenic variants investigated having a dramatic effect on activity. Of these individuals, 3/19 (including one asymptomatic sibling who is not on B_6 supplementation) were homozygous for c.347G>A (p.R116Q), present in the general population with an allele frequency of 0.0558 (ExAC) suggesting that it could be a polymorphism. Whilst previous *in vitro* studies have shown that this variant has high residual activity (80% of WT), in our study all 3 individuals were found to have low DBS PNPO activity (< 0.12 pmol DBS^{-1} h^{-1}). **Conclusion:** PNPO activity in DBS is dramatically decreased in patients homozygous for the p.R116Q variant, providing further evidence for p.R116Q as an epilepsy susceptibility locus. However, questions remain with regards to its variable pathogenicity. This variability is likely dependent upon other genetic or environmental factors. These could include variable tissue-specific expression of the PNPO protein, variants in another gene affecting B_6 homeostasis or at another epilepsy susceptibility locus, dietary vitamin B_6 intake and maternal B_2/B_6 status during pregnancy or breastfeeding.

847 - Good Initial Response to Medical Treatment in a Patient With Late-Onset Methylmalonic Aciduria and Homocystinuria due to Cobalamin D Disorder

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The Cobalamin D (CblD) disorder is a rare autosomal recessive disease of cobalamin metabolism caused by mutations in the *MMADHC* gene that can result in isolated Homocystinuria, isolated Methylmalonic aciduria, or combined Methylmalonic aciduria and Homocystinuria (MMA/HC). Only a few patients with CblD defect have been described, and those with the MMA/HC phenotype typically present with developmental delay, seizures, hypotonia, lethargy and megaloblastic anemia. We present the case report of a 12-year-old male, who started at 10 years and 5 months of age with progressive encephalopathy with regression, deterioration in school performance, behavioral and personality changes, and episodes of acute mental confusion and lethargy. Eleven months after the first symptoms

he was referred to our clinic. On our first evaluation, he presented evident disorientation, inattention and serious mental slowness. CUMANES (School-Age Neuropsychological Maturity Test) and WISC-IV (Weschler Intelligence Scale) test were performed, indicating very poor performance in visual and verbal memory. Laboratory test showed increased plasma homocysteine (HC) and methylmalonic acid (MMA) levels (143.9 µmol/L and 67.8 µmol/L), while methionine levels were within the normal range (18 µmol/L). Genetic testing showed a novel mutation in heterozygosis in the *MMADHC* gene. Treatment with intramuscular OH-Cbl, betaine and folinic acid was prompt initiated, with a rapid decrease in HC and MMA levels, and an increase in methionine levels. Soon after the treatment initiation, the patient started feeling better, presenting no more episodes of behavioral and personality changes or acute mental confusion. In 4 months, there was a rapid weight increase, from 31.8 kg (3rd-10th percentile) to 41 kg (25th-50th percentile). The neuropsychological examination 4 months after treatment initiation showed improvement in visuospatial skills, from apraxia to normal score, and cognitive speed processing (from score 57 to 67), without improvement in working memory or semantic verbal fluency. **Conclusions:** We show neuropsychological improvement in a patient with MMA/HC due to CblD disorder soon after the initiation of medical treatment. Time will determine the real extent of this improvement. As medical treatment seems to be effective, it is essential for clinicians to be aware of the possibility of this diagnosis.

848 - Early Diagnosis of Riboflavin Transporter Deficiency and Therapeutic Management—A Case Report

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Brown-Vialetto-Van Laere Syndrome was described in 1894 as a progressive neuropathy in association with childhood amyotrophic lateral sclerosis. In 2010 mutations in the *SLC52A3* were found as the etiology of this affection. This gene encodes a riboflavin transporter, which is expressed in the small intestine and the brain. The clinical findings comprise weakness, hearing loss, sensory ataxia, feeding, and respiratory difficulties. The cognition is normal, and the progressive course of disease can be modified with early therapeutic management through the administration of riboflavin. **Case report:** Female patient, 1y8 m, only child of healthy non-consanguineous parents. Pregnancy was uneventful. The child was born full-term and adequate to gestational age, receiving discharge from hospital at 2 days of life. At 8 months of age, during hospitalization to treat a respiratory infectious condition, severe anemia and dehydration, she evolved to cardiorespiratory arrest following a bronchoaspiration episode. The patient was evaluated by the Genetics Unit, due to severe hypotonia, dysphagia (using gastrostomy since 6 months of age), development delay, pyramidal syndrome and dysfunction of cranial nerves V and VII. EMG reported sensory-motor neuropathy with axonal predominance. BERA suggested bilateral brainstem alteration. Bilateral optic atrophy was reported in ophthalmologic evaluation. Cranial MRI was normal. Whole Exome Sequencing (WES) showed a homozygous missense mutation in the *SLC52A2* gene, which in combination with clinical findings and additional tests revealed the diagnosis of Riboflavin Transporter Deficiency Type 2 (OMIM 614707). Due to severity of the patient's clinical condition and knowing the natural course of the disease, treatment with oral riboflavin was started with the initial dosage of 30 mg/kg/day. Baseline acylcarnitine profile was normal. After 6 months, the dosage was increased to 60 mg/kg/day, because the patient did not show clinical improvement and had initiated seizures. Recently, it has been showed that many cases with non-specific clinical manifestations had diagnoses revealed only by WES. This is the case of Riboflavin Transporter Deficiency, a treatable disease manifesting with neuropathy and no available biochemical specific test. When available, WES can be a suitable strategy for the early diagnosis of this and other treatable diseases, allowing appropriate management and genetic counseling.

849 - High Parenteral Hydroxocobalamin Dose Strategy in 5 Patients With Different Types of Intracellular Cobalamin Deficiency: Clinical and Biochemical Evolution

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Objective: Autosomal recessive disorders of intracellular cobalamin metabolism (cblA-cblG) can give rise to deficiency of methylmalonyl-CoA mutase alone (cblA, cblB, cblD variant 2), methionine synthase alone (cblD variant 1, cblE, cblG) or both (cblC, classic cblD, cblF). X-linked intracellular cobalamin disorder is caused by mutation in the *HCFC1* (cblX) and associated with severe neurological impairment. Parenteral hydroxocobalamin (OHCbl) is the main treatment and high dose strategy may improve metabolic control and clinical outcome. **Methods**: In 5 patients with early onset of different intracellular cobalamin

disorder (patients 1-3: cblC, patient 4: cblA, patient 5: cblX), we described the clinical and biochemical evolution following high OHCbl dose strategy. **Results**: Patients 1-3 had failure to thrive, hypotonia, nystagmus and neutropenia. Hemolytic uremic syndrome, neutropenia and dermatitis enteropathica was present in patient 3. Patient 4 had hypotonia, failure to thrive and metabolic acidosis. Patient 5 had an early onset epileptic encephalopathy with high cerebro-spinal fluid glycine levels. Patients 1-3 harbored homozygous mutations (c.271 dup A) in *MMACHC*. Patient 4 had a compound heterozygous mutation (c.593_596del and c.439+4_439+7del) in *MMAA*. Patient 5 had a mutation in X-linked cobalamin (HCFC1) (c.344C>T) and a hemizygous mutation in *ATRX*. OHCbl was progressively increased to 30 mg/day in patients 1-3. In patient 4, OHCbl was increased at the age of 10 years to 20 mg/day. In patient 5, OHCbl was increased to 3 mg/day, but initially higher dose was not clinically tolerated. In patients 1-4, high OHCbl dose was given by using i-port advance injection port (50 mg/ml). In all patients, high OHCbl dose resulted in normal plasma homocysteine and or almost normal urinary methyl malonic acid. At 18 months of age the patients 1-3 had almost correct neurological examination. Only in patient 3, nystagmus persisted. Patient 4, at age of 10 years, had a normal neurological and cognitive evaluation. Patient 5 had refractory seizures and severe neurological impairment. **Discussion**: High OHCbl dose strategy led to improvement in both metabolic control and clinical outcome in all patients except in the clinical outcome of patients 5, which may be related to the presence of the hemizygous mutation in *ATRX*.

850 - A Novel homozygous Variant c.437TG in COQ4, Related to Ubiquinone Biosynthesis, Presenting With Severe Infantile Spastic Quadriplegia, Dystonia, and Seizures in Two Siblings

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Background: *COQ4* is an essential component of the multisubunit complex required for ubiquinone biosynthesis. To date <15 case reports have been published with mutations in *COQ4*, presenting mainly with severe neonatal encephalomyopathy, cerebellar hypoplasia, lactic acidosis and early death due to respiratory or cardiac failure. **Case reports:** We report two sisters of Turkish Cypricot origin homozygous for a novel *COQ4* missense variant, c.437T>G (p.F146C). They were born at term after normal pregnancy and delivery. There were no neonatal concerns, although severe developmental delay, microcephaly, squint, spastic quadriplegia and dystonia were noted from the first months of life. The older sibling presented with encephalopathic episodes from 7 months of age, whilst the younger sibling was diagnosed with epilepsy at 4 months of age, which was relatively well controlled on valproate treatment. The older sibling died at 2 years of age due to bronchopneumonia. Postmortem analysis showed cerebral and cerebellar hypotrophy and degeneration of olives and thalamus. The younger sibling suffered from severe global developmental delay and progressive dystonia. She was wheelchair bound, visually impaired and required gastrostomy by 10 years of age. Her brain MRI scan at 14 years of age showed global cerebral and cerebellar hypoplasia. Muscle histology was nonspecific. Enzymology showed normal complex I and II+III activities, but decreased complex IV activity (0.010, ref 0.014-0.034). She died at the age of 17 years due to aspiration pneumonia, left ventricular failure, arrhythmia and progressive encephalopathy. Elevated alanine with normal lactate levels were found on several occasions in older sibling. Other biochemical results were normal in both cases, including renal and liver metabolites, creatine kinase, ammonia and urinary organic acids. Potentially pathogenic *COQ4* mutations were found post mortem by whole exome sequencing. The variant is absent from all public databases and is predicted to be deleterious according to SIFT and PolyPhen; c.437 residue is also 100% conserved in 100 vertebrates. **Conclusion:** A novel c.437T>G (p.F146C) likely pathogenic variant in *COQ4* was detected in two siblings. This report expands the current phenotypic spectrum of *COQ4* deficiency presenting with a non-neonatal slowly progressive disorder, severe spastic quadriplegia, dystonia and seizures, absent lactic acidosis and low muscle respiratory chain enzyme complex IV activity.

851 - Mutations in *SLC39A14* Lead to Manganese Neurotoxicity and Childhood Onset Dystonia-Parkinsonism That may be Amenable to Chelation Therapy With Na2CaEDTA

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Objective: To identify the disease gene in a cohort of nine children with early-onset dystonia-parkinsonism associated with hypermagnesemia and hyperintensity of the globus pallidus on T1-weighted MRI. **Methods:** Whole exome sequencing in combination with autozygosity mapping and Sanger sequencing was used for gene identification. Manganese levels were determined using inductively-coupled plasma mass spectrometry. A zebrafish loss-of-function mutant was generated using CRISPR/Cas9 genome editing. **Results:** Affected children presented with loss of developmental milestones, progressive pharmacoresistant dystonia and bulbar dysfunction at ages six months to three years. MRI brain was characteristic of manganese deposition with T1-hyperintensity of the globus pallidus, striatum, and white matter including the cerebellum, spinal cord and dorsal pons, with sparing of the ventral pons. Post mortem studies revealed marked neuronal loss in the globus pallidus and dentate nucleus, and loss of myelin with coarse vacuoles in the cerebral and cerebellar white matter. Homozygous loss-of-function mutations in *SLC39A14* were identified in all patients. SLC39A14 is a divalent metal transporter facilitating uptake of manganese, zinc, iron and cadmium at the cell membrane. Blood metal analysis in affected children found an increase in manganese levels alone. Overexpression of mutant *SLC39A14* in HEK-293 cells confirmed impaired manganese uptake. Furthermore, *slc39a14* loss-of-function in zebrafish led to manganese accumulation, particularly in the brain, increased sensitivity to manganese toxicity and impaired locomotor activity. Chelation therapy with disodium calcium edetate ($Na_2CaEDTA$) significantly lowered manganese levels in our fish model and led to striking clinical improvement in at least one patient who regained the ability to walk. Further four individuals with *SLC39A14* mutations have since been identified who showed a variable treatment response. **Conclusion:** The Manganese transporter SLC39A14 joins SLC30A10 as a crucial regulator of manganese transport. Mutations in these genes impair hepatic manganese excretion with subsequent manganese accumulation in the brain causing parkinsonism-dystonia. Manganese overload responds well to chelation therapy with $Na_2CaEDTA$ leading to excellent clinical improvement in SLC30A10 deficiency but less pronounced effects in SLC39A14 deficiency.

852 - Inherited Disorders of Metal Metabolism: A Rare Case of Occipital Horn Syndrome

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Background: Menkes disease (MD) is an X-linked recessive disorder of copper transport caused by pathogenic variants in *ATP7A* gene. Children with classic Menkes disease appear healthy until 2-3 months of age, when hypotonia, seizures, and developmental regression appear. Vascular tortuosity and bladder diverticula are present in virtually all patients. If present, the hair is twisted (*pili torti*), breaks easily and has a sandpaper feel. A jowly appearance with sagging cheeks, pectus excavatum, skin laxity, and umbilical/inguinal hernias are other reported clinical features. Serum copper and ceruloplasmin levels are low. Death usually occurs by 3 years of age. A less severe variant of MD is the occipital horn syndrome (OHS). This syndrome is characterized by a longer survival, milder neurological abnormalities, and the formation of occipital exostoses (occipital horns). **Case report:** We report the case of a 7 year-old boy with developmental delay, neurological symptoms (dysarthria and previous history of distal choreoathetotic movements), coarse hair, dry lax skin, right inguinal hernia (surgically repaired), and enamel hypoplasia. Facial dysmorphisms include long face, high forehead, low-hanging columella, high-arched palate, and large ears. Growth parameters have been normal since birth and there is no history of developmental regression. He does not have joint hypermobility or restriction. Brain MRI showed an increased cervical and cerebral arterial tortuosity. Serum copper was decreased (7.6 μmol/L; normal range 14.1-30.0), as well as serum ceruloplasmin (0.16 g/L; normal range 0.2-0.6). Lateral skull X-ray demonstrated bilateral occipital exostoses. Thoracic CT angiography and abdominopelvic US were normal. *ATP7A* gene sequencing identified a hemizygous variant c.375delA (p.Ala126Glnfs*2), previously undescribed. The predicted effect of this alteration is complete loss of function; however, the patient's milder phenotype may be explained by inefficient translational reinitiation enabling some functional copper transport as previously described. **Conclusions:** Our patient showed clinical features of OHS. *ATP7A* gene sequencing identified a hemizygous *ATP7A* variant, probably pathogenic, allowing more accurate genetic counselling for this family.

853 - Menkes Disease and Cooper Sulfate Treatment

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Introduction: Menkes disease (MNKD) is a lethal and multisystemic, hereditary disease. The molecular defect in *ATP7A*, located on chromosome Xq13.3 (OMIM 300011). The

frequency is 0.8 to 2 out of 100 000 newborns. There have been several descriptions in our country, Cooper sulfate treatment is the first one carried out in Latin America. **Objective:** To value early treatment with Copper sulfate in Menkes disease (MNKD), to correlate the clinical, biochemical follow-up and molecular tests, and to evaluate the improvement. **Cases Summary: T**hree patients diagnosed with Menkes disease, two of them have first-degree consanguinity. The diagnosis was confirmed through concentrations of Copper in the blood, ceruloplasmin, and abnormal levels of: DOPA, DOPAC, DADHPG, NE [NIH Laboratory, Bethesda., USA. Dr.S.Kaler]. Patient 1 and 2: Molecular testing revealed a mutation in exon 7 (1919 del A) of the Menkes gene. The mother is carrier for the same deletion. Patient 3: identified the mutation (K865X). The first case (G.A.) died at 3 months of age, without treatment. The second (F.A.) have been treated with endovenous Copper sulfate, and subcutaneous (SC); the third patient (GC) received SC Copper sulfate when he was 18 months old. The normalization of biochemical parameters is dose dependent. **Conclusion:** Menkes patients can respond precociously to Copper treatment and the subcutaneous administration of Copper sulfate, but it is necessary to discuss with the patient's parents the possibly futile treatment, the limited benefits, potential risks and discomforts. The fact that normal enzymatic values were achieved in plasma before 3 months of age is considered biochemically effective. The treatment has no successful effect on the devastating progressive neurodegenerative of the disease. Genetic description of each patient with Menkes disease, and its correlation with the clinical and biochemical response to Copper therapy could help a better understanding of Menkes disease. The beneficial neurological effect would be influenced by the residual activity of the Copper carrier enzyme. The Copper substitutive treatment is not effective when the genic product is totally absent, but early initiation of Copper treatment may have a beneficial effect on neurodevelopment, but does not remove all the symptoms. **Acknowledgments:** Dr. Kaler, Stephen (NIH, USA), Dr. Liste, Hugo (Argentinian Air Force Hospital, Buenos Aires).

