Genetic Diagnosis in Children with Epilepsy and Developmental Disorders by Targeted Gene Panel Analysis in a Developing Country

Md Mizanur Rahman, FCPS, Kanij Fatema, FCPS
Department of Pediatric Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

Background and Purpose: In childhood epilepsy, genetic etiology is increasingly recognized in recent years with the advent of next generation sequencing. This has broadened the scope of precision medicine in intractable epilepsy, particularly epileptic encephalopathy (EE). Developmental disorder (DD) is an integral part of childhood uncontrolled epilepsy. This study was performed to investigate the genetic etiology of childhood epilepsy and DD.

Methods: In this study, 40 children with epilepsy and DD with positive genetic mutation were included retrospectively. It was done in a tertiary care referral hospital of Bangladesh from January 2019 to December 2020. Genetic study was done by next generation sequencing. In all cases electroencephalography, neuroimaging was done and reviewed.

Results: In total, 40 children were enrolled and the average age was 41.4±35.850 months with a male predominance (67.5%). Generalized seizure was the predominant type of seizure. Regarding the association, intellectual disability and attention deficit hyperactivity disorder was common. Seventeen cases had genetically identified early infantile EE and common mutations observed were SCN1A (3), SCN8A (2), SLC1A2 (2), KCNT1 (2), and etc. Five patients of progressive myoclonic epilepsy were diagnosed and the mutations identified were in KCTD7, MFSD8, and CLN6 genes. Three cases had mitochondrial gene mutation (MT-ND5, MT-CYB). Some rare syndromes like Gibbs syndrome, Kohlschütt-Tönz syndrome, Cockayne syndrome, Pitt-Hopkins syndrome and cerebral creatine deficiency were diagnosed.

Conclusions: This is the first study from Bangladesh on genetics of epilepsy and DD. This will help to improve the understanding of genetics epilepsy of this region as well as contribute in administering precision medicine in these patients. (2021;11:22-31)

Key words: Epilepsy, Gene, Mutation, Bangladesh

Introduction

Epilepsy is a major neurologic disorder in infants and children. It affects about 0.5-0.8% children and the highest incidence rate is in the first year of life. The burden of childhood epilepsy is much higher in developing countries.1,2 The etiology of childhood epilepsy is diverse and only one third of all cases are classified as specific epilepsy syndrome.3-5 With the advent of neuroimaging and electroencephalography (EEG), the etiology of epilepsy is being increasingly identified. However, in a significant number of cases, etiology remains unidentified.6-9 With the use of genetic testing, an etiology can be detected in these unknown subsets of patients.10 The International League Against Epilepsy have also opted towards the genetic classification of epilepsy.11 Compared to other age groups, children with epilepsy have a variety of developmental disorders (DD) like global developmental delay (GDD), intellectual disability (ID), autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), and etc.12 A good number of these children also have genetic etiology. About 30% of the children with epilepsy have genetic etiology. Majority of the genetic alteration related to epilepsy and DD were identified in genes encoding ion channels. These ion channels cause neuronal hyperexcitability or dysfunction in the inhibitory system and thus there is generation of seizures.13,14

In the last decade, with the invention of next generation sequencing (NGS) and advocating the concept of precision medicine, many
genes related to epilepsy and DD have been identified. NGS allows massive parallel sequencing of as many as genes as desired. Moreover, it can increase the diagnostic yield by up to 28% in patients with epileptic encephalopathy (EE). The utilization of genetic testing in pediatric epilepsy is still difficult in developing countries due to technical and financial constraints and, moreover, the list of known epilepsy genes is expanding every day. However, considering its great impact on precision medicine and genetic counseling, it became an essential part of epilepsy investigation. With this rationale, this study was done to identify the genetic characteristics of children with epilepsy and DD by NGS in suspected cases. This is the first genetic study in children with pediatric epilepsy in Bangladesh.

