



Identification of copy number variants in children and adolescents with autism spectrum disorder: a study from Turkey

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Abstract

Background Copy number variants (CNVs) play a key role in the etiology of autism spectrum disorder (ASD). Therefore, recent guidelines recommend chromosomal microarrays (CMAs) as first-tier genetic tests. This study's first aim was to determine the clinical usefulness of CMAs in children diagnosed with ASD in a Turkish population. The second aim was to describe the CNVs and clinical phenotypes of children with ASD.

Methods and Results This was a single-center retrospective cross-sectional study. Data were obtained from the medical records of children with ASD followed at Gazi University Hospital, (Ankara, Turkey). The sample consisted of 47 ASD cases (mean age: 60.34 ± 25.60 months; 82.9% boys). The diagnostic yield of the CMAs was 8.5%. Four pathogenic CNVs were identified: 9p24.3p24.2 deletion, 15q11–q13 duplication, 16p11.2 deletion, and 22q13.3 deletion. Also, four variants were found at 2q36.3, 10p11.21, 15q11.2, and Xp11.22, which were classified as variants of uncertain significance (VUS).

Conclusions The TRAP12 and PARD3 genes in CNVs classified as VUS may be worth investigating for autism. The initial identification of both clinical and biological markers can facilitate monitoring, early intervention, or prevention and advance our understanding of the neurobiology underlying ASD.

Keywords Autism spectrum disorder · Array comparative genomic hybridization · Copy number variant · Turkish children

Introduction

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder characterized by difficulties in social communication and interaction, restrictive and repetitive behavior patterns of interests and activities, and sensory processing abnormalities [1]. The prevalence of ASD is estimated to be 12%, with a male-to-female ratio of around 4:1 [2]. The clinical presentation of the disorder includes a wide range of symptoms and varying severity. ASD is often accompanied by psychiatric disorders, such as attention deficit hyperactivity disorder (ADHD), developmental delay (DD)/intellectual disability (ID), anxiety disorders, and sleep problems [3].

Studies focusing on the pathophysiology of autism have suggested several genetic, epigenetic, and environmental factors. *Although the complexity of the genetic factors involved does not allow an accurate estimate [4], twin studies have estimated that heritability in ASD ranges from 64 to 91% [5]. In 20–25% of children with ASD, a genetic cause can be identified using standard medical genetic assessments [6]. Chromosomal microarrays (CMAs) identify copy number variants (CNVs), which are DNA copies or deletions that can sometimes affect the activity of genes. CMAs have shown that 5.4–14% of ASD cases have a definite pathogenic CNV [7].* Therefore, recent guidelines recommend CMAs as first-tier diagnostic tests for unexplained ASD, ID, and multiple congenital anomalies [8]. The most common pathogenic CNVs associated with ASD are 16p11.2 deletion and duplication, 15q11.2–q13 duplication, 15q13.2–q13.3 duplication, 1q21.1 duplication, 22q11.2 deletion, 16p13.11 deletion, 7q11.23 duplication, 16p12.2 deletion, and 17q12 deletion [4, 9–11]. Approximately 17% to 42% of ASD cases have CMA findings if variants of uncertain significance (VUS) are included. Determining a genetic etiology provides clinicians with more information about the prognosis and

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the risk of recurrence of the disorder. Moreover, it can help identify and treat or prevent emerging medical conditions, directing cases and families to condition-specific resources and helping to avoid unnecessary testing [7].

CMA studies are important for determining the etiology of ASD and identifying new syndromes [4]. *Although some CNVs and their effects are well defined, more studies are needed to identify ASD-specific loci.* To contribute to this research, the first aim of this study was to determine the clinical yield of CMAs in the evaluation of ASD in Turkish children. *The second aim was to describe the CNVs and clinical phenotypes of children diagnosed with ASD.*

Method

Sample

This was a retrospective cross-sectional study. Data were retrieved from the medical records of 59 children and adolescents who referred to the Child and Adolescent Psychiatry Outpatient Clinic of Gazi University Hospital, Ankara, Turkey between August 2017 and August 2018. The inclusion criteria were an age of 1–18 years and ASD diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) criteria by two child and adolescent psychiatrists, at least one of whom had more than five years of experience treating children with ASD. Children and adolescents with ASD who did not meet the inclusion criteria, children and adolescents who met the inclusion criteria but whose genetic tests were not completed for any reason, and children and adolescents with a clear ASD etiology were excluded. Four cases were excluded because they were evaluated by only one psychiatrist. Eight cases were excluded because they never applied to the Genetics Department after being diagnosed with ASD. The families of these cases were contacted via phone and were asked to participate after being verbally informed of the aim and methods of the study. Then written informed consent was obtained from the parents of all participating cases. Thus, the final sample consisted of 47 cases.

