A Study from Turkey: Identification of Copy Number Variants in Children and Adolescents with Autism Spectrum Disorder

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Abstract

Recent studies suggest that copy number variations (CNVs) play a significant role in the aetiology of ASD. This study aims to investigate CNVs, which are thought to be an important factor in ASD etiology. In addition it was aimed to specify the clinical usefulness of chromosomal microarrays (CMA) in the examination of ASD patients in Turkish population. Of 47 children (60.34±25.60 months; 82.9% boys) with ASD were constructed the sample. The karyotype structure of all participants was found to be normal using conventional cytogenetic methods. DNA obtained from the venous blood samples of the participants was evaluated using SurePrint G3 ISCA V2 CGH 8x60K Array (Agilent Technologies Santa Clara, CA, USA). We have identified 8 CNVs, ranging in size from 55 kb to 6.5 Mb in 7 (5 boys) of 47 children with ASD of the 4 of 8 CNVs were classified as pathogenic, which were 9p24.3p24.2 deletion in 3 Mb size, 15q11-q13 duplication in 6.5 Mb size, 16p11.2 deletion in 598 kb size and 22q13.3 deletion in 55 kb size. According to results has been demonstrated that diagnostic yield of CMA in Turkish children with ASD was 8.5%. Our results indicate that CNVs contribute a part to the genetic aetiology of Turkish children with ASD. In accordance with the literature, these results emphasize the clinical importance of CMA to investigate the aetiology of ASD.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that causes functional impairment in the social, educational and occupational domains due to the difficulties in social communication and interaction, and restrictive and repetitive behaviour patterns, interests, activities and sensory processing anomalies (Association 2013). ASD is one of the most common groups of neurodevelopmental disorder that affect about 1–2% of the population, and the average male/female ratio is 4:1 (Wiśniowiecka-Kowalnik and Nowakowska 2019). ASD has a clinical presentation with a wide range of symptoms and severity. Moreover, Common co-occurring psychiatric disorders with autism include attention deficit hyperactivity disorder (ADHD), intellectual disability (ID), anxiety disorders, depression and sleep disorders (Baio et al. 2018). Studies focusing on the pathophysiology of autism have suggested several hypotheses, including genetic, epigenetic and environmental factors. (Bertoglio and Hendren 2009). Heritability in autism is estimated to be as high as 90%, but in many cases it has not been clearly defined due to heterogeneous and complex genetic factors (Stefano et al. 2019). The greatest improvement in identifying genetic causes of autism has been through the identification of known genetic mutations and disorders that may predispose to the development of autism (Miles 2011). In 20–25% of children with ASD, a genetic cause can be identified by using standard medical genetic evaluation techniques. This number increased with the use of chromosomal microarrays (CMA) (Eriksson et al. 2015). CMA is a powerful tool for genome-wide detection. It provides an advanced, high-resolution chromosome evaluation that can detect submicroscopic chromosomal rearrangements larger than 20–100 kb (Velinov 2019). Therefore, CMA has been proposed as a first-line diagnostic tool for patients with unexplained developmental delay/intellectual disability, ASD, and multiple congenital anomalies (Schaefer and Mendelsohn 2013). CMA currently identifies copy number variants (CNVs), which are DNA duplications or deletions that change the function of genes. CMA shows that 5.4–14% of individuals with ASD have a definite pathogenic CNV. The most common pathogenic CNVs identified with ASD are 16p11.2 deletion and duplication, 15q11.2-q13 duplication, 15q13.2-q13.3, 1q21.1 duplication, 22q11.2, 16p13.11 deletions, 7q11.23 duplication, 16p12.2 deletion and 17q12 deletion. Approximately 15–42% of patients with ASD have findings on the CMA if CNVs of uncertain significance are included (Hyman et al. 2020). Determining a genetic aetiology provides clinicians with more information about prognosis and risk of recurrence. Moreover, it can help identify and treat or prevent emerging medical conditions. In this way, it directs patients and families to the condition-specific resources and supports and can help avoid unnecessary testing (Hyman et al. 2020).