Methods

We are incorporating here the records of 40 children with epilepsy and DD with positive NGS. It is a retrospective, observational, mono-center study. It was done in the department of Pediatric Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. The time period was from January 2019 to December 2020. The main indication for genetic testing was idiopathic or cryptogenic epilepsy with DD. Institutional Review Board clearance and informed written consent from all the parents of the patients were taken. This study was funded by the University Grant Commission.

Initially children were evaluated through detailed history and clinical examination. History related to type of seizures, frequency, perinatal details, family history, developmental history and details of ongoing treatment were noted. A base line complete blood count, electrolyte, liver function test, renal function test, and blood glucose were performed. In every case a 30 minutes sleep and awake EEG was done. Neuroimaging (preferably magnetic resonance imaging [MRI] of brain) was done in every case. EEG reporting was done by an experienced pediatric neurophysiologist and neuroimaging was reviewed by an expert neuro-radiologist. In suspected case of metabolic disorder, metabolic tests (basic metabolic screening, tandem mass spectrometry, gas chromatography mass spectrometry) were done.

Genetic test

The genetic test was done by NGS. Selective capture and sequencing of the protein coding regions of the genome//genes were performed. DNA extracted from blood was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean >80-100X coverage on Illumina sequencing platform. The GATK best practices framework was followed for identification of variants in the sample using Sentieon (v201808.07; Sentieon, Inc., San Jose, CA, USA). The sequences obtained were aligned to human reference genome (GRCh38.p13) using Sentieon aligner and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels. Sentieon haplotype caller was used to identify variants which are relevant to the clinical indication. Gene annotation of the variants was performed using Variant Effect Predictor (VEP; European Molecular Biology Laboratory’s European Bioinformatics Institute, Hinxton, UK) program against the Ensembl release 99 human gene model. In addition to single-nucleotide variants and small Indels, copy number variants (CNVs) were detected from targeted sequence data using the ExomeDepth (v1.1.10) method. This algorithm detects rare CNVs based on comparison of the read-depths of the test data with the matched aggregate reference dataset.

The clinical significance of each variant was determined according to American College of Medical Genetics and Genomics guideline. Clinically relevant mutations were annotated using published variants in literature and a set of disease databases - ClinVar, OMIM (updated on 20th February 2020), genome-wide association study, Human Gene Mutation Database (v2019.4) and SwissVar. Common variants were filtered based on allele frequency in 1000 Genome Phase 3, gnomAD (v2.1), Exome Variant Server, Single Nucleotide Polymorphism Database (v151), 1000 Japanese Genome, and internal population database. Non-synonymous variant’s effect was calculated using multiple algorithms, such as Polymorphism Phenotyping v2, Sorting Intolerant from Tolerant, MutationTaster2, and likelihood ratio test. Only non-synonymous and splice site variants, found in the clinical exome panel consisting of 6670 genes, were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region were not reported.

Results

Demographic and phenotypical characteristics of the study subject

The phenotype and genotype of 40 patients with epilepsy and DD was recorded in this retrospective study. The age at diagnosis of the study subject was 41.4±35.850 months and age of onset of seizure was 16.2±17.145 months. More than two third of the cases were male (67.5%) (Table 1). Most common type of seizure was generalized seizure, documented in 60% of the cases. Other types of seizures were focal (32.5%), myoclonic (27.5%), infantile spasm (22.5%),
Table 1. Clinical, radiological and electrographic characteristics of the study subjects (n=40)