Procedure

The DSM-5 criteria were used to collect multiple pieces of information on the symptoms of ASD. Additionally, the symptoms were assessed using the childhood autism rating scale (CARS). The CARS is a 15-item Likert-type behavioral rating scale used to detect and quantify the symptoms of autism and to distinguish them from other developmental disabilities. Each item is scored from 1 (no signs of autism) to 4 (severe symptoms). Scores of 30.5–37 indicate mild to moderate autism, whereas scores of 37.5–60 indicate severe

autism. The scale has been reported to have good reliability and validity in Turkish children with autism [12]. In this study, it was used to determine ASD severity. The final diagnosis of ASD was established according to the DSM-5 criteria by another researcher who had more than five years of experience working with children and adolescents with ASD. DD was assessed using the Ankara Developmental Screen Inventory (ADSI) [13], which has been shown to have good reliability and validity in Turkish children. The ADSI includes 154 items aimed at determining the social, motor, cognitive, and communicative development levels of children aged 0–6 years. *It consists of four subscales—language–cognitive, fine motor, gross motor, and social skills–self-care—whose total score indicates the general developmental level of the child* [13]. ID was evaluated using the Wechsler Intelligence Scale for Children–Revised (WISC-R), which has shown good reliability and validity in Turkish children [14]. For cases who could not perform the test, a clinical diagnosis of ID was made by two child and adolescent psychiatry specialists.

After being diagnosed with ASD, all children were referred to the Medical Genetics Department of Gazi University for genetic examinations. Firstly dysmorphism examinations were performed to identify possible syndromes accompanying ASD before karyotype analysis. Karyotype analysis was then performed using conventional cytogenetic methods. All cases were found to have normal karyotype structures in terms of numerical and structural chromosomal abnormalities. CMAs were performed on DNA obtained from peripheral blood samples using the SurePrint G3 ISCA V2 CGH 8×60 K microarray (Agilent Technologies, Santa Clara, CA, USA). The obtained data were analyzed according to human genome 19 (Chr37) using Agilent CytoGenomics software. For the interpretation of the CNVs, the Database of Genomic Variants (DGV; <http://dgv.tcag.ca>), ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar>), UCSC Genome Browser (<https://genome.ucsc.edu>), Database of Genomic Variation and Phenotype in Humans using Ensembl Resources (DECIPHER; <https://decipher.sanger.ac.uk>), PubMed (<https://www.ncbi.nlm.nih.gov/pubmed>), Online Mendelian Inheritance of Man (OMIM; <https://www.ncbi.nlm.nih.gov/omim>), and in-house databases were used. *The CNVs were classified as pathogenic, likely pathogenic, VUS, likely benign, and benign according to the 2020 American College of Medical Genetics (ACMG) and Genomics and Clinical Genome Resource recommendations* [15]. *However, likely pathogenic, likely benign, and benign variants were not specified.*

Results

Of the 47 children, 8 were girls and 39 were boys. *Their ages ranged from 2 to 12 years (22–143 months). The median age was 5 years (60.34 ± 25.60 months). According to cognitive*

evaluations, ASD was accompanied by either DD or ID in 46.2% of the boys ($n = 14$ and $n = 4$, respectively) and in 62.5% of the girls ($n = 2$ and $n = 3$, respectively). Eight CNVs ranging in size from 55 kb to 6.5 Mb were identified in seven cases (five boys). The CNV detection rates in boys and girls were 12.8% and 25%, respectively. Four of the identified CNVs were classified as pathogenic: 9p24.3p24.2 deletion 3 Mb in size [arr(hg19) 9p24.3p24.2(204,193–3,221,675) × 1], 15q11–q13 duplication 6.5 Mb in size [arr(hg19) 15q11.2q13.1(22,765,628–29,253,376) × 3], 16p11.2 deletion 598 kb in size [arr(hg19) 16p11.2(29,592,783–30,190,568) × 1], and 22q13.3 deletion 55 kb in size [arr(hg19) 22q13.3(51,123,491–51,178,264) × 1]. Four VUS were detected in three children. One of them showed a paternal inheritance pattern. Parental studies were not performed for the other two cases. Table 1 shows a summary of the case characteristics and CNV findings.

Case 1 was a 6-year-old (73-month-old) girl living with her biological parents. There was no consanguinity between the parents. The girl started speaking her first words at around 36 months. The complaints upon referral to our clinic were limited eye contact, inability to imitate, difficulties in speech, stereotypy (hand clapping, head shaking), and hypersensitivity to loud sounds. She had marked tantrums and behavioral problems (e.g., hair pulling, pinching). Due to her behavioral problems, she was receiving 1 mg of risperidone daily. She had been receiving special education for three years. She was diagnosed with severe autism according to the CARS (total score: 41). She could not perform the WISC-R test. Her level of intelligence was clinically determined to be consistent with moderate intellectual disability. Her weight was in the 90th percentile, her

height was in the 75th percentile, and her head circumference was in the 50th percentile. She had no neurological deficits, and her electroencephalography (EEG) and magnetic resonance imaging (MRI) findings were normal. Phenotypic examination revealed no dysmorphic features. Her karyotype analysis was normal. Array comparative genomic hybridization (CGH) showed a deletion in the 9p24.3–p24.2 region (3017 kb). The deletion included 13 genes, the most relevant of which were *DOCK8*, *KANK1*, *SMARCA2*, and *RFX3*. Karyotype analysis and array CGH performed on both parents revealed that the CNV, which was classified as pathogenic, was inherited from her mother.

