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ORIGINAL ARTICLE

MUTATION ANALYSIS OF THE *NRXN1* GENE IN AUTISM SPECTRUM DISORDERS

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ABSTRACT

The aim of this study was to identify the sequence mutations in the *Neurexin 1* (*NRXN1*) gene that has been considered as one of the strong candidate genes. A total of 30 children and adolescents (aged 3-18) with non syndromic autism were enrolled this study. Sequencing of the coding exons and the exon-intron boundaries of the *NRXN1* gene was performed. Two known mutations were described in two different cases. Heterozygous S14L was determined in one patient and heterozygous L748I was determined in another patient. The S14L and L748I mutations have been described in the patients with autism before. Both of these mutations were inherited from their father. In this study, two of 30 (6.7%) autism spectrum disorder (ASD) patients carrying *NRXN1* gene mutations were detected. It indicates that variants in the *NRXN1* gene might confer a risk of developing nonsyndromic ASD. However, due to the reduced penetrance in the gene, the causal role of the *NRXN1* gene mutations must be evaluated carefully in all cases.

Keywords: Autism spectrum disorder (ASD); *Neurexin 1* (*NRXN1*) gene; Mutation; Sanger sequencing.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by persistent impairment in reciprocal social communication and social interaction, and restricted, repetitive patterns of behavior, interests, or activities, and with increasing prevalence in recent years [1]. Prevalence ratio of ASD was stated to be in the range of 0.6 to 2.64% [2-3]. A male predominance was observed and male-to-female ratio was 5 [4]. There is no accurate etiological factor that causes ASD; however ASD is likely to result from a complex interaction between genetic and environmental factors [3-5]. Autism spectrum disorder is one of the most heritable psychiatric disorders and heritability of ASD is 90.0% [6]. There is an increased recurrence risk of more than 20-fold in first-degree relatives [7]. Comprehensive genetic testing of children with ASD revealed a chromosomal or mendelian cause in 15.0-40.0% of the patients [8].

Classifying autism into 'essential' and 'complex' subgroups may be beneficial to understanding the genetic basis of the disorder [9]. Essential autism is usually present in about 75.0% of cases and is characterized by absence of dysmorphic features and comorbidities. In this group, there is a higher male-to-female ratio and higher sibling recurrence risk compared to the complex autism. In complex or syndromic autism, some dysmorphic features and neurological and medical symptoms such as seizures accompany autism. Because of the comorbidities, prognosis is worse in this group. The distinction between the two types of autism is important because prognosis, recurrence risk and genetic approach are different between the two groups [10].

Understanding the genetic basis of autism is challenging. The genetics of autism is an active research area and up to now, nearly all kinds of study designs have been used including family based, case-control, genome wide association studies (GWAS) and next generation sequencing (NGS). All these genetic studies reported that more than 600 genes and 2000 loci have been related to ASD and 83.4% of the detected variants are rare variants [11].

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Genetic variations detected in ASD can be classified into three subgroups: in up to 5.0% of the cases, cytogenetic anomalies can be detected with standard karyotyping, and an additional 3.0-5.0% have been found using fluorescence *in situ* hybridization (FISH); in 10.0-35.0% of cases, copy number variants (CNVs) can be found with microarray analysis and in 5.0% of the cases, single gene mutations might be found [10]. In the complex/syndromic autism group, it is relatively easier to find the genetic etiology with the help of dysmorphic features. Fragile X syndrome, Angelman syndrome or Rett syndrome are examples of syndromic ASD. Approximately 1.0-3.0% of children with ASD have been found to have fragile X syndrome. A considerable number of children being evaluated for autism *FMR1* premutations (55-200 CGG repeats) have also been found [10].

In the essential autism group, many genes have been blamed, and the likelihood of identifying a single gene mutation in an essential autism patient is extremely low. SFARIGENE [11] (https://gene.sfari.org) is a web-based database of candidate genes associated with ASD and all the genes annotated in this database are grouped in seven categories according to their relevance to ASD. A number of these genes are becoming clinically relevant. Especially the genes associated with the synaptic cell adhesion and synaptic function, such as *neurexin 1* (*NRXN1*), *neuroligin 3* (*NLGN3*), *neuroligin 4* (*NLGN4*) and *SHANK3* have attracted great attention. Neurexins function in the vertebrate nervous system as cell adhesion molecules and receptors. Neuroxin 1 is a cell surface receptor that binds neuroligins to form a calcium-dependent neurexin/neuroligin complex at synapses in the central nervous system (CNS). This complex is important for efficient neuro transmission and is involved in the formation of synaptic contacts [12]. The *NRXN1* gene is listed as a strong candidate gene in the SFARIGENE database and heterozygous deletions, and up to now, point mutations have been detected in the *NRXN1* gene in a limited number of patients with ASD [11]. Herein, in order to investigate the prevalence of *NRXN1* mutations in ASD patients, sequencing of the *NRXN1* gene was performed in 30 essential ASD patients with a normal karyotype and negative *FMR1* analysis for fragile X syndrome.

