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The Study of Whole Genome Sequencing in Monozygotic Twins with Autism Spectrum Disorder

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ABSTRACT

Objective: Autism spectrum disorder (ASD), the most well-known type of neurodevelopmental disorder, is a mental development disorder. Since there is no definitive biomarker for ASD, diagnosis is made based on the assessment of the patient's behavior. In addition to behavioral and social disorders, genetic factors are also important in ASD.

Materials and Methods: In the study, variant analyses were performed by whole genome sequencing (WGS) method, as well as evaluating the clinical features of two monozygotic twin couples (one discordant and the other concordant).

Results: According to the WGS results, thirteen high pathogenic variants were detected in twenty-nine novel candidate genes. Candidate genes include *MEAF6, OR2T8, ABI2, PDE4D, GLIS3, DRD4, LPXN, FAM186A, NEK3, GOLGA8A, SSC5D, ARMCX4, ADAR, LRP1B, DAP, LYRM7, MUC12, CNTNAP3B, TCP11L1, OR8B3, KLRC3,* and *DPP9*.

Conclusion: We speculate that clinical evaluations and examination of genetic changes are important for understanding the disease in individuals with ASD and their families.

Keywords: Autism spectrum disorder, MZ twins, whole-genome-sequencing (WGS), genetics

INTRODUCTION

Autism spectrum disorder (ASD) is a type of mental developmental disorder with characteristic features such as limitations in social communication, repetitive behaviors, insistence on sameness, and limited interests (1). The prevalence of ASD is below 1.0% worldwide. However, this rate is thought to be higher in developed countries (2). In a study that included eleven regions in the United States, the prevalence of the disease was determined as 18.5:1000 (for 8-year-old children). It has been revealed that the incidence of ASD in boys is 4.5 times higher than in girls. Symptoms of the disease appear in the early period (at the age of 1-2 years) (3, 4). Individuals with ASD have difficulties in social behavior, emotional and non-verbal communication, and relationship building. Additionally, restricted areas of interest and repetitive behavior patterns are common clinical features. Examination of social communication, limited interests, and repetitive behavioral symptoms are particularly important in diagnosis of autism (5, 6). Emotional symptoms such as depression, anxiety and attention problems, behavioral conditions such as aggression, and challenging behaviors can be noticeable in individuals with autism (7). Although imaging techniques such as magnetoencephalography (MEG), and magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computerized tomography (SPECT), and electroencephalography (EEG) are neuroimaging techniques that can be used in brain imaging with autism, the diagnosis of the disease is usually made routinely with clinical evaluations (8). Neuroanatomical differences in various parts of the brain are thought to be associated with behavioral and cognitive abnormalities, especially in individuals with ASD aged 2-3 years (9). Gastrointestinal problems, attention

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deficit hyperactivity disorder (ADHD), bipolar disorder, Tourette syndrome and tic disorders, Childhood-onset schizophrenia and epilepsy, and conditions such as sleeping, feeding, and toilet problems have been identified as comorbidities that are associated with ASD. Studies have shown that the prevalence of ASD in epilepsy is high and that it has similar etiological aspects (6,10,11). There are limited treatment options for improving the symptoms seen and for the accompanying mental or clinical manifestations that may increase the severity of the disease in ASD. As in numerous other diseases, personalized treatment and precision medicine approaches are thought to be effective in treatment of ASD.

As a result of studies on twins, it was revealed that genetic and environmental factors were related to the etiology of psychiatric diseases, including autism, and it was determined that the concordance in monozygotic (MZ) twins were higher than in dizygotic (DZ) twins. In addition, it has been stated that genetic factors may influence brain size, curvature, and subcortical gray matter in brains with ASD while environmental factors may have an effect on brain regions such as cortical thickness and cerebellar white matter (12-15). Numerous single-nucleotide polymorphisms (SNPs) or copy number variations (CNVs) have been identified in protein-coding genes, which participate in events such as neuronal development and synapse formation, in approximately 25-35% of individuals, in consequence of many studies conducted with genome sequencing analysis in ASD. In these studies, about one thousand genes were thought to be associated with the disease have been identified. When ASD-related genes are examined, it is thought that changes in many human genes such as *SHANK3, CDH8, CDH9, CDH10, CSMD1, SCNA2, CNTNAP2, MACROD2, SLC9A9*, and *BCKDK* were associated with ASD. With these analyses, it has been detected that CNVs in regions (16q11.2), (15q11-13), (Xp22.3), (15q13.1–13.2), (3p26.3 and 2p12) (16-18).

In this study, we aimed to learn more about the genetic background of the disease and to examine its contribution to the ASD phenotype by performing whole genome sequencing (WGS) analysis on two MZ twins. Besides, we examined the effects of the clinical and psychological conditions of the parents on individuals with autism by applying tests that are used to measure autism status, depression, mood, and quality of life to the families of a couple of concordant and discordant twins.

