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 of 13 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
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 (GABRG3), 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; Giovedi 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 °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

| 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 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 shown 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

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**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-eclampsia/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)          |
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 | Chromosome number | Exon | Nucleotide | change Variant information (amino acid chain) |
|----|-----------|-------------|------------------------------|--------------|------------------|------|------------|------------------------------------------|
| 01 | TENM4     | 78726217    | Missense Heterozygous        | Pathogenic   | 11               | 23   | c.3412G>A  | p.V1138Met                               |
| 02 | HECW2     | 196433210   | Missense Heterozygous        | VOUS         | 2                | 2    | c.214C>A   | p.Gln72Lys                               |
| 03 | CACNA1A   | 13209372    | Missense Heterozygous        | VOUS         | 19               | 45   | c.C6469T   | p.Arg2157Cys                             |
| 04 |           |             | Negative                     |              |                  |      |            |                                          |
| 05 |           |             | Negative                     |              |                  |      |            |                                          |
| 06 | ASXL1     | 32436347    | Missense Heterozygous        | 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    | Heterozygous                 | VOUS         | 16               | 8    | c.C673T    | p.Pro225Ser                             |
| 09 | ATP2C2    | 84453178    | Missense Heterozygous        | VOUS         | 16               | 19   | c.G1872A   | p.Met624lle                              |
| 10 | SH3TC2    | 149026671   | Missense Heterozygous        | VOUS         | 5                | 12   | c.A2954G   | p.Glu985Gly                              |
| 11 |           |             | Negative                     |              |                  |      |            |                                          |
| 12 | TSC2      | 2072243     | Missense Heterozygous        | Likely pathogenic | 16      | 20   | c.2100G>T  | p.Glu700Asp                             |
| 13 | CACNA1H   | 1211944     | Splice acceptor site variant Heterozygous | Likely pathogenic | 16      | 24   | c.4562-2A>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 mutation was 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 gene 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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