854 - Cobalamin C Disease Presenting as Hemolytic Uremic Syndrome in a Chinese Newborn

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Introduction: Cobalamin C disease (CblC) is a rare inherited disorder of vitamin B12 metabolism. Without cobalamin, accumulation of methylmalonic acid and homocysteine leads to neurotoxicity, nephrotoxicity and vascular damage. Depending on the severity of the deficiency, the clinical phenotype can range from a disastrous, heterogeneous multisystem disease presenting in the newborn period, to a milder disease with slowly progressive neurological symptoms. Hemolytic uremic syndrome (HUS), the triad of microangiopathic hemolytic anemia, thrombocytopenia and acute kidney injury is an unusual but potentially devastating presentation of CblC. This seemingly under recognized entity is believed to be secondary to hyperhomocysteinemia causing damage to the glomerular endothelium. Here we report a case of cobalamin C disease in a Chinese infant with this unusual presentation. **Case report:** A male baby presented at 45 days of age with 1 week history of worsening irritability, shortness of breath and choking during feeds. Physical examination revealed marked pallor and generalized edema. Blood tests revealed marked anemia, thrombocytopenia and renal impairment with metabolic acidosis, severe hyponatremia and hyperkalemia. Together with reticulocytosis, D dimers elevation, low haptoglobin and blood smear features of microangiopathic hemolytic anemia; the diagnosis of atypical hemolytic uremic syndrome was established. Further metabolic investigations revealed methylmalonic acid and methylcitrate in urine. Plasma amino acid profile showed elevated homocysteine and low methionine. These findings were compatible with the diagnosis of cobalamin C deficiency. The diagnosis was eventually confirmed with compound heterozygous mutations of the MMACHC gene. Upon commencement of daily subcutaneous hydroxycobalamin injections, baby's condition gradually stabilized with resolution of anemia and thrombocytopenia. Electrolytes, renal function and blood pressure also normalized. **Conclusion:** Diarrheal related hemolytic uremic syndrome (HUS) is a well-known acquired cause of acute renal injury. However, HUS occurring in early infancy (atypical HUS) is often caused by inherited genetic conditions. Cobalamin C disease is an important potentially treatable cause of atypical HUS. Early treatment with vitamin B12, folic acid, and betaine can improve the metabolic and hematologic derangements and may alter the long-term prognosis of these patients with this unusual catastrophic presentation.

855 - Brown-Vialetto-Van Laere syndrome—Life-Threatening but Treatable Vitamin B2-Transporter Defect

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Background: Brown-Vialetto-Van Laere syndrome is based on a defect in vitamin B2-transport. Three riboflavin-transporters with tissue-specific expression have been characterized so far (RFVT1, RFVT2 and RFVT3). **Patient:** The first presenting symptom of the 10-year-old girl was an ataxic gait at the age of one year. She developed muscle hypotonia and lost significant motor skills. At present, the patient is wheelchair-bound and requires enteral nutrition via a PEG tube. Due to a paresis of the diaphragm, a tracheostomy was performed and the ventilation is

supported mechanically. In contrast to the rapidly progressive course of the disease, including loss of speech and paresis of lower and upper limbs, the cognitive development was not affected. **Methods:** After suspecting a metabolic disease, whole exome sequencing was performed. Putative disease-causing mutations and their segregation in the family were confirmed by Sanger sequencing. **Results:** The patient carries two compound heterozygous mutations in the *SLC52A2* gene that have not been described before. *SLC52A2* codes for the riboflavin-transporter RFVT2, which is mainly expressed in the brain. A therapy with high-dose riboflavin was started immediately. After 5 months of therapy, the patient is now able to move the lower limbs, which was not possible for 6 years. Also, ventilation, speech and her general condition improved. **Conclusion:** Also in advanced stages of Brown-Vialetto-Van Laere syndrome, an improvement of the clinical presentation can be achieved.

856 - Prenatal Treatment of Pyridox(am)ine 5'-Phosphate Oxidase Deficiency With Normal Development at 18 Months of Age

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Introduction and objectives: Pyridox(am)ine 5'-phosphate oxidase (PNPO) deficiency is a rare autosomal recessive inborn error of vitamin B6 metabolism with less than 50 patients reported. PNPO deficiency phenotype includes frequent prematurity, early-onset neonatal severe and pharmacoresistant neonatal seizures. Despite treatment with pyridoxal phosphate (PLP), most of the neonates who have survived have exhibited severe neurocognitive outcomes. Method: We report a case of two siblings: the 3-yearold girl has neonatal encephalopathy with seizures which resolved at 3 days of life after PLP supplementation. Molecular testing confirmed the diagnosis with a homozygous mutation in the PNPO gene (c.364 -1 G>C). At 3 years of age, she has a developmental delay with persistent epilepsy despite appropriate pyridoxal phosphate therapy started at 3 days of life. Her brother was diagnosed by molecular in utero testing. Maternal treatment with pyridoxine and riboflavin was initiated at the end of the first trimester. On the day of delivery, the mother received 50 mg oral PLP supplementation. **Results:** The boy was born at term after normal pregnancy and delivery. He received immediate PLP supplementation at the dose of 40 mg/kg/day before the first hour of life. At 3 months, the dose was increased to 50 mg/kg/day. He has a normal neurodevelopment at 18 months. He has remained seizures-free until the age of 16 months where he exhibited one short seizure after a decrease of PLP dosage to 40 mg/kg/day. **Conclusions:** Normal neurodevelopmental outcome after early supplementation in PNPO patients is possible as already reported. Our case demonstrates that in utero treatment with pyridoxine and riboflavin (FMN is the cofactor of PNPO) is safe for the mother and the child and might increase prenatal PNPO fetal activity ensuring an optimal long-term outcome of the early treated newborn with PLP.

857 - Genotype–Phenotype Correlation in Biotinidase Deficiency Patients in Turkey

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Objective: Biotinidase deficiency is an autosomal recessive disorder of biotin metabolism resulting from the defect in the *BTD* gene. **Methods:** We aimed to investigate genotype-phenotype correlation of eight Turkish biotinidase deficiency patients who diagnosed within the last three years in our new and small clinic. Results: The age of the patients ranged from 1 to 134 months, male to female ratio was 5/3 and consanguinity marriage ratio was found to 7/8. Half of the patients were diagnosed with severe biotinidase deficiency and the other half with mild biotinidase deficiency. In severe biotinidase deficiency group; homozygous c.98-104del7ins3; frameshift mutation was found in three patients and homozygous c.1330G>C;p.Asp444His mutation was detected in one patient. Two of the four patients with severe biotinidase deficiency were detected by the newborn screening program and the developmental stages were normal. One patient, who was born before neonatal screening program, had severe mental and motor retardation, hearing loss, alopecia, eczema, walking, and speaking difficulties. One patient who was not detected in the screening program and was admitted due to recurrent apnea at three months of age and found severe biotinidase deficiency and died despite the biotin treatment. A second rare disorder was also considered in this patient, but no cause was found. The remaining four patients with mild biotinidase deficiency were detected by the neonatal screening program and the developmental stages were normal. In mild biotinidase deficiency group; a homozygous c.1330G>C;p.Asp444His and compound heterozygous c.470G>A;p.Arg157His/c.1439G>A;p.Gly480-Glu, c.470G>C;p.Arg157His/c.1330G>A;p.Asp444His and c.1253G>C;p.Cys418Ser/c.1330G>A;p.Asp444His mutations were found and mild clinical findings such as eczema and hair loss were observed before treatment. **Conclusion:** The newborn screening program has been very useful. Homozygous c.98-104del7ins3; frameshift and homozygous c.1330G>C; p.Asp444His mutations had been associated with severe deficiency.

858 - Screening for Biotinidase deficiency, A Collaborative Pilot Study Between Guatemala and Switzerland

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Biotinidase is an important part in human metabolism. Biotinidase deficiency is an autosomal recessive disease affecting the biotin metabolism, via alteration of the enzyme biotinidase. Biotin, acts as the prosthetic group of four carboxylases, essentials in metabolic processes like gluconeogenesis, fatty acid synthesis and amino acid catabolism. Important neurological problems such as mental retardation and skin conditions can be caused by biotinidase deficiency. Scientific efforts between Guatemala and Switzerland National Newborn Screening Program are being done to implement the Basic panel for Neonatal Screening Program in Guatemala. **Objectives:** Determine positive samples and cutoff values for Guatemalan population for Biotinidase activity in healthy newborns and babies from day 2 to day 30 of life. **Methods:** Dried blood samples (DBS) were collected randomly from 400 babies that received medical attention in the CAIMIs (for its acronym in Spanish, Maternal-Infantile Integral Attention Centers) in five departments of the country, including Escuintla, Santa Rosa, Jutiapa, Alta Verapaz and Quetzaltenango. A total of 400 samples were obtained. Samples were sent to the Swiss Newborn Screening in Zurich by express courier. The traditional method of Wolf by UV-Vis spectrophotometry Methodology was used. **Results and Discussion:** Four hundred babies were screened using DBS on filter paper. Babies had normal weigh at birth and 99.25% were breastfed. The total mean value was stablished to determine ranges for Biotinidase deficiency diagnosis as partial (< 10% d.TMW) and total deficiency (10-30% d.TMW). We found 7% (27) of the results were below 75 U for Biotinidase-Activity considered as positive. Finding 74% (20) of males and 26% (7) of females. **Conclusions:** Patients that tested as positive were partial deficiencies and a second test must be performed to exclude Biotinidase deficiency, decreased enzyme activities probably due to an inactivation during transport of the sample. International collaboration between countries can contribute to the implementation for the new programs in countries that still doesn't have a Newborn Screening Program.

859 - Effect of *BTD* Gene Variants on In Vitro Biotinidase Activity

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Introduction: Biotinidase deficiency (BD), an autosomal recessive disease, is confirmed by plasma biotinidase activity measurement and can be classified as profound (activity <10%) or partial BD (activity 10%-30%). The most frequent pathogenic variant is c.1330G>C (p.D444 H), which is associated with partial BD and considered a variant of moderate effect (the estimated residual activity is approximately half of normal). Our group identified five novel missense variants in the *BTD* gene in a cohort of patients considered to be biotinidase deficient: c.119T>C (p.L40P), c.479G>A (p.C160Y), c.664G>A (p.D222 N), c.1337T>C (p.L446P), and c.1466A>G (p.N489 S). **Objective:** To evaluate the in vitro effect of the aforementioned variants on intra- and extracellular biotinidase activity. **Methods:** HEK 293 cells were transfected with pCMV6-Entry vector containing the wild-type (wt) or mutant *BTD* gene, or empty vector. Cell culture medium and cells were collected 48 hours after transfection. Biotinidase activity was measured by colorimetric method employing biotinyl-p-amino benzoic acid as the substrate. The wt*BTD* activity was considered 100%. Severe effect was considered <10% wt*BTD* activity, moderate effect if 10%-50% wt*BTD*, and no effect if >50% wt*BTD*. Activities are presented as mean ± SD. **Results:** The c.119T>C and c.479G>A variants showed moderate effect on enzyme activity in cells (33.6 ± 12 and 14.4 ± 1.7%, respectively), and severe effect in cell culture medium (7.5 ± 0.2 and 0.3 ± 0.5%, respectively). The c.664G>A variant had no effect (cells: 112 ± 15%, culture medium: 82.6 ± 14.3%). The c.1337T>C variant showed severe effect in both cells (0%) and culture medium (2.1 ± 3.6%). The c.1466A>G variant had no effect in cells (106.8 ± 17.6%) but a moderate effect in culture medium (43.4 ± 5.3%). **Conclusion:** The severe effect of c.119T>C and c.479G>A variants and the moderate effect of c.1466A>G variant in cell culture medium are in agreement with the biotinidase activity observed in patient. They were less deleterious to intracellular than extracellular biotinidase function. However, the severe and no effect of c.1337T>C and c.664G>A, respectively, did not agree with the activity found in patients. **Support:** CAPES, CNPq, FIPE-HCPA, FAPERGS.

860 - Menkes Disease With Novel Mutation *ATP7A* Gene: c.3913G>A

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Objectives: To report a case of Menkes disease with a novel mutation. To contribute the knowledge and emphasize the importance of genetic counseling in affected families. **Methodology:** Medical record review, literature review. **Results:** Male patient. He began to present seizures and vomit with approximately 4 months, needing hospitalization. Use of Topiramate at maximum doses; Clobazam and Sabril, with discrete improvement of the spasms; also use of Domperidone and Ranitidine. Regarding

neuropsychomotor development: never presented cephalic support; evolving with dysphagia for liquids, failure to thrive, smile and visual contact losses. Also, he was submitted to a surgery for inguinal hernia repair at 7 months. At the clinical and dysmorphological examination, at 1 year and 4 months, were observed: weight 7820 g; length 79 cm; head circumference: 42 cm; regular general condition, hydrated, eupneic, irritated, inconsolable cry, axial hypotonia, Babinski sign presence, microcephaly, hair became sparse, coarse, lighter, with easy drop (characteristic changes suggesting Menkes disease), hypoplastic nails. Complementary tests: electroencephalogram showed slow base rhythm characteristic of sleep phases, moderate disorganization of the base activity and focal paroxysmal activity; cranial tomography revealed diffuse widening of cortical sulci, basal cisterns, encephalic fissures, compensatory enlargement of the supratentorial ventricular system; brain magnetic resonance imaging presented white matter signal alteration; Copper dosage: 16.1ug/dL (Reference value: 90 to 190) and ceruloplasmin: 6 mg/dL (Reference value: 18 to 45), corresponding to the minimum analytical sensitivity (lower limit of the assay); molecular analysis of DNA for the *ATP7A* gene identified a novel mutation c.3913G>A (p.Asp1305Asn), considered potentially pathogenic, compatible with the symptoms manifested by the patient. He followed with a multidisciplinary team, using Copper-histidine with Copper serum normalization. **Conclusion**: Considering that Menkes's syndrome is among the treatable metabolic diseases, it is important to include the dosage of Copper and ceruloplasmin in cases of: epilepsy; leukodystrophy; neurological regression. A novel mutation c.3913G>A was identified and considered potentially pathogenic. As it is an X linked recessive disorder, it is necessary to identify the female heterozygotes of the family for adequate genetic counseling, including information about the possibility of preimplantation diagnosis.

Z) Miscellaneous (861 to 919)

861 - Electron Microscopy Still Has a role in Diagnosis of Inborn Errors of Metabolism: Retrospective Review of Autopsies at a Teaching Hospital

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Objective: Determine the value of electron microscopy in diagnosing inborn errors of metabolism. **Methods:** A retrospective review was conducted on 900 perinatal and pediatric autopsies spanning a period of 17 years. **Results:** From 2000 to 2016, nine cases (1%) with inborn errors of metabolism were found, including 4 cases of Pompe disease, 1 case of I-cell disease, 2 cases of neonatal hemochromatosis, 1 case of bile acid synthesis defect *(delta 4-3-oxosteroid 5 beta-reductase deficiency)*, and 1 case of mitochondrial disease (Leigh syndrome). Electron microscopy was important in the diagnosis of I-cell disease and Pompe disease. This approach enabled a prenatal diagnosis to be made from a chorionic villus biopsy in two cases with a positive family history. **Conclusion:** The possibility of an inborn error of metabolism may be suggested by a history of consanguinity, adverse perinatal outcome in previous pregnancies including stillbirths, hydrops fetalis with no anatomic cause, and perinatal asphyxia. One or more of these factors was observed in 7 of the 9 cases in this study. Although many laboratories no longer have electron microscopy facilities, this modality can be extremely useful for diagnosis of inborn errors of metabolism and has a more rapid turnaround time compared to gene mutation analysis or enzyme assay.

862 - Coexistence of a Rare Disease With Novel Mutation and a Polymorphism That Cause of Speech Delay

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Introduction: Many nutritional, environmental, social, genetic, and neurometabolic reasons can lead to speech delay. Early recognition of speech problem and elucidation of its underlying cause not only help to improve speech but also benefit whole patient family. **Case:** A 4 years 7-month-old male patient was admitted with complaints of developmental delay and hypotonia. He was born vaginally at full-term from mother with polyhydramnios. He was the first child of his parent who were first-degree cousins. His parents and 2-year-old brother were healthy. He started to walk at the age of 2 years and to say the first words at the age of 2.5 years. His pathologic systemic examination findings were mild central hypotony, sacral dimple, speech delay, and relative macrocephaly. In the basale rutine and metabolic laboratory evaluation were normal. On cranial magnetic resonance imaging, widespread white matter damage, and temporal lobe subcortical cysts were present. On brain magnetic resonance spectroscopy (MRS), there was a decrease in N-acetyl aspartate (NAA) peak in the areas of white matter involvement was observed. In genetic test requested due to sacral dimples and homocysteine elevation, the methylene tetrahydrofolate reductase (MTHFR) mutation was found to be compound heterozygous A1298C/C677 T. The molecular evaluation was performed due to relative macrocephaly, neurological developmental retardation, and neuroradiological findings. Sequence analysis of the MLC1 protein revealed heterozygous mutation IVS11+6T>A (c.1059+6T>A). This mutation was interpreted as previously undefined change without clinical significance in terms of Van Der Knaap disease. **Discussion:** Neuroradiological evaluation was considered only when the patient was under pediatric neurologist. However, it was interpreted as acute disseminated encephalomyelitis, which can be encountered more frequently,

by radiologists not familiar with the disease. Van der Knaap disease was confirmed by genetic test requested because of the decrease in NAA peak in typical lesions seen as a result of especially clinical course and cerebral MRS. In addition, MTHFR mutations, were investigated because both sacral region anatomy, delayed speech and mild motor retardation. Result: Simple details such as measurement of the head circumference, control of sacral dimple can give useful information in describing a diagnosis that can affect the whole family.

