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Research Article

A Low Pass Whole-Genome Sequencing Revealed Copy Number Variations in 7 Out of 33 Bulgarian Patients Having Neurodevelopmental Disorders with Features of Autism Spectrum Disorder

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Abstract

Autism Spectrum Disorder (ASD) is a common genetically based neurobehavioral disorder in which epigenetic and environmental factors are involved. We performed a low pass whole-genome sequencing to identify Copy Number Variations (CNVs) in 33 Bulgarian patients (26 males and 7 females) sharing autistic features and clinically diagnosed with neurodevelopmental disorders. In 7 patients (~21%), 3 girls and 4 boys, large pathogenic chromosomal rearrangements were detected, including three duplications (at 8p21.3p23.3, 15q11.2q13.1, and Xq22.1q22.2) and 4 deletions (at 3q26.33q27.1, 5q14.3q21.1, 6q25.3, and 20q13.11q13.13). Moreover, in one patient the detected deletion covers ARID1B gene known to be involved in both bone and neurological development. Our data contributes to better understanding the genetic basis of ASD and the mechanisms intricated in the development of its specific characteristics and in its gender specific manifestations.

Keywords: Autism; Low pass whole-genome sequencing; Copy number variations; ARID1B gene

Introduction

Autism Spectrum Disorder (ASD) is a developmental condition affecting the whole personality as it is characterized with deficits in social communication and the presence of repetitive, restricted behavioral patterns and interests [1]. According to DSM-5, ASD belongs to the group of Neurodevelopmental Disorders (NDDs), comprising intellectual disabilities, communication disorders, attention-deficit/hyperactivity disorder, specific learning disorder, motor disorders, and other neurodevelopmental disorders. ASD appears more frequently in males than in females with a male-female ratio of 4-5:1 [2]. ASD often co-occurs together with other conditions such as epilepsy, gastrointestinal problems, motor abnormalities, sleep disorders, language disorders, and

intellectual disabilities [3]. The high rates of comorbidity and phenotypical overlap in neurodevelopmental disorders led to the term Neurodevelopmental Spectrum Disorders being proposed [4]. ASD is now considered to be a result of complex interactions between certain environmental factors and genetics [5]. Extensive genetic studies have revealed growing evidence of hundreds of genes linked to ASD confirming that ASD has a strong genetic basis and genetic heterogeneity [6]. According to its phenotype, ASD could be a distinct phenotype, or a syndromic one. It is related to the most common genetic syndromes, where it accompanies a more profound developmental disorder that includes multiple phenotypes, such as dysmorphic features, intellectual disability and epilepsy [7]. The distinct non-syndromic ASD either results of a single gene mutation leading to the development of the disease in the relatively benign form of sporadic non-syndromic ASD, or could be polygenic and multifactorial, determined by specific combinations of environmental and genetic factors [8]. Genetic variations are crucial for building the basis of ASD and have been extensively studied [9]. Copy Number Variations (CNVs) are sub-microscopic structural mutations in chromosomes being duplications, deletions, translocations, and inversions, that can imply many kilobases [10]. CNV can either be inherited or appear de novo [11]. Neurodevelopmental disorders have been linked to either increase or decrease in copy number at the same gene whereby multiple ASD specific CNVs were confirmed [12]. Several studies have highlighted certain autism-specific chromosomal regions [2]. CNVs represent a simultaneous loss or duplication of many genes which challenges the estimation of the connection of these genetic variations to ASD and the mechanism to its development [9]. Accumulating data on variants and their association with autism have been added to the database at https://gene.sfari.org/database/cnv/.

In the current study of a cohort of 33 Bulgarian individuals showing ASD features, we have estimated the presence of CNVs and their impact on the phenotype in certain comorbidities.

Materials and Methods

The present study includes a total of 33 Bulgarian patients (26 males and 7 females), clinically diagnosed with neurodevelopmental disorders. All patients manifest autistic features. The patients' guardians signed an informed consent form prior taking blood samples for genetic testing.