MATERIALS AND METHODS

Participants

Our study was conducted with concordant and discordant twins diagnosed with ASD at the Umraniye Training and Research Hospital, Child and Adolescent Psychiatry Clinic. Ethical approval of the project was taken from University of Health Sciences, Umraniye Training and Research Hospital, Clinical Research Ethics Committee (B.10.1.TKH.4.34.H.G.P.0.0.1/167, 19.12.2018). In the study, the clinical data of two twin couples, one discordant (Twin couple 1; twin 1.1 and 1.2) and the other concordant (Twin couple 2; twin 2.1 and 2.2), were examined. Then, WGS analysis was performed to investigate the genetic differences and their relationship with the disease. Toronto Alexithymia Scale (TAS-20) and Autism Spectrum Quotient (AQ) questionnaires were applied to the mothers and fathers to measure autism status, depression, mood, and quality of life in the parents of twin couples. In addition, Beck Depression Inventory (BDI) and World Health Organization Quality of Life (WHOQOL- BREF) questionnaires were completed.

Genomic Sample Collection and Preparation

Peripheral blood samples (~2 ml) were collected into a tube with EDTA. The total DNA was extracted from 200 µL blood samples according to the manufacturer's instructions (Cat. No. 11796828001, Roche Applied Sciences, Germany), and the DNAs were kept in a freezer at -20^{0}C until sequencing. Through spectrophotometric analysis (DENOVIX DS-11 FX, USA), the concentration of the samples was determined as 200 ng/µL. DNA fragments were ligated with adaptor oligonucleotides to form paired-end DNA libraries with an insert size of 500 base pair (bp).

Whole Genome Sequencing (WGS)

The samples were run on the Illumina Novaseq platform (NovaSeq™ 6000 Sequencing System, Cat. No. 20012850, US) on S1 flow cell that has 2 lanes; the data is from the two lanes. In the current study, which used the Illumina NovaSeq6000 system, an average length of 100 bp, a sequence depth of 12 Gb per sample and 100×10^6 paired end were read. A total of 265,815 unigenes were detected with an average contig length of 201 bp.

Bioinformatic Analysis

The pool has been created as two forward fast adaptive shrinkage thresholding algorithm and quality (FASTQ) files and two reverse FASTQ files for each sample (as each sample has 4 FASTQ files: 2 forward and 2 reverse (paired-end)). Variant call format (VCF) and PLINK files were created. In preparing the VCF files, we included all possible variants right after standard genome analysis toolkit (GATK, 4.2.0.0) bioinformatics analyses on purpose, in which only a minimum of standard quality control was applied. These VCF files were intended to provide a comprehensive pool of variants, from which further quality controls can be applied manually to filter for higher quality variants. The effects (mutations) and classifications (localization) of variants in genome wide were annotated by ANNOVAR (Annotate Variation). Assuming that the disease is caused by different genotypes between affected and unaffected individuals, MZ couples were compared among themselves to identify differences (variants). Subsequently, overlapping of identified variants shared by the two families was found. The data was analyzed using R Bioconductor (V.3.13; it works with R V.4.1.0). In the study, filtering was performed so that the quality deep (QD) value was between 27-33.

RESULTS

Clinical, Developmental and Diagnostic Evaluation of Twins

When the clinical characteristics of twin individuals were examined, it was observed that while all individuals were found to

have an early birth time and a low birth weight, none of them had epilepsy (Table 1). According to the developmental evaluations of the twins, it was observed that only the individual with severe autism (Twin 2.2) did not speak, and it was determined that walking was delayed in the twin 2 couple, and they did not have toilet training (Table 2). Diagnostic features were divided into social disability, communicative limitation, and repetitive interests and limitations categories and evaluated in three individuals with autism other than the healthy individual. As a conclusion, it was determined that the symptoms were directly proportional to the severity of autism, and it was noticed that the regression of the symptoms was more pronounced with special education, especially in twin 1.1. When the autism-behavior-checklist (ABC) and childhood-autism–rating-scale (CARS) scores were examined in individuals with autism, it was revealed that these values were increased with the severity of autism (Table 3).

Parental Information

While the parents of the twin couple were alive and married, it was determined that there was no consanguinity between the parents. While there was no individual with any psychological illness in the family and relatives of twin 1, it was stated that one of the relatives of twin 2 had a late speaking individual. As a result of the TAS-20 evaluation, possible alexithymia was detected only in the mother of twin 1 (59 points), while alexithymia was detected in other parents (Twin couple 1 father, 75 points; twin couple 2 mother, 71 points; twin couple 2 father, 71 points). According to BDI examinations, mothers of twin couple 1 (24 points) and 2 (29 points) had moderate depression in both. As a result of the WHOQOL-BREF test, the psychological evaluation of both mothers was below 50% (Twin couple 1 mother, 45,8%; twin couple 2 mother, 41,7%). However, in the mother of twin 2, the value of all categories was below 50%.