863 - Potential Therapeutic Effect of Pyridoxal Phosphate on Collagen Type VI-Related Myopathies

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Introduction: Primary hyperoxaluria type I (PH1) is a deficiency of the Alanine glyoxylate Aminotransferase (AGT) enzyme caused by pyridoxal 5 phosphate dependent autosomal recessive disease. Congenital muscular dystrophies (CMDs) include disorders which show the symptoms of muscle weakness and joint contractures at early ages. CMDs can present with different degrees of severity: a more severe form known as Ullrich Congenital Muscular Dystrophy (UCMD) and a less severe Bethlem myopathy. **Case:** The female patient aged 12.5 who was referred for the definitive metabolic diagnosis of the renal kidney caused by hyperoxaluria. She was diagnosed with UCMD with a mutation in *COL6A2* gene. Upon examination, she had hyperemic dry lesions on cheeks, hands and legs. She is able to remain standing when supported and mobilized by a wheelchair. She has thoracolumbar scoliosis and equinus contracture. Molecular screening was made due to hyperoxaluria, and a homozygous mutation of combined AGT Pro 11 Leu/Ile 340 Met was found. After 5 mg/kg/day pyridoxal phosphate, 2 liter/day water, sodium/potassium citrate treatment, her skin symptoms improved, urine oxalate/creatinine ratio decreased from 156.2 to 38.31 (n<48 mg/g). Her asymptomatic parents and elder sister were eliminated molecularly for possibility of UCMD, but they were carriers for the mutation. **Discussion:** Her dermatological symptoms improved due to the primary hyperoxaluria treatment. However, it was remarkable that the progressive muscle weakness caused by UCMD and present since the age of 2, which was detected via muscle biopsy and molecular examination, improved significantly and that the measurements of creatine phosphokinase being > 600 U/L previously decreased to normal values. As there is no information on any previous effect of pyridoxine on CMD, it was considered that a beneficial effect was incidentally observed for the second disorder. Except being used as a cofactor for PH1 treatment, pyridoxal phosphate influences more than 150 enzymatic reactions. It is the known immunomodulating effect that might positively affect apoptosis considered to influence the muscular symptoms of CMD. **Conclusion:** Current knowledge reveals the need for further studies regarding the response to pyridoxine in collagen type VI-related myopathies which are only treated symptomatically and have no specific treatment.

864 - Toe Walking Caused by Inborn Error of Metabolic Diseases Following a Specific Case

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Introduction: As a result of literature research on the case of toe walking, neural tube defects refer generally to orthopedic and neurologic case reports. The case of toe walking is selected to present the information obtained from our patient group monitored for 6 years and to attract attention towards Methylene Tetra Hydro Folate Reductase (MTHFR) polymorphisms. **Case:** A 10-year-old female patient has applied for toe walking and mild neurologic retardation. Her family history was unremarkable, except stroke background in her grandfather. Her prominent clinical findings were obesity, sacral dimple and toe walking. The laboratory assessment showed that moderate high lipid levels and MTHFR A1298C heterozygote mutation. Hypoplastic disc, spondylolisthesis and filar lipoma were detected in spinal MRI. **Discussion:** The final diagnoses of 20/8000 patients (11 female, 9 male) monitored in our clinic due to the symptoms of toe walking were as follows: 3 late diagnosed phenylketonuria (PKU), 2 metachromatic leuko-dystrophy (MLD), 1 L (OH) glutaric aciduria, 3fatty acids oxidation defect (FAO), 1 mitochondrial disease and 10 MTHFR. A problem was detected on medium chain fatty acids in the patients with FAO. The complaint was considered to be muscle cramp due to increased muscle enzymes and aches throughout the complaint period. Considering neurological findings, the difficulties in walking were apparent in PKU and MLD patients. The short Achilles tendon was included in neurological findings in the patients diagnosed with L2 glutaric aciduria and mitochondrial diseases. From MTHFR patients, syringomyelia was found in one patient with sacral central line asymmetry and filar lipoma was detected in the remaining patients with deep sacral center line whereas spondylolisthesis was observed in 2 patients and the tethered cord presence was found in 1 patient. Toe walking refers to a finding defined as early symptom of autism. MTHFR was associated with autism. The autism like symptom was found only in one patient. **Conclusion:** The treatment for toe walking would not be a simple case to be recovered locally (by means of Achilles tendon surgery). It would be vital to find out the underlying reason.

865 - The Relationship Between Marfanoid Habitus and Methylentetrahydrofolate Reductase C677 T or A1298C Polymorphism

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Aim: Methylentetrahydrofolate reductase (MTHFR) converts 5,10 Methylentetrahydrofolate into 5 Methylentetrahydrofolate which is the active form of folate. 5-MTHF is the methyl provider for DNA methylation and methionine synthesis. MTHFR gene polymorphism is related with connective tissue disorders. With this study, we aim to find more clues on the effects of MTHFR polymorphisms which is not fully identified yet on connective tissue and to have better experience on differential diagnosis of these polymorphisms. **Materials and Method**: 48 patients with marfanoid habitus and diagnosed with MTHFR gene polymorphism were included to this study. Anthropometric measurements, eye, skeletal, cardiovascular evaluations and family histories of these patients were recorded. Fibrillin 1 gene mutation analysis and homocysteine levels were recorded. **Findings:** The study includes 20(%41,7) female and 28(%58,3) male patients (M/F:1,4). Average age of the patients was 13.51 ± 3.56. The study group was divided according to their FBN gene mutations into two groups. First group had 26 patients with possible pathogen FBN1 gene mutation and were planned to have family screening, the other group had 22 patients with benign FBN1 gene mutation. After the comparison, according to pathogen FBN1 mutation, we found that Walker-Murdoch sign (including only long fingers and joint hyperelasticity) was significantly higher in the group with FBN1 and MTHFR mutation. No statistically significant difference was found after we compared all parameters according to exonic and intronic possible pathogenic *FBN1* mutations. However, when we compared the patients with possible intronic *FBN1* gene mutations and the ones who do not, we found that wrist sign was statistical higher at the first group. Homocysteine levels weren't statistically different in any group. **Conclusion:** *MTHFR* and *FBN1* gene polymorphisms are located at other gene locuses and has different histopathological pathways, however they present with similar clinical findings. And also if they exist together they amplify their effects on tissues which support our study results such as high joint hyperelasticity ratio. In the elder group with combined *FBN1* and *MTHFR* gene mutations the possibility of finding severe ocular, cardiovascular and skeletal symptoms are higher. Findings in our study may supply information to predict severe complications in these genetic conditions.

866 - Epidemiological Study of Inborn Errors of Intermediary Metabolism in a Single Specialized Reference Center in Mexico

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Objective: To describe some epidemiological characteristics of inborn errors of intermediary metabolism (IEiM) patients from a highly specialized pediatric hospital in Mexico in the last 25 years. **Methods:** An observational, longitudinal, retrospective study was performed. The cohort consisted of all patients with IEiM who were consecutively evaluated at the Inborn Errors of Metabolism and Screening Laboratory (LEIMyT) of the National Institute of Pediatrics, in Mexico City from January 1992-April 2017. For diagnosis, a multiplex laboratory platform was used, conformed by high performance liquid chromatography, gas chromatography-mass spectrometry, tandem mass spectrometry and other specialized tests. **Results:** 659 Mexican patients were evaluated in the last 25 years. Thirty-seven different IEiM were found; 204 (31%) patients were diagnosed at neonaepidemiolotal age through newborn screening and 455 (69%) were high risk patients with suggestive symptoms. The IEiM found were: 400 amino acid disorders; 213 organic acidemias; 40 carbohydrate metabolism disorders and 6 fatty acid oxidation disorders. The most frequent evaluated disorder was HPA/PKU (138 cases) followed by propionate defects (92 patients with methylmalonic acidemia and 39 with propionic acidemia) and urea cycle disorders (23 patients with citrullinemia, 21 with argininemia, 16 with ornithine transcarbamylase deficiency and 5 with argininosuccinic aciduria). **Conclusion:** A wide variety of IEiM were found and as expected, amino acid disorders were the most frequent one. Remarkably, fatty acid oxidation disorders are relatively rare among this group of Mexican patients. As in other countries where expanded newborn screening is not mandatory, we still observe a high number of late diagnosed symptomatic patients (69%), compared with those detected by newborn screening. Like other developing countries, it is necessary to promote expanded newborn screening programs as well as continue with clinical training for all the pediatricians in order to achieve earliest diagnosis and management of these diseases.

867 - A Novel *MC4 R* Gene Mutation Associated With Early Onset Childhood Obesity—A Case Report

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The melanocortin-4-receptor gene (MC4 R) is a key regulator of energy homeostasis, food intake and body weight. MC4 R gene mutations are the most common cause of monogenic obesity. Monogenic obesity is described as rare and severe early-onset obesity with abnormal feeding behavior and endocrine disorders. MC4R-linked obesity is characterized by the variable severity of obesity and almost no notable additional

phenotypes. Mutations in the MC4 R gene are involved in 2-3% of obese children and adults; the majority of these are heterozygous. Authors describe a case report of a two-year-old boy with early onset severe obesity, hyperfagia, body mass index (BMI) 25,3 kg/m^2 (> 97th percentile), BMI SD score + 5,34 and increased linear growth. There was also a morbid obesity phenotype in the patient's mother. Direct sequencing of the MC4 R gene was performed. A novel mutation -22 A/T in 5'UTR region was identified in the patient. We assume that this as-yet-undescribed mutation is linked to severe obese phenotype and might be a cause of monogenic obesity.

868 - Analysis of the *NKX2.5* Gene in Mexican Patients With Primary Congenital Hypothyroidism due to Thyroid Dysgenesis

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Mexico has a high incidence of primary congenital hypothyroidism (CH) due to thyroid dysgenesis (TD) as 1:1000 live born is affected, although the environmental and/or genetic etiology factors are still unknown. **Objective:** To define if genetic variants in the *NKX2.5* gene contribute to TD in a sample of Mexican CH patients. **Methodology**: Sequencing of the two exons and borders intron/exon of the *NKX2.5* gene (NG_013340.1, NM_004387.3) was performed in 131 unrelated Mexican CH patients. The diagnosis of CH and classification of TD relied on serum THS/T3/T4 profile and thyroid scintigraphy (n = 98) or ultrasonography (n = 33) [ectopy n = 60, athyrosis n = 54, and hypoplasia n = 17]. The genetic variants observed in this study were compared in main genotype databases. **Results:** One male patient with ectopic thyroid was identified as heterozygous for the variant c.355G>T (rs137852684) or p.(Ala119Ser). Also, the following variants were observed: c.63A>G (rs2277923) or p.(Glu21 =) (15 homozygous and 48 heterozygous patients), the c.543G>A (rs72554028) or p.(Gln181 =) and c.898T>C or p.(Leu300 =) (rs761208569), each one present in two heterozygous females with ectopy thyroid gland. **Discussion:** The variant c.355G>T it is catalogued as pathogenic allele in dbSNP, but their damaging effect in literature is still controversial. Dentice et al 2006, observed one heterozygous patient with ectopic thyroid and her mother with autoimmune hypothyroidism with this variant and functional studies reveals a reduced binding DNA capacity for the mutant NKX2-5 protein, so it was considered as pathogenic. In 2012, van Engelen et al observed this variant in one familial and one sporadic case with congenital heart disease (CHD); this last also had a thyroid nodule. As the p.(Ala119Ser) variant, was observed in healthy relatives of the familial case, besides it did not segregate with CHD and functional studies were normal, it was considered as a benign. This variant in ExAC has a very low allelic frequency (0.0013-0.0026) in different populations. In our case, the parents have normal thyroid function but their molecular analysis and also in healthy controls is ongoing. The c.63A>G variant has been associated with CH due to thyroid hypoplasia its study in normal individuals will allow us to define if it is a TD-risk variant in our population. In summary, it seems that pathogenic variants in the *NKX2.5* do not play a relevant role in the etiology of TD in Mexican population.

869 - Inhibitor-Induced In Situ Chaperone Therapy: A Novel Drug Targeting Strategy for Treating Metabolic Disorders

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Background: Metabolic disorders resulting from missense mutations often lead to protein misfolding and/or instability. Mutant enzymes that reach the folded/assembled state may have partial activity but are usually thermolabile and vulnerable to proteolysis. Protein stability is often improved significantly by binding of protein/enzyme substrate analogs, product(s), or allosteric modulators. Screening for chaperone drugs that bind enzymes at sites away from their catalytic sites to stabilize structurally compromised variants has been a major rational for drug development. Alternatively, stabilizing molecule(s) such as downstream pathway intermediates may provide some stabilizing effect. In this study, we tested the hypothesis that the use of inhibitory drugs of downstream pathway reaction(s) would cause the accumulation of pathway intermediates, including the product(s) of the upstream mutant enzyme, that in turn could bind to the unstable enzyme and confer stability, improve its function, and so can provide clinical benefit. **Methods:** Three biochemical pathways were targeted: fatty acid b-oxidation, and the Phe and Leu catabolic pathways. Patient fibroblasts deficient in enzymes of these pathways were cultured in the presence of downstream reaction inhibitors, trimetazidine, nitisinone (NTBC), and epigallocatechin gallate (EGCG), respectively. Immunostaining and western blotting, biochemical parameters, protein expression, and/or enzyme activity were monitored in cell extracts. **Results:** Immunostaining and western blotting indicated that the VLCAD protein variant presence in cultured patients fibroblasts improved significantly in a dose dependent fashion with the trimetazidine treatment. VLCAD activity in patient cells also increased comparably. Likewise, phenylalanine hydroxylase and tyrosine aminotransferase immuno-signal increased up to two-fold in variant cells from patients with PKU when NTBC was present compared to without treatment. EGCG improved the protein signal in IVD, MCCC, and HMGCL in IVD deficient cells. The improvement varied with the apparent severity of the enzyme variants' stability. **Conclusion:** Treatment of metabolic genetic disorders with

inhibitors of downstream reactions improved the presence of enzyme variants catalyzing upstream reactions, providing proof of concept for further investigation of the efficacy of such drugs and the validity of this novel drug targeting approach.

870 - Clinical Features and Long-Term Developmental Trends Of Pediatric Nonspecific Mitochondrial Disease

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Recent papers indicate that mitochondrial dysfunction can cause a higher proportion of uncategorized nonspecific encephalopathy as well as named disorders. This study aimed to evaluate the clinical features and long-term developmental trends in pediatric patients with nonspecific mitochondrial disease (MD). Data of 189 pediatric patients with nonspecific MD (85 males, 45%) were obtained from hospital records and the results of developmental evaluations using the developmental quotient (DQ). Additionally, disease-related clinical variables were reviewed. The age at onset of symptoms was 1.2 $\pm$ 1.6 years (0-9.1 years) and the nature of the initial symptoms varied, with developmental delay (83 patients, 44%) and seizures (74 patients, 39%) being the most frequent. The time from the first clinical presentation until the confirmative diagnosis of MD was 2.8 $\pm$ 2.7 years (0.03-16.9 years), regardless of the initial symptoms. In terms of severity of the clinical manifestations, all patients had involvement of the central nervous system, 48% (91) of patients had elevated serum lactic acid level, followed by seventy of 91 (37%) had mildly elevated levels, and 21 of 91 (12%) moderately and severely elevated levels of serum lactic acid. MRI studies of 56% patients (104 of 188) were abnormal and showed a variety of findings including atrophy or abnormal signals in different areas of the brain. Long-term follow-up over the course of 7.70 years showed declining trends over all studied periods. In patients diagnosed via syndromic diagnosis, nonsignificant differences in Development quotient (DQ) were observed between patients with nonspecific MD and those with Leigh syndrome. Age at the first symptom onset showed positive correlation with the level of developmental function at the post-diagnostic visit. Lead time to diagnosis was negatively associated with DQ at all-time points, but not reach statistical significance. Follow-up revealed consistent patterns of significant developmental deterioration of DQs during lead time, whereas no significant differences after diagnosis were observed. DQs might be a candidate as a predictor or a measure in pediatric MD by giving the functional level of patients, which is a very meaningful value in pediatrics. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2016R1A2B4011052).

871 - Clinical Spectrum and Outcome of AIP Patients Without Heme-Arginate

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Background: Acute Intermittent Porphyria (AIP) is a hepatic porphyria caused by low levels of Porphobilinogen Deaminase enzyme (PBGD). **Objective:** To review clinical and biochemical spectrum of the disease at the time of diagnosis and outcome without Heme arginate treatment in a developing country like India. **Materials and method:** We evaluated 7 unrelated patients (2 M, 5F) with AIP. The mean age at presentation was 16 years. Biochemical workup done to ascertain diagnosis. **Result:** All patients had typical manifestation of AIP—abdominal, neurological and cardiovascular symptoms. Most common presenting symptoms were: Abdominal pain (n = 7), vomiting (n = 5), psychosis (n = 5), weakness (n = 4), convulsions (n = 4), constipation (n = 4), tachycardia and hypertension (n = 3). 1 patient had anemia, 1 had peripheral neuropathy. All the patients showed significantly reduced activity of PBGD enzyme (mean 7.84 $\pm$ 2.38 nmol/L/s, around 30-60% of normal). They showed significantly elevated levels of urinary precursors ALA and PBG and increased levels of porphyrin isomers in urine. There was significant elevation or Uro porphyrin isomers in urine with an increased Uroporphyrin I >>> Uroporphyrin III. 4 patients were documented to have hyponatremia and deranged liver function tests during the acute episodes. Serum ferritin levels were also elevated in these patients, suggesting hepatopathy. These patients were managed on high dose IV glucose along with supportive anti- convulsants therapy and managed for hyponatremia. Symptoms of abdominal pain, vomiting, weakness and pain in limbs resolved gradually. None of the patients received Hematin or Heme arginate for therapy as it is not available and is not affordable to the patients here. However, with high dose IV glucose, acute symptoms resolved. Improvement in biochemical parameters was documented in 1 patient with decrease in levels of ALA, PBG and porphyrin isomers after glucose infusion. Her abdominal pain resolved immediately whereas weakness in limbs got better gradually. **Conclusion:** AIP is an acute hepatic porphyria which presents with life threatening episodes and needs treatment with hemearginate to avoid recurrent episodes. However, due to unavailability of hemearginate in India, we are managing our patients symptomatically and with IV glucose therapy with good results. Only one patient has developed chronic neuropathy and is wheelchair bound. There are no deaths in our cohort so far.

872 - Founder Effect and Genotype/ Phenotype Correlation in Colombian Patients With Hypophosphatasia

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Hypophosphatasia is a rare inherited disorder of bone and mineral metabolism, caused by a deficiency of tissue-nonspecific alkaline phosphatase (TNSALP) secondary a mutations in the *ALPL* gene. Until now more than 330 mutations of *ALPL* gene have been identified. The strong allelic heterogeneity results in clinical heterogeneity, ranging from stillbirth with a poorly mineralized skeleton to pathologic skeletal fractures which develop in late adulthood only. We found that mutation c.892G>A (p.E298 K) is the most frequent in Colombian patients, previously reported in a Russian patient. We investigated whether it had a unique origin or rather multiple origins due to recurrence of de novo mutations. Our results show that all the p.E298 K mutations are carried by a common ancestral haplotype. We conclude a founder effect in Colombia and provide new evidence of the clinical variability in HPP patients.