Researching and reporting data from CMA (Stefano et al. 2019; Velinov 2019; Wiśniowiecka-Kowalnik and Nowakowska 2019) are important to identify the aetiology of ASD and identify new syndromes. However, there is still a relevant pathophysiological gap between ASD and CMA findings. In this study, we aimed to determine the clinical usefulness of CMA in the evaluation of ASD patients in Turkish population. Array based comparative genomic hybridization (CGH) is a platform used to identify CNV in a Turkish patient with ASD.

Methods

The study was planned as a retrospective-cross-sectional study. The sample was consisted of 47 children (82.9% boys) with a mean age of 60.34 months (*SD* = 25.60) who applied to the Gazi University Faculty of Medicine Child and Adolescent Psychiatry Outpatient Clinic with ASD symptoms (e.g., poorly integrated verbal and non-verbal communication, simple motor stereotypies) between August 2017 and August 2018 in Ankara. DSM-5 criteria were used for collecting multiple source of information on the symptoms of ASD. In addition, the autistic symptoms of these children were assessed with Childhood Autism Rating Scale (CARS). CARS is a 15- item behaviour-rating scale designed to detect and quantify symptoms of autism as well as to distinguish them from other developmental disabilities. Each item on the CARS is scored on a Likert scale, from 1 (no signs of autism) to 4 (severe symptoms). Good evidence of reliability and validity has been reported in Turkish children with autism (Sucuoglu et al. 1996). The final diagnosis of ASD was given according to DSM-5 criteria by another researcher, who had at least five years of experience working with children and adolescents with ASD. Developmental delay in children with ASD were evaluated using Ankara Developmental Screen Inventory (ADSI), was developed by Savasır et al. (1998) and had been reported that the reliability and validity of the inventory was sufficient for Turkish Children. ADSI consists of 154 items that are answered as "Yes-No-I don't know" by asking their mothers in order to evaluate the social, motor, cognitive and communicative development levels of children in the 0–6 age group. The questions are organized to represent different but related areas of development (Language-Cognitive, Fine Motor, Gross Motor, Social Skills-Selfcare. The total development score reflects the child's general developmental level and is obtained from the sum of four subscales (language-cognitive development, fine motor development, gross motor development, social interaction subscales) (Savasir et al. 1994). While a clinical diagnosis of intellectual disability (ID) was made by two different child and adolescent psychiatry specialists in patients who could not comply with the test, ID was evaluated with the Wechsler Intelligence Scale for Children-Revised (WISC-R) in patients with compliance. Good reliability and validity evidence for the WISC-R have been reported in Turkish population (Savaşır and Şahin 1995). After explaining the aim and method of the study verbally, a written informed consent form and genetic consent form were obtained from all parents of participants who agreed to participate in the study. After the diagnosis of ASD all children who agreed to participate in the study were referred to Gazi University Medical Genetics Department for genetic examination.

Dysmorphic examinations were performed in all cases referred to the genetic department in terms of possible syndromes accompanying ASD before karyotype analysis. Then karyotype analysis was performed. All patients had normal karyotype structure in terms of numerical and structural chromosomal abnormalities that can be detected with conventional cytogenetic methods. CMA was performed from DNA obtained from the peripheral blood samples of the patients using the SurePrint G3 ISCA V2 CGH 8x60K Array (Agilent Technologies Santa Clara, CA, USA). The obtained data were analysed according to human genome 19 (Chr37) using Agilent CytoGenomics software. For the interpretation of the CNVs, Database of Genomic Variants (DGV) (www.dgv.tcag.ca), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), UCSC Genome Bioinformatics (https://genome.ucsc.edu), Decipher (Database of Chromosome Imbalance and Phenotype in Humans using Ensembel Resources) (https://decipher.sanger.ac.uk), PubMed (The U.S. National Library of Medicine) (https://www.ncbi.nlm.nih.gov/pubmed), OMIM (Online Mendelian Inheritance of Man) (https://www.ncbi.nlm.nih.gov/omim) and also in house databases were used. The CNVs were classified as 1. Benign, 2. Likely benign, 3. Unknown clinical significance, 4. Likely pathogenic, 5. Pathogenic (Kearney et al. 2011). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Results