| Baseline information and investigation of the studied subject | Value |
|---------------------------------------------------------------|-------|
| Age at diagnosis (months)                                      | 41.4±35.85 |
| Age of onset (months)                                          | 16.2±17.15 |
| Sex                                                           |       |
| Male                                                          | 27 (67.5) |
| Female                                                        | 13 (32.5) |
| Seizure type                                                   |       |
| Focal                                                         | 13 (32.5) |
| Generalized                                                   | 24 (60.0) |
| Infantile spasm                                               | 9 (22.5) |
| Myoclonic                                                     | 11 (27.5) |
| Other                                                         | 1 (2.5) |
| Status epilepticus                                            | 5 (12.5) |
| Associated features                                           |       |
| Neuroregression                                               | 12 (30.0) |
| Visual impairment                                             | 6 (15.0) |
| Hearing Impairment                                            | 1 (2.5) |
| Spasticity                                                    | 15 (37.5) |
| Hypotonia                                                     | 3 (7.5) |
| Dystonia                                                      | 3 (7.5) |
| Ataxia                                                        | 4 (10.0) |
| Hyperactivity                                                 | 9 (22.5) |
| Autism spectrum disorder                                      | 5 (12.5) |
| Intellectual disability                                       | 18 (45.0) |
| Skin features                                                 | 4 (10.0) |
| Microcephaly                                                  | 10 (25.0) |
| Dysmorphism                                                   | 6 (15.0) |
| Birth and family characteristics                               |       |
| Perinatal insult                                              | 8 (20.0) |
| Consanguinity                                                 | 9 (22.5) |
| Sib affected                                                  | 5 (12.5) |
| Sib death                                                     | 5 (12.5) |
| Positive family history, other than sib                        | 6 (15.0) |

Values are presented as mean±standard deviation or number (%). EEG, electroencephalography; CSWS, continuous spike wave in slow wave sleep; MRI, magnetic resonance imaging.

status epileptics (12.5%), and others (2.5%). Regarding the associated neurodevelopmental status, neuro-regression was present in 30% cases, most commonly motor and cognitive regression. Visual and hearing impairment were observed in 15% and 2.5% cases, respectively. While 37.5% of the subjects had spasticity, 7.5% had dystonia, 7.5% had hypotonia, and 10% had ataxia. A notable number of patients had psychiatric comorbidities: 22.5% with ID, 22.5% with ADHD, and 12.5% with ASD.

Regarding the mentionable physical features, 25% individuals had microcephaly, 15% had dysmorphism (mainly facial) and 20% had skin abnormality (café au lait spot, shagreen patch, adenoma sebaceum, and etc.). Most of the study subjects had uneventful birth history, while 20% had perinatal insult, most common being perinatal asphyxia, sep-sis, birth trauma and neonatal hyperbilirubinemia. Consanguinity was present in 22.5% of the cases. About 12.5% cases had affected sib with same type of illness and another 12.5% had history of sib death, while 15% had other family members affected with similar type of illness. DD was present in all the cases. Global delay was present in 72.5% of the patients, while 20% only had cognitive delay, 5% had motor delay, and 5% had speech delay only (Table 1).

**Electrophysiological and imaging findings of the studied subjects**

EEG was abnormal in all the study subjects. Most common EEG change was focal epileptic discharge (32.5%). Other abnormalities detected were generalized discharge (30%), epileptic syndrome (EE) (17.5%), hypsarrhythmia (12.5%), continuous spike wave in slow wave sleep (CSWS) and others (2.5%). Regarding MRI of the brain, most patients had abnormality in the imaging (80%). The commonest abnormality was cortical atrophy (45%). Other changes were
Table 2. Genetic mutation of cases with early infantile epileptic encephalopathy (n=17)