873 - Analysis of the Mutation Spectrum of the *FBN1* Gene in Russian Patients With Marfan Syndrome

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Purpose: to develop a method for direct diagnosis of Marfan syndrome to detect mutations in the *FBN1* gene in Russian patients. Materials and methods: The DNA extraction was done using a set of reagents "DLAtomTM DNA Prep100". The analysis of nucleotide sequences of the *FBN1* gene was performed by direct automatic sequencing. **Results:** We formed a sample of 10 unrelated probands with clinical signs of Marfan syndrome and members of their families. 5 patients inherited the disease, and the remaining 5 had isolated cases. 9 different mutations were found in the heterozygous state in 9 probands. Four of them were detected for the first time, including the neonatal form of Marfan syndrome: *Trp988X* (exon 25), *c.4210G> C* (exon 34), *c.1979delC* (exon 17), and *c.3285C>G* (exon 27); five have been described previously. In exon 25 of the 10th patient we discovered a replacement of *c.2956G> A (rs 112877030)* with an unknown clinical value. To clarify the association of this finding with the disease in the family, we studied DNA samples of sick and healthy relatives of the proband. The results obtained allowed considering this substitution as an informative marker for the diagnosis of Marfan syndrome in the given family. Later, it was proved that this replacement belonged to the polymorphic variant and was not a mutation. Apparently, mutation *c.1979delC* (exon17) causes the formation of severe skeletal and cardiovascular changes, whereas mutation *c.4210G>* C (exon 34) is responsible for mild pathology of the musculoskeletal system and typical lesion of vision. Mutation *p.Trp988X* (exon 25) causes relatively light bone deformities, no ocular pathology and severe changes in the cardiovascular system followed by early death. Mutation *c.3285C>G; p.Cys1095Trp* (exon 27) promotes the development of severe neonatal form of the disease. This mutation arose de novo. Mutation *c.7606G> A* (exon 62) entails severe skeletal pathology, cardiovascular system with early manifestation of the aortic aneurysm, no eye damage and expressive personal characteristics of patients such as anxiety, increased suspiciousness, and conflict. Mutation *p.5912G> C* (exon 48) described previously leads to the development of severe symptoms of Marfan syndrome leading to short life expectancy. Thus, direct diagnosis of Marfan syndrome is extremely important for effective medical and genetic counseling of families, as well as for the prevention of severe clinical disorders and disability of patients.

874 - Development of a Platform (Database Management System) for Information Management of the Expanded Newborn Screening Program in Nuevo León, Mexico

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Introduction: One of the main success factors in newborn screening (NBS) programs is the expeditious location of the suspected case for its timely diagnosis and treatment. Information and communication technologies (ICTs) provide systems that facilitate this function, as well as give a tool that helps analyze the information and thus evaluate the effectiveness of the program. Objective: Develop and implement a platform available to all NBS program managers from anywhere in the state of Nuevo Leon Mexico, to establish a rapid communication network for the location and reference of cases. **Method:** A joint work was done between programmers, laboratory and

program managers. The instrument should have demographic information of the newborn, data of the mother and whatever was necessary for the evaluation: time of taking and sending of samples, reception in the laboratory, delivery of results, confirmation of diagnosis and initiation of treatment (automatic notification for follow-up, missing data, card repeated and others). **Results:** It was possible to construct a semaphorized system (alert system) based on the population cut off values facilitating the rapid detection of the suspicious case. After two years of its implementation, 60 000 samples of the different public hospitals in the metropolitan and rural areas have been registered. The information is available in real time form the moment of sampling in the hospital, reducing the time of location of suspected cases as well as perform confirmation test that let time reduction to initiate treatment. **Discussion:** There are different database management systems (work platforms), however, the advantages of a customized platform, such as this, addresses the requirements and needs according to our facilities. **Conclusions:** The incorporation of information and communication technologies in the management of the newborn screening program has improved the effectiveness of this in Nuevo Leon, Mexico.

875 - The Hematologic Manifestations of Inborn Errors of Metabolism: They are More Than Expected

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Hematological problems are some of the most frequently observed findings of inherited metabolic diseases. These may be seen together with other systemic findings or sometimes as the first and only diagnostic finding of disease. Early determination of hematological findings has a positive effect on the prognosis of metabolic diseases. The aim of this study is to evaluate the incidence of hematological findings in inherited metabolic diseases since there are a few studies about the true incidence in literature. Three hundred eighteen patients who were being followed-up within the previous 6 months at Gazi University Department of Pediatric Nutrition and Metabolism, Turkey, were included in the study. Since patients were in different age groups, hematological findings were compared with normal values for each patient's age group. The hematological findings were classified under seven main groups; anemia of chronic disease, iron deficiency anemia, vitamin B12 deficiency anemia, hemophagocytosis, leukocytosis and thrombocytosis[ük1]. Metabolic diseases were classified according to the textbook of Inborn Metabolic Diseases: Diagnosis and Treatment. Nine hundred twenty-two hematological examinations of the 318 patients were included to the study, and 283 hematological findings were determined, 127 anemia of chronic disease, 80 iron deficiency anemia, 56 cytopenia and four vitamin B12 deficiency anemia. Leukocytosis (n = 1), thrombocytosis (n = 5), and hemophagocytosis (n = 9) were also observed[ük2] . It was determined that although anemia of chronic disease and nutritional anemia are the most common hematological findings, these may be diagnosed late, while neutropenia, thrombocytopenia, pancytopenia and hemostasis disorders may be diagnosed earlier. Metabolic diseases must be considered in the evaluation of cytopenias, particularly in cases with an atypical cause that are resistant to treatment and have additional accompanying findings. Our study is the most comprehensive one in the literature, and we think it would positively contribute to the monitoring and prognosis of congenital metabolic diseases.

876 - Results From 15 Years of a Pioneer Free Service for Aid With Inborn Errors of Metabolism in Brazil

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The Inborn Errors of Metabolism Information Service (SIEM, in Portuguese) is a free service that provides assistance in diagnosing, managing and supplying general information on Inborn Errors of Metabolism (IEM). Once contacted by a health professional, a standard form is completed with relevant clinical information which is registered in a database and analyzed by a specialist who will suggest diagnostic hypothesis, further laboratory and/or imaging markers, and, if necessary, emergency management. This cross-sectional study aims to analyze the results obtained by the SIEM from October 2001 until April 2017. The data was collected from the Service's data bank. During this period, 3277 cases were registered. Most cases (89.4%) were registered by professionals that sought diagnostic and management assistance followed by 199 registrations (6.1%) seeking general information on IEM. 134 cases (4.1%) were registered for support for patients that had already been diagnosed with an IEM. The main medical specialties that contacted the SIEM are Pediatricians and/or Neonatologists (35.8%), Medical Geneticists (21.1%) and Neurologists and/or Neuropediatricians (20.1%); non-medical health professionals are responsible for 288 (8.9%) contacts. The purpose of the

contacts was chiefly in regard to patients with symptoms that suggested an IEM (86.5%); the most frequent symptoms registered include Neuropsychomotor Development Delay (43.3%), Seizure (38.7%), Hypotonia (38.5%), Hepatomegaly (21.1%) and Vomit (20.3%). The onset of symptoms was mostly up to 1 year of age (68.4%). 2658 cases (81.1%), out of which, 329 (12.4%) were confirmed as an IEM distributed as such: 136 (41.3%) amino acid and peptide metabolism deficiency; 60 (18.3%) lysosomal metabolism deficiency; 39 (11.6%) energetic metabolism deficiency; 34 (10.4%) carbohydrate metabolism deficiency; 19 (5.9%) fatty acids and ketone bodies metabolism deficiency; 16 (4.9%) peroxisomal metabolism deficiency; 25 (7.6%) from other IEM. With such heterogeneous pathologies, the IEM occur fairly frequently as a group of metabolic conditions yet are rare when considered individually. Diagnosis is challenging due to non-specific symptoms with early onsets; however, it needs to be established with proper timing for an adequate and accurate management of the patient's clinical status. The SIEM is an important tool for the endeavor of propagating information and proper orientation on inherited metabolic diseases.

877 - Increased Susceptibility for Mood Disorders and Altered Stress Adaptation in Male Mice With Suboptimal Mitochondrial Function

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An increasing body of evidence points toward the involvement of suboptimal mitochondrial function (SMF) in depression and other mood disorders. Several clinical and preclinical studies show that depression is associated with altered mitochondrial structure and function. Furthermore, stress, a major risk factor for depression, directly influences mitochondrial function. There is a surprising high association between mitochondrial disease and depression as well, which is estimated to be around 50%. This is significantly higher than the expected 16% depression prevalence in the general population. These findings suggest that SMF is causal in depression, however, this notion has not been substantiated yet. In this study, we tested the hypothesis that SMF influenced the animals' stress response and impacted various biological domains linked to the pathobiology of depression. To test this hypothesis, a new genetically engineered mouse model was used. These animals have a deficiency of the NDUFS4 protein (Ndufs4def mice), a structural protein of complex I (CI), an essential component of the electron transport chain and oxidative phosphorylation. This deficiency leads to a 25% reduction of CI activity in the brain compared to WT mice. Despite this reduction, Ndufs4def mice exhibited no differences in body weight and temperature, physical activity (distanced travelled and velocity in the open filed), and motor coordination (Rotarod-test) compared to their WT littermates. After exposure to a chronic variable stress protocol, a well-validated animal model for depression, Ndufs4def mice showed increased anxiety like behavior (open field) as well as a disturbed day-night rhythm compared to WT animals, a symptom that can also be seen in individuals with depression. To provide mechanistic insights, we assessed the activation of brain nodes implicated in the pathobiology of depression. The expression of the protein FOS, a surrogate marker of neuronal activation, revealed distinct activation of several brain regions of WT and Ndufs4def mice. These results indicate a difference in functional coupling within and an altered balance between major brain networks, a well-established phenomenon in individuals with depression. In conclusion, here we report on distinct chronic stress-evoked responses in various biological domains in Ndusf4def and WT mice, supporting our hypothesis that suboptimal mitochondrial function mediates the impact of stress on mental health.

878 - Managing the Crisis: Rethinking the Provision of Specialized Metabolic Services for Underserved Populations

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Objectives: The problems generated by the paucity of biochemical geneticists have been greatly exacerbated by expanded newborn screening, and the number of board-certified metabolic specialists is not increasing to meet the clinical demand. This has created a crisis for both patients and providers. We developed a telehealth Clinical Consultation Support Service (CCSS) to improve access to specialized metabolic expertise for clinical geneticists and other providers who need assistance with complicated metabolic cases, and to assist established clinics with coverage during faculty vacations, respite, and/or during metabolic position vacancies. **Methods:** Non-urgent consultations are provided during scheduled conference times, or on-demand; there is 24/7 availability for emergency cases. Most discussions occur by email or phone, adapted to be HIPAA-compliant. The CCSS is a peer-to-peer support service; direct patient care is not provided. It is not intended to replace the traditional delivery of metabolic care but to fill clinical deficiencies. The pilot for the CCSS was originally funded through grants from the New England Genetics Collaborative and private investment capital. The CCSS now provides metabolic support to clinics in the United States and international medical community. **Results:** To date, GMCE has provided 127 telehealth consultations for one or multiple patients. The nature of the consultations has included diagnosis (32), management (59), diagnosis and management (24), and disease counseling (3). CCSS contracts are in place with 5 academic US hospitals and Tbilisi, Georgia.

Conclusions: GMCE's CCSS is addressing an acute need for metabolic services in an underserved area of medicine.

879 - Acylcarnitines in Cerebrospinal Fluid

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Introduction: The application of the Amino acids and Acylcarnitines profile is very beneficial in the study of ECM, even as confirmatory for certain pathologies. The study of the cerebrospinal fluid provides information on diagnosis and progression of pathologies related to the Central Nervous System. **Materials and Methods:** We used 18 control samples, 3 of patients with encephalopathies and 4 with Multiple Sclerosis. They were processed using a derivative method and internal standards from Cambridge Isotope Laboratories. The treated samples were analyzed on a Waters XEVO TQ-S mass spectrometer and evaluated with Neolynx Research 3.5 software. **Results:** In the studied pathologies, the concentrations of Free Carnitine and Short Chain Acylcarnitines were markedly higher than the values of the controls. Mean values for μmol / L of C0 = 1.40, C2 = 0.76, C3 = 0.05 and C4 = 0.10 were obtained for controls; For the pathological samples of Encephalopathies and Multiple Sclerosis the values were C0 = 4.08 and 5.84; C2 = 2.73 and 3.87; C3 = 0.37 and 0.44; And for C4 = 0.16 and 0.23, respectively. **Conclusion:** Our laboratory receives samples of different etiologies, often without differentiating if it is only neurometabolic pathologies, in this way when obtaining a supposedly pathological result we can provide a better contribution to the diagnosis of the patient.

880 - Cleidocranial Dysplasia and Hypophosphatasia in a Child With RUNX2 Mutation

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Cleidocranial dysplasia (CCD; OMIM 119600) is a rare skeletal dysplasia arises from mutations causing haploinsufficiency of *RUNX2* gene, coding an osteoblast transcription factor specific for bone and cartilage tissue. Hypophosphatasia (HPP; OMIM241500) is a rare disease associated with mutations of *ALPL* gene, resulting in deficiency of the tissue-nonspecific alkaline phosphatase (TNALP) activity and thereby impaired bone mineralization. A subset of patients with CCD have radiographic and biochemical features that overlap with HPP [Unger et al., 2002; El-Gharbawy et al., 2009]. It has been shown that *ALPL* expression is activated by *RUNX2* (according to SIGNOR database). Runx2 deficient mice (+/−) have features of CCD with reduced alkaline phosphatase activity. We have observed a girl with signs of both diseases (HPP and CCD). She was born at 36 weeks by Caesarean section, weight 2190 g and length 47 cm[KK1], with APGAR scores 5/5. From the first day, she presented with respiratory failure (needed in respiratory support), hypotonia, hyporeflexia, soft bones of the skull, divergence of sagittal suture, and chest hypoplasia. Biochemical signs of HPP has been revealed: low levels of TNALP (64-107 units/L) and parathyroid hormone (0.3-7.6 pg/mL), moderately increased calcium and phosphorus in serum. Cranial deformation was developed and ultrasound signs of nephrocalcinosis were identified. At the age of 13 months she had low weight and height (8100 g, 72 cm), severely delayed motor development, narrow deformed thorax with deployed lower ribs. The clavicles were underdeveloped, formed only in the central parts, thinned. Most of symptoms corresponded to HPP, so the *ALPL* gene analysis was performed by the direct sequencing of the exons and the nearby regions. No pathogenic mutations were found, and the synonymous change c.330C>T (p.Ser110=) was revealed. Whole[KK2] exome sequencing was performed for the patient. There was found missense amino acid change Arg258Trp (Chr6:45,399,744, C→T, GRCh37/hg19) in the exon 4 of gene *RUNX2*. According to PolyPhen algorithm this amino acid change is probably pathogenic. We have assumed that *RUNX2* mutation may lead to a decreased *ALPL* expression since its transcription is controlled by *RUNX2*. So clinical and biochemical signs of HPP could manifested in a number of CCD patients including our case. This observation raises the problem of whole exome sequencing in patients with overlapped phenotypes.

881 - Trimethylaminuria or Fish Odor Syndrome: First Molecular Study in an Argentinean Cohort

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Primary genetic Trimethylaminuria (TMA) or Fish Odor Syndrome (FOS) is a disease inherited in an autosomal recessive fashion caused by *FMO3* gene mutations that result in loss of function of FMO3 enzyme activity. More than 300 SNPs of the *FMO3* have been reported and over 40 of these polymorphisms have been linked to TMA. **Objective**. To establish the molecular basis of the TMA disease in Argentina as a first step in the investigation of this disease. **Subjects**. Eight unrelated patients with clinical suspicion of TMA were included. **Method**. We amplified and sequenced the eight coding exons and flanking regions of FMO3 gene. **Results.** The results were grouped base on the type of genetics variants detected in the patient studied: 1) Only missense changes: one patient results homozygous for a new variation at FMO3 gene, the p.Arg387His mutation

located at exon 7. Three bioinformatic algorithms indicated a deleterious effect of this new change on the FMO3 enzyme meanwhile a fourth one predicts a tolerable effect on this protein. 2) Polymorphic and intronic variants: a- In 2 patients, the polymorphisms associated with TMA, p.Glu158Lys and p.Glu308Gly were identified in one allele. Additionally, the probands were heterozygous and homozygous, respectively for the c.485-22 G>A (IVS4-22G>A) variant in intronic region (it was reported that it could potentially affect the splicing). In one allele of one patient was detected also a synonymous polymorphism, p.S147 S. This synonymous polymorphism was reported that results in the strengthening of a potential SRP40 site in exon 4. b- Other 2 patients resulted heterozygous for three changes: p.Glu158Lys in exon 4, c.485-21 G>A (IVS4-22G>A) and c.627+10 (IVS5G>C) in intronic regions. The functional relevance of the IVS5G>C is uncertain. Both patients also carried the p.S147Spolymorphism in one allele. 3) No genetic variant**:** No change at FMO3 gene were found in three investigated individuals. **Conclusions.** The molecular analysis showed a new variation (p.Arg387His) at FMO3 gene in both alleles of one patient. The determination of the complete genotype in 4 patients (2nd group) is at present underway. It is unknown if the polymorphisms found in these probands are in cis configuration. Additional studies will be needed to establish the exact molecular diagnosis in this cohort. However, this work represents the initial stage of the first investigation of this socially distressing condition carried out in Argentina.

