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Original Article

Investigation the etiology of syndromic autism with targeted gene analysis

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Abstract

Aim: In this study, we aimed to determine the etiology of syndromic autism with analyzing 50 genes with a targeted gene panel. Cases with a pre-diagnosis of genetic syndrome including autism spectrum disorder were analysed in the current study.

Materials and Methods: The targeted gene panel containing fifty genes causing syndromic autism was sequenced using the Illumina NextSeq550 platform. Forty-nine cases with autism spectrum disorder and syndromic clinical findings were analysed after excluding chromosomal abnormalities, microdeletion/duplication syndromes and Fragile X syndrome.

Results: Pathogenic/likely pathogenic variants or variants of unknown clinical significance were detected in 26.5% (13/49) of the cases. One case was diagnosed with KBG Syndrome with a de novo pathogenic variant detected in the *ANKRD11* gene. Other two pathogenic/likely pathogenic variants were detected in *DHCR7* and *AMT* genes, two cases were accepted as carriers for these genes. Eleven variants of unknown clinical significance were detected in the *VPS13B, SETD2, DHCR7, GRIP1, MED12, ALDH5A1, CREBBP, NSD1* and *CHD7* genes.

Conclusion: In this study, the diagnosis rate was 2%, and the rate of pathogenic/likely pathogenic variant detection rate was 6%, after excluding the cases diagnosed with microdeletion/duplication syndromes and Fragile X Syndrome. Our study is the first study in the literature with 50 genes targeted panel investigating the association of autism spectrum disorder and syndromic disorders with high phenotypic diversity.

Keywords: Autism spectrum disorder; syndromic autism; targeted gene analysis

INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that children have deficiencies in social communication and restrictive, repetitive behavioral patterns (1). Although autism can be diagnosed at any age, it is defined as a developmental disorder as symptoms usually occur in the first two years of life.

Although the exact causes of ASD are not known, it is reported that genetic factors and environmental factors may be effective together. Some factors that increase the risk of developing ASD are: having a sibling with ASD, having older parents, having certain genetic diseases (such as Down syndrome, Fragile X syndrome and Rett syndrome), a very low birth weight history. The symptoms, characteristics and functional effects of ASD vary from person to person. For many children with ASD, the onset of symptoms occurs at the age of 12-24 months (2). It is reported that the loss language and/or social communication skills are typical (3).

Autism is a syndrome, and it is known to have many different etiologies. The term syndromic or secondary autism is used to refer to autism that has a single identified cause, such as Fragile X syndrome (FXS) and tuberous sclerosis. However, none of these etiologies are specific to autism because each encompasses a variable proportion of individuals with and without autism. Single gene mutations and chromosomal abnormalities are reported as the causative agent in approximately 10% of individuals with autism (4), and this rate increases even more when the array-CGH (comparative genome hybridization) test is also performed.

Chromosomal abnormalities and potential candidate genes play a powerful role in the disruption of neuronal connections and the synaptic/dendritic network. Metabolic and mitochondrial diseases can have toxic effects on

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neurons, resulting in neuronal loss and altered modulation of neurotransmission systems (5). In this study, we aimed to present the results of the syndromic autism panel (50 genes) analyzed in cases with findings such as dysmorphic appearance, congenital anomalies, growth retardation and who have a pre-diagnosis of syndromic autism after exclusion of chromosomal anomalies, Fragile X syndrome and microdeletions /duplications.

MATERIALS and METHODS

Cases

Forty-nine cases who presented with a pre-diagnosis of syndromic autism were referred to the Genetic Diseases Diagnosis Center from the Child Psychiatry clinic and who were analysed with the syndromic autism targeted gene panel between 10.2018-10.2020 were included in our study. Written informed consent forms were obtained from the parents.

Targeted NGS Panel

Genomic DNA was isolated from the 2 ml peripheral blood sample taken from the EDTA tube from the cases according to the protocol of the kit (EZ1 DNA Investigator Kit, Qiagen, Hilden, Germany). Quality control of isolated DNA samples was determined using NanoDrop (Thermo Fisher Scientific, Waltham, MA). Samples with A260/280 values between 1.8 and 2.0 were included in the study.

