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Detection of Clinically Relevant Genetic Variants in Children with Autism Spectrum Disorder by Whole Exome Sequencing

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Abstract

Autism spectrum disorder (ASD) is a neurodevelopment disorder which is related with a good number of genetic mutations. There is a lack of data regarding the genetic framework of children with ASD in Bangladesh. Hence, this study was conducted to detect clinically relevant genetic variants of ASD. A total of13 children with ASD aged between 2-10 years were included in this study, DSM-5 was used to confirm the diagnosis of ASD and to exclude other neurodevelopment, emotional, and behavioral disorders. Whole Exome Sequencing was done under the supervision of a geneticist. Mean age of participants was 5.54±3.13 years with male predominance (61.5%). Positive family history of neurodevelopment disorder was present in 46.2%. The average loss of acquired skill of study participants was 26.45±14.50 months. Most of the patients had delayed development of vocalization (92.3%), body gesture (61.5%), and spontaneous phrases (69.2%). Attention deficit hyperactivity disorder (ADHD) was the most common (46.2%) co-morbidity Majority patients (76.92%, n = 10) had presence of gene mutation, wherein 38.46% (n = 5) had variants of uncertain clinical significance (VOUS), 30.77% (n = 4) had likely pathogenic, and only 1 patient (7.69%) had pathogenic gene mutation. In this study, a good number of pathogenic genes related to ASD namely *TENM4, ASXL1, CHD3, TSC2 and CACNA1H* were detected. Larger multicenter study is recommended.

Keywords: Autism spectrum disorder neurodevelopment disorder; Whole exome sequencing; genetic variants

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Introduction

Autism spectrum disorder (ASD) is a group of highly heterogeneous neurodevelopmental disorders affecting 1 in 59 children aged 8 years. Boys are four times more likely to be affected than girls (Baio *et al.*, 2018). It is characterized by impaired reciprocal social interaction and communication, as well as restricted repetitive interests and behaviors (American Psychiatric Association, 2013). The symptoms could develop gradually from early childhood, affecting daily functioning and persisting throughout one's life (Stefanatos, 2008). Given the variety of phenotypes and severity, it's believed that genetic factors play pivotal role in the pathogenesis of ASD, in combination with developmental and environmental factors (Anagnostou *et al.*, 2014). The rapid progression of genomics technologies, coupled with expanding cohort sizes, have led to significant progress in characterizing the genetic architecture of complex disorders (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Sanders *et al.*, 2015; de Lange *et al.*, 2017; Deciphering Developmental Disorders Study, 2017; Marshall *et al.*, 2017).

To date, studies have mainly focused on genotyping array technologies to survey common variants and large rare copy number variations (CNVs), as well as whole exome sequencing (WES) to scan rare protein coding variants. In recent years, a good number of genetic mutations were detected in relation to ASD (Sanders *et al.*, 2015; Deciphering Developmental Disorders Study, 2017; Jin *et al.*, 2017). The common genes associated with ASD are sodium voltage-gated channel alpha subunit 2 (*SCN2A*), calcium voltage-gated channel subunit alpha1 E *(CACNA1E),* calcium voltage-gated channel auxiliary subunit beta 2 (*CACNB2*), potassium voltage-gated channel subfamily Q members 3 and 5 (*KCNQ3 and KCNQ5*), potassium voltage-gated channel subfamily D member 2 (*KCND2*), glutamate receptor signaling protein SH3 and multiple ankyrin repeat domains 3 (*SHANK3*), synaptic RasGTPase activating protein 1 (*SYNGAP1*), gamma-aminobutyric acid type A receptor gamma3 subunit (*GABRG*3), *MeCP2, UBE3A*, chromodomain helicase DNA binding protein 8 (*CHD8*), activity dependent neuroprotectorhomeobox (ADNP), pogo transposable element derived with ZNF domain (*POGZ*), fragile X mental retardation protein (*FMRP*), and RNA binding forkhead box (*RBFOX*) genes (Carney *et al.*, 2003; Samaco, Hogart and LaSalle, 2005; Durand *et al.*, 2012; Schmunk and Gargus, 2013; Giovedí *et al.*, 2014; Stessman *et al.*, 2017; Tran *et al.*, 2019).

This study was undertaken to analyze the genetic framework of children with ASD. This will highlight the complexity of the genetic landscape of the disease and also would shed light on some of the biological pathways at risk in ASD.

Materials and methods

This was a descriptive cross sectional study conducted from July 2019 to June 2020 in the Department of Pediatric Neurology, Bangabandhu Sheikh Mujib Medical University (BSMMU). Thirteen children aged 2-10 years diagnosed as ASD were enrolled for the study. Formal ethical approval was taken from the Institutional Review Board (IRB) of BSMMU. Informed written consent was taken from the parents or care givers of the study participants.

