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Novel Insights from Clinical Practice

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Warburg Micro Syndrome 1 due to Segmental Paternal Uniparental Isodisomy of Chromosome 2 Detected by Whole-Exome Sequencing and Homozygosity Mapping

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Established Facts

- Warburg micro syndrome is an autosomal recessive disorder characterized by microcephaly, intellectual disability, ocular abnormalities, spastic diplegia, and microgenitalia.
- Autosomal recessive disorders are generally inherited from both parents but rarely from only one parent due to uniparental isodisomy.
- Single-nucleotide polymorphism array and short tandem repeat polymorphism tests are widely used in the diagnosis of uniparental disomy.

Novel Insights

- Homozygosity mapping analysis was applied to next-generation sequencing data to investigate isodisomic segments of the uniparental disomic chromosome and provided valuable information.
- A rare mechanism of uniparental disomy, paternal meiosis I nondisjunction, was detected.
- The first case of WARBM1 as a result of uniparental isodisomy of chromosome 2 is reported.

Keywords
Chromosome 2 · Homozygosity mapping · *RAB3GAP1* · Segmental paternal uniparental isodisomy · Warburg micro syndrome · Whole-exome sequencing

Abstract
Warburg micro syndrome (WARBM) is a rare autosomal recessive disorder characterized by microcephaly, cortical dysplasia, intellectual disability, ocular abnormalities, spastic diplegia, and microgenitalia. WARBM has 4 subtypes arising from pathogenic variants in 4 genes (*RAB18*, *RAB3GAP1*, *RAB3GAP2*, and *TBC1D20*). Here, we report on a patient with a homozygous pathogenic c.665delC (p.Pro222HisfsTer30) variant in the *RAB3GAP1* gene identified by whole-exome sequencing (WES) analyses. Only his father was a heterozygous carrier, and homozygosity mapping analysis of the WES data revealed large loss-of-heterozygosity regions in both arms of chromosome 2, interpreted as uniparental isodisomy. This uniparental disomy pattern could be due to paternal meiosis

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I nondisjunction because of the preserved heterozygosity in the pericentromeric region. This report provides novel insights, including a rare form of UPD, usage of homozygosity mapping analysis for the evaluation of isodisomy, and the first reported case of WARBM1 as a result of uniparental isodisomy.
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Warburg micro syndrome (WARBM) is characterized by microcephaly, microphthalmia, microcornea, congenital cataracts, optic atrophy, cortical dysplasia, in particular corpus callosum hypoplasia, severe intellectual disability, spastic diplegia, hypogonadism, and microgenitalia and is inherited in a rare autosomal recessive pattern [Morris-Rosendahl et al., 2010]. WARBM has 4 subtypes (WARBM1–4; OMIM 600118, 614225, 614222, and 615663, respectively) arising from pathogenic variants of 4 different genes (*RAB3GAP1*, *RAB3GAP2*, *RAB18*, and *TBC1D20*). WARBM is referred to as Ras-related protein Rab-18 (RAB18) deficiency and is caused by the absence of RAB18 itself or its function. Because Rab3 GTPase-activating protein catalytic subunit (RAB3GAP1), Rab3 GTPase-activating protein non-catalytic subunit (RAB3GAP2), and TBC1 domain family member 20 (TBC1D20) are essential for the regulation of RAB18, biallelic loss-of-function variants in the genes that encode these proteins lead to clinically indistinguishable phenotypes. Biallelic pathogenic variants in *RAB3GAP1* are the most frequently reported cause of RAB18 deficiency [Handley and Sheridan, 2018].

Uniparental disomy (UPD) is described as the presence of 2 chromosomes inherited from only 1 parent and is most commonly caused by trisomy rescue as a result of chromosomal nondisjunction. While the situation in which both chromosomes originate from identical sister chromatids is defined as isodisomy, the presence of both homologs of one parent is called heterodisomy. UPDs commonly raise clinical concern because of imprinted regions or pathogenic variants in individual genes [Nussbaum et al., 2015]. In some disorders, the expression of the disease phenotype depends on the paternal or maternal inheritance of the mutant allele or abnormal chromosome. This case is defined as parent-of-origin effect and is the result of genomic imprinting. Imprinting disorders have not been described for UPDs of some chromosomes including chromosome 2, regardless of paternal or maternal, while UPDs of chromosomes 6, 7, 11, 14, 15, and 20 are related to imprinting disorders due to their parent-of-origin [Bastepe et al., 2001; Liehr, 2010; Mulchandani et al., 2016]. An increasing number of uniparental isodisomy (UPiD) cases have been reported in autosomal recessive diseases because of the more frequent use of new generation techniques such as single-nucleotide polymorphism array and next-generation sequencing [Niida et al., 2018]. Here, we report on a patient with a homozygous pathogenic variant in the *RAB3GAP1* gene due to segmental UPiD of chromosome 2 found by homozygosity mapping analysis of whole-exome sequencing (WES) data in addition to segregation analyses.