MATERIALS AND METHODS

Thirty children and adolescents (aged 3-18) diagnosed with autism, who were followed at the Ege University Child and Adolescent Psychiatry Clinic, Autism and Developmental Disorders Department, Ege University School of Medicine, Izmir, Turkey, were included in this study. The patients' autism diagnosis was made using (DSM-IV-TR) [13] criteria by two expert child and adolescent psychiatrists, who have been working in the autism area for the past 15 years. The Childhood Autism Rating Scale (CARS) Turkish Version [14,15] was used for all children in this study. The CARS is widely used to determine the presence and degree of autism. The CARS is an autism diagnostic schedule covering 14 functional areas that may be compromised in autism, and a final general category referring to 'degree of autism' [14]. The 15 items in the scale are: relating to people; imitative behavior; emotional response; body use; object use; adaptation to change; visual response; listening response; perceptive response; fear or anxiety; verbal communication; non-verbal communication; activity level; level and consistency of intellective relations; general impressions. The examiner assigned a score of 1 to 4 for each item: 1 indicates behavior appropriate for age level, while 4 indicates severe deviance with respect to normal behavior for age level. The total score range is between 15 and 60. Scores of 30 to 36 indicate mild to moderate autism and scores above 36 indicate severe autism.

Chromosomal abnormalities and fragile X syndrome were excluded in all patients by karyotyping and *FMR1* gene CGG expansion analysis, respectively. The *NRXN1* gene mutation analysis was performed by sequencing of the coding exons and exon-intron boundaries of the gene at the Department of Medical Genetics, Molecular Genetics Laboratory, Ege University, Izmir, Turkey. Genomic DNA was isolated from peripheral blood cells by standard techniques. All polymerase chain reaction (PCR) products were sequenced by the BigDye termination method using a DNA sequencing kit (Perkin-Elmer, Foster City, CA, USA) and analyzed using The ABI PRISM® 3100 sequence analyzer (Applied Biosystems, Foster City, CA, USA).

RESULTS

Thirty patients were included in this study. Twenty-five (83.3%) were boys and five (16.7%) were girls. The mean age was 8.43 ± 4.06 years (minimum 3; maximum 18). Three patients had a positive family history of ASD. Based on the autism severity ranking considering the CARS points, the majority of the features in the working group was determined as severe. Sequencing of the *NRXN1* gene revealed two different mutations, namely, S14L and L748I (Table 1).

Table 1. Clinical characteristics and genetic results of the patient group. (ASD: autism spectrum disorder; CARS: Childhood Autism Rating Scale.)

Patient	Sex-Age	Family History of ASD	CARS	CARS Scores	Mutations	Mother	Father
P1	M-17		37	severely autistic		–	–
P2	M-4		30	mild/moderately autistic	S14L/-	–	S14L/-
P3	M10		49.5	severely autistic		–	–
P4	F-5		46	severely autistic		–	–
P5	M-15		31	mild/moderately autistic		–	–
P6	M-6		44	severely autistic		–	–
P7	M-5		41.5	severely autistic		–	–
P8	M-9		38.5	severely autistic		–	–
P9	M-7		41.5	severely autistic	L748I/-	–	L748I/-
P10	M-11		42.5	severely autistic		–	–
P11	F-8		47.5	severely autistic		–	–
P12	M-10		32	mild/moderately autistic		–	–
P13	M-15		26	mild/moderately autistic		–	–
P14	M-4		51	severely autistic		–	–
P15	M-6		47	severely autistic		–	–
P16	M-6	positive	41.5	severely autistic		–	–
P17	M-10		37	severely autistic		–	–
P18	F-6		37	severely autistic		–	–
P19	M-10		38	severely autistic		–	–
P20	M-8		43	severely autistic		–	–
P21	M-9		37	severely autistic		–	–
P22	M-8	positive	31.5	mild/moderately autistic		–	–
P23	M-18		50	severely autistic		–	–
P24	M-4		46.5	severely autistic		–	–
P25	F-14	positive	31	mild/moderately autistic		–	–
P26	M-9		35	mild/moderately autistic		–	–
P27	M-8		30	mild/moderately autistic		–	–
P28	M-3		53	severely autistic		–	–
P29	M-4		39	severely autistic		–	–
P30	F-4		42	severely autistic		–	–