Case 2 was a 9-year-old (110-month-old) boy living with a foster family. Information before the age of 1 year was not available. The boy was diagnosed with ASD in a different hospital around the age of 2 with complaints of inadequacy in social interactions, no speech, and tantrums. Upon referral to our clinic, tantrums, difficulty making friends at school, inappropriate gestures, and limited interests were reported. He was using 5 mg of aripiprazole daily for his tantrums. He had been receiving special education for seven years. He was diagnosed with mild to moderate autism according to the CARS (total score: 34). He could not perform the WISC-R test. His level of intelligence was clinically determined to be consistent with mild intellectual disability. His weight was in the 40th percentile, his height was in the 85th percentile, and his head circumference was in the 15th percentile. No deficit was detected in his neurological examination. However, his EEG revealed a focal epileptic disorder originating in the left frontotemporal region. On phenotypic examination, grooves in the gum between the upper incisors, clinodactyly of the fifth finger of the right hand, syndactyly

Table 1 CNVs in the children with ASD

Gender/ Age	Diagnosis	Cytoband	Start-stop (bp) (hg19)	Size (kb)	Number of the genes	Relevant genes	Del/ Dup	Inheritance	Interpre- tation
F/6	ASD + moderate ID	9p24.3-p24.2	204,193–3,221,675	3017	13	DOCK8, KANK1, SMARCA2, RFX3	Del	Maternal (from mildly affected mother)	Pathogenic
M/9	ASD + mild ID	15q11.2-q13.1	22,765,628–29,253,376	6488	135	ATP10A, CYFIP1, GABRB3, MAGEL2, UBE3A	Dup	Unknown	Pathogenic
M/5	ASD + ADHD	16p11.2	29,592,783–30,190,568	598	31	TAOK2, KCTD13, MAPK3, SEZ6L2	Del	De novo	Pathogenic
M/4	ASD + DD	22q13.33	51,123,491–51,178,264	55	2	SHANK3	Del	De novo	Pathogenic
F/9	ASD + mild ID	2q36.3	230,479,578–230,951,771	472	4	DNER, TRIP12	Dup	Paternal	VUS
M/7	ASD	10p11.21	35,072,047–35,281,153	209	1	PARD3	Dup	Unknown	VUS
M/3	ASD + DD	15q11.2	22,833,122–23,217,514	384	5	TUBGCP5, CYFIP1, NIPA2, NIPA1	Dup	Unknown	VUS
		Xp11.22	50,056,856–50,674,794	618	4	SHROOM4	Dup	Unknown	VUS

F Female, M Male, ASD Autism Spectrum Disorder, ID Intellectual Disability, ADHD Attention Deficit Hyperactivity Disorder, DD Developmental Delay, Del Deletion, Dup Duplication, VUS Variants of unknown significance

of the second and third toes of both feet, and bilateral sandal gap deformity were observed. His karyotype analysis was normal. Array CGH revealed a duplication (6488 kb) in the 15q11.2–q13.1 region. The duplication included 135 genes, the most relevant of which were *ATP10A*, *CYFIP1*, *GABRB3*, *MAGEL2*, and *UBE3A*. Since the biological parents could not be reached, it was not possible to determine whether the CNV, which was classified as pathological, was inherited.

Case 3 was a 5-year-old (58-month-old) boy living with his biological parents. There was no consanguinity between the parents. The boy started to speak his first words at 20 months and simple, two-word sentences at around 30 months. His family referred to our clinic because of hyperactivity. He had severe tantrums, inappropriate facial expressions, restricted joint attention, excessive motor activity, delayed echolalia, and tactile hypersensitivity. He was using 1 mg of risperidone daily due to his behavioral problems, hyperactivity, and tantrums. He had been receiving special education for two years. He was diagnosed with mild to moderate autism according to the CARS (total score: 31.5). According to the ADOS, his general development age was 49–54 months, his language–cognitive development age was 45–48 months, his fine motor development age was 45–48 months, his gross motor development age was 61–66 months, and his social skills–self-care development age was 48–54 months. His development was found to be on par with that of his peers. He was diagnosed with concomitant ADHD. His weight was in the 99.4th percentile, his height was in the 89.5th percentile, and his head circumference was in the 98.5th percentile. He had no neurological deficits, and his EEG and MRI findings were normal. His phenotypic examination revealed syndactyly in the second and third toes of both feet and an Angelman phenotype. His karyotype analysis was normal. Array CGH revealed a deletion (598 kb) in the 16p11.2 region. The deletion included 31 genes, the most relevant of which were *TAOK2*, *KCTD13*, *MAPK3*, and *SEZ6L2*. The CNV, which was classified as pathogenic, was determined to be *de novo*.