882 - Delineation of Clinical and Molecular Phenotype of Aicardi-Goutieres Syndrome in Arab Population

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Aicardi-Goutieres syndrome (AGS) is a genetically heterogeneous encephalopathy, patients typically present with early onset encephalopathy. Cerebrospinal fluid showed persistent lymphocytosis and Brain CT showed calcification in the basal ganglia. Patients' presentation and their family history suggested autosomal recessive and dominant inheritance. We report 23 patients from Middle East descent with AGS, although some patients presented late after initial normal development, but the majority of the cases present early in the neonatal period with subacute severe encephalopathy. Patients exhibit primarily neurological symptoms including, irritability, developmental delay, acquired microcephaly, hypo- or hypert-onia, poor feeding and neonatal seizure. Other systemic symptoms include hepatosplenomegaly and hematological abnormalities and chilblain skin lesions. The course of the disease varies; while some patients die early after severe subacute encephalopathy, in other patients the disease progresses to certain point after which the condition stabilizes. We described the genes mutations related to AGS in our cohort including *RNASEH2B, RNASEH2A, RNASEH2C, TREX1, SAMHD1*, *and IFIH1*. The most frequently observed genes associated with AGS were *RNASEH2B* followed by *RNASEH2A*. Radiological features of AGS included bilateral basal ganglia calcification that might extend to the deep white matter. Brain MRI might show high signal in the anterior and posterior poles of the lateral ventricles. In severe cases, patients showed frontotemporal leukodystrophy and diffuse cerebral atrophy in MRI. Cerebellar atrophy, brain stem and corpus callosum involvement were observed. This study shed light on genotype, phenotype, and the course of AGS in Arab population.

883 - Cobalamin C Disease Presenting With Neonatal Nephrotic Syndrome: A Case Report

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Objective: Combined Methyl malonic acidemia and Homocystinuria cblC type (also known as cobalamin C disease or cblC) is the most common inherited disorder of vitamin B12 metabolism and is due to mutations in the *MMACHC* gene located on chromosome 1p34.1 which causes methyl cobalamin and adenosylcobalamin (cofactors of methionine synthase & methylmalonyl-coA mutase respectively) deficiencies. Clinical presentation and severity of cblC disease vary considerably from severe burden of disease, even death at early age (average survival less than 10 months) to late presenting disabilities. Biochemical and clinical phenotypes of this disease are distinct from isolated MMA (mutase defect, cblA, cblB) or classical homocystinuria. So far, more than 55 different mutations of the *MMACHC* gene have been identified, with the most frequent (42%): c.271dupA associated to early onset of the disease. The wide range of types and age of presentation ranging from prenatal (IUGR, Dysmorphia, microcephaly, congenital heart disease), Infantile (feeding problems, failure to thrive, seizure, and neurological deterioration) or non-infantile (neurologic regression and psychiatric symptoms) makes cblC disease diagnosis challenging that may be due to *MMACHC* mutations or other factors like ethnicity, diet and individual genetic variations. Review of literature has shown reports of late onset cblC disease presenting with isolated pulmonary hypertension and hemolytic uremic syndrome. We aim to report a rare type of early-onset cblC disease presentation with neonatal nephrotic syndrome. **Methods:** This is a case report of cblC disease. **Reports:** The patient had early onset presented with hypertension and nephrotic syndrome. Molecular analysis revealed homozygosis for c.616C>T mutation in the *MMACHC* gene, that to our knowledge has not been reported for early onset of the disease. **Conclusion:** Considering the diverse symptoms of

cblC disease, it should be included as a differential diagnosis in any unusual disease.

884 - Non-Alcoholic Steatohepatitis as a Form of Presentation of Inherited Metabolic Disorders

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Background: Non-alcoholic steatohepatitis (NASH) represents a condition with a wide spectrum of diagnosis and it is essential not to miss a treatable condition. In secondary nonalcoholic steatohepatitis (NASH) both hepatic substance accumulation, resulting in inflammatory injury, and a direct injury from toxic components are present, leading to hepatocellular injury. The presence of hepatomegaly may be an important clue for this group of diagnosis. **Methods:** The authors reviewed the clinical records of all the patients with elevated hepatic transaminases and echographic pattern of liver steatosis (defined as bright or liver hyperechogenicity), without signs or symptoms of other system involvement, referred to Pediatric Gastroenterology or Metabolic Diseases consultation between 2000-15. **Results:** Nine cases were included (4F:5 M) with a median age of 9 years [14M-16Y]. Concerning the reason for consultation referral: 7 have fortuitous finding of elevated hepatic enzymes; 1 had elevated transaminases and family history of Wilson disease; 1 was referred because of hepatomegaly and persistent elevation of liver enzymes. Only one patient had BMI>P95; 5 had clinical hepatomegaly and none had stigma of chronic liver disease. On presentation, alanine aminotransferase and aspartate aminotransferase elevation ranged between 1,5 to 3,5 above normal (N). None had elevated bilirubin, 1 had gamma-glutamyl transferase and alkaline phosphatase elevation (1,5xN). Cholesterol profile was high in 1 case (IMC P50) and triglycerides in 2 cases. Two had total cholesterol, LDL cholesterol and apolipoproteina B below 5th percentile. On ultrasound, all had diffuse steatosis and hepatomegaly without splenomegaly. Liver biopsies ($n = 6$) showed mixed diffuse steatosis ranging from mild to severe, 3 with periportal fibrosis and one with PAS-positive material. In this series, the diagnosis were: Wilson disease (3), glycogenosis type IX (3), hypobetaliproteinemia (2) and lysosomal acid lipase deficiency (1). Eight had genetic studies confirming the diagnosis. **Conclusions:** Inborn errors of metabolism must be considered in the differential diagnosis of NASH mainly in non-obese children and even in the absence of systemic involvement. The presented cases have liver storage diseases, either from cholesterol, copper or glycogen, reflecting the pathophysiology of NASH. An early diagnosis, will allow an appropriate follow-up and management of these diseases.

885 - Proteome of Plasmodium Falciparum Suffers Oxidative Damage in Erythrocytes With Sickle Trait

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Objective: Quantify oxidative damage suffered by *P. falciparum* during intra-erythrocytic developmental cycle in red blood cells with sickle trait. **Methods:** Donnors with HbS were diagnosed by hemoglobin electrophoresis in agarose gels. Synchronic cultures at high parasitemia levels of *P. falciparum* 3D7 clone in controls (HbAA) and sickle cell trait (HbAS) were collected. Then, the parasite proteome was obtained for rings, trophozoites and schizonts phases. Finally, to measure oxidative damage carbonyl index was determinate by quantitative dot-blot assay. **Results**: A significant increase in the oxidation of the parasite proteome throughout its asexual phases was observed in HbAS erythrocytes. In particular, during ring and trophozoite phases, carbonyl indexes were 4.8 and 6.5 times greater than obtained for controls in HbAA erythrocytes, respectively. It shows that individuals with sickle cell trait creates a hostile environment causing oxidative damage on parasite proteins. Furthermore, have been hypothesized that redox imbalance can affect the traffic of proteins of export of Plasmodium and the remodeling of the membrane of the parasitized erythrocyte. **Conclusion:** Proteome of *P. falciparum* suffer oxidative damage *in vitro* during early phases of intra-erythrocytic developmental cycle in red blood cells with sickle trait. **Acknowledgments**: Colciencias and the University of Cartagena for the financial support to Gant 1107-569-33704. To Dr. Sara Robledo, University of Antioquia, for gentle donation of P. falciparum 3D7 clone.

886 - Understanding the Biology of the "Pterinergic metabolism" in the Brain: Neopterin acts as an Endogenous Cognitive Enhancer

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Introduction: Neopterin is a byproduct of the tetrahydrobiopterin (BH4) de novo biosynthetic pathway. Increased levels of this metabolite in biological fluids have been used for decades as a sensitive biomarker of inflammatory disorders. The biological role of this pteridine remains undefined; however, due to its capacity to increase hemeoxygenase-1 content, it has been proposed as a protective agent during cellular stress.

Objectives: Investigate the potential role of neopterin on learning and memory processes in adult and aged rodents, under basal and inflammatory conditions. **Material and Methods:** Neopterin (0.4 and/or 4 pmol) was injected intracerebroventricularly before or after the training sessions of step-down inhibitory avoidance and fear conditioning tasks, respectively. Memory-related behaviors were assessed in Swiss and C57BL/6 mice, as well as in Wistar rats. Moreover, the putative effects of neopterin on motor and anxiety-related parameters were addressed in the open field and elevated plus-maze tasks. The effects of neopterin on cognitive performance were also investigated after intraperitoneal lipopolysaccharide (LPS) administration (0.33 mg/kg) in interleukin-10 knockout mice (IL-10−/−)**. Results and Discussion**: It was consistently observed across rodent species that neopterin facilitated aversive memory acquisition by increasing the latency to step-down in the inhibitory avoidance task. This effect was related to a reduced threshold to generate the hippocampal long-term potentiation (LTP) process, and reduced IL-6 brain levels after the LPS challenge. However, neopterin administration after acquisition did not alter the consolidation of fear memories, neither motor nor anxiety-related parameters. **Conclusions:** Neopterin enhances learning and memory in different species of rodents under normal and inflammatory conditions, probably by inducing an antioxidant/anti-inflammatory state, and by facilitating LTP generation. Finally, it is also feasible to propose that glial cells release neopterin in order to protect neurons during cellular stress.

887 - Recurrent Acute Liver Failure in a Family With *NBAS* Gene Mutation and Successful Liver Transplantation: First Cases From Turkey

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Acute liver failure is a currently unresolved and a life-threatening problem in children. Hundreds of causes and a high rate of mortality were reported, however etiological evaluation revealed nothing in about half of cases. The frequently detected causes are inherited metabolic diseases, toxins, drugs, and infections. When the reason of hepatic insufficiency cannot be obtained, it will be difficult to establish an appropriate treatment. Deciding to do a liver transplantation in a child with a nonspecific diagnosis is a difficult and a complex problem. The neuroblastoma amplified sequence (*NBAS)* gene mutations were related with RALF and SOPH syndromes previously. Also, there are very limited number of reports dealing with only acute liver failure and *NBAS* gene mutations in the literature. Here, we report two Turkish siblings born from consanguineous parents, that were hospitalized several times with acute attacks of hepatic insufficiency and encephalopathy. The male sibling died at the age of 26 months without a specific diagnosis, but with the suspicion of Wilson disease. Postmortem liver biopsy was compatible with microvesicular fatty degeneration and fibrosis. The clinical picture and the laboratory findings of the other sibling suggested the diagnosis of 3-OH 3 methyl-Co A lyase deficiency, although a mutation was not detected. After consecutive plasmapheresis, at the age of 5 years the female sibling was successfully transplanted from a living donor. 5 years after the transplantation whole exome next generation sequencing revealed a new homozygous c.3602A>C mutation in the *NBAS* gene. This mutation was also present in heterozygous state in both parents. Our patients did not share the other commonly observed features of systemic forms of *NBAS* gene mutation; short stature, optic atrophy, cardiomyopathy, gastrointestinal disorders, autoimmune defects, Pelger Huet anomaly, renal and skeletal problems, encephalopathy, and epilepsy. However, they had similar findings of the hepatic phenotype, in which the first attack was usually seen in infancy, triggered with an infection or a vaccination, and mimics the picture of fatty acid oxidation defects. The patient could fully recover from this hepatoencephalopathic attacks or died. Conservative approaches and liver transplantation are the recommended therapeutic options. Our patient is completely well at the 7th year of transplantation. A functional study is under investigation for the new mutation.

888 - Utility of WHOLE EXOME SEQUENCING (WES) in the Diagnosis of Lysosomal Storage Disorders (LSDs)

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WES is the current test of choice for patients with complex phenotypes and previous uninformative genetic testing. Although LSDs are well-defined disorders, they have overlapping phenotypes and despite biochemical analyses and NGS panels being available, clinicians might opt for WES analysis when unable to arrive at a specific diagnosis. The high cost and large time to diagnosis of step-wise single gene testing or NGS panels; unavailability of enzyme analysis locally; and the possibility of expanding the analysis to a larger set of genes, makes WES a good option for patients with LSDs. In this study, we analyzed the reported WES cases to date with respect to 41 LSD genes, to determine if WES is a good diagnostic tool for LSD cases. The cases were then reviewed to identify those with a confirmed or possible diagnosis. **Results:** On review, 107 cases had at least one reported variant (a pathogenic (PV), likely pathogenic (LPV) or variant of unknown significance (VUS)) in an LSD gene. **Confirmed genetic diagnosis:** In

53 out of 107 cases a diagnosis was confirmed, as 49 cases were homozygous/compound heterozygous for a PV/ LPV in an autosomal recessive (AR) gene; 1 case was hemizygous for an LPV in an X-linked (XL) gene and 3 cases were compound heterozygotes for a PV and a VUS. **Possible genetic diagnosis (VUS):** In 48 out of 107 cases a diagnosis of LSD might be possible, as 41 cases were homozygous/compound heterozygous for a VUS in an AR gene and 7 cases were hemizygous for a VUS in an XL gene. **Single variant, unconfirmed diagnosis, carrier status:** In 13 cases, only 1 variant was detected in an AR gene. For 7 cases, deletion/duplication analysis was recommended due to significant overlap of patient symptoms with the disease. In 4 cases, the LSD variant was identified in an unaffected relative, segregated in the family and had significant overlap with the affected index's symptoms. Two cases were incidental carriers of an LSD variant and had another diagnostic variant. Approximately 49.5% of cases had a confirmed diagnosis of LSD on WES. These results show that despite a distinct phenotype and availability of biochemical testing, many patients with LSDs remain undiagnosed. As most LSD genes are reasonably well covered on WES, are well-characterized and have biochemical testing to help reclassify VUS, WES can be considered a good alternative to single gene or panel testing for patient with LSD phenotypes when cost, time and logistic barriers for testing are present.

889 - PGAP3-Related Hyperphosphatasia With Mental Retardation Syndrome: Report of 10 New Patients and a Homozygous Founder Mutation

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Hyperphosphatasia with mental retardation syndrome (HPMRS) is caused by recessive mutations in genes involved in the glycosylphosphatidylinsitol pathway, including *PGAP3*. Herein, we describe 10 patients from 8 Egyptian families presenting with developmental delay, severe intellectual disability, distinct facial dysmorphism and increased alkaline phosphatase. Eight patients had cleft palate, four had postnatal microcephaly and five had seizures. Neuroimaging findings showed thin corpus callosum in 9 patients, mild ventriculomegaly in 3 patients and variable degrees of cerebellar vermis hypoplasia in 4 patients, a finding not previously reported in patients with HPMRS. Additional manifestations included double row teeth, hypogenitalism and congenital heart disease. Biallelic loss of function mutations in the *PGAP3* gene were detected in all patients. Nine patients were homozygous for the c.402dupC (p.M135Hfs*28) mutation strongly suggesting a founder effect. On the other hand, one patient had a novel mutation, c.817_820delGACT (p.D273Sfs*37). To our knowledge, this is the largest series of patients with HPMRS from same ethnic group. Our results reinforce the distinct clinical and facial features of *PGAP3*-related HPMRS which are the clue for targeted genetic testing. Moreover, we present additional unreported clinical and neuro-imaging findings and a novel mutation thus expanding the phenotypic and mutational spectrum of this rare disorder.

890 - Mutation Screening Using Neurogenetics Next Generation Sequencing (NGS) Gene Panel in Patients With Normal Metabolic Profiling

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Background: Epilepsy, hypotonia, and developmental delay are common signs and symptoms of neurogenetic disorders with or without recognizable metabolic abnormalities. Neuroimaging and "candidate" metabolic studies often fail to identify a specific etiology. Next generation sequencing (NGS) is now recognized as a robust and reliable method for molecular diagnosis. Objective: To identify the molecular causes in patients presenting with various neurogenetic/neurometabolic phenotypes. **Methods:** Neuroimaging, and metabolic studies (MS/MS based acylcarnitines, very long chain fatty acids, urine organic acids, quantitative plasma amino acids, creatine and transferrin glycosylation profiles) were followed by molecular analysis of gDNA extracted from dry blood spots (DBS) using an Ion AmpliSeq based "753 gene" panel. **Results:** A pathogenic or likely pathogenic mutation was identified in 62% (77/125) of these individuals who had normal metabolic profiling. Six novel variants (*FOLR1*, *SUOX*, *SPAST*, *ALDH5A1*, *MUSK*, *SPTAN1*) were found. In particular, the *MUSK* mutation was found in a newborn with vocal cord paralysis, a very rare and treatable presentation of congenital myasthenia syndrome. **Conclusion:** Mutation analysis using NGS based neurogenetic panel is superior to metabolic screening of common metabolic disorders.

891 - IEM unit in a Pediatric Hospital of CABA, 2015-2016 Experience

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IEM are a heterogeneous group of genetically determined, underdiagnosed pathologies. They often simulate common medical conditions and are not suspected by pediatricians. Their suspicion and early detection allow treatment, medical follow-up and the pertinent genetic advice. The IEM Office was created to cover needs in the context of a High Complexity Pediatric Hospital (HNRG). **Objective:** To describe the experience since the creation of the IEM Office at the Medical Genetics Section of the HNRG. **Material and methods**: Retrospective- descriptive study of the activities performed in the first year: number of consultations, practices and diagnoses, medical training and teaching. **Results:** The office has a professional trained in IEM. 2051 outpatient and inpatient visits were performed. E-mail and cell phone were enabled, evacuating consultations at work and nonworking hours. The office of remote communication and a network were created. Samples for specific diagnostic studies were derived to other centers without cost for the patient. The following diseases were diagnosed among others: 9 mitochondrial diseases, 10 alterations of vitamin metabolism, 2 adrenoleukodystrophies, 1 gangliosidosis, 3 disorders per contiguous gene, 3 deficiencies of coenzyme Q10, 1 creatine deficiency, 1 Pelizaeuz Merchbacher disease, 3 bile acid synthesis disorders 2 sphingolipidoses, 2 glycogenosis and 1 xanthomatosis. Patients already diagnosed were monitored. Teaching activities included: IEM course of the University specialist career, interdisciplinary athenaeum and seminars, fellowship physicians training and lectures. **Conclusions:** We emphasize the need to raise awareness about IEM in order to increase their suspicion in acute or chronic conditions, to optimize access and correct use of diagnostic resources, and to perform adequate outpatient or inpatient follow-up. We warn about the need of increase human, technical and equipment resources to consolidate the office as an initial step of a reference center of IEM within our Hospital.