The QIAseq Targeted DNA Panel (Qiagen, Hilden, Germany) kit was used according to the manufacturer's instructions for NGS. In all cases, 50 genes included in the syndromic autism panel (*ADNP, ADSL, ALDH5A1, AMT, ANKRD11, ARID1B, BRAF, CACNA1C, CDKL5, CHD2, CHD7, CNTNAP2, CREBBP, DHCR7, EHMT1, FOXG1, HOXP1, HPRT1, MAGEL2, MECP2, MED12, MID1, NHS, NIPBL, NRXN1, NSD1, PCDH19, POGZ, PQBP1, PTEN, PTPN11, RAD21, RAI1, SCN1A, SCN2A, SETD2, SLC6A1, SLC6A8, SMC1, SLC6A8, SMC TSC1, TSC2, UBE3A, VPS13B, ZEB2*) were sequenced using Illumina NextSeq550 (Illumina Inc., San Diego, CA, USA) technology after library creation and barcoding with all exons (covering intron regions 5 bp). Quality control of the prepared libraries was done with the Qubit dsDNA BR Assay system (Invitrogen, Carlsbad, CA).

NGS Data Analysis

Illumina NextSeq550 Software was used for data analysis, Qiagen QCI Analysis for the evaluation of quality parameters, Qiagen Clinical Insight and Qiagen Ingenuity software for filtering variants, and IGV 2.9.2 program for visual evaluation of the data. According to the ACMG-2015 guideline, variants are classified as pathogenic/likely pathogenic, variant of unknown clinical significance (VUS), and likely benign/benign.

Variant Databases and Pathogenicity Classification

Variant databases: 1000 Genomes Project(http://browser.1000genomes.org/index.html), NCBI dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), NHLBI Exome Sequencing Project (ESP) Exome Variant Server (http://evs.gs.washington.edu/EVS/), Genome Aggregation Database(gnomAD;http://gnomad.broadinstitute.org), ClinVar (http://www.ncbi.nlm.nih .gov/clinvar/), Human Genome Mutation Database (HGMD Professional 2020; http://www.biobase-international.com/).

Pathogenicity scoring programs: PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT and Provean (http://provean.jcvi.org/protein_batch_submit.php?species=human), Mutation Taster (http://www.mutationtaster.org/).

This study is approved by the Ethical Committee of our university with the number 2021/129 and performed in consonance with the principles of the Declaration of Helsinki.