The parents of the children were interviewed face to face using a structured questionnaire. The socio-demographic information, presenting complaints, history of present illness, birth history, developmental history, family history, history of comorbidities like seizure, hyperactivity, sleep and

feeding pattern were recorded. General examination and neurological examination was done. Diagnosis of ASD was done according to DSM-5 criteria. (Chiche, 2016).

For genetic study, 3 mL of blood sample was taken from anti-cubital vein with full aseptic precaution from each child. Serum was separated from blood samples at collection place. Sample was stored at -20 ^{0}C temperature until transported to genetic laboratory, Neurogen Technologies Limited (A genetic lab) in Bangladesh for Whole Exome Sequencing. A geneticist supervised the whole process. The statistical analysis was carried out by using the Statistical Package for Social Sciences version 24.0 for Windows.

Results and discussion

The phenotype and genotype of 13 children with ASD was obtained. Mean age of the participants was 5.54±3.13 years. There was a male predominance (61.5%). Maximum children were from urban area (69.2%) and belonged to middle class (69.2%). Most of the subject never went to school (69.2%). Father and mother of study participants were mostly literate (92.3 and 100%). Most fathers (84.6%) were service

Table 1. Socio-demographic Profile of Study Participants (n = 13)

Variables Age (in years)	n (%)
Sex	5.54±3.13
Male	8 (61.5)
Female	5 (38.5)
Residence	
Rural	1 (7.7)
Urban	9 (69.2)
Semi Urban	3 (23.1)
Educational status of children	
Never went to school	9 (69.2)
Goes to special school	2 (15.4)
Conventional normal school	2 (15.4)
Education of father	
Literate	12 (92.3)
Illiterate	1 (7.7)
Education of mother	
Literate	13 (100)
Illiterate	0 (0)
Occupation of father	
Service holder	11 (84.6)
Business	2 (15.4)
Occupation of mother	
Service holder	4 (30.8)
House wife	9 (69.2)
Socio-economic status	
Lower	0 (0)
Middle	9 (69.2)
Higher	4 (30.8)

holder while most mothers were homemaker (69.2%) (Table 1). Positive family history of neurodevelopmental disorder was present in 46.2%. Maximum children were delivered at term (69.2%) by LUCS (61.5%). Mean birth weight was 2861.54±450.07 g. Almost 1/4th patients (23.1%) needed infection care with antibiotics during neonatal period while 2 (15.4%) children needed NICU admission and 1 (7.7%) child required oxygen supplement due to breathing difficulty at birth (Table 2).

The average loss of skill or regression of study participants was 26.45±14.50 months. Most of the patients (76.9%) developed social smile in time whereas most had delayed development of vocalization, body

Table 2. Risk factors among study participants (n=13)

Risk factors	n (%)
Family history of ASD	1 (7.7)
Family history of neurodevelopmental disorder	6 (46.2)
History of consanguineous parents	1 (7.7)
Maternal drug history	0 (0)
Maternal pre-ecclampsia/ecclampsia	0 (0)
Maternal Diabetes	1 (7.7)
Maternal radiation exposure	0 (0)
Maternal infection during pregnancy	1 (7.7)
Mode of delivery	
Normal vaginal delivery (NVD)	5 (38.5)
Lower Uterine cesarean section (LUCS)	8 (61.5)
Birth history	
Term	9 (69.2)
Pre term	4 (30.8)
Birth weight (in g)	2861.54±450.07
Breathing with oxygen at birth	1 (7.7)
NICU admission needed	2 (15.4)
Infection care with antibiotics	3 (23.1)
Neonatal Seizure	1 (7.7)
Neonatal Jaundice	1 (7.7)

gesture, and spontaneous phrases, sentences making and telling full name (92.3, 61.5, 69.2%, respectively) (Table 3). Attention deficit hyperactivity disorder (ADHD) was most common (46.2%) comorbidity (Table 4). Genetic study by WES of the studied participants showed that majority (76.92%) had presence of gene mutation, wherein 30.77% had likely pathogenic, 38.46% had variants of uncertain clinical significance (VOUS) and only 1 patient (7.69%) had pathogenic genetic mutation. (Table 5). The genetic mutations found in the WES are showed in Table 6.

ASD is a neurodevelopmental disorder with clinical and genetic heterogeneity. Although environmental factors play some roles but genetic factors are the key cause of ASD. With the advent of genetic test, in the last few decades significant advancement was observed in the genetic test of ASD (Tan *et al.*, 2017). The developed country had made significant progress in WES diagnosis of ASD, while due to financial

constrain countries like Bangladesh did not have genetic profile of children with ASD (Anagnostou *et al.*, 2014; Al-Mubarak *et al.*, 2017). With this purpose, this study was done to explore the genetic spectrum of children with ASD in Bangladesh.