Case 4 was a 4-year-old (45-month-old) boy living with his biological parents. There was third-degree consanguinity between the parents. The boy had a history of prenatal hypoxia. He started babbling after the age of 1 year, but language development stopped around the age of 2 years. His parents referred to our clinic with complaints of absence of speech. He had been receiving special education for two years. Limited emotional reactions, inadequacy in interaction, insistence on sameness, limitations in imitation skills, and repetitive movements were observed. He was diagnosed with severe autism according to the CARS (total score: 47.5). According to the ADOS, his general development age was 21–23 months, his language–cognitive development age was 9–11 months, his fine motor development age was 14–16 months, his gross motor development age was

36–38 months, and his social skills–self-care development age was 12–14 months. He was diagnosed with concomitant DD. His weight was in the 50th–75th percentiles, his height was in the 99th percentile, and his head circumference was in the 25th–50th percentiles. He had no neurological deficits, and his EEG and MRI findings were normal. Posterior rotated ears were observed on phenotypic examination. His karyotype analysis was normal. Array CGH revealed a deletion (55 kb) in the 22q13.33 region. The deletion included two genes, the most relevant of which was *SHANK3*. The CNV, which was classified as pathogenic, was determined to be *de novo*.

Case 5 was a 9-year-old (104-month-old) girl living with her biological parents. There was no consanguinity between the parents. The girl spoke her first words at the age of 3 years. The complaints upon referral to our clinic were limited eye contact, delayed language development, repetitive movements (hand clapping, head shaking), and self-harm. Due to her behavioral problems, she was using 10 mg of aripiprazole daily. She had been receiving special education for six years. She was diagnosed with severe autism according to the CARS (total score: 51.5). She was unable to perform the WISC-R test. Her level of intelligence was clinically determined to be consistent with moderate mental retardation. Her weight was in the 50th percentile, her height was in the 75th percentile, and her head circumference was in the 50th percentile. Her neurological examination showed hypotonia. Her EEG and MRI findings were normal. Arched eyebrows, synophrys, a high nasal bridge, a short philtrum, and clinodactyly of the fifth fingers of both hands were observed on phenotypic examination. Her karyotype analysis was normal. Array CGH showed a duplication (472 kb) in the 2q36.3 region. The deletion included four genes, the most relevant of which were *DNER* and *TRIP12*. Karyotype analysis and array CGH performed on both parents showed that the CNV, which was classified as a VUS, was inherited from her father.

Case 6 was a 7-year-old (84-month-old) boy living with his biological mother. There was no consanguinity between the parents. The boy spoke his first words when he was 14 months old and started using simple, two-word sentences at around 30 months. Upon referral to our clinic, the complaints were inappropriate gestures, difficulty holding conversations, limited interests, and insistence on routines. He had been receiving special education for five years. His total intelligence score according to the WISC-R test was 85, which was clinically consistent with ASD. He was diagnosed with mild to moderate autism according to the CARS (total score: 34). His weight was in the 96th percentile, his height was in the 99th percentile, and his head circumference was in the 33rd percentile. His neurological examination revealed no deficits. However, his EEG showed inappropriate discharges in the temporal region, and he was followed

up clinically for temporal lobe epilepsy. On MRI, hypoplasia was detected in the splenium part of the corpus callosum (CC). On phenotypic examination, anteverted ears and short thumbs were detected bilaterally. His karyotype analysis was normal. Array CGH revealed a duplication (209 kb) in the 10p11.21 region. The duplication included only the *PARD3* gene, which is not known to be associated with ASD. Karyotype analysis and array CGH were performed only on the mother. It could not be determined whether the CNV, which was classified as a VUS, was inherited.

Case 7 was a 3-year-old (39-month-old) boy living with his biological parents. There was no consanguinity between the parents. The parents referred to our clinic with complaints of language development delay. A lack of joint attention, limited emotional reactions, hyposensitivity to pain, and repetitive movements were observed. He was diagnosed with autism for the first time in our clinic. His total CARS score (48) indicated severe autism. According to the ADOS, his general development age was 12–14 months, his language–cognitive development age was 9–11 months, his fine motor development age was 9–11 months, his gross motor development age was 21–23 months, and his social skills–self-care development age was 9–11 months. He was clinically diagnosed with concomitant DD. His weight was in the 90th percentile, his height was in the 90th percentile, and his head circumference was in the 75th percentile. No neurological deficits were detected, and his EEG and MRI findings were normal. A phenotypic examination revealed no dysmorphic features. His karyotype analysis was normal. Array CGH revealed duplications in the 15q11.2 and Xp11.22 regions (384 kb and 618 kb, respectively). The duplication in the 15q11.2 region included five genes, the most relevant of which were *TUBGCP5*, *CYFIP1*, *NIPA2*, and *NIPA1*, whereas the duplication in the Xp11.22 region included four genes, the most relevant of which was *SHROOM4*. It was not possible to determine whether the CNV, which was classified as a VUS, was inherited, as the parents did not consent to undergo genetic testing.

Discussion

In this study, we performed an array CGH to evaluate the genetic construction of CNVs in Turkish children and adolescents diagnosed with ASD. We identified pathogenic CNVs in 8.5% of the children. This rate is consistent with previous studies, which have usually reported diagnostic yields of around 9% [7]. This study contributes to an increasing number of CNV studies on ASD cases in Turkey.

