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, Kohlschütter-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).[15] 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).[16] 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.[16,17] 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.[18,19] 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.[20] 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.[21-25] 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.[26-29] 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 (22.5)
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)

Table 1. Continued

Baseline information and investigation of the studied subject	Value
Developmental status	
Normal	1 (2.5)
Speech delay only	2 (5.0)
Motor delay only	2 (5.0)
Cognitive delay	8 (20.0)
Global delay	29 (72.5)
Metabolic test	
Normal	38 (95.0)
Abnormal	2 (5.0)
EEG	
Focal discharge	13 (32.5)
Generalized discharge	12 (30.0)
Epileptic encephalopathy	7 (17.5)
Hypsarrhythmia	5 (12.5)
CSWS	2 (5.0)
Others	1 (2.5)
MRI of brain	
Normal	8 (20.0)
Cortical atrophy	18 (45.0)
White matter hyper/hypo/demyelination	5 (12.5)
Cerebellar atrophy	3 (7.5)
Neuronal migration defect	2 (5.0)
Corpus callosal agenesis/hypoplasia	2 (5.0)
Calcification	1 (2.5)
Periventricular leukomalacia	1 (2.5)

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

status epilepticus (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
Pathogenic and likely pathogenic variants										
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>T(p.Pro1732Le)	SCN1A	Heterozygous	ADD	Dravet syndrome	P
5	13	M	2	11	c.1303G>T(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.Arg1872Gin)	SCN8A	Heterozygous	ADD	Infantile epileptic encephalopathy-13, Benign familial infantile seizure-5	LP
8	17	M	9	5	c.1301A>G(p.Tyr434Cys)	NTRK2 mutation	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.Arg1872Gin)	SCN8A	Heterozygous	ADD	Early infantile epileptic encephalopathy-13	LP
Variance of uncertain significance										
11	5	F	1	31	c.3845G>A(p.Gly1282Glu)	DOCK7	Heterozygous	ARD	Early infantile epileptic encephalopathy-23	VOUS
12	60	F	X	7	c.325C>T(p.Pro1095Ser)	CDKL5	Hemizygous	XLD	Early infantile epileptic encephalopathy-2	VOUS
13	30	F	16	14	c.2965A>T(p.Asn989Tyr)	GRIN2A	Heterozygous	ADD	Focal epilepsy and speech disorder with mental retardation	VOUS
			X	7	c.325C>T(p.Pro1095Ser)	CDKL5	Hemizygous	XLD	Early infantile epileptic encephalopathy-2	VOUS
14	12	M	2	17	c.2710G>A(p.Gly904Ser)	SCN2A	Heterozygous	ADD	Early infantile epileptic encephalopathy-11	VOUS
15	12	F	9	10	c.1277C>A(p.Ala426Glu)	*SPTAN1*	Heterozygous	ADD	Early infantile epileptic encephalopathy 5	VOUS
			2	12	c.1726A>G(p.Ser576Gly)	*SCN9A*	Heterozygous	ADD	Generalized epilepsy with febrile seizures plus, type 7 and Familial febrile seizures-3B	VOUS
16	53	M	X	24	c.3592G>A(p.Glu1198Lys)	SMC1A	Hemizygous	XLR	Early infantile epileptic encephalopathy 85 with or without midbrain defect	VOUS
17	7	M	11	10	c.1460C>T(p.Ser487Phe)	SLC1A2	Heterozygous	ADD	Early infantile epileptic encephalopathy-41	VOUS
			X	12	c.1261G>A(p.Ala421Thr)	THOC2	Hemizygous	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). *SNC8A* 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), *KCNT1* 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

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.Thr7Ile)	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
					c.1111C>A(p.Leu371Met)	MT-CYB	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	Chromo-some	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)	ROGD I	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
			2	Exon 11	c.1811G>A (p.Arg604His)	SCN1A	Heterozygous	ADD	Generalized epilepsy with febrile seizure plus-2, familial febrile seizure-3A	VOUS
7	108	M	6	7	c.564del (p.Tyr188Ter)	CSNK2B	Heterozygous	ADD	Poirier-Bienvenu neurodevelopmental syndrome	VOUS
8	17	M	5	2	c.(1705+1_17061)_(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	Hemizygous	XLR	Cerebral creatine deficiency syndrome 1	VOUS
10	36	M	1	12	c.1510G>T (p.Glu504Ter)	AMPD2	Homozygous	ARD	Pontocerebellar hypoplasia type-9	VOUS
11	108	F	6	15	c.2390C>G (p.Ser797Cys)	KCNQ5	Heterozygous	ADD	Mental retardation- 46	VOUS
12	12	F	19	5	c.532dupT (p.Cys178Leufs22)	SCN1B	Heterozygous	ADD	Generalized epilepsy with febrile seizure plus (GEFS+)	VOUS
			3	3	c.1546A>G (p.Arg516Gly)	SETD2	Heterozygous	ADD	ASD	VOUS
13	84	M	2	7 5	c.2161A>G (p.Met721Val) C787C>T (p.Arg263Cys)	ZNF142	Heterozygous	ARD	Neurodevelopmental disorder with impaired speech and hyperkinetic movement	VOUS
14	36	M	12	Intron 7	c.1746+1G>T (5slice 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	Heterozygous	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*, *SCN1B*, *SETD2*, *ZNF142*, and *DNM1L* 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.[30] 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.[31] 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.[15,32-38] Children under 2 years of age have even higher yields.[39] The common pathogenic variants found were *SCN8A*, *SCN2A*, *SCN1A*, *KCNQ2*, *STXBP1*, *GRIN2A*, and *CHRNA4* genes. Common genetic mutations which are linked with EE are *SCN1A*, *STXBP1*, *CDKL5*, and etc. While some new genes that were identified to be associated with EE are *GABRB3*, *ALG13*, *DNM1*, and etc.[40,41]