Genetic Assessment of WGS

According to the identical variants between the twins were examined, there were seven high pathogenic variants out of 64,867 variants, of which 17,936 were genic, when the identical alleles were examined, out of 23,362 variants, of which 5,823 were genic, six high pathogenic variants were detected. As a result of the comparison of the variants of the twin couples among themselves, fifteen of the 265,815 variants, of which 45,626 were genic in the discordant twin couple (Twin couple 1), were determined as high pathogenic. According to the examination of the different variants in the concordant twin couple (Twin couple 2), it was determined that fourteen out of 268,928 variants, 45,521 of which were genic variants, were high pathogenic variants. After filtering data of twins, *MEAF6, OR2T8, ABI2, PDE4D, GLIS3, DRD4, LPXN, FAM186A,*

Table 1. Clinical characteristics of twins.

	Twin 1.1	Twin 1.2	Twin 2.1	Twin 2.2
Age	15		6	
Gender	Female		Female	
Diagnosis-Severity Mild Moderate Severe Healthy	Mild	Healthy	Moderate	Severe
Birth Time Pre-term (< Week 37) Term (Week 37-41) Post-term (≥ Week 42)	Preterm	Preterm	Preterm	Preterm
Birth Weight (g) Very low (<1500 g) Low (<2500 g) Normal (2500-3999 g) High (>4000 g)	Very low	Very low	Very low	Very low
Epilepsy Yes No	No	No	No	No
History of Incubator Yes No	Yes	No	Yes	Yes

Table 2. Developmental information of twins.

	Twin 1.1	Twin 1.2	Twin 2.1	Twin 2.2
Unsupported Sitting Early (<month 7) In time (month 7-9) Late (>month 7-9)	In time	In time	In time	In time
Babbling Early (<month 3) In time (month 3) Late (>month 3) No Babbling	In time	Early	In time	In time
Teething Early (<month 6) In time (month 6-8) Late (>month 6-8)	Late	Late	Late	Late
Walking Early (<month 11) In time (month 11-15) Late (>month 11-15) No Walking	In time	In time	Late	Late
Talking Yes No Regression	Yes	Yes	Yes	No
Toilet Training Early (<years 2-3) Normal (years 2-3) Late (≥years 4) No Toilet Training	Normal	Normal	No Toilet Training	No Toilet Training

Table 3. Total CARS and ABC scores of twins with autism.

	Twin 1.1	Twin 2.1	Twin 2.1
CARS Score	23.5	36	47.5
ABC Scores			
Sensory	7	13	26
Relating	20	26	38
Stereotypes and object use	4	34	34
Language	0	26	18
Self-Help And Social	7	15	18
Total Score	**38**	**114**	**134**

CARS, Childhood Autism Rating Scale; ABC, Autism Behavior Checklist.

NEK3, GOLGA8A, SSC5D, ARMCX4, ADAR, LRP1B, DAP, LYRM7, MUC12, CNTNAP3B, TCP11L1, OR8B3, KLRC3, and *DPP9* genes have been identified as candidate genes in ASD (Figure 1 and 2, Table 4).

DISCUSSION

In the large-scale association studies, genetic heterogeneity and environmental factors make it difficult to reach clear conclusions for disease etiology, especially for psychiatric diseases.