We have identified 8 CNVs, ranging in size from 55 kb to 6.5 Mb in 7 (5 boys) of 47 children with ASD. The CNV detection rates in boys and girls with ASD were 12.8 and 28.5% respectively. Of the seven children with CNV with autism, three were accompanied by ID, two with global developmental delay and one with attention deficit hyperactivity disorder ADHD. Table 1 shows the summary of the sample characteristics and CNV findings. Of the 8 CNVs detected, 4 were classified as pathogenic, which accounts for 8.5% of the entire sample. Pathogen CNVs, which were 9p24.3p24.2 deletion in 3 Mb size [arr(hg19) 9p24.3p24.2(204193–3221675)x1], 15q11-q13 duplication in 6.5 Mb size [arr(hg19) 15q11.2q13.1(22765628–29253376)x3], 16p11.2 deletion in 598 kb size [arr(hg19) 16p11.2(29,592,783 – 30,190,568)x1] and 22q13.3 deletion in 55 kb size [arr(hg19) 22q13.33(51123491–51178264)x1] (Table 1). In 3 children, four variants of unknown significance were detected and one was shown to be inherited from paternal. Parental studies have not been performed in the other two patients.

Table 1
CNVs in the children with ASD

Patient No	Gender/Age	Diagnosis	Cytoband	Start-stop (bp)(hg19)	Size (kb)	Genes	Del /Dup	Inhe
Pt 2	F/9	ASD + mild ID	2q36.3	230479578–230951771	472	DNER, TRIP12, FBXO36, SLC16A14	Dup	Pate
Pt 6	F/6	ASD + moderate ID	9p24.3-p24.2	204193–3221675	3,017	DOCK8,KANK1,DMRT1,DMRT2, SMARCA2,VLDLR,KCNV2, KIAA0020, C9orf66,DMRT3,FLJ35024	Del	Mat (fro mild affe mot
Pt 7	M/4	ASD + DD	22q13.33	51123491–51178264	55	SHANK3, ACR	Del	Unk
Pt 23	M/7	ASD	10p11.21	35072047–35281153	209	PARD3	Dup	Unk
Pt 24	M/9	ASD + ID	15q11.2-q13.1	22765628–29253376	6,488	TUBGCP5,CYFIP1,NIPA2,NIPA1,MKRN3,MAGEL2,NDN,C15orf2, SNRPN, PAR5, IPW, PAR1, PAR4, UBE3A…	Dup	Unk
Pt 29	M/3	ASD + DD	15q11.2	22833122–23217514	384	TUBGCP5, CYFIP1, NIPA2, NIPA1, WHAMML1	Dup	Unk
			Xp11.22	50056856–50674794	618	SHROOM4, LOC347376, BMP15, CCNB3, DGKK	Dup	Unk
Pt 36	M/5	ASD + ADHD	16p11.2	29,592,783 – 30,190,568	598	SPN, QPRT, KIF22, MAZ, C16orf53, MVP, KCTD13, DOC2A, ALDOA, PPP4C, TBX6, YPEL3, MAPK3, SLC7A5P1, C16orf54, ZG16, PRRT2, CDIPT, LOC440356, SEZ6L2, ASPHD1, TMEM219, TAOK2, HIRIP3, INO80E, C16orf92, FAM57B, GDPD3, LOC100271831	Del	De n

Pt: Patient, Del: Deletion, Dup: Duplication, VUS: Variant of unknown significance

Discussion

In this study, copy number variants of children and adolescents diagnosed with ASD utilizing array CGH were determined. This study is to our knowledge the second one in Turkey, contribute to the increasing number of CNV studies conducted with ASD patient. We identified pathogenic CNV in 8.5% of children with ASD using the array CGH. The overall diagnostic yield in our study was 15% which was consistent with other studies (Görker et al. 2018; Siu et al. 2016).