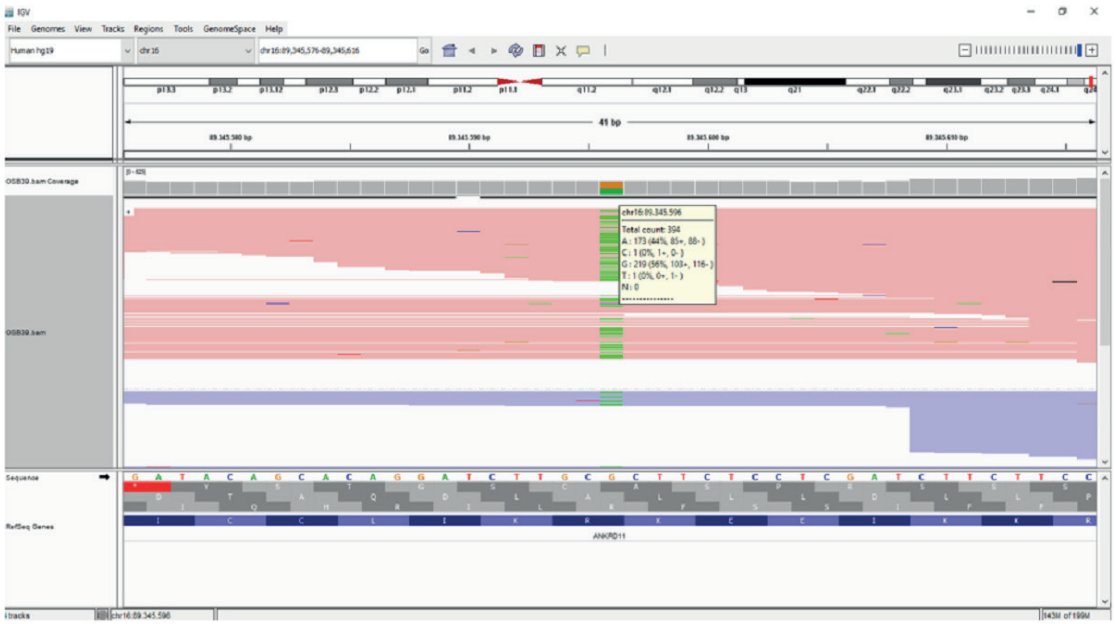

Figure 1. The Integrative Genomic Viewer image of the case diagnosed with KBG Syndrome showing heterozygous de novo pathogenic NM_013275.6:c.7354C>T p.(Arg2452Cys) variant in the ANKRD11 gene

Table 1. Cases with pathogenic/likely pathogenic variants and variants of unknown clinical significance in the current study

Case	Age/ Gender	Clinical findings	Gene	Variant	Protein	dbSNP	Pathogenicity (ACMG-2015)	Segregation	Phenotype
1	4/M	Atypic autism, epilepsy, intellectual disability	ANKRD11	NM_013275.6:c.7354C>T	p.(Arg2452Cys)		Pathogenic (PS2,PM1,PM2,PM5, PP3,PP5,PS2,BP1)	de novo	KBG Syndrome (Autosomal dominant)
2	9/F	Autism, dysmorphic appearance, intellectual disability	DHCR7	NM_001360.2:c.854_856delTCT	p.(Phe285del)		Likely pathogenic (PM1,PM2,PM4)	-	Smith-Lemli-Opitz syndrome (Autosomal recessive)
3	6/F	Atypic autism, dysmorphic appearance, sensorineural hearing loss, intellectual disability	AMT	NM_000481.3:c.878-1G>A	-	rs181134220	Pathogenic (PVS1, PM2, PP3, PP5)	-	Glycine encephalopathy (Autosomal recessive)
4	17/F	Autism, ADHD	MED12	NM_005120.2:c.2555T>G	p.(Val852Gly)	-	VUS (PM2, PP3)	de novo	Lujan-Fryns syndrome, Ohdo syndrome, X-linked, Opitz-Kaveggia syndrome (X linked recessive)
5	5/F	Atypic autism	VPS13B	NM_017890.5:c.10640C>T	p.(Thr3547Ile)	rs781253026	VUS (PM2, PP3)	Maternally inherited	Cohen Syndrome (Autosomal recessive)
6	2 months/F	CHARGE?	SETD2	NM_014159.7:c.1477C>G	p.(Arg493Gly)	-	VUS (PM2, BP4)	-	Luscan-Lumish syndrome (Autosomal dominant)
7	4/F	Rett Syndrome?	DHCR7	NM_001360.2:c.988G>A	p.(Val330Met)	rs139724817	VUS (PM2, PP2, PP3)	-	Smith-Lemli-Opitz syndrome (Autosomal recessive)
			GRIP1	NM_021150.4:c.160G>A	p.(Val54Ile)	rs199768740	VUS (PM1, PM2)	-	Fraser syndrome 3 (Autosomal recessive)
8	14/M	Autism	MED12	NM_005120.3:c.5716C>G	p.(Pro1906Ala)	rs1028187089	VUS (PM2, BP4)	-	Lujan-Fryns syndrome, Ohdo syndrome, X-linked, Opitz-Kaveggia syndrome (X linked recessive)
9	7/M	Atypic autism	ALDH5A1	NM_170740.1:c.244G>A	p.(Ala82Thr)	rs1300964978	VUS (PM2, PP2, PP3)	-	Succinic semialdehyde dehydrogenase deficiency (Autosomal recessive)
10	8/M	Atypic autism	CREBBP	NM_004380.3:c.1655C>A	p.(Pro552Gln)	rs1398406959	VUS (PM2, PP2, PP3)	-	Rubinstein-Taybi syndrome 1 (Autosomal dominant)
11	2/M	Autism	NSD1	NM_022455.4:c.1070A>G	p.(Asn357Ser)	rs573536540	VUS (PM1, PM2, PP2, BP4)	-	Sotos syndrome 1 (Autosomal dominant)
12	11/M	Autism	CHD7	NM_017780.4:c.413T>C	p.(Phe138Ser)	-	VUS (PM2, PP2, PP3)	-	CHARGE syndrome (Autosomal dominant), Hypogonadotropic hypogonadism 5 with or without anosmia (Autosomal dominant)
13	9/M	Autism, dyslexia	CHD7	NM_001316690.1:c.1424T>A	p.(Met475Lys)	-	VUS (PM2, PP2, BP4)	-	CHARGE syndrome (Autosomal dominant), Hypogonadotropic hypogonadism 5 with or without anosmia (Autosomal dominant)