DNA was extracted from collected lymphocyte cells and subjected to spectrophotometry for qualitative/quantitative assessments.

The patients' DNA samples were screened for large chromosomal rearrangements along the genome by the use of low pass whole-genome sequencing (WGS) suitable for detection of copy number variations ≥100 Kb (VistaTM Chromosome Sequencing – 100K, BGI Clinical Laboratories, BGI).

The interpretation and classification of the detected genetic variants refers to the guidelines of the American College of Medical Genetics and Genomics (ACMG), taking into account the patient's clinical manifestation.

The study was conducted in accordance with the Declaration of Helsinki, and the pro-tocol was approved by the Ethics Committee of Sofia University "St. Kliment Ohrid-ski", Protocol No 93-M-412/1.10.2024, date 10/1/2024.

Results

A total of 33 Bulgarian patients sharing autistic features, clinically diagnosed with autism (17), neurodevelopmental disorder (10), and intellectual developmental delay (6) were screened for copy number variations along the whole genome. In 7 patients (~21%), 3 girls and 4 boys, large chromosomal rearrangements were detected (Table 1). These 7 variants are classified as pathogenic according to the ACMG guidelines. From the rest 26 probands, 9 turned out to be negative for copy number variations and 17 of them carry different Variants of Uncertain Significance (VUS) (data not shown, but available upon request). The detected pathogenic CNVs represent 3 duplications and 4 deletions. The duplicated regions affect chromosomes 8 (3 copies), 15 (4 copies) and X (2 copies in a male patient). The deletions are located in chromosomes 3, 5, 6 and 20, respectively. The genes involved in the pathogenic CNVs are represented in Table 1.

S. No.	Disorder	Gender	Mutation	Size
1	Autism	Female	46,XX,dup(8p21.3p23.3).seq[GRCh37/hg19](10,132-19,484,185)×3	19.47 MB
2	Autism	Female	46,XX,del(20q13.11q13.13).seq[GRCh37/hg19](41,997,980-47,920,477)×1	5.92 MB
3	Retardation in neuropsychological development	Male	46,XY,del(5q14.3q21.1).seq[GRCh37/hg19](88,393,429-98,769,766)×1	10.38 MB
4	Retardation in neuropsychological development	Male	46,XY,dup(Xq22.1q22.2).seq[GRCh37/hg19](102,450,374-103,300,417)×2	-
5	Retardation in neuropsychological development; epilepsy	Female	46,XX,dup(15q11.2q13.1).seq[GRCh37/hg19](21,885,000-28,989,181)×4	-
6	WAGR, epilepsy, intellectual disability	Male	46,XY,del(3q26.33q27.1).seq[GRCh37/hg19](181,279,161-183,234,086)×1	-
7	Moderate retardation, limbs anomaly	Female	46,XX,del(6q25.3).seq[GRCh37/hg19](157,513,616-158,380,719)×1	867.10 KB containing ARID1B

Table 1: The genes involved in the pathogenic Copy Number Variations (CNVs) detected by a low pass whole-genome sequencing in 7 out of 33 Bulgarian patients having neurodevelopmental disorder with features of autism spectrum disorder.

Discussion

Copy number variations are frequent large chromosomal rearrangements, accounting for 5-10% of the human genome [13, 14]. About 1 % of CNVs are defined as rare and occur in single families or individuals either de novo or are inherited [15]. Such mutation encompasses dose dependent genes or include regulatory elements that are associated with neurodevelopmental disorders such as ASD, ADHD, epilepsy, schizophrenia, bipolar disorders, developmental delay and intellectual disability [16].

In the present study, 21% of the cases were tested positive for pathogenic CNVs, which exceeds the values reported in the published data. These proportions might be due to the small group of patients tested or to the fact that genetics of autism is still poorly understood.