In this descriptive cross sectional study mean age of participants was 5.54±3.13 years with male predominance (61.5%). Most of the patients were from urban areas and from middle class. Due to language and communication impairment, majority of the study subject were not school going (69.2%). However, 15.4% of the study subjects went to normal school and another 15.4% went to special school. As most of the study participants were from urban area, most of the parents were literate in this study group and holding service was the main profession of most of the father (84.6%).

Epigenetic deregulation is an important factor causing autism. (Gardener, Spiegelman and Buka, 2009). In this study, the risk factors of the study subjects were evaluated. Regarding the family history, most

Table 3. Neuro-Developmental status of studied children (n = 13)

Variables	n (%)
Loss of skill or regression (in month)	26.45±14.50
Milestone social smile	
Delayed	3 (23.1)
Age appropriate	10 (76.9)
Vocalization	
Delayed	12 (92.3)
Age appropriate	1 (7.7)
Polysyllabic consonant babbling	
Delayed	13 (100)
Age appropriate	0 (0)
Body gesture	
Delayed	8 (61.5)
Present	3 (23.1)
Absent	2 (15.4)
Spontaneous (not echoed) phrases	
Delayed	9 (69.2)
Present/age appropriate	4 (30.8)
Sentences	
Delayed	9 (69.2)
Present/age appropriate	4 (30.8)
Tells full name	
Delayed	9 (69.2)
Present/age appropriate	4 (30.8)

Table 4. Co-morbidities in the study subject (n = 13)

Co-morbidities	n (%)
ADHD	6 (46.2)
Sleep disturbance	5 (38.5)
ID	4 (30.8)
Epilepsy	1 (7.7)
Dysmorphism	0 (0)

Table 5. Genetic profile of the studied subject (n = 13)

Types of Gene mutation	n (%)
Positive	10 (76;92)
1. Likely pathogenic	4 (30.77)
2. Variants of uncertain clinical significance (VOUS)	5(38.46)
3. Pathogenic	1 (7.69)
Negative	3 (23.07)

significant risk factor was positive family history of other neurodevelopmental disorder (NDD) which was found in 64.2% of the subject. Other risk factors identified were family history of ASD, consanguinity of parents, maternal diabetes and maternal infection. Similar finding was found in related study where positive family history of NDD and maternal diabetes were risk factor of ASD (Gardener, Spiegelman and Buka, 2009).

Hindrance in the perinatal period significantly contributes in the pathogenesis of ASD(Atladóttir *et al.*, 2010; Gardener, Spiegelman and Buka, 2011). In this study, it was found that, about 30.8% of the subject were born preterm, 15.4% had NICU admission (15.4%), 23.1% had neonatal infection requiring antibiotics. Pinto Martin JA *et al.* in their study have mentioned that there is significant association of preterm birth with ASD (Pinto-Martin *et al.*, 2011). In another related study notified that ASD was prevalent in low birth weight babies and infants having neonatal jaundice (Amin *et al.,* 2011).

Table 6. Clinically relevant variants detected by WES (n = 13)

ID	Gene name	Coordinated	Mutation types/ variants type	Significance	Chromo some number	Exon	Nucleotide	change Variant information (amino acid chain)
01	TENM4	78726217	Missense Heterogygus	Pathogenic	11	23	c.3412G>A	p.Va-line11138Met
02	HECW2	196433210	Missense Heterogygus	VOUS	2	2	c.214C>A	p.Gln72Lys
03	CACNA1A	13209372	Missense Heterogygus	VOUS	19	45	c.C6469T	p.Arg2157Cys
04				Negative				
05				Negative				
06	ASXL1	32436347	Missense Heterogygus	Likely pathogenic	20	12	c.3635C>T	p.Ser1212Phe
07	CHD3	7911466	Missense	Likely pathogenic	17	40	c.6061G>A	p.Ala2021Thr
08	ATP2C2	84422438	Heterogygus	VOUS	16	8	c.C673T	p.Pro225Ser
09	ATP2C2	84453178	Missense Heterogygus	VOUS	16	19	c.G1872A	p.Met624Ile
10	SH3TC2	149026671	Missense Heterogygus	VOUS	5	12	c.A2954G	p.Glu985Gly
11				Negative				
12	TSC2	2072243	Missense Heterogygus	Likely pathogenic	16	20	c.2100G>T	p.Glu700Asp
13	CACNA1H	1211944	Splice accep-tor site variant Heterozygous	Likely pathogenic	16	24 *	c.4567-2A>G	

Regarding the clinical features, delayed onset of vocalization was observed in about 92.3% of the study subject. Other important parameter were delayed polysyllabic babbling, abnormal body gesture in 61.5%, delayed onset of phrases (69.2%), delayed onset of sentence making (69.2%) and inability to tell his/her own name (69.2%). In related studies, the core symptoms mentioned were reciprocal social deficit, communication impairment, rigid ritualistic interests. Thus, the clustering of symptoms are very importing for diagnosis of ASD along with the suggestive age (Nazeer and Ghaziuddin, 2012).