892 - Screening *ALPL* Gene Differences by Next Generation Sequence Techonology in Patients Having Low ALP Levels

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Introduction: Hypophosphatasia(HPP) is a rare genetic disorder caused by mutations in the *ALPL* gene encoding the alkaline phosphatase. Clinical manifestations range from extreme life-threatening lethal forms with severely impaired bone mineralization, muscular hypotonia, respiratory insufficiency and seizures to mild symptoms. **Methods:** Patients who admitted to our hospital at any complaint from Jan 2014 and Apr 2016. 30000 patients were evaluated with biochemically and 1000 patients had low levels of ALP with repetitive times. 34 patients who had severe musculoskeletal pain, recurrent fractures and teeth anomalies. Four patient had low levels of ALP levels and had heterozygote mutations in the *ALPL* gene. One of them was a 1-year-old girl with dysplastic hips, nephrocalcinosis, hypercalciuria, and irregular teeth. Her biochemical analysis with substrates were consistent with HPP. Her mutation analysis showed p.Ala116Thr c.346G>A heterozygote mutation in the *ALPL* gene. The other one was 2-year-old boy who had delayed closure in cranial sutures and irregularity in teeth. He had p.Gly82Arg (c.244G>A) heterozygote mutation in ALPL gene. The fathers of children also had low levels of ALP and had some teeth anomalies. Third patient was a 26 year old woman with severe musculoskeletal pain and had also heterozygote mutation with p.His482Tyr (c.1444C>T) changes. Fourth patient was a 46-year-old woman who had severe musculoskeletal pain, tooth loss before thirty and p.Asn493Ser (c.1478A>G) heterozygote mutation was found in the *ALPL* gene. **Results:** HPP is a rare and heterogeneous inherited disorder of bone and mineral metabolism. Transmission can be autosomal recessive or autosomal dominant manner. In these patients, it was thought that children had autosomal dominant form as their father had low levels of ALP. The fourth patient had odongtohypophosphatasia form of the disease. In case of bone, muscle, teeth symptoms and some neurologic problems with low ALP levels, hypophospatasia should be thought. Since the clinical expression is highly variable depending on the mutations, mutation analysis should be studied when HPP is suspected.

893 - The Added Burden of Common Childhood Presentations in Children With Inborn Errors of Metabolism: A Length of Stay Comparison

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Children with inborn errors of metabolism (IEM) can be at greater risk of acute metabolic decompensation as a consequence of common childhood presentations to hospital. The aim of this study was to determine if children with IEMs, admitted for common childhood conditions, had an increased length of stay (LOS) compared to children without IEMs. A retrospective analysis of acute care admissions for children

with an underlying IEM to the Children's Hospital at Westmead was undertaken from 2004 to 2013. This identified 366 admissions for 141 children for common childhood presentations identified according to Diagnosis Related Groups (DRG) coding. This represented 20% of acute care admissions for the IEM cohort. The LOS between the IEM cohort and the state average LOS for each DRG was compared using the Wilcoxon signed rank test. Children with IEMs, having an admission coded by DRG as representing respiratory symptomatology/infections, whooping cough and acute bronchiolitis, viral illness, febrile convulsions and seizures had an increased LOS between 0.2 -1.5 days (P value < .05) greater than the state pediatric reference LOS. These accounted for 55% (202) of total admissions. In contrast, children admitted for bronchitis & asthma, fever of unknown origin, kidney & urinary tract infections, tonsillectomy, adenoidectomy or myringotomy, and esophagitis & gastroenteritis did not have a LOS statistically different to the state based pediatric population for admissions coded with the same DRG. These data show that children with an IEM have an increased LOS for particular childhood presentations. Further studies are required to determine if better metabolic control can improve LOS in this cohort.

894- Perinatal Intracranial Hemorrhage in Ehler Danlos Type 6, Report of 2 Cases

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Objective: To describe 2 cases of genetically confirmed Ehler Danlos syndrome type 6 presenting with perinatal intracranial hemorrhage. **Method**: case review and literature review. **Results**: Two unrelated cases with confirmed homozygous pathogenic variant in the *Plod1* gene in whom the reason for referral was assessment of global developmental delay, attributed to a history of perinatal intracranial hemorrhage. Case 1 was referred at age 4 years with marked motor delay and a history of vp shunted post hemorrhagic hydrocephalus; her developmental delay was presumed to be purely related to her neonatal grade iv intraventricular hemorrhage. there was a family history of a male sibling death at age 9 months. Physical exam was remarkable for profound axial hypotonia, head lag, scoliosis; pectus excavatum and marked joint hyperlaxity. Ehler danlos syndrome was suspected and genetic testing for plod1 gene confirmed a homozygous pathogenic variant c.955C>T (p.Arg319). Case 2 was referred at 19 months of age with global developmental delay attributed to fetal intraventricular hemorrhage. Parents are cousins and the family history was negative. His physical exam was remarkable for macrocephaly, bilateral undescended testes, marked head lag with axial hypotonia and marked wrist, thumb and ankle hyperlaxity. Ehler danlos type 6 was suspected and testing for plod1 gene confirmed a homozygous pathogenic variant c.955C>T (p.Arg319). **Conclusion:** Perinatal intracranial hemorrhage can be a very early manifestation of Ehler danlos type 6. It is important to recognize that in order to avoid overlooking the etiological diagnosis of Ehler danlos type 6 syndrome which will have significant implications on the patient as far as prognostication and the need for future follow up of known complications of Ehler danlos type 6. In addition, there are implications on family counseling and risk of recurrence in future pregnancies. the author could not find any literature report of this early manifestation of Ehler danlos type 6.

895 - Hematological findings in inborn errors of metabolism

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Introduction: Biochemical diagnosis of inborn errors of metabolism (IEM) is performed through complex laboratory analyses in highly specialized centers. Peripheral blood film observation (PBF) is a routine laboratory practice in children and can provide valuable, quick and inexpensive information to guide the diagnosis of IEM. **Aim:** To emphasize the usefulness of the cytomorphological examination in PBF in patients with suspicion of IEM, in relation to the clinical presentation of the cases diagnosed in our Center. **Method**: Review of clinical histories and evaluation of Methanol-Giemsa stained PBF for the observation of erythrocyte and leukocyte cytomorphology by an optic microscope in patients with suspected IEM. **Results**: 1. Female 28 days old who presents with food rejection, failure to thrive and hypotonia. PBF: severe anemia. Diagnosis: methylmalonic acidemia; 2. Female 6 months old with decreased βgalactosidase activity. Possible diagnoses: Morquio B disease, Galactosialidosis or Gangliosidosis. PBF: vacuolated lymphocytes and neutrophils with thick granulations, which rules out diagnosis of MPS IVB. Diagnosis: Galactosialidosis; 3. Female12 year old with short stature, umbilical hernia and severe dysostosis. PBF: Neutrophils with irregularly distributed azurophilic granules. Diagnosis: Mucopolysaccharidosis type IVA; 4. Female 9 month old, with cherry red spot, hypotonia and hepatomegaly. PBF: vacuolated lymphocytes and neutrophils with thick granulations. Diagnosis: GM1 Gangliosidosis; 5. Female. PBF: vacuolated lymphocytes and atypical neutrophils and eosinophils. Diagnosis: Multiple Sulfatase Deficiency. **Conclusion**: Cytomorphological findings in PBF observed in patients with pathologies such as MMA, Galactosialidosis, MPS IVA, GM1 gangliosidosis and multiple sulfatase deficiency, demonstrate the usefulness of PBF evaluation to complement biochemical studies in patients with suspected or diagnosed IEM. The analysis is inexpensive, quick and available at any primary health care center.

896 - The Case That Received Enzyme Replacement Treatment With Congenital Hypophosphatasia

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Hypophosphatasia is a congenital metabolic disease depending on lack of serum alkaline phosphatase (ALP) activity regarding mutations in the gene encoding isoenzyme not specific for tissue. Mineralization defect in bones and teeth, affected respiratory functions, seizure, hypotony, bone pain, and nephrocalcinosis can be observed. Along with low determined ALP, not low calcium and phosphorus values and increased plasma Pridoxal 5' Phosphate (PLP) levels are identifiers. Enzyme replacement treatment entered into application. This study aimed to emphasize that patient diagnosed in neonatal period should take part in the definitive diagnosis and share current treatment experience. There was postnatal respiratory distress, soft calvarium, angles in both forearms, seizure in follow-up. In the workups; serum ALP low 10 U/L (N:70 U/L), serum calcium high 13,5 mg/dl (N:9-10.9), serum phosphor normal 4.9 mg/dL (N:3.4-5.9), in the direct graphy, bone structure radiolucent, fracture and callus formations were found. Plasma PLP high 762 μg/L (N:5-50) and urine phosphoethanolamine (PEA) high 1015 μmol/L (N:15-341) and in the *ALP* gene analysis, p.R184W/p.G288A combined heterozygote mutation was determined. On the 60th day of life, the patient was diagnosed with hypophosphatasia and initiated to have 3 mg/kg/dose asphotase alpha subcutaneous three times a week. Patient had received five dose enzyme replacement treatment and died at home following aspiration. While the disease has different symptoms and distinct clinical forms inherited by autosomal dominant and autosomal recessive ways; congenital hypophosphatasia should take place in the definitive diagnosis in the presence of multiple system findings such as skeleton deformity, spontaneous fractures and along with hypotony, respiratory distress, and seizure. In addition, p.G288A change determined in the patient is a new identified alteration.

897 - Inborn Errors of Metabolism: The Impact in Neonatal and Pediatric Intensive Care

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Study's Objective: To evaluate the significance of inborn errors of metabolism (IEM) amongst intensive care admissions in our Centre, concerning prevalence, diagnosis and expended resources. **Methods:** Retrospective analysis of the clinical records of patients with a confirmed IEM diagnosis admitted to the intensive care units (ICUs) of a third-level center (2 neonatal and 1 pediatric), from January 2000 to December 2016. **Results:** Over the past 17 years, 61 patients with IEM represented 0.6% of the total number of admissions (92/15984), with 22 patients having more than 1 admittance. The median length of IEM patients' ICUs stay was 6 days. Most admissions (61/92) occurred in the 1st year of life, with more than 1/3 in the neonatal period. The diagnosis was already known in 45.7% admissions. For 34.4% of the patients, the diagnosis was achieved during the stay. The 61 IEM patients presented: amino acid and peptide metabolism (22), energy metabolism (12), carbohydrate metabolism (10), lysosomal (6), fatty acid metabolism (3), glycosylation (3), peroxisomal (3) and other disorders (2). Concerning the neonatal period (37 stays, 36 patients), the most common reasons for admission were: neurologic abnormalities (12) and respiratory distress (8). Six patients had an IEM diagnosis prior to admission: 2 had a prenatal diagnosis and 4 had a positive newborn screening. Concerning the total 92 stays, there were 32 admissions for respiratory and/or cardiac insufficiency, 15 for neurologic abnormalities (5 in coma) and 5 for liver failure. There were 19 admissions to post-surgery surveillance and 3 to begin a therapeutic protocol for the IEM. Invasive mechanical ventilation was needed in 66.3% of the episodes, inotropes in 25%, red blood cells transfusion in 23.9% and continuous renal replacement therapy in 6.5%. Mortality rate was 34.4%, represented by patients with: mitochondrial diseases (6), Zellweger syndrome (2), congenital disorders of glycosylation (2), ornithine transcarbamylase deficiency (2), Niemann-Pick C disease (2), metilmalonic aciduria (2) and other disorders (5). **Conclusion:** Although being rare in ICUs, recognizing IEM is important, once some specific therapeutics are available, which can be lifesaving. Definitive diagnosis is essential, not only for a targeted treatment but also, to the genetic counseling of affected families. The high mortality can reflect the complexity of many IEM, underscoring the importance of palliative care when the prognosis is poor.

898 - A Colombian Reference Center Model in Inborn Errors of Metabolism

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Colombia is a transcontinental country largely situated in the Northwest of South America, with an estimated 49 million people in 2017, Colombia is the third-most populous country in Latin America, after Brazil and Mexico. In 2016, the National Administrative Department of Statistics (DANE) reported that 28.0% of the population were living below the poverty line, of which 8.5% in "extreme poverty.

Since 1993 a new health system was introduced, this new system has widened population coverage by the social and health security system from 21% in 1993 to 96% in 2012, however just since 2010 rare diseases are recognized as being of special interest and rules are adopted to guarantee social protection by the Colombian State to the population suffering from orphan diseases and their caregivers. Right now, the discussion is around the newborn screening and reference centers. The Hospital Universitario San Ignacio, which was recently ranked among Latin America's twenty best hospitals by the influential business journal América Economía, is a 4th level university hospital in Bogotá, Colombia. It is located on the campus of the Pontificia Universidad Javeriana and is home to this school's faculties of Medicine, Nursing, and Dentistry. As part of the hospital, is the Inborn Errors of Metabolism Clinic (CEIM), which is a reference center for rare diseases since 2012, the group of the CEIM was organized around two institutes that are currently integrated into the San Ignacio Hospital in terms of services: The Institute of Human Genetics and Inborn Errors of Metabolism. Each one has more than thirty years of experience in the study of these diseases. They were joined by specialists from different disciplines who have managed patients with metabolic diseases and who are convinced that a specialized clinic is the best way to provide the comprehensive service that these patients require. Each year the clinic receives patients from different parts of the country, and even nearby countries; as a university hospital receives undergraduate students, medical doctors with previous training in pediatrics, medical genetics or child neurology and dietitians. We want to describe all the activities developed in inborn errors of metabolism by a Colombian reference center.

899 - ERNDIM Diagnostic Proficiency Testing is an Important Tool in Determining Quality of Laboratory Diagnosis in a Wide Range of Inborn Errors of Metabolism

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ERNDIM (www.erndim.org) aims to improve the quality of diagnosis and monitoring in patients with inborn errors of metabolism (IEM) through quality assurance programs and educational activities. Diagnostic proficiency testing schemes focus on the ability of laboratories to identify and interpret abnormalities in natural urine samples reflecting a wide range of IEMs. During the past decade 264 samples from 83 different conditions, were distributed to up to 105 laboratories mainly from Europe but also worldwide, by five centers in the Czech Republic, France, Netherlands, Switzerland, and United Kingdom and latterly from a central provider (CSCQ). Six samples a year, one common to all centers, were distributed together with clinical information. Laboratories choose and perform the tests (limited amount of urine) needed to reach a diagnosis using analysis of one or more of amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines and pyrimidines. Performance is evaluated according to scores for analysis and interpretation and lack of critical error, in line with harmonization with other genetic disciplines. Diagnostic proficiency, based on analytical findings and interpretation, ranged widely from below 50% for extremely challenging samples to 100% for more straightforward ones. Taken together, diagnostic proficiency (average % of total points possible for all participating laboratories within all schemes for all samples) was: amino acid disorders, n disorders/samples = 27/71, range 26-100, mean 78%; organic acid disorders, n = 33/89, range 14-100, mean 87%; mucopolysaccharide disorders, n = 5/34, range 67-93, mean 81%; oligosaccharide disorders, n = 6/19, range 10-99, mean 65%; purine/pyrimidine disorders, n = 8/22, range 12-93, mean 69%; miscellaneous disorders, n = 6/7, range 17-91, mean 63%; no IEM n = 21, range 65-95, mean 85%. When the sample was re-distributed in a subsequent survey from the same center, performance improved in 21 cases (average 15%) with no improvement seen in 13. Although difficult to interpret this suggests improvement in performance. In conclusion, ERNDIM diagnostic proficiency testing is a valuable activity which can: inform accreditation; help to assess individual laboratory performance; and identify methodological and technical challenges contributing to improved diagnostic approaches. Further, clinicians must be aware that laboratory testing for IEM is fraught with difficulties and results need to be viewed with caution.

900 - Continuous Age - and Sex-Corrected Reference Ranges of Plasma Alkaline Phosphatase (ALP)

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Background: Low plasma levels of ALP may be a marker of the disorder hypophosphatasia. Since both age and sex may influences the plasma levels appropriate reference ranges are necessary for correct screening of cases. Custom reference limits do not fulfill this requirement, since mainly elevated levels have been of clinical interest. **Objectives:** To develop continuous

reference age- and sex-adjusted ranges by mathematical modeling after appropriate Box-Cox transformations, that correct for variable skewness. **Material and Methods:** First sample results from 52 073 female and 49 786 male reference individuals without known hypophosphatasia were extracted after outlier exclusion (2273 females and 1756 males) from the laboratory productions in Oslo University Hospital. A modified quantile regression method was used to establish mathematical models for expectation values and dispersions as a function of age and sex, by which each individual value could be converted to a Z-score. By retransformation percentile curves were obtained. **Results:** The two sexes exhibit homologous functional form with respect to age, but with pubertal decline that started approximately 4-5 years earlier in girls. In both genders, common lower reference limits (LRL) increase from 54-60 U/L to approximately 90 U/L the first month of life and slowly approaches 100 U/L at 5 years age. Here girls exhibit a transient increase before falling from about 9-10 years of age. The corresponding decline in boys starts at 14 years. After 22 years LRL are little affected by age and sex and stabilizes at approx. 30-35 U/L. Our data demonstrate that existing and widely used discrete reference limits give rise to unacceptable rates of both false positives and false negatives in different age ranges. **Conclusion:** The introduction of Z-scores for patient values better adapted to the normal biological variation with age and sex. These also may be used to establish age- and sex- adjusted decision limits that identify cases with hypophosphatasia with optimal test accuracy. The diagnosis can be further verified by measurement of urinary phosphoethanolamine.