Case Presentation

A 20-month-old male patient was referred to our clinic for genetic evaluation due to his neurodevelopmental delay and congenital anomalies. He is the second child of healthy and non-consanguineous parents (father 39 and mother 28 years old). Family history did not include any other individual who had developmental delay and similar congenital anomalies. The pregnancy was uneventful and delivery was at term, but he had asphyxia at birth. His weight was 3,000 g (–0.89 SD), length was 50 cm (0 SD), and head circumference was 34 cm (–1.22 SD) at birth. In his initial examination, when he was 20 months old, his weight was 11.5 kg (–0.28 SD) and head circumference was 43.5 cm (–3.46 SD). His height could not be measured properly due to the spasticity in his lower limbs. In addition to spasticity, he had global neurodevelopmental delay, hypotonia, microcephaly, ptosis, bilateral congenital cataracts, microphthalmia, microcornea, strabismus, operated lacrimal duct obstruction, large ears, micropenis, bilateral cryptorchidism, and hammer great toes (Fig. 1). Brain MRI showed dilatation of the third and lateral ventricles and corpus callosum hypoplasia. According to clinical and imaging findings, the patient was diagnosed as WARBM. When the patient reapplied for diagnosis at the age of 14 years, all his anthropometric measurements were below the 3rd centile, the spasticity had progressed and was observed in all 4 limbs; however, the lower limbs were affected more severely (Fig. 1). The patient still had not achieved any developmental progression, and his speech was limited to a couple of words. The clinical diagnosis was still compatible with WARBM, and therefore, WES analysis was planned for the patient to evaluate the genes of WARBM and the genes related to the clinics of the differential diagnosis.

Methods and Results

WES analysis was only conducted for the proband with genomic DNA extracted from peripheral blood lymphocytes. BGI Group (Shenzhen, China) performed the main part of WES, including targeted capture, massive parallel sequencing (MGISEQ-2000 platform), alignment, mapping, variant calling, single nucleotide variants and insertion/deletion filtering, and annotation.

WES analysis revealed the homozygous c.665delC (p.Pro222HisfsTer30) variant in the *RAB3GAP1* gene

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Fig. 1. A, **B** Facial gestalt of the patient at the age of 20 months (**A**) and 14 years (**B**). **C** Spasticity affecting lower limbs. **D** Hammer great toes.