| Case | Age (months) | Sex | Chromosome | Location: exon | Variance | Gene | Zygocity | Inheritance | Disease | Significance |
|------|--------------|-----|-------------|----------------|----------|------|----------|-------------|---------|--------------|
| 1    | 3            | M   | 20          | 4              | c.794C>T; (p.Ala265Val) | KCNQ2 | Heterozygous | ADD | Early infantile epileptic encephalopathy 7 | P |
| 2    | 11           | M   | 1           | 10             | c.244G>C; (p.Gly82Arg) | SLC1A2 | Heterozygous | ADD | Early onset epileptic encephalopathy | P |
| 3    | 10           | M   | 2           | 26             | c.4907G>A; (p.Arg1636Gln) | SCN1A | Heterozygous | ADD | Dravet syndrome | P |
| 4    | 11           | F   | 2           | 26             | c.5195C>G; (p.Trp1732Le) | SCN1A | Heterozygous | ADD | Dravet syndrome | P |
| 5    | 13           | M   | 2           | 11             | c.1303G>A; (p.Glu435Ter) | SCN1A | Heterozygous | ADD | Early infantile epileptic encephalopathy-6 (Dravet syndrome) | P |
| 6    | 12           | M   | 9           | 15             | c.1421G>A; (p.Arg474His) | KCNT1 | Heterozygous | ADD | Early infantile epileptic encephalopathy-14 | LP |
| 7    | 11           | M   | 12          | 27             | c.5615G>A; (p.Arg1872Gln) | SCN8A | Heterozygous | ADD | Infantile epileptic encephalopathy-13, Benign familial infantile seizure-5 | LP |
| 8    | 17           | M   | 9           | 5              | c.1301A>G; (p.Tyr434Cys) | NTRK2 | Heterozygous | ADD | Early infantile epileptic encephalopathy-58 | LP |
| 9    | 12           | F   | 9           | 15             | c.1421G>A; (p.Arg474His) | KCNT1 | Heterozygous | ADD | Early infantile epileptic encephalopathy-14 | LP |
| 10   | 12           | M   | 12          | 27             | c.5615G>A; (p.Arg1872Gln) | SCN8A | Heterozygous | ADD | Infantile epileptic encephalopathy-13 | LP |
| 11   | 5            | F   | 1           | 31             | c.3845G>A; (p.Tyr1282Glu) | DOCK7 | Heterozygous | ARD | Early infantile epileptic encephalopathy-23 | VOUS |
| 12   | 60           | F   | X           | 7              | c.325C>T; (p.Pro1095Ser) | CDKL5 | Heterozygous | XLD | Early infantile epileptic encephalopathy-2 | VOUS |
| 13   | 30           | F   | 16          | 14             | c.2965A>G; (p.Asp989Tyr) | GRIN2A | Heterozygous | ADD | Focal epilepsy and speech disorder with mental retardation | VOUS |
| 14   | 12           | M   | 2           | 17             | c.2710G>A; (p.Glu904Ser) | SCN2A | Heterozygous | ADD | Early infantile epileptic encephalopathy-7 | VOUS |
| 15   | 12           | F   | 9           | 10             | c.1277C>T; (p.Ala426Glu) | SPTA1 | Heterozygous | ADD | Early infantile epileptic encephalopathy-5 | VOUS |
| 16   | 53           | M   | X           | 24             | c.3592G>A; (p.Ala1198Lys) | SMC1A | Heterozygous | XLR | Early infantile epileptic encephalopathy 85 with or without midbrain defect | VOUS |
| 17   | 7            | M   | 11          | 10             | c.1460C>T; (p.Glu487Phe) | SLC1A2 | Heterozygous | ADD | Early infantile epileptic encephalopathy-41 | VOUS |
| X    | 12           |     |             |                | c.1261G>A; (p.Ala421Thr) | THOC2 | Heterozygous | XLR | X linked mental retardation | VOUS |

M, male; ADD, autosomal dominant disorder; P, pathogenic; F, female; LP, likely pathogenic; ARD, autosomal recessive disorder; VOUS, variant of uncertain significance; XLD, X linked dominant disorder; XLR, X linked recessive disorder.

White matter abnormality, neuronal migration defect, corpus callosal abnormality and periventricular leukomalacia (Table 1).

**Early infantile EE with genotype**

In the studied subjects, 17 cases were identified as early infantile epileptic encephalopathy (EIEE). Three cases had *SCN1A* mutation...
and the disease was EIEE 6 (Dravet syndrome). SLC8A4 mutation was observed in two cases and related disease was EIEE 13 while two cases had SLC1A2 mutation (related disease EIEE 41). Other mutations detected in this category were CDKL5 two cases (EIEE 2), KCN7 two cases (EIEE 14), NTRK2 (EIEE 58), DOCK7 (EIEE 23), GRIN2A, SPTAN1 (EIEE 5), SCN9A (GEFS+ 7), SCN2A (EIEE 11), SMC1A (EIEE 85) and etc. (Table 2).