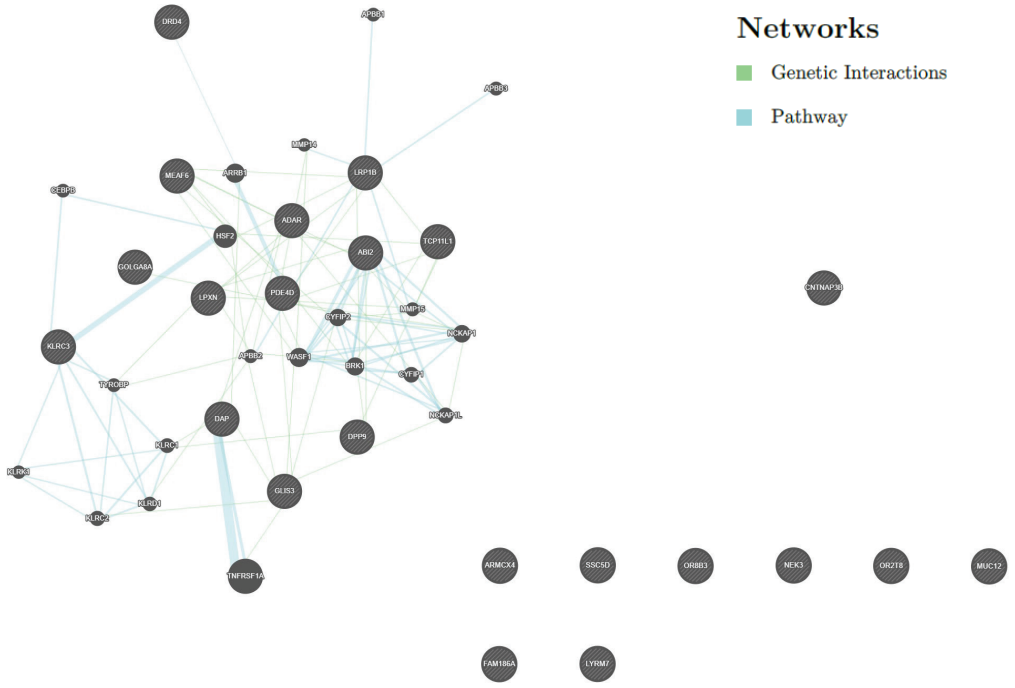

Figure 1. Genetic interaction and pathway networks of candidate genes obtained by WGS analysis. Green lines show genetic interaction, blue lines show pathway networks (https://genemania.org/).

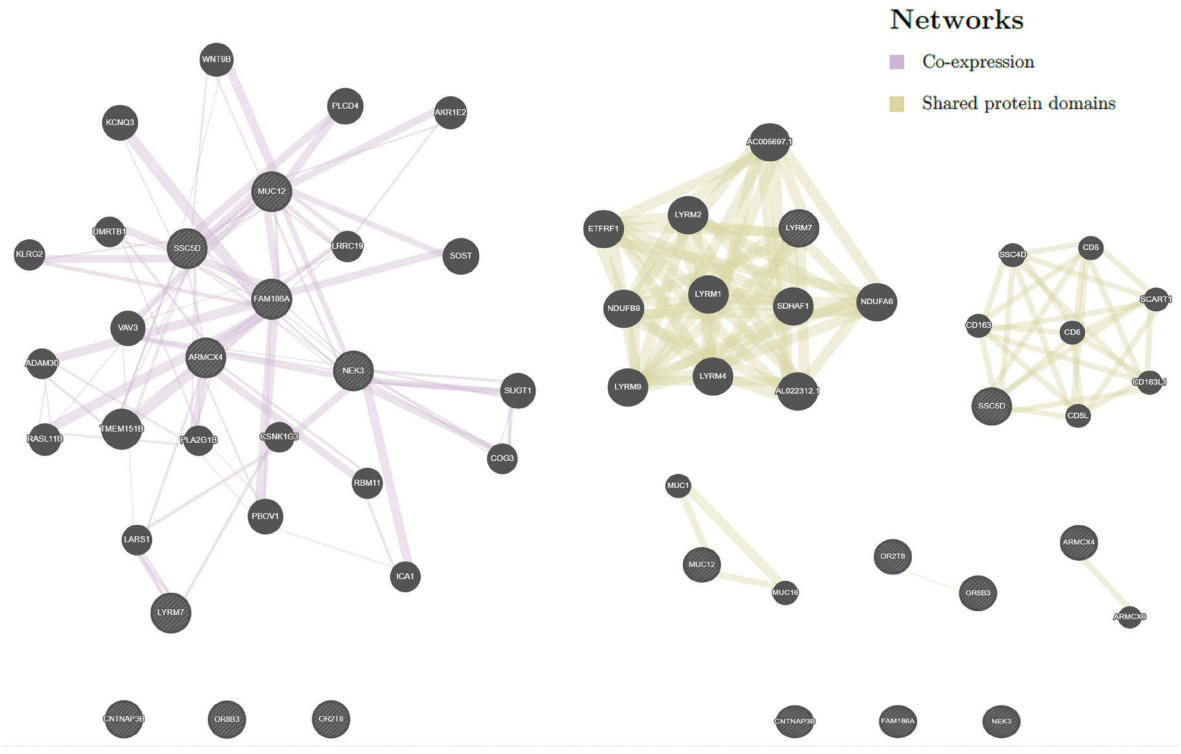

Figure 2. Co-expression and shared protein domains networks of candidate genes obtained by WGS analysis. Violet lines show co-expression, yellow lines show shared protein domains networks (https://genemania.org/).

Table 4. Comparison results of variants obtained by WGS analysis.