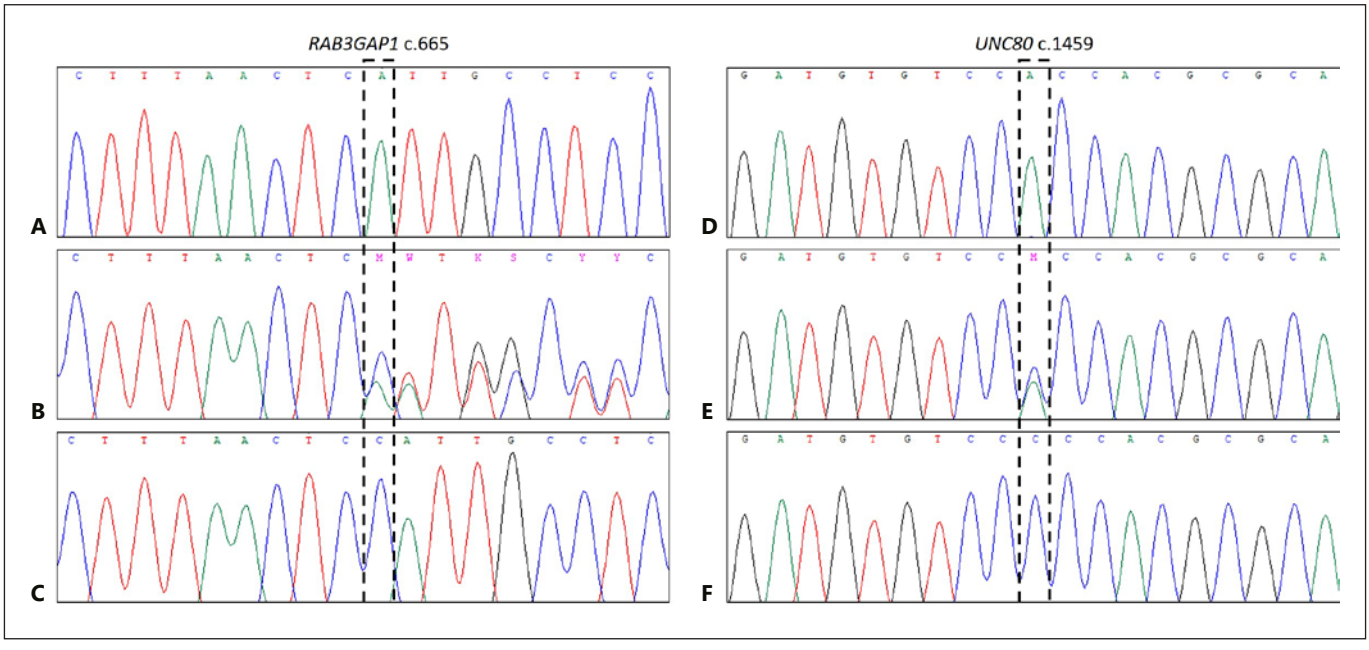


Fig. 2. A–C Sanger sequencing of the *RAB3GAP1* (exon 8) gene. The chromatograms show the homozygous c.665delC in the proband (**A**), the heterozygous c.665delC in the father (**B**), and the normal sequence in the mother (**C**). **D–F** Sanger sequencing of the *UNC80* gene. The chromatograms show the homozygous c.1459C>A in the proband (**D**), the heterozygous c.1459C>A in the father (**E**), and the normal sequence in the mother (**F**).

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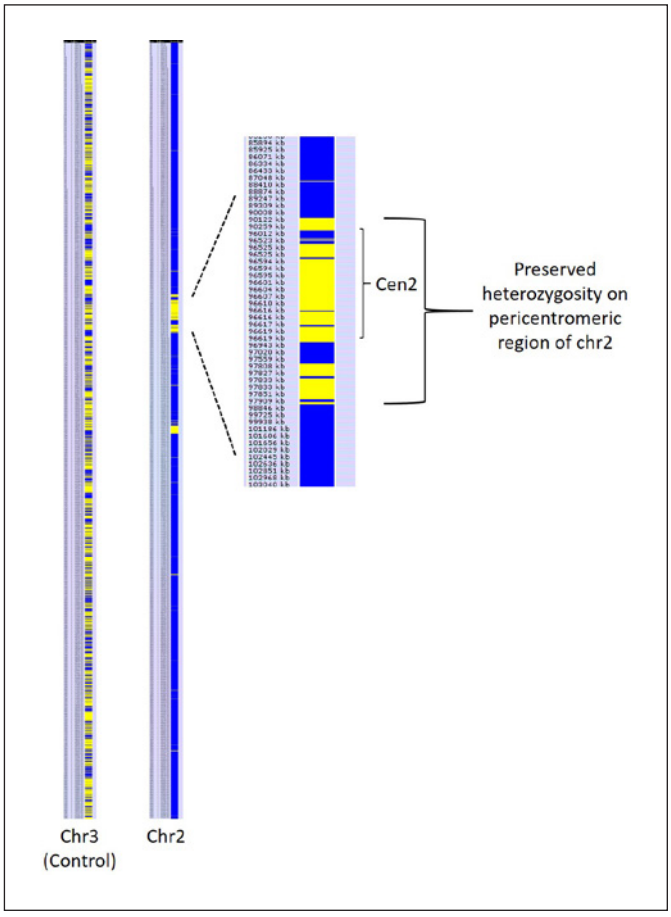


Fig. 3. Output of the homozygosity mapping module of HomSI tool applied on WES data of the patient for chromosome 2 (Chr2) and chromosome 3 (Chr3; control). The figure shows the loss-of-heterozygosity regions starting from the pericentromere to the telomeres in both arms of chromosome 2 and the approximately 7.8-Mb region with preserved heterozygosity in the pericentromere. Blue color indicates homozygosity, yellow indicates heterozygosity. Cen2, centromere of chromosome 2.

(NM_012233.3) located in 2q21.3 which was also confirmed by Sanger sequencing. Interestingly, segregation analysis by Sanger sequencing showed that the variant was inherited only from the father in a heterozygous state; the mother was no carrier. Another rare homozygous variant, c.1459C>A (p.Pro487Thr) in the *UNC80* gene (NM_032504.1) located in 2q34, was detected by WES and also confirmed by Sanger analysis. This variant also showed only paternal segregation (Fig. 2). This situation was considered to be compatible with paternal UPiD of chromosome 2.