Progressive myoclonic epilepsy (PME) cases with genotype

Five patients with suspected PME had identifiable gene mutation. Among them, two had likely pathogenic mutation of CLN6 gene mutation. Other three cases had mutation of KCTD7 and MFSD8 gene mutation, but these were variant of uncertain significance. All PME cases were autosomal recessive in inheritance pattern (Table 3).

Mitochondrial disorders with genotype

Three patients had mutation in the mitochondrial gene. In the first case the genes were MT-ND5(+) and MT-CYB(+); this case was diagnosed as Leigh syndrome. Rest of the two cases were also diagnosed as Leigh syndrome, but the genes involved were CYB and MT-ND5, respectively. Zygocity was homoplasmic in all the cases (Table 4).

Other cases with genotypes

There were two cases with neurocutaneous syndromes (NCS). One was tuberous sclerosis with TSC1 gene mutation, and another

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**Table 3.** Genetic mutation of cases with progressive myoclonic epilepsy (n=5)

| Case | Age (months) | Sex | Chromosome | Location: exon | Variance | Gene | Zygocity | Inheritance | Disease | Significance |
|------|--------------|-----|-------------|----------------|----------|------|----------|-------------|---------|--------------|
| Pathogenic and likely pathogenic variants | | | | | | | | | | |
| 1 | 84 | M | 15 | 7 | c.794_796del (p.Ser265del) | CLN6 | Homozygous | ARD | Neuronal ceroid lipofuscinosis-6 | LP |
| 2 | 48 | M | 15 | 7 | c.794_796del (p.Ser265del) | CLN6 | Homozygous | ARD | Neuronal ceroid lipofuscinosis-6 | LP |
| Variance of uncertain significance | | | | | | | | | | |
| 3 | 84 | M | 7 | 4 | c.505C>T(p.Arg169Trp) | KCTD7 | Homozygous | ARD | Progressive myoclonic epilepsy-3, with or without intracellular inclusions | VOUS |
| 4 | 108 | F | 7 | 2 | c.190A>G (p.Thr64Ala) | KCTD7 gene (CLN14) | Homozygous | ARD | Progressive myoclonic epilepsy-3 | VOUS |
| 5 | 48 | M | 4 | 9 | c.850G>C (p.Ala284Pro) | MFSD8 | Homozygous | ARD | Neuronal ceroid lipofuscinosis-7 | VOUS |

M, male; ARD, autosomal recessive disorder; LP, likely pathogenic; VOUS, variant of uncertain significance.

**Table 4.** Cases with mitochondrial genetic mutation (n=3)

| Case | Age (months) | Sex | Chromosome | Location: exon | Variance | Gene | Zygocity | Inheritance | Disease | Significance |
|------|--------------|-----|-------------|----------------|----------|------|----------|-------------|---------|--------------|
| Pathogenic and likely pathogenic variants | | | | | | | | | | |
| 1 | 12 | M | M14766 | - | m.14766C>T(p.Thr71Ile) | CYB | Homoplasmic | Mitochondrial | Leigh syndrome | LP |
| Variance of uncertain significance | | | | | | | | | | |
| 2 | 24 | F | - | - | c.25A>G (p.Thr9Ala) | MT-ND5 | Homoplasmic | Mitochondrial | Leigh syndrome | VOUS |
| | | | | | | | | | |
| 3 | 72 | M | - | c.475T>C (p.Tyr159His) | MT-ND5 | Homoplasmic | Mitochondrial | Leigh syndrome due to complex 1 deficiency | VOUS |