	CHROM: POSX	REF	ALT	GT1	GT2	AF	CH	EFF	CD	AT	RS	GENE INFO
Twin Couple 1	chr1:37498409	TTTA	T,*	2/2	0/0	0.25, 0.25	T	Moderate	Protein coding, downstream gene variant, pseudogene, intron variant	c.534-1676_534-1674del |n.*2523_*2525delTAA c.534-1676_534-1674del c.534-2494_534-2492del c.533+3392_533+3394del n.607-1676_607-1674del n.607-1808_607-1806del n.696-1676_696-1674del	35576307	MEAF6:64769
	chr1:247921552	A	G	1/1	0/1	0.625	G	Moderate	Protein coding	c.535A>G|p.Thr179Ala	4584426	OR2T8:343172
	chr1:247921607	T	G	1/1	0/1	0.625	G	Moderate	Protein coding	c.590T>G|p.Met197Arg	4474294	OR2T8:343172
	chr2:203395448	T	TATATATATATACAC, TATATACACACAC, TAC	0/0	0/1	0.125, 0.25, 0.25	TAC	Moderate	Protein coding, intron variant	c.708-208_708-207ins c.708-208_708-207ins c.726-207_726-206ins c.726-172_726-171dup c.708-207_708-206ins c.708-172_708-171dup c.540-207_540-206ins c.540-172_540-171dup c.708-207_708-206ins c.708-172_708-171dup c.573-207_573-206ins c.573-207_573-206ins c.573-172_573-171dup	750379504	ABI2:10152
	chr5:59266270	AATAT	A,AAT	2/2	1/2	0.375, 0.625	A	Moderate	Protein coding, upstream gene variant, intron variant	c.90-50306_90-50303del c.90-60156del c.-722_-721del c.-722del c.456-60156_456-60155del c.456-60156del c.90-60156_90-60155del c.90-60156del c.48-60156_48-60155del c.264-60156_264-60155del c.264-60156del c.273-60156_273-60155del c.273-60156del	150096498	PDE4D:5144
	chr5:59276122	GAA	GA,G	1/1	0/1	0.625, 0.25	G	Moderate	Protein coding, intron variant	c.90-60156_90-60155del	753205660	PDE4D:5144
	chr7:100999201	G	A	1/1	0/0	0.25	A	Moderate	Protein coding, upstream gene variant, intron variant	c.8638G>A|p.Glu2880Lys	.	.
	chr9:4118111	G	T	0/1	1/1	0.875	T	Moderate	Protein coding	c.1367C>A|p.Pro456Gln c.902C>A|p.Pro301Gln	6415788	GLIS3:169792
	chr11:640090	G	C	1/1	0/1	0.75	C	Moderate	Protein coding	c.841G>C|p.Ala281Pro c.*4460C>G c.*4460C>G	3889692	DRD4:1815
	chr11:1095015	T	C	1/1	0/1	0.75	C	Moderate	Protein coding	c.4772T>C|p.Ile1591Thr	.	.

Table 4. Comparison results of variants obtained by WGS analysis. (Countinued)

	CHROM: POSX	REF	ALT	GT1	GT2	AF	CH	EFF	CD	AT	RS	GENE INFO
Twin Couple 1	chr11:58555768	GCACA	G,GCA	1/2	0/2	0.375, 0.5	G	Moderate	Protein coding	|c.234-832_234-829del c.234-832_234-831del c.219-832_219-829del c.219-832_219-831del c.234-832_234-829del c.234-832_234-831del c.219-832_219-829del c.219-832_219-831del c.159-832_159-829del c.159-832_159-831del	71454340	LPXN:9404
	chr12:50352315	A	G	0/1	1/1	0.875	G	Moderate	Protein coding	c.4517T>C|p.Leu1506Pro	10876022	FAM186A:121006
	chr13:52133615	T	TCACACA	1/1	0/1	0.625	TCACACA	Moderate	Protein coding, upstream gene variant, pseudogene, intron variant	c.1436+68_1436+73dup c.1385+68_1385+73dup c.-6139_-6134dup n.*892_*893ins c.1436+68_1436+73dup c.1436+68_1436+73dup c.1385+68_1385+73dup n.1811+68_1811+73dup	3831081	NEK3:4752
	chr15:34386710	C	G	0/1	1/1	0.875	G	Moderate	Protein coding, non-coding transcript exon variant, upstream gene variant, pseudogene	c.200G>C|p.Arg67Pro n.-4560G>C n.-4560G>C n.2571G>C	147828722	GOLGA8A:23015
	chr16:4920371	G	GAAAGAA AGAAAGA AAGAAAG AAAGAAA GAAAGAA AGAAAGA AA, GAAAGAA AGAAAGA AAGAAAG AAAGAAA GAAAGAA AGAAAGA AAGAAA,*	1/1	0/1	0.375, 0.125, 0.125	GAAAGA AAGAAAG AAAGAAA GAAAGAA AGAAAGA AAGAAAG AAA	Moderate	Protein coding	c.63-9423_63-9422ins c.63-9423_63-9422ins c.63-9423_63-9422ins c.63-9423_63-9422ins	.	.
	chr19:55518098	G	A	1/1	0/1	0.875	A	Moderate	Protein coding	c.3822G>A|p.Met1274Ile	4801331	SSC5D:284297
	chrX:101494140	A	G	0/1	1/1	0.75	G	Moderate	Protein coding, intron variant, pseudogene	c.5551A>G|p.Ile1851Val n.1549-4005A>G n.1440-4005A>G n.574-4005A>G n.1482-4005A>G	5951336	ARMCX4:100131755