Homozygosity mapping analysis was performed on the WES data of the patient via the "homozygous stretch identifier from next-generation sequencing data (HomSI)" tool [Görmez et al., 2014] in order to determine isodisomic and heterodisomic segments of chromosome 2. Analysis of the WES data with the HomSI tool revealed loss of heterozygosity starting from the pericentromeric region to the telomeres in both arms of chromosome 2, and a region of approximately 7.8 Mb with preserved heterozygosity in the pericentromere (Fig. 3). Sequencing of the pericentromeric region is notoriously challenging because of the repetitive sequences in this region. However, here we have listed some of the single-nucleotide polymorphisms in heterozygous state which are common in the population and located in the pericentromeric region of both arms of chromosome 2 with deep read depth and high variant quality score (Table 1). This result was interpreted as UPiD of chromosome 2. The region of heterozygosity in chromosome 2 was regarded as a result of segmental uniparental heterodisomy (UPhD).

Other rare homozygous variants which were detected in morbid OMIM genes, classified as variants of unknown significance, and identified in isodisomic segments of chromosome 2 are presented in Table 2. There were no other variants which were classified as pathogenic or likely pathogenic.

Discussion

The homozygous c.665delC (p.Pro222HisfsTer30) variant detected in the *RAB3GAP1* gene in the WES analysis of our patient is expected to generate a premature stop codon by frame shifting. This variant is not found in the population databases (GnomAD). The same variant was reported as pathogenic in a Turkish WARBM patient who was previously evaluated without details of the clinical findings [Handley et al., 2013]. Finally, this variant is classified as pathogenic according to the guidelines for the interpretation of sequence variants of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [Richards et al., 2015], and the outcome was evaluated as consistent with WARBM1.

In our patient, segmental paternal UPiD of chromosome 2 was detected via homozygosity mapping analysis of WES data and segregation analyses. Since the mechanism of the homozygous pathogenic variant was UPiD, and only one of the parents was heterozygous carrier, the recurrence risk of the disease is below 1% instead of 25% expected in classical Mendelian inheritance of autosomal recessive disorders [Niida et al., 2018]. Information and

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Table 1. List of some SNPs located in the pericentromeric heterozygous state region of chromosome 2 with read depths, variant quality scores, and population frequencies

Chromosomal locus	Base pairs (hg19)	Reference	Alternative	Read depth	Allele frequency	Variant quality score	dbSNP	Frequency in GnomAD
2p11.2	90,121,845	A	G	506	0.44	8472.77	rs2693227	0.1906
2p11.2	90,121,849	A	G	524	0.45	8628.77	rs1852250	0.1933
2p11.2	90,121,926	G	T	625	0.54	8415.77	rs1852249	0.1884
2p11.2	90,121,985	A	G	628	0.47	7384.77	rs2693226	0.1643
2q11.1	96,594,697	G	C	112	0.6	1771.77	rs72931795	0.4195
2q11.1	96,594,723	A	G	131	0.52	2497.77	rs78476446	0.4191
2q11.1	96,594,735	C	T	137	0.47	2506.77	rs75927751	0.4186
2q11.1	96,594,742	C	T	145	0.43	2534.77	rs75729585	0.4181

hg19, human genome version 19; dbSNP, single nucleotide polymorphism database; GnomAD, genome aggregation database.

Table 2. Other rare homozygous variants detected in morbid OMIM genes and classified as variants of unknown significance according to ACMG NGS guidelines [Richards et al., 2015] identified in isodisomic segments of chromosome 2

Gene (RefSeq transcript)	Variant	dbSNP	Frequency in GnomAD	OMIM phenotype	MIM Number	Inheritance
ADCY3 (NM_004036.5)	c.2753C>T (p.Thr918Met)	rs776200284	0.0000718	Obesity, susceptibility to; BMIQ19}	617885	AR
IFIH1 (NM_022168.4)	c.2962G>T (p.Val988Leu)	rs74162090	0.000453	Aicardi-Goutieres syndrome 7 Singleton-Merten syndrome 1	615846 182250	AD AD
SCN9A (NM_002977.3)	c.2123A>G (p.His708Arg)	N.A.	N.A.	Epilepsy, generalized, with febrile seizures plus, type 7 Erythermalgia, primary Febrile seizures, familial, 3B HSAN2D, autosomal recessive Insensitivity to pain, congenital Paroxysmal extreme pain disorder Small fiber neuropathy Dravet syndrome, modifier of	613863 133020 613863 243000 243000 167400 133020 607208	AD AD AD AR AR AD AD AD
SPEG (NM_005876.5)	c.5891A>G (p.Glu1964Gly)	rs55760738	0.0000565	Centronuclear myopathy 5	615959	AR
TTN (NM_001256850.1)	c.95303G>A (p.Cys31768Tyr)	rs201112096	0.0000872	Cardiomyopathy, dilated, 1G Cardiomyopathy, familial hypertrophic, 9 Muscular dystrophy, limb-girdle, autosomal recessive 10 Myopathy, myofibrillar, 9, with early respiratory failure Salih myopathy Tibial muscular dystrophy, tardive	604145 613765 608807 603689 611705 600334	AD AD AR AD AR AD
UNC80 (NM_032504.1)	c.1459C>A (p.Pro487Thr)	N.A.	N.A.	Hypotonia, infantile, with psychomotor retardation and characteristic facies 2	616801	AR