Patient 6 with ASD and moderate ID had a large deletion at chromosome 9p24.3-p24.2 involving the DOCK8, KANK1, DMRT1, DMRT2 and SMARCA2 genes where various studies suggested that, contribute to the autistic spectrum disorder phenotype (Szatmari et al. 2007; Vanzo et al. 2019). The DMRT1, DMRT2, and DMRT3 genes are shown to be significant for sexual determination and development while DOCK8 and KANK1 genes have been shown that expressed in fetal brain tissue and to be involved in neurodevelopment. It has been reported that deletion in DOCK8 significant for intellectual disability, developmental

delay, and/or ASD (Coe et al. 2014). Vanzo et al. (2019) reported that paternally inherited KANK1 deletion might be related clinical features of ASD, motor delay, and ID (Vanzo et al. 2013). Another study suggested that KANK1 is maternally imprinted and deletion of the paternal copy of the KANK1 gene at 9p24.3 was associated with congenital cerebral palsy (Lerer et al. 2005). On the other hand many study suggests that KANK1 deletions are not clinically relevant for cerebral palsy (CP) ASD, or any other phenotype (Coe et al. 2014; MacDonald et al. 2014; Wallis et al. 2020). Although our patient, who had KANK1 deletion inherited by the mother, had diagnoses of moderate ID and ASD, she exhibited no features of CP. Heterozygous variants of SMARCA2 gene caused Nicolaides-Baraitser syndrome (NCBRS) characterized by mental retardation, seizures and short stature (Van Houdt et al. 2012). However, the relationship of the entire gene deletion of SMARCA2 with NCBRS is not clear. Although our results supports evidence of the pathogenic role of the 9p24.3 deletion, we are not able to arrive at any conclusion on determining specific contribution of deletion genes to the phenotype of our patient.

In patient 7 with ASD and global development delay, we have found a 58kb deletion in 22q13.33, involving the gene SH3 and multiple ankyrin repeat domain SHANK3 which encodes a scaffolding protein that actively roles in neuronal development (Naisbitt et al. 1999). SHANK3 is highly expressed in brain regions, including the striatum and medial prefrontal cortex, which are responsible for the regulation of emotional and social behaviours and reward systems. (Herbert 2011; Jin et al. 2018). Deletions or sequence variants of the SHANK3 gene required for the diagnosis of Phelan McDermid Syndrome (PMS) are associated with the resulting neuropsychiatric symptoms (De Rubeis et al. 2018; Sorte et al. 2013). ID, ASD features, hypotonia, epilepsy and severely delayed or absent speech are often exhibited in patient with PMS (Kolevzon et al. 2014; Sarasua et al. 2014). In our patient, developmental delay (DD) accompanied autism, but there was no epileptic disorder detected so far. PMS has also been suggested as a risk factor for bipolar disorder and its associated cognitive and behavioural regression (Kohlenberg et al. 2020; Verhoeven et al. 2020). We detected that this deletion may cause to the ASD and DD seen in patient 7. Identification of initially both clinical and biological markers will potentially facilitate in monitoring, early intervention or prevention and promote to our understanding of the neurobiology underlying these disorders.