M, male; LP, likely pathogenic; F, female; VOUS, variant of uncertain significance.
case was neurofibromatosis with *NF1* gene mutation. Other syndromic cases diagnosed were Gibbs syndrome with *AHDC1* mutation, Kohlschütter-Tönz syndrome (KTS) with *ROGD1* mutation, Cockayne syndrome A with *ERCC8* mutation, Rett syndrome with *MECP2* mutation, Poirier-Bienvenu neurodevelopmental syndrome with *CSNK2B* syndrome, Pitt-Hopkins syndrome with *NCF4* mutation and cerebral

Table 5. Genetic mutation of other cases (n=15)

| Case | Age (months) | Sex | Chromosome | Location: exon | Variance | Gene | Zygocity | Inheritance | Disease | Significance |
|------|--------------|-----|-------------|----------------|----------|------|----------|-------------|---------|--------------|
| Pathogenic and likely pathogenic variants | | | | | | | | | | |
| 1 | 28 | F | 9 | 15 | c.1525C>T (p.Arg509Ter) | TSC1 | Heterozygous | ADD | Tuberous sclerosis 1 | P |
| 2 | 132 | M | 17 | 43 | c.6569delG (p.Gly2190AlafsTer10) | NF1 | Heterozygous | ADD | Neurofibromatosis type-1 | P |
| 3 | 84 | F | X | 4 | c.1158_1198del (p.Leu386Fs) | MECP2 | Homozygous frame shift deletion | XLD | Rett syndrome | P |
| 4 | 96 | M | 18 | 2 | c.277G>T (p.Gly93Ter) | NCF4 | Heterozygous | ADD | Pitt-Hopkins syndrome | P |
| 5 | 48 | M | 16 | 11 | c.229_230del (p.Leu77Alafs) | ROGD1 | Homozygous | ARD | Kohlschütter-Tönz syndrome (KTS) | LP |
| Variance of uncertain significance | | | | | | | | | | |
| 6 | 36 | F | 21 | Intron 8 | c.1239+1G>A (5splice site) | Dyrk1A | Heterozygous | ADD | Mental retardation-7 | P |
| 7 | 108 | M | 6 | 7 | c.564del (p.Tyr188Ter) | CSNK2B | Homozygous | ADD | Poirier-Bienvenu neurodevelopmental syndrome | VOUS |
| 8 | 17 | M | 5 | 2 | c.1705+1_17061 \( \cdot \) (1845+1_1846+1)del (Exonic deletion) | ERCC8 | Homozygous | ADD | Cockayne syndrome A | VOUS |
| 9 | 24 | M | X | 9 | c.1319G>A (p.Arg440His) | SLC6A8 | Hemiogyous | XLR | Cerebral creatine deficiency syndrome 1 | VOUS |
| 10 | 36 | M | 1 | 12 | c.1510G>T (p.Glu504Ter) | AIMP2 | Homozygous | ARD | Pontocerebellar hypoplasia type-9 | VOUS |
| 11 | 108 | F | 6 | 15 | c.2390C>G (p.Ser797Cys) | KCNQ5 | Homozygous | ADD | Mental retardation- 46 | VOUS |
| 12 | 12 | F | 19 | 5 | c.532dupT (p.Cys178Leufs22) | SCN1B | Homozygous | ADD | Generalized epilepsy with febrile seizure plus (GEFS+) | VOUS |
| 13 | 84 | M | 2 | 7 | c.2161A>G (p.Met721Val) C787T>C (p.Asp263Cys) | ZNF142 | Heterozygous | ARD | Neurodevelopmental disorder with impaired speech and hyperkinetic movement | VOUS |
| 14 | 36 | M | 12 | Intron 7 | c.1746+1G>T (5splice site) | DNM1L | Heterozygous | ADD/ARD | Encephalopathy with defective mitochondrial and peroxisomal fission 1 | VOUS |
| 15 | 36 | M | 1 | 6 | c.4183C>A (p.Gln1395Lys) | AHDC1 | Homozygous | ADD | Xia-Gibbs syndrome | VOUS |