Case 1, diagnosed with concomitant moderate ID, had a large deletion in chromosome regions 9p24.3–p24.2 involving the *SMARCA2*, *DOCK8*, *RFX3*, and *KANK1* genes. Several studies have suggested that these genes

contribute to the ASD phenotype [16–19]. A previous study found that the deletion of *DOCK8* is associated with ID, DD, and/or ASD [20]. Another study reported that deleterious variations in *RFX3* in cases of monogenic ID, ADHD, and ASD may play a critical role in neurodevelopmental disease pathogenesis [18]. Vanzo et al. reported that paternally inherited *KANK1* deletions might be related to clinical features of ASD, motor delay, and ID [19]. Similarly *SMARCA2* mutations have been reported in several cases diagnosed with ID and ASD, to our knowledge, this is the first study to report a *SMARCA2* deletion in a case diagnosed with ASD and moderate ID [17]. Although our findings support a pathogenic role of the 9p24.3 deletion, we cannot draw conclusions regarding the specific contribution of the deleted genes to the phenotype of Case 1.

Case 2 carried a 6488-kb duplication at 15q11.2–q13.1 involving the 15q11–q13 duplication syndrome (Dup15q syndrome). This region contains several genes that are critical for brain development and synaptic function, especially *ATP10A*, *CYFIP1*, *GABRB3*, *MAGEL2*, and *UBE3A*, which have been associated with autism [21–24]. Dup15q syndrome is considered the most common CNV contributor to the etiology of ASD, accounting for an estimated 1–3% of all cases [25]. In most cases, the duplication is inherited from the mother or occurs de novo on the maternally inherited chromosome, with a broad spectrum of neurodevelopmental phenotypes [23]. In our case, it was not possible to examine the biological parents because the case was adopted. Among the dysmorphic features, we observed clinodactyly in the right hand and syndactyly and gap deformity in both feet, which have also been previously observed in Dup15q syndrome [26]. The *GABRB3* gene plays a role in GABAergic neurotransmission, and genetic variants within the GABA receptor gene complex at 15q11–q13 have been linked to epilepsy and ASD in both humans and animal models [24]. Therefore, it is plausible that the *GABRB3* gene duplication contributed to both ASD symptoms and focal epileptic disorder in Case 2.

Case 3 had a de novo deletion of at least 598 kb in the 16p11.2 region, which includes the *TAOK2*, *KCTD13*, *MAPK3*, and *SEZ6L2* genes. Deletions of 16p11.2 are de novo in approximately 80% of the cases [27]. Case 3 had obesity, macrocephaly, and syndactyly in the second and third toes of both feet. The case was diagnosed with concomitant ADHD, which is quite common in patients with 16p11.2 deletions (19–38%) [28]. Besides ASD, deletions in this region have been associated with macrocephaly, intellectual disability, schizophrenia, learning disabilities, DD, and behavioral problems [29]. Reciprocal 16p11.2 CNVs exhibit mirrored phenotypes: obesity/underweight and macrocephaly/microcephaly in deletion and duplication carriers, respectively [30, 31]. In line with previous reports, our

case showed phenotypic features such as behavioral problems, ADHD, obesity, macrocephaly, and ASD.

Case 4, diagnosed with concomitant DD, had a 58-kb deletion at 22q13.33 involving SH3 and multiple ankyrin repeat domain SHANK3. Deletions or sequence variants of the SHANK3 gene are required for the diagnosis of Phelan–McDermid Syndrome (PMS). As in our case, PMS patients often present with ID, ASD, dysmorphic facial features (posterior rotated ears), obesity, and severely delayed or absent speech [32].

Any variant for which there is evidence both for and against pathogenicity is classified as a VUS according to the ACMG guidelines [15]. In this study, we detected four VUS in three cases. A paternally inherited 472-kb duplication at 2q36.3 involving the *DNER* and *TRIP12* genes was identified in Case 5. A previous study identified four *TRIP12* de novo single-nucleotide variants among 64 candidate genes for ASD that were re-sequenced in 5979 individuals, as well as two missense and two truncating variants in individuals with mild to moderate ASD and ID [33]. As in Case 5 in our study, studies providing detailed clinical information on cases with *TRIP12* mutations found that autism was accompanied by mild ID, hypotonia, arched eyebrows, synophrys, a high nasal bridge, a short philtrum, and clinodactyly [34–36]. Although it is unknown whether the VUS duplication associated with *TRIP12* contributed to the clinical features of Case 5, we suggest that this gene may be a candidate gene for autism. Further investigations are required to determine the clinical significance of this duplication.

In Case 6, we identified a de novo 209-kb duplication at 10p11.21 harboring only one gene, *PARD3*, which encodes the polarity protein partitioning defective 3 homolog (Pard-3) [37]. Cell polarity has a key role for maintaining several structural tasks such as, asymmetric cell division, cell migration and neuronal parts specification [38]. An association has been found between defects in cell polarity and diseases such as schizophrenia, autism, and epilepsy, whose pathophysiology is assumed to impair neuronal or synaptic development [39]. In Case 6, who had abnormal activities originating in the temporal lobe were detected on EEG, and CC hypoplasia was detected on cranial MRI. Moreover, obesity, bilateral anteverted ears and short thumbs were observed. CC hypoplasia is defined as a CC that is fully formed but thinner than expected for the person's age and gender [40]. One of the most consistent findings in MRI studies on individuals with autism is a reduction or thinning of the CC [41]. Although CC hypoplasia has been reported in children with 16p11.2 deletions and duplications [42], to our knowledge, our study is the first to report an association between *PARD3* duplication and CC hypoplasia. *PARD3* has been shown to play a role in cortical neurogenesis in mice by regulating the balance between proliferation and differentiation [43].