M: Male, F: Female, dbSNP: database of single nucleotide polymorphisms, ACMG: American College of Medical Genetics, VUS: Variant of Unknown Clinical Significance, ADHD: Attention Deficit Hiperactivity Disorder

RESULTS

Forty-nine cases, 29 male and 20 female, who were examined with a pre-diagnosis of syndromic autism between October 2018 and October 2020 were included in the current study. The mean age was 6.83, the ages of the cases ranged from 2 months to 17 years. The cases referred with different syndromic pre-diagnosis such as Rett syndrome, Rubinstein-Taybi syndrome, CHARGE syndrome, Asperger Syndrome, and Cohen Syndrome. In addition, some cases were investigated for findings such as dysmorphic appearance, extremity anomalies, congenital anomalies, growth retardation, hypotonia and epilepsy with autism. No pathology was found in chromosome analysis, Fragile X mutation analysis and array-CGH tests of all cases.

Pathogenic/likely pathogenic or variant of unknown clinical significance was detected in 13 (26.5%) of 49 cases analysed with the syndromic autism panel. Three pathogenic/likely pathogenic variants were detected in the *ANKRD11, DHCR7* and *AMT* genes in 3 cases, and 11 variants of unknown clinical significance detected in the *VPS13B, SETD2, DHCR7, GRIP1, MED12, ALDH5A1, CREBBP, NSD1* and *CHD7* genes in 10 cases (Table 1).

Case 1, who was investigated for atypical autism, epilepsy, motor and metal retardation, was diagnosed with KBG syndrome with a heterozygous de novo pathogenic variant (c.7354C>T) in the *ANKRD11* gene (Figure 1). Cases 2 and 3 also had dysmorphic appearance and intellectual disability with autism; these cases were heterozygous for autosomal recessively inherited *DHCR7* and *AMT* genes.

DISCUSSION

Autism spectrum disorders are complex disorders. Although many studies have been conducted on the genetic basis of autism, it has been reported that there is a specific genetic etiology for only 15% of the cases (6). Although the importance of de novo mutations is emphasized in trio studies using whole exome sequencing that include the mother-father-child, no specific cause for autism has been identified (7,8). These exome analysis studies also highlighted the heterogeneous genotype of autism and reported that genes claimed to play a role in the etiology which has also roles in common pathways.

The advantages of targeted gene sequencing are that the number of patients may be higher in the analysis and the number of patients who can be diagnosed may be higher comparing with whole exome sequencing. With the targeted gene panel, a greater depth of read on the analysis can be achieved at a lower cost. Increased depth will facilitate the detection of small insertions and deletions that may be overlooked by exome sequencing. Targeted sequencing also avoids the problem of random results. Clinical interpretation of new variants remains difficult, but will become increasingly easy with the constant development of databases.