F, female; ADD, autosomal dominant disorder; P, pathogenic; M, male; XLD, X linked dominant disorder; ARD, autosomal recessive disorder; LP, likely pathogenic; VOUS, variant of uncertain significance; XLR, X linked recessive disorder; ASD, autism spectrum disorder.
creatine deficiency syndrome with SLC6A8 mutation. In two cases of epilepsy, mutation of KCNQ5 and DYRK1A mutation were observed, which are related to mental retardation. Other mutations observed were in AMPD2, KCNQ5, SCN18, SETD2, ZNF142, and DNMT1 gene (Table 5).

**Discussion**

In recent years, there was an emergence of genomic technologies, including chromosomal microarrays and NGS. This accelerated the understanding and application of genetic test in epilepsies and DD. Moreover, these modalities of test highlighted the critical pathways for epileptogenesis in addition to ion channels, which caused significant advancement in precision medicine. Our study was done in this respect and may add additional information in the genetics of epilepsy and DD of a developing country like Bangladesh.

Genetic testing and counseling for epilepsy is incorporated in routine practice in many advanced centers worldwide. NGS panel testing is mostly used in this respect. The high diagnostic yield and cost effectiveness of this test made NGS very useful. In previous studies, yields of 10-48.5% have been reported by NGS panel in epilepsy patients. Children under 2 years of age have even higher yields. In this study, 17 cases (42.5%) were genetically diagnosed as EIEE. Most common type was EIEE 6 (Dravet syndrome) where there is predominance of Dravet syndrome with SCN1A mutation. There were SCN8A, SLC1A2, CDKL5, and KCNT1 mutation related to EIEE. We observed SCN8A, SLC1A2, CDKL5, and KCNT1 mutation related to EIEE. These are common mutations found in related study. We found some rare genetic mutations in the study group like NTRK2 related to EIEE 58, DOCK7 mutation related to EIEE 23, SPTAN1 (EIEE 5), SCN9A (GEFS+ 7), SCN2A (EIEE 11), SMIC14 (EIEE 85), and etc.44-46

PME are a group of neuro-regressive disorders characterized by myoclonus, multiple seizure type, progressive regression, and cerebral and/or cerebellar atrophy. Genetic tests play important role in diagnosing and classifying PME. We have summarized five cases of PME with genotypes in this study. Two cases were PME3 with mutation in KCTD7. The phenotype of these two cases were typical of that of the reported cases, onset in infancy, frequent myoclonic seizure along with regression and ataxia. We also identified two cases of NCL 6 with CLN6 gene mutation and one case of NCL7 with MFSD8 mutation. In related study by Zhang et al., the most common gene identified was PPT1 which was not identified here. While other gene mutations were similar to their study findings like their 2nd prevalent gene was KCTD7.

All three cases with mitochondrial gene mutation were Leigh syndrome. Here, two different genes were identified: MTND5 and MTCYB. Mitochondrial disorders have heterogeneous phenotype with involvement of multiple organs; however, epilepsy is a common feature here. Two of our cases had infantile spasms, and the rest had focal with secondary generalized epilepsy. This coincides with the study done by Lee et al. The mutations found in this study were c.25A>G(p.Thr9Ala), c.1111C>A(p.Leu371Met), m.14766C>T(p.Thr7Ile), c.475T>C(p.Tyr159His). We did not find any case of classic m.3243A>G mutation for mitochondrial encephalomyopathy, lactic acidosis, and stroke-like syndrome which is closely related to status epilepticus.