Patient 24 carried a 6,488 kb duplication on the long arm of chromosome 15 (15q11.2q13.1), involving the 15q11-q13 duplication syndrome (Dup15q syndrome). Duplication of this region is associated with incomplete penetration and various clinical depicts, often including ASD, ID, epilepsy, DD, and behavioural problems (Napoli et al. 2018). Dup15q syndrome is generally considered to be the most recurrent copy number variant in its aetiology in ASD, and it accounts for an estimated 1–3% of all cases (Depienne et al. 2009). This region contains several genes that are critical for brain development and synaptic function, particularly including Gamma-Aminobutyric Acid Type A Receptor Subunit Beta-3 (GABRB3) and Ubiquitin Protein Ligase E3A (UBE3A) (DiStefano et al. 2016). The GABRB3 gene was shown that plays a role in GABAergic neurotransmission and also were assumed that genetic variants within the GABA receptor gene complex at 15q11-q13 might play a role in ASD (Buxbaum et al. 2002). Ubiquitin protein ligase is encoded from UBE3A and is making a major contribution in regulating synaptic function and development (Saravanapandian et al. 2020). Moreover UBE3A modulates synaptic plasticity, learning and memory formation (Sun et al. 2015). Previous studies have indicated that the UBE3A gene is an important candidate gene for autism [45], consistent with the literature, it is plausible that duplication of these genes (GABRB3 and UBE3A) contributes to ASD symptoms in patient 24.

Patient 36 was found to have a deletion of at least 598 kb within the 16p11.2 region. We examined 16p11.2 microdeletion in both parents and it was found that this deletion in the patient was de-novo. Most often, deletions of 16p11.2 are de novo, as reported in our study; it was estimated that approximately to 75% of the children with 16p11.2 deletion did not inherit (Miller et al. 2015). CNVs involving region 16p11.2 has repeatedly been reported in approximately 1% of people with a diagnosis of ASD (Steinman et al. 2016). As in our patient, ADHD is quite common in those with 16p11.2 deletion (19% -38%) (Niarchou et al. 2019). Beyond the ASD, deletions in this region have been associated with macrocephaly, intellectual disability, schizophrenia, learning disabilities, developmental delays and behavioural problems. Although there is strong evidence that carriers of 16p11.2 deletions are at increased risk of psychiatric disorders, 16p11.2 deletions are also shown to be associated with early-onset obesity (Bachmann-Gagescu et al. 2010; Goldenberg 2018; Steinman et al. 2016). The patient, whose de-novo deletion was detected at 16p11.2, had no dysmorphic appearance, the developmental stages evaluated by ADSI were appropriate for her age, only hyperactivity and destructive behaviours were observed. Despite in our patient's body mass index was in the normal range, for this patient and others with this microdeletion, attention should be paid to suspicious for obesity.

If a CNV is not known to be associated with the abnormal phenotype and does not encompass genes known to be associated with genetic conditions, it is typically reported as a variants of uncertain clinical significance (VOUS) by the testing laboratory (Kearney et al. 2011). In this study, VOUS accounted for 50 % of all CNV detected. In this study, a paternally inherited 472-kb duplication in 2q36.3, encompassing DNER, TRIP12, FBXO36 and SLC16A14 genes, has been identified in a 9-year old girl with ASD and mild ID. TRIP12, which encodes a member of the HECT domain E3 ubiquitin ligase family, related CNVs have been reported in current studies. In one of these, a *de novo* ~ 5.4 Mb CNV deletion at 2q36.2q36.3 was specified in a person with severe intellectual disability (Doco-Fenzy et al. 2008). Another study identified a de novo ~ 60 kb deletion containing the first non-coding exon of TRIP12 and FBXO36 in a patient with ASD (Pinto et al. 2014). In recent study, four TRIP12 de novo single nucleotide variants have been identified among 64 candidate genes for autism spectrum disorder (ASDs) that were re-sequenced in 5,979 individuals, and two missense and two truncating variants were identified in individuals with mild to moderate ASD and ID (O'roak et al. 2014). E3 ubiquitin ligase TRIP12 has crucial role both nervous system development and function, and TRIP12 haploinsufficiency lead to neurodevelopmental disorders beginning in childhood (Zhang et al. 2017). It is unknown whether the clinical features of patient 2 are due to duplication classified as VOUS, which was found to be associated with the TRAP12 gene. Further investigations are required to determine the clinical significance of this duplication.