EIEEs are a group of severe epilepsy where there are intractable seizures and unremitting interictal paroxysmal epileptiform activity, which consequently impair neurodevelopmental outcome during the first year of life. Genetic causes are considered in the absence of structural brain abnormalities or inborn errors of metabolism.[2,11] In this study, 17 cases (42.5%) were genetically diagnosed as EIEE. Most common type was EIEE 6 (Dravet syndrome) where there is *SCN1A* mutation (three cases). Related studies on EIEE also showed predominance of Dravet syndrome with *SCN1A* mutation.[42,43] There was phenotype and genotype correlation in all three cases with onset in less than 1 year and typical seizure with developmental regression. We observed *SCN8A*, *SLC1A2*, *CDKL*, and *KCNT1* mutation related to EIEE. These are common mutations found in related study.[42] 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), *SMC1A* (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.[47,48] Genetic tests play important role in diagnosing and classifying PME.[49] 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.,[50] 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*.[50]

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.[51] 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.[52] 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.[53]

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 *NF1* 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.[54] A case of KTS was diagnosed with definite phenotype of amelogenesis imperfecta, intractable seizure, and GDD with *ROGD1* mutation. It is a rare cause of epilepsy and very few cases have been identified till date.[55] 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.[56]

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:

618732).[57] These features coincide with the previously reported case.[58] 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.[59] There was a case of cerebral creatine deficiency syndrome with mutation of *SLC6A8*+ 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.[60]

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.[61] 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.[61] 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.[62] Similarly, in cases with *KCNQ2* gene mutation, sodium channel blocker was used.[63]

The mean age of the studied subject was 41.4±35.850 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.[42,64] 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.[64] 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.,[42] 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.[65] In this study, the abnormalities detected were focal epilepsy (32.5%), generalized discharge (30%), EE (17.5%), hypsarrhythmia (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.,[42] only 38.9% had abnormal MRI of the brain. They also reported the finding as noncontributory as most patients had cortical atrophy.[42] 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.

Acknowledgements

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

1. Banerjee PN, Hauser WA. Incidence and prevalence. In: Engel P, Pedley T, ed. *Epilepsy: A Comprehensive Textbook.* 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2008;45-56.

2. Sharma S, Prasad AN. Genetic testing of epileptic encephalopathies of infancy: an approach. *Can J Neurol Sci* 2013;40:10-6.

3. Camfield P, Camfield C. Incidence, prevalence and aetiology of seizures and epilepsy in children. *Epileptic Disord* 2015;17:117-123.

4. Sillanpää M, Kälviäinen R, Klaukka T, Helenius H, Shinnar S. Temporal changes in the incidence of epilepsy in Finland: nationwide study. *Epilepsy Res* 2006;71:206-15.

5. Saarinen MM, Sillanpää M, Schmidt D, Virta LJ. Long-term changes in the incidence of childhood epilepsy. A population study from Finland. *Epilepsy Behav* 2016;58:81-5.

6. Cowan LD. The epidemiology of the epilepsies in children. *Ment Retard Dev Disabil Res Rev* 2002;8:171-81.

7. Wirrell EC, Grossardt BR, Wong-Kisiel LC, Nickels KC. Incidence and clas-

sification of new-onset epilepsy and epilepsy syndromes in children in Olmsted County, Minnesota from 1980 to 2004: a population-based study. *Epilepsy Res* 2011;95:110-8.

8. Mann JR, McDermott S. Maternal pre-eclampsia is associated with childhood epilepsy in South Carolina children insured by Medicaid. *Epilepsy Behav* 2011;20:506-11.

9. Syvertsen M, Nakken KO, Edland A, Hansen G, Hellum MK, Koht J. Prevalence and etiology of epilepsy in a Norwegian county-a population based study. *Epilepsia* 2015;56:699-706.

10. Ream MA, Mikati MA. Clinical utility of genetic testing in pediatric drug-resistant epilepsy: a pilot study. *Epilepsy Behav* 2014;37:241-8.

11. Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia* 2010;51:676-85.

12. Zavadenko NN. Neurodevelopmental disorders in children with epilepsy: intellectual disability and autism spectrum disorders. *Ep and Par Cond* 2017;9:64-71.

13. Symonds JD, Zuberi SM, Johnson MR. Advances in epilepsy gene discovery and implications for epilepsy diagnosis and treatment. *Curr Opin Neurol* 2017;30:193-9.