Several repetitive CNVs have been associated with ASD, comprising various ASD-risk genes, thus highlighting some autism-specific chromosomal regions [2,15]. The results of a whole genome studies of thousands of individuals showed that all regions of any chromosomes except chromosome Y are affected by duplications and deletions [10]. The most common CNVs associated with ASD include segments of the long arm of chromosomes 1, 7, 15, 22, and of the short arm of chromosomes 2 and 16 [9]. Among the 33 patients participating in our study, 7 were found to have major chromosomal rearrangements, representing 3 duplications and 4 deletions. In our cohort of 3 duplication-positive patients, the duplicated regions affect chromosomes 8 (3 copies), 15 (4 copies) and X (2 copies in a male patient). The deletions were located in chromosomes 3, 5, 6 and 20, respectively. In a cohort of 887 families from Simons Simplex Collection, CNVs appear more frequent in females than in males, who in turn represented more de novo deletions than duplications [17]. Our results did not show a gender dependent tendency probably due to the small number of the selected population.

CNVs have been detected in specific loci and they were described to be associated with contiguous genes syndromes, where ASD is as a commonly recurring component [2]. Only two of our patients had ASD alone, while in the others it was combined with retardation in neuropsychological development, epilepsy, WAGR syndrome, and intellectual disability.

An 867.10Kb deletion in chromosome 6, encompassing ARID1B gene was detected in the present sample. According to the Simons Foundation Autism Research Initiative (SFARI) gene database, there are more than 1000 gene candidates associated with ASD (About the Gene Scoring Module), among which ARID1B gene is enlisted being a high confidence syndromic one https://gene.sfari.org/database/human-gene/ARID1B. ARID1B encodes for a protein being a subunit of several different nucleosome remodelling complexes that belong to the family SWItch/Sucrose Non-Fermentable (SWI/SNF). SWI/SNF complexes regulate gene expression through chromatin remodelling including nucleosome rearrangement which enables binding of specific transcription factors for gene activation or repression [18]. Haploinsufficiency of ARID1B gene was described to be associated with agenesis of

the corpus callosum, intellectual disability and features of ASD [19]. Growth delay is a common characteristic of patients with ARID1B mutations, which may be associated with dysregulation of the Wnt/β-catenin signalling pathway, involved in bone growth [20]. Our patient also had a limb anomaly along with ASD manifestations and moderate mental retardation. It was reported that Arid1b haploinsufficiency leads to imbalance of excitation/inhibition in the mouse brain, caused by loss of parvalbumin-positive interneurons, and that using a GABA positive allosteric modulator for treatment effectively improves various behaviors associated with ASD and intellectual disability [21]. It is highly recommended more profound clinical studies to be performed for refinement of positive allosteric modulators of the GABAA receptor as an effective treatment for some Arid1b haploinsufficiency-related behavioral phenotypes [22].

Although the small number of the cohort is of limited statistical power to discover significant findings, our data contribute to the enrichment of accumulating information on the involvement of CNVs in ASD. We identified three duplications (at 8p21.3p23.3, 15q11.2q13.1, and Xq22.1q22.2) and 4 deletions (at 3q26.33q27.1, 5q14.3q21.1, 6q25.3, and 20q13.11q13.13), that have been classified as pathogenic according to the ACMG guidelines. Also, the deletion including ARID1B gene detected in our study, corresponds to the existing knowledge about its involvement in both bone and neurological development. Further investigations are needed for the elucidation of the genetically based mechanisms intricated in the development of the specific characteristics of this disease and in its gender specific manifestations.

Conclusion

In summary, we identified 7 different types of CNVs (3 duplications and 4 deletions) associated with ASD, and the obtained data confirms the involvement of ARID1B gene in neurodevelopmental disorders.

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Author Contributions

L. B. T. conceptualization, supervision, writing the original draft

M. Z. conceptualization, supervision, writing the original draft

T. T. – conceptualization, data curation, formal analysis, validation, visualization, writing – review and editing

A. T. – conceptualization, supervision, writing the original draft

Declaration of Interests

The authors declare no competing interests.

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