DISCUSSION

The ASD is a neurodevelopmental disorder characterized by deficits in social communication and the presence of repetitive patterns of behavior, activity and interests [16]. The heritability of ASD is 90.0% and there is an increased recurrence risk in first-degree relatives of more than 20-fold [7,8]. The aim of this study was to better understand the role of the *NRXN1* gene that is listed as one of the two strong candidate genes in SFARIGENE (an important database for autism) [12].

Neurexin proteins are the cell surface receptors that tie neuroligin (NLGN). The Ca^{2+}-dependent neurexin/neuro-ligin complex is present at synapses in the CNS is required for efficient neuro-transmission, and is involved in the formation of synaptic contacts. The *NRXN1* is a gene that expresses NRXN1 (OMIM:600565) protein, located on chromosome 2 at position 2p16.3, has 22 exons, encodes 1477 amino acids, has 7505 bp. Small and large deletions in the *NRXN1* gene, could play a role in the etiology of ASD. Additionally, missense and nonsense mutations in the *NRXN1* gene could play a role in the pathogenesis of this disorder [17,18].

Mild/moderately and severely autistic patients were involved in our study. There were more male cases than female cases in the study parallel to the rates notified in

the autism literature [3,10]. Two known mutations were described in two different cases. S14L (SFARIGENE Variant ID: GEN179R001) in patient P2 and L748I (SFARIGENE Variant ID: GEN179R007) in patient P9. S14L [17] and L748I [18] mutations have been described in patients with autism before. Both of these mutations were inherited from their father. The fathers of the children who carried the mutations were reevaluated psychiatrically and autism-spectrum quotient (AQ) questionnaire [19,20], which has been used extensively to measure the broader autism phenotype (BAP), was applied to the fathers. The father of patient P2 did not have any psychiatric diagnosis and symptoms. His total AQ score was 22 (social skill: 6 points, attention switching: 3 points, attention to detail: 4 points, communication: 4 points, imagination: 5 points). This may indicate a reduced penetrance in the *NRXN1* gene. Reduced penetrance in the *NRXN1* gene has been reported before [18-21]. The father of patient P9 had attention deficit and hyperactivity disorder (ADHD) symptoms and mild obsessions. His total AQ score was 13 (social skill: 4 points, attention switching: 4 points, attention to detail: 3 points, communication: 1 points, imagination: 1 points). The ADHD and sub-threshold obsessive compulsive symptoms in one father (father of P9) was determined as remarkable. Genetic relatives of people with autism may show milder expression of characteristic traits for autism, referred to as the BAP [22].

Up to now, the S14L mutation has been detected in four subjects with ASD and not in 1201 controls [17]. The S14L mutation was described in two siblings in two families and the mutations in these two families were inherited from their father. In one of family, there was a boy who met the autism diagnostic interview-revised (ADI-R) criteria and his sister with central auditory processing problem, and decreased social interaction. Their father, who carried a heterozygous S14L mutation, had a learning disability. In the other family there was also a boy who met the ADI-R criteria and his brother had learning disability and hyperactivity [17]. The L748I mutation was previously identified in two families with ASD. There were four ASD patients in these two families; the L748I mutation was detected in three of four ASD patients, and also detected in one of two unaffected individuals. Because of that, this mutation was evaluated as an ASD susceptibility allele showing incomplete penetrance [18].

CONCLUSIONS

Two of 30 (6.7%) ASD patients were diagnosed with *NRXN1* mutations in this study. It indicated that variants in the *NRXN1* gene might confer a risk in developing non syndromic ASD. However, due to the reduced penetrance in the gene, the causal role of the *NRXN1* gene mutations must be evaluated carefully in all cases.