Moreover several studies have suggested that *PARD3* variants may be associated with ASD [44, 45]. In light of previous findings, we suggest that the duplication of *PARD3* in Case 6 may be linked to newly identified phenotypical features. Future studies should comprehensively investigate the possible association between *PARD3* duplication and ASD.

In Case 7, who had concomitant DD, we identified two VUS duplications in the 15q11.2 and Xp11.22 regions, sized 384 kb and 618 kb, respectively. The 15q11.2 region includes *TUBECP5*, *CYFIP1*, *NIPA2*, and *NIPA1*, all of which are variously involved in axonal growth and neural connectivity [46]. Previous studies have reported cases of 15q11.2 microduplications related to ASD, DD, and epilepsy [46, 47]. Moreover, duplications of *SHROOM4*, which encodes a regulator of cytoskeletal architecture, have been suggested as candidates for DD [48]. In a previous study, a case with *SHROOM4* duplication, diagnosed with ASD and DD, was reported, similar to the phenotypic characteristics of the Case 7 [49]. VUS not only adds the current knowledge, but also proposes potential risk factors for ASD. Therefore, our results will contribute to interpreting the association of *TUBECP5*, *CYFIP1*, *NIPA2*, *NIPA1* and *SHROOM4* variants with ASD in future studies.

This study has certain limitations. The first limitation is the relatively small sample size. Second, as few parents consented to undergo array CGH testing, information about inheritance was not sufficient to help in the interpretation of CNVs, particularly VUS. The reason for the parents' reluctance may have been a failure to explain to them the purpose of the test in an appropriate manner. Another limitation of our study is that we did not use control CNV data from normal individuals in the same population. Our results should be validated by a multi-center study with a larger Turkish ASD cohort and CNV data from population-matched controls for interpretation.

Conclusion

In this study, we sought to comprehensively describe the phenotypic characteristics of children with ASD in whom either pathogenic CNVs or VUS were detected. The diagnostic yield of the CMAs was 8.5%. Our results suggest that the *TRAP12* and *PARD3* genes in CNVs classified as VUS may be worth investigating for ASD. The initial identification of both clinical and biological markers may facilitate monitoring, early intervention, or prevention and advance our understanding of the neurobiology underlying ASD.

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Author contribution Conceptualization: Eİ, FEP, MAE, EG. Data acquisition: AÖ, Eİ, EG. Formal analysis: AÖ, GK; Writing—original draft preparation: AÖ; Writing—review and editing: AÖ, GK, Eİ, MAE, EG, FEP. Supervision: Eİ, MAE, EG, FEP.

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Data availability The data sets used in this study can be obtained from the corresponding author upon reasonable request.

Declarations

Conflicts of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Consent to participate Informed consent was obtained from all participants included in the study.

Ethical approval This study was approved by the Clinical Research Ethics Committee of Gazi University's Faculty of Medicine (No. 431).