Comorbid disorders are common in cases of ASD. In this study, 46.2% of the children had ADHD, 38.5% had sleep disturbances and 30.8% had ID and 7.7% had epilepsy. In a large population based study by Mohammadi MR et al, 86% of the children with ASD had comorbidities. The most common one was ID observed in 70.3%. Other comorbid conditions were epilepsy, enuresis and ADHD with prevalence rates of 29.7%, 27% and 21.62%, respectively (Mohammadi *et al.*, 2019).

WES is an efficient diagnostic tool to detect the genetic mutation of ASD (Du *et al.*, 2018). In this study, interestingly, in more than two third of the subjects genetic test were positive. The pattern of positivity was as follows: likely pathogenic 30.77%, variant of uncertain clinical significance (VOUS) (38.46%) and pathogenic 7.69%. The pathogenic and likely pathogenic genetic mutations we found here were TENM4, ASXL1, CHD3, TSC2, CACNA1H etc.

One important gene related to ASD in this study was *CHD3*. Chromodomain helicase DNA binding protein 3 (*CHD3*) participates in the remodeling of chromatin by deacetylating histones. Two de novo missense variants and one de novo in-frame deletion variant were identified in the *CHD3* gene in ASD probands following WES (Iossifov *et al.*, 2014; Yuen *et al.*, 2017). In the case in this study, missense heterozygous mutation was detected which was likely pathogenic. *ASXL1* gene has been reported in previous studies in link with ASD (De Rubeis *et al.*, 2014).

One case with missense mutation of *TSC2* gene was identified here. Tuberous sclerosis is caused by mutations in one of two tumor suppressor genes: TSC1 (9q34) and TSC2 (16p13.3) (Maheshwar *et al.*, 1997). One case of *CACNA1C* pathogenic mutationwas found here. This gene is rarely associated with ASD. In a previous study, this mutation was identified in 6 cases out of 461 individuals with ASD (Splawski *et al.*, 2006). One case of mutation of teneurintransmembrane protein 4 (*TENM4*) gene was identified in this study. This geneis expressed primarily in the brain and has been detected in several transcriptomes derived from neuronal tissues. The link to ASD of this gene is very rare (Hor *et al.*, 2015).

Other VOUS genetic mutations found in this study were *HECW2, CACNA1A, ATP2C2, SH3TC2, KANK1, FOXP1* etc. One of our case had *HECW2* mutation. This gene mutation is associated with ID, epilepsy, visual impairment apart from ASD (Berko *et al.*, 2017). While, *CACNA1A* gene is another gene related to ASD found in one of the cases in this study. Previous study detected that a single nucleotide polymorphism (SNP) in *CACNA1A* confers risk to ASD (Li *et al.*, 2015). The *ATP2C2* gene is closely related to language impairment and dyslexia, some links with ASD has also been observed (Eicher and Gruen, 2015). Two cases of *ATP2C2* mutation were also detected here where both had VOUS and missense mutation. There was one case of *SH3TC2* gene mutation in this study. This gene encodes SH3 domain and tetratricopeptide repeats-containing protein 2 and is linked to peripheral neuropathy (Senderek *et al.*, 2003).

Again, there was one case with *KANK1* mutation in this study. This gene has potential impact on neurodevelopment. Vanzoet et al reported a case series of ASD with *KANK1* mutation (Vanzo *et al.*, 2019). Another mutation which was observed in this case series was in *FOXP1*. Haploinsufficiency of the forkhead-box protein P1 (*FOXP1*) gene leads ASD traits, intellectual disability, language impairment, and psychiatric features (Siper *et al.*, 2017).

Conclusion

This is probably the first case series of children with ASD with whole exome sequencing. Although, the number of cases are small, but it will highlight the genetic profile of ASD children of this zone and initiate further studies in this field. The pathogenic genes detected in relation to ASD were *TENM4, ASXL1, CHD3, TSC2,* and *CACNA1H.*

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Conflicts of Interest

The authors declare no conflict of interest

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