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REFERENCES

1. Diagnostic and Statistical Manual of Mental Disorders, 5th Ed. American Psychiatric Association (APA). American Psychiatric Publications, Arlington, VA, USA, 2013. (http://www.dsm5.org/psychiatrists/practice/dsm.)
2. Fombonne E. Epidemiology of pervasive developmental disorders. Pediatr Res. 2009; 65(6): 591-598.
3. Kim YS, Leventhal BL, Koh YJ, Fombonne E, Laska E, Lim EC, *et al.* Prevalence of autism spectrum disorders in a total population sample. Am J Psychiatry. 2011; 168(9): 904-912.
4. Fuentes J. Autism spectrum disorders: Ten tips to support me. J Am Acad Child Adolesc Psychiatry. 2014; 53(11): 1145-1146, 1146 e1-3. doi: 10.1016/j.cbpa.2014. 07.017.
5. Levy SE, Mandell DS, Schultz RT. Autism. Lancet. 2009; 374(9701): 1627-1638.
6. Santangelo SL, Tsatsanis K. What is known about autism: Genes, brain, and behavior. Am J Pharmacogenomics. 2005; 5(2): 71-92.
7. Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, *et al.* Autism as a strongly genetic disorder: Evidence from a British twin study. Psychol Med. 1995; 25(1): 63-77.
8. Schaefer GB, Mendelsohn NJ, Professional P, Guidelines C. Clinical genetics evaluation in identifying the etiology of autism spectrum disorders. Genet Med. 2008; 10(4): 301-305.

9. Miles JH, Takahashi TN, Bagby S, Sahota PK, Vaslow DF, Wang CH, *et al.* Essential versus complex autism: Definition of fundamental prognostic subtypes. Am J Med Genet A. 2005; 135(2): 171-180.
10. Miles JH. Autism spectrum disorders – A genetics review. Genet Med. 2011; 13(4): 278-294.
11. Banerjee-Basu S, Packer A. SFARI Gene: An evolving database for the autism research community. Dis Model Mech. 2010; 3(3-4): 133-135.
12. Reissner C, Klose M, Fairless R, Missler M. Mutational analysis of the neurexin/neuroligin complex reveals essential and regulatory components. Proc Natl Acad Sci USA. 2008; 105(39): 15124-15129.
13. Diagnostic and Statistical Manual of Mental Disorders, 4th Ed. (Text Revision) (DSM-IV-TR). American Psychiatric Association, Washington, DC, USA, 2000.
14. Schopler E, Reichler RJ, DeVellis RF, Daly K. Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). J Autism Dev Disord. 1980; 10(1): 91-103.
15. Sucuoglu B, Oktem F, Akkok F, Gokler B. A study of the scales for the assessment of the children with autism. (Turkish) J Psychiatry Psychopharmacol Psychol. 1996; 4(2): 116-121.
16. World Health Organization. ICD-10: International Statistical Classification of Diseases and Related Health Problems, 10th revision, 2nd Ed. WHO, Geneva, Switzerland, 2002. (http://www.who.int/iris/handle/10665/42980).
17. Feng J, Schroer R, Yan J, Song W, Yang C, Bockholt A, *et al.* High frequency of neurexin 1β signal peptide structural variants in patients with autism. Neurosci Lett. 2006; 409(1): 10-13.
18. Kim H-G, Kishikawa S, Higgins AW, Seong I-S, Donovan DJ, Shen Y, *et al.* Disruption of neurexin 1 associated with autism spectrum disorder. Am J Hum Genet. 2008; 82(1): 199-207.
19. Baron-Cohen S, Wheelwright S, Skinner R, Martin J, Clubley E. The autism-spectrum quotient (AQ): Evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. J Autism Dev Disord. 2001; 31(1): 5-17.
20. Köse S, Bora E, Erermis S, Aydın C. Psychometric features of Turkish version of autism-spectrum quotient. (Turkish) Anadolu Psikiyatri Derg. 2010; 11: 253-260.
21. Duong L, Klitten LL, Møller RS, Ingason A, Jakobsen KD, Skjødt C, *et al.* Mutations in NRXN1 in a family multiply affected with brain disorders: NRXN1 mutations and brain disorders. Am J Med Genet B Neuropsychiatr Genet. 2012; 159B(3): 354-358.
22. Sasson NJ, Faso DJ, Parlier M, Daniels JL, Piven J. When father doesn't know best: Selective disagreement between self-report and informant report of the broad autism phenotype in parents of a child with autism. Autism Res. 2014; 7(6): 731-739.