Table 4. Comparison results of variants obtained by WGS analysis. (Countinued)

	CHROM: POSX	REF	ALT	GT1	GT2	AF	CH	EFF	CD	AT	RS	GENE INFO
Twin Couple 2	chr1:154593135	CA	C	0/1	1/1	0.875	C	Moderate	Protein coding, intron variant	c.2271-2727del c.1386-2727del c.2271-2727del c.2214-2727del c.1386-2727del	556341696	ADAR:103,ADAR:103
	chr2:140272258	AACACACACAC	A, AACACAC ACACAC, AACACAC ACACACA CACAC	0/3	2/3	0.25, 0.375, 0.25	A	Moderate	Protein coding, intron variant	c.13143-1922_13143-1913del c.13143-1924_13143-1923dup c.13143-1922_13143-1913del c.13143-1924_13143-1923dup c.13143-1930_13143-1923dup	138326343	LRP1B:53353
	chr4:2042401	C	T	1/1	0/1	0.875	T	Moderate	Protein coding	c.149C>T|p.Pro50Leu c.50C>T|p.Pro17Leu	570712	C4orf48:401115|NELFA:7469
	chr5:10731085	G	T	0/1	1/1	0.625	T	Moderate	Protein coding, intron variant	c.152+17090C>A c.152+17090C>A c.152+17090C>A	93417	DAP:1611
	chr5:10739201	CAAA	C	0/0	1/1	0.5	C	Moderate	Protein coding, intron variant	c.152+8971_152+8973del c.152+8971_152+8973del c.152+8971_152+8973del	558306009	DAP:1611
	chr5:131197846	CTGTG	C,CTG	1/1	0/1	0.625, 0.25	C	Moderate	Protein coding, intron variant, pseudogene	c.245-1684_245-1681del c.245-1682_245-1681del c.245-1649_245-1646del c.245-1647_245-1646del c.163-1649_163-1646del c.163-1647_163-1646del n.199-1649_199-1646del	72182125	LYRM7:90624,LYRM7:90624
	chr6:51804371	GGGC	G,*	0/0	1/1	0.25, 0.25	G	Moderate	Protein coding, intron variant	c.8303-13001_8303-12999del c.8303-13001_8303-12999del c.8303-13001_8303-12999del c.8303-13001_8303-12999del	.	.
	chr7:101004095	C	T	0/1	1/1	0.5	T	Moderate	Protein coding	c.13532C>T|p.Thr4511Ile	201694075	MUC12:10071
	chr9:41938349	G	T	1/1	0/1	0.875	T	Moderate	Protein coding	c.2132C>A|p.Ser711Tyr	62536540	CNTNAP3B:728577
	chr11:1095015	T	C	0/1	1/1	0.75	C	Moderate	Protein coding	c.4772T>C|p.Ile1591Thr	.	.
	chr11:33047216	G	GA	0/1	1/1	0.875	GA	Moderate	Protein coding, intron variant	c.163+3280_163+3281ins c.163+3293dup c.163+3293dup	11385765	TCP11L1:55346,TCP11L1:55346
	chr11:124397010	C	T	0/1	1/1	0.625	T	Moderate	Protein coding	c.342G>A|p.Met114Ile	530992	OR8B3:390271
	chr12:10420546	C	T	0/1	1/1	0.625	T	Moderate	Protein coding	c.5G>A|p.Ser2Asn	2682489	KLRC3:3823

Table 4. Comparison results of variants obtained by WGS analysis. (Countinued)