References

- American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders (5th edn.). <https://doi.org/10.1176/appi.books.9780890425596>.
- Wiśniowiecka-Kowalik B, Nowakowska BA (2019) Genetics and epigenetics of autism spectrum disorder: current evidence in the field. *J Appl Genet*. <https://doi.org/10.1007/s13353-018-00480-w>
- Maenner MJ, Shaw KA, Baio J, et al. (2020) Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2016. *MMWR Surveill*. <http://dx.doi.org/https://doi.org/10.15585/mmwr.ss6904a1>
- Vicari S, Napoli E, Cordeddu V et al (2019) Copy number variants in autism spectrum disorders. *Prog Neuropsychopharmacol Biol Psychiatry* 92:421–427. <https://doi.org/10.1016/j.pnpbp.2019.02.012>
- Takumi T, Tamada K (2018) CNV biology in neurodevelopmental disorders. *Curr Opin Neurobiol* 48:183–192. <https://doi.org/10.1016/j.conb.2017.12.004>
- Huguet G, Bourgeron T (2013) The genetic landscapes of autism spectrum disorders. *Annu Rev Genomics Hum Genet* 14:191–213. <https://doi.org/10.1146/annurev-genom-091212-153431>
- Hyman SL, Levy SE, Myers SM (2020) Identification, evaluation, and management of children with autism spectrum disorder. *Pediatrics*. <https://doi.org/10.1542/peds.2019-3447>
- Miller DT, Adam MP, Aradhya S et al (2010) Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet* 86(5):749–764. <https://doi.org/10.1016/j.ajhg.2010.04.006>
- Carter MT, Scherer SW (2013) Autism spectrum disorder in the genetics clinic: a review. *Clin Genet* 83(5):399–407. <https://doi.org/10.1111/cge.12101>
- Luo R, Sanders SJ, Tian Y et al (2012) Genome-wide transcriptome profiling reveals the functional impact of rare de novo and recurrent CNVs in autism spectrum disorders. *Am J Hum Genet* 91(1):38–55. <https://doi.org/10.1016/j.ajhg.2012.05.011>
- Woodbury-Smith M, Scherer SW (2018) Progress in the genetics of autism spectrum disorder. *Dev Med Child Neurol* 60(5):445–451. <https://doi.org/10.1111/dmcn.13717>
- Sucuoglu B, Oktem F, Akkok F, Gokler B (1996) A study of the scales for the assessment of the children with autism. *Psikiyatri Psikoloji Psikofarmakoloji* 3P(4):116–121
- Savasir I, Sezgin N, Erol N (1994) Handbook of Ankara developmental screening inventory. Turkish Psychologists Association Publication, Ankara
- Savaşır I, Şahin N (1995) Wechsler çocuklar için zeka ölçeği (WISC-R) el kitabı. Türk Psikologlar Derneği Yayınları, Ankara
- Riggs ER, Andersen EF, Cherry AM et al (2020) Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genet Med* 22(2):245–257. <https://doi.org/10.1038/s41436-019-0686-8>
- Capkova P, Srovnal J, Capkova Z et al (2019) MLPA is a practical and complementary alternative to CMA for diagnostic testing in patients with autism spectrum disorders and identifying new candidate CNVs associated with autism. *PeerJ*. <https://doi.org/10.7717/peerj.6183>
- Xiong J, Chen S, Pang N et al (2019) Neurological diseases with autism spectrum disorder: role of ASD risk genes. *Front Neurosci* 13:349. <https://doi.org/10.3389/fnins.2019.00349>
- Harris HK, Nakayama T, Lai J et al (2021) Disruption of RFX family transcription factors causes autism, attention-deficit/hyperactivity disorder, intellectual disability, and dysregulated behavior. *Genet Med* 23:1028–1040. <https://doi.org/10.1038/s41436-021-01114-z>
- Vanzo RJ, Martin MM, Sdano MR, South ST (2013) Familial KANK1 deletion that does not follow expected imprinting pattern. *Eur J Med Genet* 56(5):256–259. <https://doi.org/10.1016/j.ejmg.2013.02.006>
- Benítez-Burraco A, Fernández-Urquiza M, Jiménez-Romero MS (2020) Language impairment with a partial duplication of DOK8. *Mol Syndromol* 11(5–6):243–263. <https://doi.org/10.1159/000511972>
- Guffanti G, Strik Lievers L, Bonati MT et al (2011) Role of UBE3A and ATP10A genes in autism susceptibility region 15q11-q13 in an Italian population: a positive replication for UBE3A. *Psychiatry Res* 185(1–2):33–38. <https://doi.org/10.1016/j.psychres.2010.04.057>
- Sledziowska M, Galloway J, Baudouin J (2020) Evidence for a contribution of the *Nlgn3/Cyfp1/Fmr1* pathway in the pathophysiology of autism spectrum disorders. *Neuroscience* 445:31–41. <https://doi.org/10.1016/j.neuroscience.2019.10.011>
- Al Ageeli E, Drunat S, Delanoë C et al (2014) Duplication of the 15q11-q13 region: clinical and genetic study of 30 new cases. *Eur J Med Genet* 57(1):5–14. <https://doi.org/10.1016/j.ejmg.2013.10.008>
- Buxbaum JD, Silverman JM, Smith CJ et al (2002) Association between a GABRB3 polymorphism and autism. *Mol Psychiatry* 7(3):311–316. <https://doi.org/10.1038/sj.mp.4001011>
- Depienne C, Moreno-De-Luca D, Heron D et al (2009) Screening for genomic rearrangements and methylation abnormalities of the 15q11-q13 region in autism spectrum disorders. *Biol Psychiatry* 66(4):349–359. <https://doi.org/10.1016/j.biopsych.2009.01.025>
- Wisniewski L, Hassold T, Heffelfinger J, Higgins JV (1979) Cytogenetic and clinical studies in five cases of inv dup(15). *Hum Genet* 50(3):259–270. <https://doi.org/10.1007/BF00399391>
- Miller DT, Chung W, Nasir R et al (2009) 16p11.2 recurrent microdeletion. [Updated 2015 Dec 10]. In: Adam MP, Ardinger HH, Pagon RA et al (eds) GeneReviews® [Internet]. University of Washington, Seattle, Seattle (WA)