901 - Clinical Profile of Patients With Inborn Errors of Metabolism Treated at the Pediatric Clinic of Metalobic Diseases of the Federal University of Rio de Janeiro

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Introduction: The Inborn errors of metabolism (IEM) are individual rare, but collectively numerous. Mostly are transmitted in an autosomal recessive manner. The changes occur at the molecular level, causing absence or deficiency in the activity of a specific enzyme, leading to the block of several metabolic pathways. They can be divided into the following three diagnostic groups: disorder that give rise to intoxication (group 1), disorders involving energy metabolism (group 2) and disorders involving complex molecules (group 3). Several metabolic disorders may have their natural history substantially altered by the introduction of a treatment in its initial phase. Many IEMs can be management with diet and co factors replacement, and the metabolic physician is fundamental for a good evolution of the patient. **Purpose:** To analyze the clinical profile of patients with inborn errors of metabolism treated at the Pediatric Clinic of Metabolic Diseases of the Federal University of Rio de Janeiro. **Methodology:** A retrospective longitudinal study with data collected from patients diagnosed with IEMs between January 2016 and April 2017 attended on outpatient clinic of metabolic diseases of the Federal University of Rio de Janeiro. The sample consisted of 54 medical records of individuals aged between one and 19 years. Data were analyzed for the following aspects: age, gender, gestational age, onset of symptoms, age of diagnosis, if there was an initial diagnosis of neonatal sepsis, clinical diagnosis, molecular diagnosis, need for support treatment and newborn bloodspot screening test. **Results:** Of 54 patients analyzed, 59% were female, with a mean age of 7.4 years. The majority of the sample was at term, and in 37% the onset of symptoms was between 1 and 12 months of age. Regarding the etiology, the majority of the patients had diseases of group 1 (46%). Only 9% of the sample had altered newborn screening test and 22% had a molecular diagnosis performed after the suspicion. **Conclusion:** IEMs represent a major challenge for clinicians and pediatricians worldwide because of the large number of genetic defects in various metabolic pathways and variable clinical manifestations. The prognosis and development of the affected child depends on a rapid and effective treatment, particularly in the diseases treatable by diet. However, in our country, difficult and limited access to complementary tests makes it impossible to make an early diagnosis and institute appropriate treatment.

902 - Diagnostic Investigation of Inborn Errors of Metabolism in Patients With Intellectual Disabilities in the State of Bahia

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Objective: To investigate the Inborn Errors of Metabolism (IEM) in individuals with intellectual disability (ID) with no

defined etiology, from Monte Santo and other cities in Bahia who are accompanied in referral services. **Methods:** This is a convenience sample, whose selection of patients was performed in two different research fields: one in partnership with the Health Department of the municipality of Monte Santo and another with the Medical Genetics Service and the Psychiatry Outpatient Clinic of the University Hospital Complex Professor Edgar Santos (COM-HUPES / UFBA). The design for each research field was differentiated, but the inclusion and exclusion criteria were the same. In Monte Santo, individuals were selected in the context of the Family Health Program by previously trained Community Health Agents, from the active search of suspected cases and through a simplified questionnaire called "A-GEN SHEET". While in the COM-HUPES / UFBA the screening was done through the revision of the medical records and selection of new cases during care. All suspected ID cases were confirmed by neuropsychological assessments and evaluated by medical geneticists to rule out causes other than genetic. Clinical and epidemiological data were collected from the selected patients, as well as biological samples for the following initial investigations: dosage of phenylalanine, glycosaminoglycans (quantitative and qualitative) and chitotriosidase. **Results:** To date 85 patients were selected from Monte Santo, 53.9% were male, with a mean age of 15.6 years old, 26% inbreeding and 56% had a family history. In this group, two female patients were diagnosed phenylketonuria (PKU) and that there was a family history of PKU. In the reference outpatient clinics, 67 patients were selected, with a mean age of 11.6 years old, 80% male, 10% referred inbreeding and family history. To date, no patients in this sample group presented alterations after the tests performed. **Conclusion:** The results are preliminary, the selected tests are screening, but they have already been shown to be important for the investigation of IEM in groups with a high consanguinity rate and positive family history.

903 - Laboratory Protocol for Genetic Diseases in Individuals With Autism Spectrum Disorder Associated With Intellectual Disability

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Objective: To investigate genetic cause in individuals with autism spectrum disorder (ASD) associated with intellectual disability (ID) with no defined etiology, using a laboratory protocol simple and accessible to the scope of the public services. **Methods:** A systematic review of the literature was carried out to identify the main genetic diseases associated with TEA, and then an optimized diagnostic investigation protocol was developed accessible to public services. The protocol is being tested in groups of patients with ASD from the Hospital Universitário Professor Edgar Santos (COM-HUPES/UFBA) and the Interdisciplinary Laboratory of Autism Research (LABIRINTO) of the Bahian School of Medicine. Clinical and epidemiological data are collected from selected patients, as well as biological samples for biochemical and molecular biology research. Patients are being screened for ASD by the neuropsychological tests ABC (Autism Behavior Checklist) and M-CHAT (Checklist for Autism in Toddlers-modified), and the diagnosis is being made by specialists through anamnesis and psychiatric examination. Only cases of ASD level 1 or 2, according to DSM-5, and with other neurological comorbidities are being selected. **Results:** The laboratory protocol established after the systematic review included the following laboratory tests: karyotype; Molecular X-fragile research; Dosage of phenylalanine and quantitative dosage of amino acids, glycosaminoglycans (quantitative and qualitative), chitotriosidase and organic acids. To date, 29 patients were selected, of which 82.7% were males, mean age was 9.5 years, there was no report of consanguinity. To date, no patient has undergone changes to the tests performed, and dosages of amino acids and organic acids have not yet been made. **Conclusion:** The sample number is still small, but it is expected that with the establishment of a rational protocol of investigation it will be possible to guide public health teams in the etiological investigation of TEA and thus be able to offer genetic counseling and treatment/support strategies more appropriate for patients and relatives.

904 - Inherited Duplication Disrupting the Gene *MYH10*: A Case Characterized by Microcephaly, Severe Developmental Delay, and Dysmorphic Features

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We describe the clinical and genetic characteristics of a child with a duplication disrupting the gene *MYH10*. The patient, third child, born at term, female of healthy nonconsanguineous parents. The pregnancy was characterized by mother smoking and anhydramnios. The parents coming for low-income, low-

education, with the father working as a waiter. Birth weight was 2900 g (10th centile), length 48 cm (10th centile), and head circumference 32 cm (<3 rd centile). At the age of 11 months, she was hospitalized to investigate microcephaly, dysmorphic features (fine hair, tip narrow nose with flattened nasal root and wings, thin upper lip, and edentulous), severe developmental delay (bubbling, grasping objects, sitting, rolling, and crawling still not acquired) and axial hypertonia. Metabolic screening, EEG, audiometric examination, echocardiogram and abdominal ultrasound were normal. Brain MRI showed brain atrophy, mainly of the frontal lobes. In consideration of developmental delay and facial dysmorphism, CGH-Array was performed. This showed a paternal 73 kb duplication between nucleotides 8,386,920 and 8,460,142, in the p13.1 band of chromosome 17, involving the gene *MYH10*; which encodes the non-muscle myosin heavy chain IIB critical for heart and brain development. Loss of *MYH10* function in mice results in embryonic lethality, hydrocephalus and neuronal migration defects but the cognitive and behavioral phenotype of heterozygous mice has not been reported yet. Heterozygous de novo mutations in association with human neurological disease have been previously described in only 3 cases, characterized by loss of function of the protein: a nonsense mutation, a missense mutation (c.838C>T, p.Arg280Cys) and a nonsense truncating variant p.E908X (c.2722G > T), all having microcephaly and severe developmental delay with marked cerebral and cerebellar atrophy complicated by ex-vacuo hydrocephalus or basal ganglia and thalami involvement. Neither human *MYH10* gain of function nor duplications, similar to our case, have yet been described in literature or reported in the Database of Genomic Variation and Phenotype in Humans using Ensemble Resources. Nonetheless, considering the function of the gene and the pathogenic role of its mutation, we can hypothesize a possible intrafamilial expression variability seen the patient's clinical phenotype and its genotype. More cases need to be reported to establish with certainty this pathogenic correlation.

905 - Use of Proton Nuclear Magnetic Resonance (H1-NMR) for Diagnosis of Inborn errors of Metabolism

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Introduction: The Inborn Errors of Metabolism (IEM) of small molecules are traditionally diagnosed using specific analytical tools according to the molecules to be analyzed, thus GC-MS is used for Organic Acidurias and HPLC for Aminoacidopathies. H1-NMR has been proposed as an alternative that allows the simultaneous evaluation of metabolites characteristic of different disorders (Aminoacidopathies, Organic Acidemias, carbohydrates disorders, etc.) in less time and using small quantity of sample. **Objective:** This work aims to evaluate the diagnostic potential of H1-NMR in IEM through the analysis of urine samples obtained from patients with Organic Acidemias and Aminoacidopathies. **Methodology:** 95 urine samples [classified as either IEM patients (n = 19) or healthy individuals (N = 78) by GC/MS] were analyzed by H1-NMR. Briefly, 580 µL urine was mixed with 180 µL phosphate buffer solution (1.5 M, pH 7.0) and TSP-D_4 0.1% as a frequency standard. H1-NMR spectra were acquired on either a Bruker 300 MHz or a Bruker 400 MHz spectrometer at 297°K using the 1-D NOESY pulse sequence coupled with water pre-saturation. Each spectrum was reduced by averaging (binning) the data over every spectral region of 0.04 ppm. The spectral region 4.3-5.6 ppm, containing the residual HOD and urea peaks, was removed prior data analysis. The data obtained was statistical evaluated for Principal Components Analysis (PCA). **Results:** The H1-NMR profile of the analyzed samples consists of approximately 271, signals with a displacement between 0.0 and 10 ppm. Initial PCA analysis between control and affected samples did not show statistical differences. However, qualitative analysis of the profiles revealed that pathological samples showed a higher abundance of the signals in the aliphatic zone (~0.8-4 ppm) when was compared with control profiles. **Conclusion**: Although H1-NMR profiles were not highly discriminatory, the combined study of H1-NMR and GC-MS of urine samples obtained from patients being studied for inborn errors of metabolism may provide a new approach that allows better identification and characterization of this entities.

906 - Effect of pH on an Oxalate Oxidase Method for Plasma Oxalate

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Aim: The aim was to determine the effect of the diluent on the measured concentration of oxalate in plasma. Background: Published methods for the measurement of oxalate in plasma recommend the dilution of plasma in acid, prior to an ultrafiltration step, to limit the spontaneous conversion of L-ascorbic acid to oxalate. Method validation revealed unacceptable recovery of spiked oxalate from plasma. It was hypothesized that the diluent and therefore pH was critical for the generation of accurate and unbiased results. **Methods:** Plasma samples were diluted 1:1 with 0.24 M HCl and filtered through a 30 kDa filter. Oxalate in the resulting ultra-filtrate was measured using the Trinity Biotech oxalate kit, which is based on an oxalate oxidase reaction. To assess accuracy, plasma samples were spiked with doubling concentrations of an oxalate certified reference material (CRM). The samples were prepared using one of 3 diluents of increasing pH (0.24 M HCl, 0.01 M HCl and H20). The pH values of the resulting extracts were 2.0, 5.5 and 7.0 respectively. Recovery was calculated. Bias

was investigated by measuring oxalate CRM at 3 concentrations (5.7, 57 and 142 μmol/L). The pH of the CRM was modified by addition of 2 μL concentrated HCl, 0.01 M NaOH or 1 M NaOH to 400 μL. Linearity, precision and stability were also assessed using various diluents. **Summary:** Bias and recovery were optimal using H20 (pH 7) as a diluent, and appeared to be inversely related to the pH of the diluent. It is suggested that this is due to protein precipitation prior to filtration resulting in loss of oxalate and also due to suboptimal oxalate oxidase activity at acidic pH.

907 - Inborn Errors of Metabolism Presenting With Non-Immune Hydrops Fetalis: Novel Molecular Findings and Useful Clues for the Diagnosis Approach

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Some inborn errors of metabolism (IEM) manifest prenatally with non-immune hydrops fetalis (NIHF). The frequency of IEM in hydropic fetus is usually underestimated due to inadequate investigation approach. The aim of this presentation is to describe eight Brazilian newborns with NIHF caused by different IEM, emphasizing some findings that can contribute to the diagnosis during the NIHF evaluation, and showing novel molecular variants. The diagnosis was based on biochemical and/or molecular analysis, and the metabolic investigation started after the exclusion of the main etiologies of NIHF. We identified six patients with lysosomal storage disorders (LSD)—gangliosidosis GM1 [3], infantile sialic acid storage disease (ISSD) [1], galactosialidosis [1], MPS VII [1]; and two cases of congenital glycosylation defect (CDG)—PMM2-CDG. The familial history analysis showed two families with previous child with hydrops of "unknown" etiology due to incomplete investigation. The placenta evaluation was performed in four cases of LSD (gangliosidosis GM1 [2], ISSD [1] and galactosialidosis [1]) and it was observed vacuolization of trophoblast and/or stromal cells leading to hypothesis of LSD in all of them. The babygram of seven infants showed findings suggestive of LSD (osteopenia, coarse trabeculation, metaphyseal fraying and periosteal cloaking) in the infant with ISSD, and subtler signs in the baby with galactosialidosis. The two infants with PMM2-CDG presenting with delayed pubis ossification. Molecular tests detected 10 confirmed or likely pathogenic variants, including four previously not described. A novel variant in *PMM2* (c.97C>T; p.Gly33Ter) was confirmed in two not related families, suggesting a common ancestral and a possible founder effect. A uncommon variant in *PMM2* (c.193G>T; p.Asp65Tyr) was identified in one family, reinforcing the hypothesis of Iberian origin of this variant. Finally, the variant c.1498A>G (p.Thr500Ala) in *GLB1*, classically associated with Morquio B, was related to gangliosidosis GM1 for the first time. The results highlight the importance to include a systematic approach to investigate NIHF, including radiological and placental examination, because they may provide some clues related to metabolic etiology. The study expands the molecular data about the diseases and give new information about genotype-phenotype correlation related to *GLB1*.

908 - Episodic Vomiting as a Manifestation of Glycerol Kinase Deficiency

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Isolated glycerol kinase deficiency (GKD) is a very rare X-linked disorder of glycerol metabolism characterized biochemically by elevated plasma and urine glycerol levels, and clinically by variable neurometabolic manifestations, depending on the age of onset, infantile, juvenile, and adult. As a result, complex GKD usually presents as an infantile syndrome commonly including vomiting, failure to thrive and acidosis, which may progress to lethargy, seizures, psychomotor retardation and coma. Isolated GKD may also present as a childhood metabolic crisis with developmental decompensation or be discovered as a relatively benign asymptomatic form in adulthood. Both types of isolated GKD are thought to result from different genetic mutations affecting the activity or regulation of GK. Symptoms depend on the size of deletion. **Objective:** Describing a Clinical Case Study related to the Deficit of Glycerol Kinase in infancy. Materials and **Method:** Observational, Digitalized clinical history. **Results:** An 8-year-old patient who first consulted in our hospital at the age of 6 years. The patient required 2 hospitalizations due to secondary dehydration, cyclic vomiting associated with severe ketoacidosis and hyponatremia, and the reversing of the condition with the intravenous replacement of electrolytes and expansions with physiological solution. In the last year, we took account of 4 admissions in emergency. Basic biometrics: 50th percentile weight, size 25-50, PC +1 SD. Laboratory: Hyponatremia (Na 124mEq/L), metabolic acidosis (pH 7.06; Bic. 3.6), urine ketones (++),

triglycerides (418 mg/dL). A metabolic disease was suspected and thus forwarded to the laboratory of congenital errors, resulting in acylcarnitines and amino acids within normal limits and urinary organic acids with very marked glycerol. Studies rule out adrenal hyperplasia and Duchenne disease. The mother reported that her maternal uncles and 2 of her siblings had hypertriglyceridemia without results or improvement of their respective condition with the pharmacological and dietary treatments they received. **Conclusion:** GKD is a rare form of congenital error. One shall not stop thinking about it in the presence of Hyperglycerolemia, since this deficiency causes episodes of vomiting, lethargy and hypotonia. The diagnosis is based on the detection of high concentrations of glycerol in serum and urine and is confirmed by DNA analysis. The treatment consists of preventive care.

909 - Lactose Intolerance as an Inborn Error of Metabolism

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Lactose intolerance is an enzymatic defect, or the absence of, resulting in inability to digest it. In some perspective should be seen as a public health problem. Children and even adults might develop allergies, neurologic and cognitive symptoms. These could end in absenteeism in school and work. Reposition of the lactase enzyme by public health system should be considered in poor populations. This paper will review some articles about lactose intolerance, public health and inborn error of metabolism.