	CHROM: POSX	REF	ALT	GT1	GT2	AF	CH	EFF	CD	AT	RS	GENE INFO
Twin Couple 2	chr16:4920371	G	GAAAGAA AGAAAGA AAAGAAG AAAGAAA GAAAGAA AGAAAGA AA, GAAAGAA AGAAAGA AAGAAAG AAAGAAA GAAAGAA AGAAAGA AAGAAA,*	0/2	0/3	0.375, 0.125, 0.125	GAAAGA AAGAAAG AAAGAAA GAAAGAA AGAAAGA AAGAAAG AAA	Moderate	Protein coding, intron variant	c.63-9423_63-9422ins c.63-9423_63-9422ins |c.63-9423_63-9422ins	. .	.
	chr19:4719326	TTAAATAAA	T, TTAAATA AATAAA	2/2	0/2	0.25, 0.375	T	Moderate	Protein coding, intron variant	c.56+517_56+524del c.56+513_56+516dup c.56+517_56+524del c.56+513_56+516dup	150534589	DPP9:91039
	chrX:101494140	A	G	0/1	1/1	0.75	G	Moderate	Protein coding, intron variant, pseudogene	c.5551A>G|p.Ile1851Val n.1549-4005A>G n.568-4005A>G n.1440-4005A>G n.574-4005A>G n.1482-4005A>G	5951336	ARMCX4:100131755

* CHROM & POSX, Chromosome and position; REF, Reference Allele; ALT, Alternate Allele; GT 1, Genotype for 1. twin; GT 2, Genotype for 2. twin; AF, Allele Frequency; CH, Changing; EFF, Effect; CD, Protein Coding or non-coding region; AT, Alteration; RS, dbSNP ID; GENE INFO, Pairs each of gene symbol, gene id.
* CHROM & POSX, Chromosome and position; REF, Reference Allele; ALT, Alternate Allele; GT 1, Genotype for 1. twin; GT 2, Genotype for 2. twin; AF, Allele Frequency; CH, Changing; EFF, Effect; CD, Protein Coding or non-coding region; AT, Alteration; RS, dbSNP ID; GENE INFO, Pairs each of gene symbol, gene id.

However, it may be possible to obtain definitive findings with genetic or epigenetic studies with twins. Twin studies have some advantages over studies with non-twins. Twin studies can sometimes be developed into longitudinal studies. Before and after diagnosis, severity of disease, speaking, age of onset, the profile of symptoms, response to a variety of drugs might potentially need to be considered. With next-generation sequencing technology, it is possible to match clinical features with genetic changes and to detect epigenetic differences. Twin studies, with this technology, make it possible to detect differences in several aspects such as somatic mutations, DNA methylation, histone modifications, CNVs, single nucleotide variants (SNVs), changes in introns, synapse formation, and regulation of neural networks including microglia (12). However, the detection of discordant twins also play a considerable role in understanding the etiology of psychiatric diseases.

Not a little evidence demonstrates the importance of complex genetic factors in ASD development. Examples of candidate genes in our study include *Abl Interactor 2* (*ABI2*), a protein coding gene. *ABI2* is a gene associated with autosomal recessive limb-girdle muscular dystrophy type 2H. In a study investigating the genetic background of autism, it was seen that the *ABI2* gene was among the genes with *de novo* missense mutations discovered in consequence of the evaluation of the exome sequencing results (19). As for that to a study conducted with 192 relatives with non-syndromic intellectual disability, homozygous loss-of-function mutations were found in nine genes, including the *ABI2* gene, in 43 families (20). *MYST/Esa1 Associated Factor 6* (*MEAF6*) is a gene that encodes a nuclear protein involved in transcriptional activation, with a pseudogene for this gene on chromosome 2. This gene aberration was observed in our study. Genes expressed at various levels in schizophrenia and schizoaffective disorder were investigated with microarray datasets; it was determined that *MEAF6* expression levels were low in parvalbumin positive neurons of the 3rd layer of the dorsolateral prefrontal cortex in patients (21). *Mucin 12 (MUC12)* gene encodes an integral membrane glycoprotein that play a crucial role in forming protective mucous barriers on epithelial surfaces and have been implicated in epithelial regeneration and differentiation. In an exome sequencing study conducted with individuals with autism, a *de novo* nonsense variant in the *MUC12* gene was identified in a case with ASD (20). In a different study, in which postzygotic mutations were analyzed with whole exome sequencing (WES) in ASD, six non-synonymous postzygotic mosaic mutations (PZM) were identified in the *MUC12* gene (22). *Dopamine Receptor D4 (DRD4)* gene encodes the D4 subtype of the dopamine receptor. The D4 subtype is a G-protein coupled receptor which inhibits adenylyl cyclase. This gene contains a polymorphic number (2-10 copies) of tandem 48 nt. repeats; the sequence shown contains four repeats. A high prevalence of rare *DRD4* alleles in children diagnosed with ADHD was reported. As to examining whether the *DRD4* alleles overlap in ADHD and ASD, it was reported that rare variants were not observed in individuals with ASD (23). It is known that dopamine receptors are involved in the control of behav-

ior-related signals and are associated with attention disorders. Although the *DRD4* gene, which is associated with the post-synaptic effect of the dopamine hormone, participates in many neural pathways, these gene polymorphisms are thought to be associated with psychiatric disorders (24).