28. Niarchou M, Chawner SJRA, Doherty JL et al (2019) Psychiatric disorders in children with 16p112 deletion and duplication. *Transl Psychiatry* 9(1):8. <https://doi.org/10.1038/s41398-018-0339-8>
29. Rein B, Yan Z (2020) 16p11.2 copy number variations and neurodevelopmental disorders. *Trends Neurosci* 43(11):886–901. <https://doi.org/10.1016/j.tins.2020.09.001>
30. Jacquemont S, Reymond A, Zufferey F et al (2011) Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. *Nature* 478(7367):97–102. <https://doi.org/10.1038/nature10406>
31. Qureshi AY, Mueller S, Snyder AZ et al (2014) Opposing brain differences in 16p11.2 deletion and duplication carriers. *J Neurosci* 34:11199–11211. <https://doi.org/10.1523/JNEUROSCI.1366-14.2014>
32. De Rubeis S, Siper PM, Durkin A et al (2018) Delineation of the genetic and clinical spectrum of Phelan-McDermid syndrome caused by SHANK3 point mutations. *Mol Autism* 9:31. <https://doi.org/10.1186/s13229-018-0205-9>
33. O’Roak BJ, Stessman HA, Boyle EA et al (2014) Recurrent de novo mutations implicate novel genes underlying simplex autism risk. *Nat Commun* 5:5595. <https://doi.org/10.1038/ncomms6595>
34. Bramswig NC, Lüdecke HJ, Pettersson M et al (2017) Identification of new TRIP12 variants and detailed clinical evaluation of individuals with non-syndromic intellectual disability with or without autism. *Hum Genet* 136(2):179–192. <https://doi.org/10.1007/s00439-016-1743-x>
35. Zhang J, Gambin T, Yuan B et al (2017) Haploinsufficiency of the E3 ubiquitin-protein ligase gene TRIP12 causes intellectual disability with or without autism spectrum disorders, speech delay, and dysmorphic features. *Hum Genet* 136(4):377–386. <https://doi.org/10.1007/s00439-017-1763-1>
36. Donoghue T, Garrity L, Ziolkowski A, McPhillips M, Buckman M, Goel H (2020) Novel de novo TRIP12 mutation reveals variable phenotypic presentation while emphasizing core features of TRIP12 variations. *Am J Med Genet Part A* 182A:1801–1806. <https://doi.org/10.1002/ajmg.a.61618>
37. Shi SH, Jan LY, Jan YN (2003) Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. *Cell* 112(1):63–75. [https://doi.org/10.1016/s0092-8674\(02\)01249-7](https://doi.org/10.1016/s0092-8674(02)01249-7)
38. Hakanen J, Ruiz-Reig N, Tissir F (2019) Linking cell polarity to cortical development and malformations. *Front Cell Neurosci* 13:244. <https://doi.org/10.3389/fncel.2019.00244>
39. Sterling N, Duncan AR, Park R et al (2020) De novo variants in MPP5 cause global developmental delay and behavioral changes. *Hum Mol Genet* 29(20):3388–3401. <https://doi.org/10.1093/hmg/ddaa224>
40. Paul LK, Brown WS, Adolphs R et al (2007) Agenesis of the corpus callosum: genetic, developmental and functional aspects of connectivity. *Nat Rev Neurosci* 8(4):287–299. <https://doi.org/10.1038/nrn2107>
41. Wegiel J, Flory M, Kaczmarek W et al (2017) Partial Agenesis and Hypoplasia of the Corpus Callosum in Idiopathic Autism. *J Neuropathol Exp Neurol* 76(3):225–237. <https://doi.org/10.1093/jnen/nlx003>
42. Steinman KJ, Spence SJ, Ramocki MB et al (2016) 16p112 deletion and duplication: characterizing neurologic phenotypes in a large clinically ascertained cohort. *Am J Med Genet A* 170(11):2943–2955. <https://doi.org/10.1002/ajmg.a.37820>
43. Liu WA, Chen S, Li Z et al (2018) PARD3 dysfunction in conjunction with dynamic HIPPO signaling drives cortical enlargement with massive heterotopia. *Genes Dev* 32(11–12):763–780. <https://doi.org/10.1101/gad.313171.118>
44. Chen CH, Chen HI, Chien WH et al (2017) High resolution analysis of rare copy number variants in patients with autism spectrum disorder from Taiwan. *Sci Rep*. <https://doi.org/10.1038/s41598-017-12081-4>
45. Kosmicki JA, Samocha KE, Howrigan DP et al (2017) Refining the role of de novo protein-truncating variants in neurodevelopmental disorders by using population reference samples. *Nat Genet* 49(4):504–510. <https://doi.org/10.1038/ng.3789>
46. Picinelli C, Lintas C, Piras IS et al (2016) Recurrent 15q112 BP1-BP2 microdeletions and microduplications in the etiology of neurodevelopmental disorders. *Am J Med Genet B* 171(8):1088–1098. <https://doi.org/10.1002/ajmg.b.32480>
47. Lusk L, Vogel-Farley V, DiStefano C, Jeste S et al (2016) Maternal 15q duplication syndrome. In: Adam MP et al (eds) *GeneReviews®*. University of Washington, Seattle
48. Evers C, Mitter D, Strobl-Wildemann G et al (2015) Duplication Xp1122-p14 in females: does X-inactivation help in assessing their significance? *Am J Med Genet A* 167A(3):553–562. <https://doi.org/10.1002/ajmg.a.36897>
49. Froyen G, Van Esch H, Bauters M et al (2007) Detection of genomic copy number changes in patients with idiopathic mental retardation by high-resolution X-array-CGH: important role for increased gene dosage of XLMR genes. *Hum Mutat* 28(10):1034–1042. <https://doi.org/10.1002/humu.20564>

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