910 - Autosomal Recessive Infantile Osteopetrosis: Three Cases With Three Novel Mutations

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Objectives: Osteopetrosis is a rare inherited metabolic bone disorder characterized by extensive sclerosis of skeleton, visual and hearing impairment, hepatosplenomegaly and pancytopenia with marked anemia. It has two major clinical forms: the autosomal dominant adult (benign) form which is associated with milder symptoms often appearing in later childhood or adulthood, whereas the autosomal recessive infantile (malignant) form has a more severe presentation in very early childhood and if untreated it is typically fatal during infancy. **Methods:** The clinical and molecular data of three osteopetrosis patients evaluated retrospectively. **Results:** All three infants were admitted with low grade of fever and abdominal mass in the first three months of life. They were born from consanguineous, healthy Turkish parents. On physical examination, the patients were pale, severely malnourished, and had growth retardation and hepatosplenomegaly. Hematological tests revealed anemia and thrombocytopenia and the peripheral blood smear test showed leukoerythroblastosis. Radiographic investigation was compatible with diffuse sclerosis of bones. The striking feature of bone marrow aspiration was hypocellularity. The clinical, hematological and radiological findings pointed out the diagnosis of osteopetrosis. Three novel mutations were detected with genetic analysis. IVS5-1G>C (c.485-1G>C) and p.Q671*(c.2011C>T) mutations were found in *CLCN7* gene and IVS12-1G>A (c.1464-1G>A) mutation was identified in *TCIRG1* gene. **Conclusion:** Malignant infantile osteopetrosis is caused by the failure of osteoclast development or function due to the mutations in at least 10 different genes. Nearly the cause of half of the infantile autosomal recessive osteopetrosis are mutations in the *TCIRG1* gene. This is followed by the mutations in the *CLCN7* gene which is responsible for 13% of cases. Different from the literature, *CLCN7* gene mutations are the most frequently detected mutations in our Turkish patient group.

911 - Urinary Amino Acids: Should We Screen Our Requests?

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Objective: In pediatrics a 'metabolic screen' is often carried out on patients with potential inborn errors of metabolism. Many of these investigation panels are initiated by general pediatricians in peripheral hospitals with the samples being sent to tertiary laboratories. One test sometimes included is a urinary amino acid profile. The UK National Metabolic Biochemistry Network, which consists of 24 specialist laboratories, has provided guidance on which amino acid disorders require urine amino acids for diagnosis. A subsequent survey showed varying consensus in terms of urinary amino acid analysis. Our biochemistry laboratory processes every sample it receives from across the south of England and we wanted to look at the number of urinary amino acid tests requested in one year and if these were all clinically indicated in terms of diagnosis and monitoring. This is being carried out as part of a quality improvement project considering both the financial and workload implications of these requests. **Method:** In the first stage of the project we gathered data retrospectively from a 1-year period of all the urinary amino acid profiles analyzed in our lab from external sources and reviewed this to determine the total number of pediatric samples analyzed. Then these were reviewed to obtain the number of normal and abnormal results. This initial phase has been completed. The second stage of the audit will be to review the referral information in the context of the test results, for both the positive and the negative samples. This will be used to determine if the requests were

appropriate and the time and cost savings if inappropriate samples had not been not processed. **Results:** In one year, 1403 urinary amino acid profiles were sent for analysis at our lab. Of these 1202 were pediatric samples and 779 were from sources outside our Trust. A total of 749 were then analyzed to give 475 normal and 274 abnormal profiles. During the second stage of the project we anticipate that some of the abnormal profiles will be re-graded as not significant or non-diagnostic the clinical context. **Conclusion:** The information gathered in the next phase will be important in determining if these tests are being sent appropriately and whether we need to 'screen' requests before processing them so that only those with clinical indications are tested. We analyse a large volume of these samples and few are diagnostically beneficial at the potential expense of finite specialist resources.

912 - 10 Years of Rare Inborn Errors of Metabolism and Rare Metabolic Disorders Research Funding in the European Union

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Objective: The objective is to provide insight on the relevant European public funding that has been allocated to rare inborn errors of metabolism and rare metabolic disorders research at the European Union level during the last decade, from the perspective of long- and short-term research outcomes, and the added value brought to the European patients and society. **Methods:** Collaborative health research projects that have been supported by the European funds were searched from 2007 up to April 2017; projects were considered eligible for inclusion if they addressed a single and/or a group of rare metabolic disorders and rare inborn errors of metabolism. In the European Union, a disease is defined as rare when it affects fewer than 5 people in 10,000. **Results:** Eight collaborative research projects that have been funded by the European Framework Programs were selected for the in-depth analysis. Among these, the European 7th Framework Program (2007-2013) supported 7 projects with a total funding of over 30 million euros, and the Horizon 2020 (2014-04/2017) has so far supported 1 project with nearly 6 million euros. The identified projects target the following disease entities: acute intermittent porphyria, alkaptonuria, alpha-mannosidosis, and lysosomal storage diseases (with the focus on Gaucher disease, Pompe disease, mucopolysaccharidosis VI and multiple sulfatase deficiency). The innovative diagnostic approaches and nature of therapies developed, many with clinical success, were evaluated. Several research projects supported by the European Union clearly constitute milestones in the management of rare inborn errors of metabolism and rare metabolic disorders, thus they are flagged and highlighted as "success story". **Conclusion:** In conclusion, systematic review of selected projects and assessment of their outcomes has emphasized the significant added value of the European Union funding in advancing metabolic medicine research, both in terms of the contribution to improve the clinical management as well as the health and social outcomes of European patients. **Disclosure Statement:** The authors do not have a conflict of interest. The views expressed in this publication are the sole responsibility of the authors and do not necessarily reflect the views of the European Commission. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of the following information.

913 - Thinking of an Inborn Error of Metabolism in Manaus-AM

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Objectives: To highlight the clinical and epidemiological characteristics of patients referred for investigation of innate metabolic error. Ask what makes you think of innate metabolism error? **Methodology**: Survey of the clinical-epidemiological data of patients referred for investigation of innate metabolism error at the genetics outpatient clinic in Manaus. Used archive of the EIM Brazil Network and MPS Brazil Network from 2010 until April 2017 and the local medical records. **Results**: A total of 140 patients were treated for investigation. The age range varied from newborn to adulthood. 77 male and 63 female. The most prevalent symptoms were delayed neuropsychomotor development, hypotonia and seizures. Viewed consanguinity in 34. 5 indigenous investigated. Due to the ease of transport the most requested exams were amino acid dosage and acylcarnitine profile in addition to the enzymatic dosages. 23 presented alterations in the examinations, 14 for diseases of the lysosomal deposit. **Conclusion**: We conclude that the signs and symptoms reported are pertinent to conducting research into an inborn error of metabolism. However, there is a need for greater dissemination of information about inborn errors of metabolism for general practitioners, especially pediatricians for more early research and for more patients.

914 - A Multi-Specialty Autism Clinic

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Clinics devoted to autism spectrum disorders (ASDs) are usually devoted to diagnosis and behavioral interventions, or the application of genetic diagnostic methods to patients with ASDs. We established an autism multi-specialty clinic in 2008 to evaluate, manage, and understand the biomedical aspects of the ASDs. The diagnosis of autism is done by others,

using standard methods. The genetic evaluation of patients with ASDs typically includes chromosome microarray, karyotype, fragile X analysis, and a panel of autism-related genes. Biochemical evaluation can include routine chemistry analysis, plasma amino acids, acylcarnitines, urine organic acids, creatine metabolites and nucleotides. A specific "cause" might be found in 20%-40% of patients. These tests do not address the numerous co-morbidities found in patients with autism, and how addressing them might ameliorate the autism symptoms. Our clinic addresses the common experience of intestinal dysfunction in children with autism—gastroesophageal reflux, abnormal stooling, bowel inflammation, and food intolerance. Dysbiosis is being investigated. Significant food and intestinal issues are present in perhaps a third of our current patients. Disturbed sleep and increased irritability are common. All these aspects are treatable. The clinic core includes a metabolic geneticist, neurologist, gastroenterologist, dietician, and coordinator. An immunology component is being added because of the discovery of autoantibodies to brain components (Cunningham panel), and connections between mitochondrial and immune dysfunction. There is no obvious relationship between genetic and metabolic "causes" of autism, and the presence or absence of intestinal, mitochondrial, or immunologic dysfunction. The clinic is affiliated with research projects devoted to mitochondrial dysfunction, oxidative stress, folic acid metabolism, and cerebral folate deficiency, and immune dysfunction. The clinic meets one full day weekly. Follow-up visits and phone calls are done separately. In a typical recent year there were 1216 outpatient visits. Roughly 5% of the patients are admitted for overnight EEG, often accompanied by brain MRI and lumbar puncture. Patients come from the USA and abroad. The current waiting list is more than a year, emphasizing the desire and need for comprehensive assessment and treatment.

915 - High Frequency of Duplication of 24 bp in Indigenous of Northern Brazil

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Introduction: The Quitotriosidase (QT) is a chitinase secreted by activated macrophages with the capacity to degrade chitin. Function of the QT is linked to defense against pathogens containing chitin and involvement in diseases with macrophage activation. Elevated plasma levels of QT are used as a biochemical marker to monitor the treatment of Gaucher disease (GD). The gene QUIT1 is on chromosome 1q31-1q32, containing 12 exons and encoding a protein found in plasma of 50KDa and 39KDa isoform (stored in the lysosomes). Although plasma levels of QT are related to various diseases, there are some polymorphisms in the gene that cause QT deficiency. A duplication of 24 base pairs (bp) in exon 10 is the most common, generating an alternative splice site in exon 10, resulting in the absence of 87 nucleotides. **Objectives:** To identify the genotype and allelic frequency of duplication of 24 bp in exon 10 in the QT gene in the population of Belém (Pará, Brazil), indigenous populations and in patients with GD. **Material and Methods:** DNA sample was extracted of seven patients with Gaucher Disease, 166 subjects of the Belém population and 182 Amerindians: Kaapor (n: 13), Xikrin (n: 13), Araweté (n: 13), Parakanã (n: 38), Arara (n: 35), Gavião (n: 42), Asurini (n: 28). The presence or absence of duplication of 24 bp was detected by SSCP (Simples Stranded Conformational Polymorphisms), PCR and sequence analysis. **Results:** The presence of the allele for duplication of 24pb was high in indigenous populations: 92% for Ka'apor, 76% for Arara, 71% for Parakanã, 69% for Araweté, 68% for Asurini, 50% for Xikrin and 45% for Gavião. In the population of Belém, the frequency was 24% and in patients with GD was 7%. **Conclusion:** Studies determining the frequency of gene polymorphisms of QT in different populations reflect a clinical importance, since the high activity of the enzyme QT is associated with many diseases. This is the first study that addresses the frequency of polymorphisms in the QUIT gene in indigenous populations, calling attention to the high frequency of the duplication of 24pb in these populations (92%, 76%, 71%, 69%, 68%, 50% and 45%) compared to that found in the population of Belém (24%). These results suggest that in the case of indigenous patients with GD, the investigation of duplication of 24 pb is essential, and other markers for monitoring of enzyme replacement therapy may be considered.

916 - WWOX-Related Encephalopathy Mimicking GLUT-1 Deficiency: Expanding the Phenotype

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Aims: To describe a case of early infantile epileptic encephalopathy (IEE) due to *WWOX* gene homozygous deletion misdiagnosed initially as GLUT-1 deficiency. **Methods:** Clinical history assessment with charts reviews. **Results:** Patient was the only child born to nonconsanguineous parents after an uneventful pregnancy except for an enlarged nuchal translucency seen at 11 weeks echography but not confirmed in the next. At 36 weeks of pregnancy, it was proceeded a cesarean delivery due to acute fetal distress. He was born with 2836 g, 47 cm, head circumference at the 50th percentile, Apgar 1' 7 / 5' 9 and mild respiratory distress. He remained in intensive care unit for four days, and he was discharged with good breast feeding. Hypononia was noticed since the second month of life. Skull echography was normal, a positive Benedict test and increased glycine levels were found in the first urine sample. With 4 months, he presented his first seizure—left eye blink and limb adduction, without satisfactory response to the use of phenytoin and phenobarbital. His seizures subsided after a 48

hours fasting with glycosated serum plus a few dosis of Valproic Acid. He presented clonic movements a week later. A month later he presented in status again with low glucose ratio in a liquor sample, not confirmed after that. Extensive biochemical and metabolic investigation were all within normal range—acylcarnitine profile or biotinidase deficiency were not searched. Brain MRI has showed a mild volumetric reduction and corpus callosum thinning. With 8 months old, he was with 42 cm of cephalic circumference (< 3 rd percentile), no remarkable dysmorphisms, axial hypotonia, appendicular hypertonia, bilateral equinovarus and Babinski's sign, with poor response at use of Topiramate, Clobazam and Canabidiol. Brainstem Evoked Response Audiometry was normal. Molecular genetic panel to treatable IEMs and *SLC2A1* gene sequencing were normal. Clinical exome analysis has found a deletion on *WWOX* gene in homozygosis. **Conclusion:** The low glucose ratio in the first CSF sample was a confounding factor for *GLUT1* deficiency, an important differential diagnosis. There is a report of another patient with heterozygous deletion of *WWOX* and a mutation on the second allele whom presented also IEE and acquired microcephaly, despite of other cases of mutations presented spinocerebelar ataxia, which suggests a genotype phenotype correlation.

917 - Exome Sequencing for Understanding the Genetics of Complex Diseases

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There are thousands of known associations between genetic variants and complex human phenotypes, and the rate of novel discoveries is increasing. One of those is absence of ulna and fibula with severe limb deficiency. It is a rare autosomal recessive disorder characterized by severe malformations of upper and lower limbs with severe lyhypoplastic pelvis and abnormal genitalia. The disorder is believed to represent a defect of dorso-ventral patterning and outgrowth of limbs and Charcot-Marie-Tooth (CMT) disease type 4C. It is caused by homozygous or compound heterozygous mutation in the *SH3TC2* gene; affected individuals had peripheral motor and sensory neuropathy and reduced Median nerve, consistent with a demyelinating process. A 5-year-old male patient, mestizo race, with clinical diagnosis of arthrogryposis, congenital hip dislocation, bilateral varus-equine-foot, right tibiae torsion, tendon transposition, multiple genital plasty, resection of face tumor pilomaxitroma, pectum excavatum, obesity, learning disturbance, normal brain magnetic resonance, normal karyotype, normal metabolic studies is reported. Exome sequencing was performed for this patient on the iIlumina platform. 2 pathogenic variants on WNT7A gene located at chromosome 3 (position 13860881, Clin Var rs387907231), and SH3TC2 gene located at chromosome 5 (position 148407326, ClinVar rs80338925) both reported as pathogenic were identified. According to the signs and symptoms of the patient, and using the base of Data GeneMANIA and evaluation on networks and expression, co-expression, it is found an interaction between the 2 genes associated with the structure of the extracellular matrix, metabolic processes of collagen, growth factor, and skeletal system development brain development. Intersecting the compendium of identified genetic associations with maps of regulatory activity across the human exome has revealed that phenotype-associated variants are highly enriched in candidate regulatory elements.

918 - Evolution and Correlation of Number of Published Papers About Inborn Errors Of Metabolism in PubMed, Scielo.org, and Scielo.br From 20th TO 21st Century

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Number of papers about Inborn Errors of Metabolism (IEM) in medical databases is widely variable and has increased over time, just like the proportion of Brazilian papers on this issue. This work describes the evolution and correlation of the number of paper in PubMed, Scielo.org and Scielo.br databases between 20th and 21st centuries. Using INBORN+ERRORS+METABOLISM as keywords, 2667 papers have been retrieved in PubMed, 107 in Scielo.org and 23 in Scielo.br. Comparing the number of published papers on IEM, no difference between PubMed and Scielo.br have been observed using chi square test (neither looking at 21st X 20th century nor comparing 2001-Today X All time before 2010, $P = .093$ and $P = .500$, respectively). However, number of papers in PubMed was significantly higher than that in Scielo.org ($P < .001$) considering both centuries (all time), likely as a result of a "dilution" of Brazilian papers (accounting for 21.5% of the total amount of papers in Scielo.org). The results may reflect a relevant proportion of Brazilian papers (from Brazilian journals or indexed in Scielo.br) in the total of papers in Scielo.org, as well as that the evolution and increase in papers on IEM in Scielo.br correlates and resembles that of PubMed.

919 - Carnitine and TMAO Metabolism in Children With Various Inborn Errors Of Metabolism Receiving Oral or Intravenous Carnitine

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L-Carnitine is used in the treatment of several inborn errors of metabolism (IEM). Trimethylamine-N-oxide (TMAO) is a gut derived carnitine metabolite which has recently been associated with cardiovascular risk, thus raising concerns about the

long-term safety of carnitine supplementation. We aim to study the metabolism of carnitine and its derivatives including TMAO in children with various inborn errors of metabolism receiving oral L-carnitine or intravenous carnitine. **Methods:** We measured the plasma and urine levels of Carnitine, Trimethylamine (TMA) and TMAO. The urine and plasma samples were collected within 2 days, which allowed us to determine the metabolites fractional excretion (FE). We included 15 patients with several IEM including MCADD, 3-MCC deficiency, Propionic Acidemia (PA), Ethylmalonic Encephalopathy, Isovaleric Acidemia, Glutaric Acidemia type II (GA2), MMA, Primary Carnitine Deficiency, Beta Ketothiolase deficiency and TMLHE deficiency. **Results:** The oral dose of L-Carnitine was not correlated with plasma or urine levels of Carnitine or TMAO. The plasma TMAO level correlated strongly ($R = 0.92$) with the urine TMAO levels, but this wasn't the case for Carnitine ($R = 0.27$). Given this close correlation between the plasma and urine TMAO levels, the FE was close to 1 for most patients. The Carnitine FE was much lower across all patients. There were two patients with significantly higher Carnitine FE, the patient with GA2 and the patient with Primary Carnitine Deficiency. The explanation is obvious in the latter, as the OCTN2 transporter is deficient. The first case is likely related to decreased energy affecting renal tubular reabsorption. The TMAO FE and Carnitine FE were not correlated. The patients with PA had remarkably high levels of plasma TMAO. The ratio TMAO/TMAO+TMA was close to 1 for most patients except for the two patients receiving oral antibiotics. The plasma TMAO level reduced significantly in one patient with MCADD after IV Carnitine infusion (20x fold decrease). **Conclusion:** Given the close correlation of plasma and urine TMAO levels, we suggest urine TMAO levels could be used as a marker of TMAO plasma levels. Carnitine likely has a very efficient renal retention system compared to TMAO despite sharing OCTN2 transporter. Oral antibiotics affect the TMAO/TMA ratio likely due to gut flora disturbances. Patients with PA are at risk for high plasma TMAO levels. IV carnitine infusion can significantly decrease plasma TMAO level.