In this study, two genetically diagnosed NCS were identified. Although NCS are commonly found in children with epilepsy, most of the cases are not genetically diagnosed. One case was tuberous sclerosis with TSC1 gene mutation and another case was NF with NFI gene mutation. We found some rare syndromes in relation to epilepsy like Gibbs syndrome with AHDC1 mutation. This patient had infantile spasm, dysmorphism, GDD, hypotonia and failure to thrive. These features have similarity with the case reported by Gumus. A case of KTS was diagnosed with definite phenotype of amelogenesis imperfecta, intractable seizure, and GDD with ROGDI mutation. It is a rare case of epilepsy and very few cases have been identified till date. We also found a case of cockayne syndrome A with ERCC8 mutation with the typical phenotype of microcephaly, failure to thrive, ID, short stature, seizure and abnormal behavior.

A case of casein kinase 2 beta gene causing Poirier- Bienvenu neurodevelopmental syndrome was identified in this study. The patient was a boy with ID, hypotonia, and generalized seizure (OMIM ID: 607000).
These features coincide with the previously reported case. Another rare case identified was of Pitt-Hopkins syndrome with NCF4 mutation. This case had focal epilepsy, ID, stereotypes, and visual impairment. The phenotype and genotype of this case coincided with case reported by Rosenfeld et al. There was a case of cerebral creatine deficiency syndrome with mutation of SLCG6A8+ in exon 9 in this study. It is an X linked recessive disorder characterized by speech and language delay, cognitive delay, autistic behavior, infantile spasm, generalized hypotonia, and microcephaly.

Although phenotypical heterogeneity complicates the use of precision medicine in genetic epilepsy, the genetic diagnosis of epilepsy has shown significant and dramatic changes in its treatment. On the basis of genotype, we applied the precision medicine in some of our cases. Like in cases with Dravet syndrome (SCN1A gene mutation), we avoided sodium channel blocker as that is the challenge in treatment of this syndrome, instead of selecting antiepileptic drugs. In contrary to this, sodium channel blockers are suggested in cases of SCN8A encephalopathy, like high dose phenytoin, which has also been applied in our cases. Similarly, in cases with KCNQ2 gene mutation, sodium channel blocker was used.

The mean age of the studied subject was 41.4±35.85 months, while the age of onset of seizure was far earlier (16.2±17.145 months). A male predominance was observed like other similar studies. Generalized seizure was the most common type of seizure and the 2nd most common type was focal seizure. All the patients had DD, most common being GDD, while 30% had neuroregression. DD was also very common in study done by Balciuniene et al. Regarding the associated neuropsychiatric disorders, ID, hyperactivity, and ASD was observed in a significant number of patients. In a study done by Arafat et al., 26.4% patients had mild ID, 29.4% patients had moderate ID and 44.2% had severe ID. Most of the patients had uneventful birth history in this study. Family history revealed that 22.5% patients had consanguinity of parents, while 15% had family members (other than sib) affected and 12.5% had history of sib death with similar type of illness.

EEG is very important to diagnose and classify epilepsy syndromes. In this study, the abnormalities detected were focal epilepsy (32.5%), generalized discharge (30%), EE (17.5%), hypersynchrony (12.5%), CSWS and others. Neuroimaging was mostly abnormal, most common being cortical atrophy. On the contrary, in a related study by Arafat et al., only 38.9% had abnormal MRI of the brain. They also reported the finding as noncontributory as most patients had cortical atrophy. However, some of our findings were contributory in diagnosis of the syndrome like TSC, NF1, Rett syndrome, neuronal migration defect, and etc.

NGS is an evolving diagnostic test in pediatric genetic epilepsy. The value of this test is much more than just diagnosis as there is scope of precision medicine based on this. Although the financial burden is tremendous, considering the diagnostic yield, targeted therapy and minimization of related test, the application of NGS is increased in the recent years. This NGS based study will thus highlight the pediatric genotype of Bangladesh and may improve the understanding of genetics of EIEE, PME, mitochondrial epilepsy and other syndromes with epilepsy and DD. Furthermore, it will be a potential source of further prospective study.

Limitation of the study
As this is a retrospective study, this does not portray the total NGS yield in suspected case. Furthermore, in most of the cases, parental genetic study was not done.

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Conflict of Interest
The authors declare that they have no conflicts of interest.

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