Considering the relationship of the exon 3-7 repeat allele in the *DRD4* gene with autistic symptoms in twins with ADHD was investigated, it was suggested that high repeat alleles may increase the risk of autistic symptoms. As a result, it was reported that this gene may be associated with the possibility of autistic features in the phenotype (25). According to another study, it was stated that *DRD4* polymorphisms of oppositional defiant disorder, separation anxiety disorder, obsession-compulsions, and repetitive behaviors may be related to the severity of the symptoms of the disease. It has been reported that the symptoms are more severe in the phenotype. In addition, it has been determined that oppositional defiant disorder symptoms are more severe in patients who are homozygous with the *DAT1* 10-repeat allele and who are carriers of the *DRD4* 7-repeat allele. These results support the idea that *DRD4* polymorphisms may be a candidate biomarker associated with autism severity (26). In another study, the *DRD4* gene repeat allele was examined in ASD individuals, as well as in their parents, and the children were examined in terms of opposition, separation anxiety, and repetitive behaviors (27). Consequently, the 7-repeat genotypes were found to be associated with oppositional defiant disorder, obsessive-compulsive disorder, and tic severity, it was concluded that genotype research in families could help to establish biomarkers for the evaluation of prognosis for behavioral disorders in patients with ASD.

Phosphodiesterase 4D (PDE4D) regulates cyclic adenosine monophosphate (cAMP) signaling and plays a crucial role in sex-specific signaling regulation in ASD. In a study investigating the behavioral and biochemical effects of *CC2D1A* deficiency in male and female mice in intellectual disability and autism spectrum disorder, it was shown that, unlike females, *PDE4D* was hyperactive in *CC2D1A*-deficient male mice, resulting in a decrease in cAMP response element-binding protein signaling (28).

Armadillo Repeat Containing X-Linked 4 (ARMCX4) is a member of the ARMadillo repeat-containing proteins gene family on the X chromosome (29). In a study examining genetic aberrations with WES in childhood-onset schizophrenia (COS) patients, variants of this gene were identified in twelve male (30). To date, there is no study describing the relationship of this gene with autism. However, this gene variant (c.5551A>G|p.Ile1851Val) was detected in our study.

ADAR enzymes are important in the healthy development of the brain. *ADAR* gene has been linked with Fragile X and ASD (31). In a study in which ASD-related genes were examined by transcriptome analysis, it was suggested that *ADAR* enzymes may cause deterioration in the cells due to insufficient regulation in inhibitory neurons (32).

Low-density lipoprotein (LDL) receptor related protein 1B (LRP1B) gene belongs to the receptor gene family. These receptors play a wide variety of roles in normal cell function and development due to their interactions with multiple ligands. A study conducted with array-comparative genomic hybridization (aCGH) analysis examined a 23-year-old individual with episodes of unexplained severe mental retardation, autism spectrum disorder, congenital malformations including hypospadia and omphalocele, and episodes of high blood pressure. In the study, mutations in eight genes, including the *LRP1B* gene, were detected in an individual with autism accompanied by mental retardation (33).

Death associated protein (DAP) gene has been found associated with ASD as a biomarker by Carvalho et al. (34). Also, in the Center for Health Assessment of Mothers and Children of Salinas [CHAMACOS], associations of prenatal *DAP*s with lower IQ, poorer attention (35), and other genes, such as *GLIS3, LPXN, FAM186A, NEK3, GOLGA8A, SSC5D, LYRM7, CNTNAP3B, TCP11L1, OR8B3, KLRC3*, and *DPP9* had been reported. As a consequence of studies on ASD up to now, indels, SNVs, and CNVs in many genes have been found to be associated with the disease. Genetic variations revealed by whole genome sequencing and whole exome sequencing studies on concordant and discordant twins are important in understanding the genetic background of the disease. Even with great strides in understanding the genetic basis of ASD by sequencing of multiple cohorts in todays, many causes underlying autism remains undiscovered.

CONCLUSION

Our study provides evidence that WGS data can aid in the detection and clinical evaluation of individuals with ASD and their families. According to analysis of sequence, rs5951336 variant in ARMCX4 gene were detected in our MZ twins. The diagnostic yield and clinical utility should increase as more undetected structural genetic variants are discovered and characterized and as additional individuals with ASD are studied. Several genome sequences may help to resolve the role of common variants in ASD, and integrating these data with those on rare variants will aid understanding of penetrance, variable expressivity, and pleiotropic effects. As a result, genetic susceptibility to ASD may be different for each individual. This makes that individual a prime candidate for the precision medicine era.

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