Autism is a syndrome with its wide phenotypic spectrum, and it is known to have many different factors in etiology. The term syndromic or secondary autism is used to refer to autism that has a single defined cause, such as Fragile X syndrome and tuberous sclerosis. However, none of these etiologies are specific to autism because each encompasses a variable proportion of individuals with and without autism. In our study, we aimed to report the rate of diagnosis that can be detected with a targeted panel containing 50 genes in cases with a pre-diagnosis of syndromic autism. Pathogenic variant was detected in 3 (6.1%) of 49 cases included in the study, while 1 case (2%) was diagnosed with a definite diagnosis.

Case 1, who had a heterozygous de novo pathogenic variant in the *ANKRD11* gene, which shows autosomal dominant inheritance, was diagnosed with KBG syndrome. This case had atypical autism, speech retardation, obsessive disorder, growth retardation, and epilepsy. The patient also had a round face, flat forehead, flat eyebrows, long palpebral fissures, long filtrum, thin lips, micrognathia, and prominent ears, among dysmorphic features. It has been reported that KBG syndrome is generally undiagnosed due to its nonspecific findings and phenotype variation (9). "KBG" represents the initials of the first families diagnosed with this disease (10). KBG syndrome (OMIM 148050) is a rare genetic disease with macrodontia of the upper santral incisors, prominent facial features, short stature, skeletal anomalies, hearing loss, developmental delay and intellectual disability. Approximately 200 KBG patients have been reported to date and various diagnostic criteria have been proposed by different researchers according to their study groups (11,12). The final diagnostic criterias were defined as growth retardation/learning disability, speech delay or major behavioral problems with at least two major criterias or one major and two minor criterias (13). Although two patients in one study showed an atypical phenotype (short stature) without intellectual disability or hearing loss, molecular analysis identified two rare *ANKRD11* variants with an uncertain pathogenicity of KBG syndrome (14). At first examination of case 1, we did not consider KBG Syndrome in our case, as he did not show typical features. The pre-diagnosis of atypical autism was analyzed in the syndromic autism panel due to his developmental delay and mild dysmorphic features, and thus a diagnosis was made. Due to the phenotypic diversity in KBG Syndrome, other variants in addition to *ANKRD11* variants may contribute to the phenotype, and perhaps a whole exome analysis may be required to exclude this possibility in the future.

The other two cases (Cases 2 and 3) were detected with pathogenic/likely pathogenic variants and were accepted as carriers for Smith-Lemli-Opitz syndrome and glycine encephalopathy due to the autosomal recessive inheritance pattern. Identified syndromes, chromosomal abnormalities, and de novo copy number variations (CNVs) account for approximately 10-20% of autism cases (15). The results of our study show that it is not easy to detect the presence of a genetic syndrome in cases with autism. Therefore, it is important to apply the syndromic autism panel to cases determined by a good clinical selection.

In a study, screened 237 newborns for metabolic diseases with a diagnosis of autism reported that 6 patients had also a diagnosis of metabolic diseases with autism (16). In a study using targeted gene analysis, it was reported that a patient diagnosed with autism spectrum disorder was diagnosed with succinic semialdehyde dehydrogenase deficiency with this gene panel containing neurological diseases (17). To the best of our knowledge, this is the first study in the literature analysing the syndromic autism panel that includes fifty genes.

CONCLUSION

The etiology of autism spectrum disorder and syndromic autism is quite wide and should not be considered as a single disease. Various studies provide some clues to unravel the complexity of autism pathogenesis. The diagnosis rate was 2% and the variant detection rate was 26.5% with analysing 50 genes with targeted gene analysis in the current study. Cases with fragile X syndrome or microdeletion syndrome were not included in our study. These results show that the cases should be carefully evaluated with clinical findings and more genes should be analyzed in larger samples.

Competing Interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical Approval: The study was approved by the Ethics Committee of the Trakya University, Faculty of Medicine (March 8, 2021, TUTF-BAEK-2021/129).

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