AD, autosomal dominant; AR, autosomal recessive; dbSNP, single nucleotide polymorphism database; GnomAD, genome aggregation database; N.A., not applicable.

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genetic counseling were provided to the family about this outcome.

Nondisjunction occurring during meiosis I or II can be distinguished by whether the pericentromeric component is heterozygous (meiosis I) or homozygous (meiosis II). In the case of crossing over in meiosis I, chromosomes affected by UPD are formed as segmental isodisomic/heterodisomic elements [Niida et al., 2018]. It is thought that the segmental uniparental isodisomy/heterodisomy pattern determined in the patient was due to nondisjuntion in paternal meiosis I, because the centromeric region was heterozygous (Fig. 3). Our result which is consistent with paternal meiosis I nondisjunction is remarkable in terms of the rarity of this condition (3.5% of the UPiD cases reported by Niida et al. [2018]). A chromosome pair with a few (or even no) recombinations, or with recombination close to the centromere or telomere, may be more susceptible to nondisjunction than a chromosome pair with a more typical number and distribution of recombination events [Nussbaum et al., 2015]. In the presented patient, the heterodisomy/isodisomy distinction portions near the centromere in both arms of chromosome 2 show the possible recombination sites, possibly due to the aforementioned reason, predisposing to nondisjunction formation in paternal meiosis I, thus leading to the emergence of UPD of chromosome 2 by trisomy rescue mechanism.

One of the variants located in the isodisomic segments of UPD2 detected in the present patient, the c.1459C>A (p.Pro487Thr) variant in the *UNC80* gene, is rare and considered to be a disease-causing variant in in silico prediction tools (Table 2). The domains of the protein encoded by *UNC80* have not been studied sufficiently yet [Boerkoel and du Souich, 2017]. Additionally, it was reported that biallelic loss-of-function pathogenic variants in the *UNC80* gene lead to "Hypotonia, infantile, with psychomotor retardation and characteristic facies 2 (OMIM 616801)" [Stray-Pedersen et al., 2016]. In this study, it was not investigated whether the c.1459C>A variant detected in the *UNC80* gene leads to loss of function. If this variant leads to loss of function of the protein, this would result in a complex, clinically indistinguishable presentation, as the clinical aspects of pathogenic variants in the *UNC80* gene, including hypotonia, strabismus, oral motor dysfunction, postnatal growth retardation, and severe developmental retardation can also be found in WARBM. It is therefore very difficult to infer from the clinic whether this disease is present. Finally, the variant is classified as variant of unknown significance with the available data. However, it should be kept in mind that in cases of UPD with long isodisomic segments, there may be more than one rare homozygous variant that could be disease causing.

In conclusion, in cases where the patient's clinic suggests a clear genetic disease, the use of more comprehensive new-generation analysis methods instead of conventional tests performed in the first step may increase the chance of giving the correct diagnosis for the patients. In addition to the standard diagnostic applications, more comprehensive tests can provide additional information, allowing different analyses including homozygosity mapping, which the conventional tests cannot provide.

UPDs of chromosomes not related to imprinting disorders are detected occasionally. This rare condition is of particular importance as it increases the risk of autosomal recessive diseases in children of unrelated couples and alters their genetic counseling. The present case, the first reported case of WARBM1 as a result of UPiD of chromosome 2, should guide clinicians to analyze next-generation sequencing data in detail when a clear molecular diagnosis through conventional approaches is not possible.

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Statement of Ethics

Informed consent was obtained from the parents of the patient for genetic testing and publication of the clinical pictures.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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There was no funding relevant to this study.

Author Contributions

All authors participated in acquisition, analysis, and interpretation of data for the work. A.S., G.K., and F.E.P. drafted the work and revised it critically for important intellectual content. A.S. and F.E.P. approved the final version to be published. F.E.P. is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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