In patient 23, we have identified a de novo 209-kb duplication in 10p11.21, harbouring only one gene PARD3 that encodes polarity protein partitioning defective 3 homolog (Pard-3), a regulator of cell polarity (Shi et al. 2003). Cell polarity is very important in maintaining various structural functions such as cell migration, axon and dendrite specification, and asymmetric cell division (Hakanen et al. 2019). The association between defects in cell polarity and diseases such as schizophrenia, autism, and epilepsy, which are assumed to be impaired neuronal or synaptic development in their pathophysiology, is shown (Sterling et al. 2020). For example, a study conducted with schizophrenia patients in Korea showed an association between schizophrenia and PARD3 gene polymorphisms (Kim et al. 2012). Moreover five study were demonstrated six variants linking PARD3 to ASD (Chen et al. 2017; Griswold et al. 2015; Iossifov et

al. 2014; Kosmicki et al. 2017; Wang et al. 2016). In the light of previous findings we suggest that despite being of VOUS, this duplication linking PARD3 may might still deserve further investigations for any possible association with ASD.

In patient 29, we identified two duplications which are classified VOUS, in the 15q11.2 and Xp11.22 regions, sized 384 kb and 618 kb, respectively. 15q11.2 regions contains four evolutionarily conserved non imprinted genes: TUBGCP5, CYFIP1, NIPA2, and NIPA1 which are all variously involved in axonal growth and neural connectivity (Picinelli et al. 2016). Several publications have described patients with 15q11.2 micro duplications involving this region related with ASD, DD, motor and/or expressive language delay, epilepsy and learning disabilities (Finucane et al. 2016; Picinelli et al. 2016). Additionally in recent reports it have been defined duplications of Xp11.22 associated with ID, ASD and epilepsy (Evers et al. 2015). VOUS not only adds the current knowledge and but also proposes a potential risk factors for ASD. Parental results can facilitate interpretation of the VOUS (Jang et al. 2019). However, both CNVs in our patient with ASD and mild developmental delay were not tested in terms of parental origin and this was accepted as a limitation of our study.

A possible limitation of our study is the relatively small number of participants. Also, only a few of the family members consented to the parental array CGH testing and, therefore, there was insufficient information about inheritance to help the interpretation particularly for VOUS.

The reason why parents reluctant to genetic testing may be due to the failure to explain the purpose of the test in an appropriate way to the parents. Another demerit of our study, control CNV data from normal individuals in the same population were missing. Our results should be validated in a larger and multicentre Turkish ASD cohort with CNV data from population-matched controls for interpretation.

Conclusion

Genetic testing in children with ASD can give the chances of providing an etiological explanation to parents. Further it could be lend enhanced opportunities for prognosis, the risk of recurrence for future siblings, as well as the development of targeted advanced treatment choices. Our results indicate that CNVs contribute a part to the genetic aetiology of ASD in which relatively paucity data from Turkey. In accordance with the literature, these results emphasize the clinical importance of CMA to investigate the aetiology of ASD.

Declarations

Data Availability: Data sets used and analyzed during the current study can be obtained from the corresponding author upon reasonable request.

Declarations of interest: None. The authors declare that they have no conflict of interest

Compliance with Ethical Standards:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study procedure was reviewed and approved by the Clinical Research Ethics Committee of Gazi University Faculty of Medicine.

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

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None.

Authors' contributions

Conceptualization: Elvan İşeri (Eİ), Ferda Emriye Perçin (FEP), Mehmet Ali Ergün (MAE) and Esra Güney (EG). Data acquisition: Ahmet Özaslan (AÖ), Eİ and EG. Formal analysis: AÖ and Gülsüm Kayhan (GK) Writing– original draft: AÖ. Writing– review & editing: AÖ, GK, Eİ, MAE, EG and FEP. Supervision: Eİ, MAE, EG and FEP.

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