14. Orsini A, Zara F, Striano P. Recent advances in epilepsy genetics. *Neurosci Lett* 2018;667:4-9.

15. Mercimek-Mahmutoglu S, Patel J, Cordeiro D, et al. Diagnostic yield of genetic testing in epileptic encephalopathy in childhood. *Epilepsia* 2015;56:707-16.

16. Freed D, Aldana R, Weber JA, Edwards JS. The Sentieon Genomics Tools-a fast and accurate solution to variant calling from next-generation sequence data [Internet]. BioRxiv, 2017 [cited 2017 May 12]. Available at : https://doi.org/10.1101/115717.

17. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* 2010;26:589-95.

18. McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F. Deriving the consequences of genomic variants with the Ensembl API and SNP effect predictor. *Bioinformatics* 2010;26:2069-70.

19. Zerbino DR, Achuthan P, Akanni W, et al. Ensembl 2018. *Nucleic Acids Res* 2018;46(D1):D754-61.

20. Plagnol V, Curtis J, Epstein M, et al. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. *Bioinformatics* 2012;28:2747-54.

21. Landrum MJ, Lee JM, Benson M, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res* 2016;44(D1):D862-8.

22. McKusick VA. *Mendelian inheritance in man: a catalog of human genes and genetic disorders*. Baltimore: Johns Hopkins University Press, 1998.

23. Welter D, MacArthur J, Morales J, et al. The NHGRI GWAS catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res* 2014;42 (Database issue):D1001-6.

24. Stenson PD, Mort M, Ball EV, et al. The human gene mutation database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Hum Genet* 2017;136:665-77.

25. Mottaz A, David FP, Veuthey AL, Yip YL. Easy retrieval of single amino-acid polymorphisms and phenotype information using SwissVar. *Bioinformatics* 2010;26:851-2.

26. 1000 Genomes Project Consortium, Auton A, Brooks LD, et al. A global reference for human genetic variation. *Nature* 2015;526:68-74.

27. Exome Variant Server. NHLBI Exome Sequencing Project (ESP) [Internet]. Seattle: University of Washington [cited 2021 Jan 12]. Available at : http://evs.gs.washington.edu/EVS/.

28. Nagasaki M, Yasuda J, Katsuoka F, et al. Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. *Nat Commun* 2015;6:8018.

29. Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res* 2001;29:308-11.

30. Myers CT, Mefford HC. Advancing epilepsy genetics in the genomic era. *Genome Med* 2015;7:91.

31. Pal DK, Pong AW, Chung WK. Genetic evaluation and counseling for epilepsy. *Nat Rev Neurol* 2010;6:445-53.

32. Møller RS, Larsen LH, Johannesen KM, et al. Gene panel testing in epileptic encephalopathies and familial epilepsies. *Mol Syndromol* 2016;7:210-9.

33. Trump N, McTague A, Brittain H, et al. Improving diagnosis and broadening the phenotypes in early-onset seizure and severe developmental delay disorders through gene panel analysis. *J Med Genet* 2016;53:310-7.

34. Lemke JR, Riesch E, Scheurenbrand T, et al. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. *Epilepsia* 2012;53:1387-98.

35. Carvill GL, Heavin SB, Yendle SC, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. *Nat Genet* 2013;45:825-30.

36. Kodera H, Kato M, Nord AS, et al. Targeted capture and sequencing for detection of mutations causing early onset epileptic encephalopathy. *Epilepsia* 2013;54:1262-9.

37. Wang J, Gotway G, Pascual JM, Park JY. Diagnostic yield of clinical next-generation sequencing panels for epilepsy. *JAMA Neurol* 2014;71:650-1.

38. Della Mina E, Ciccone R, Brustia F, et al. Improving molecular diagnosis in epilepsy by a dedicated high-throughput sequencing platform. *Eur J Hum Genet* 2015;23:354-62.

39. Oates S, Tang S, Rosch R, et al. Incorporating epilepsy genetics into clinical practice: a 360°evaluation. *NPJ Genom Med* 2018;3:13.

40. Epi4K Consortium; Epilepsy Phenome/Genome Project, Allen AS, et al. De novo mutations in epileptic encephalopathies. *Nature* 2013;501:217-21.

41. EuroEPINOMICS-RES Consortium; Epilepsy Phenome/Genome Project; Epi4K Consortium. De novo mutations in synaptic transmission genes

including DNM1 cause epileptic encephalopathies. *Am J Hum Genet* 2014;95:360-70.

42. Arafat A, Jing P, Ma Y, et al. Unexplained early infantile epileptic encephalopathy in Han Chinese children: next-generation sequencing and phenotype enriching. *Sci Rep* 2017;7:46227.

43. Gokben S, Onay H, Yilmaz S, et al. Targeted next generation sequencing: the diagnostic value in early-onset epileptic encephalopathy. *Acta Neurol Belg* 2017;117:131-8.

44. Perrault I, Hamdan FF, Rio M, et al. Mutations in DOCK7 in individuals with epileptic encephalopathy and cortical blindness. *Am J Hum Genet* 2014;94:891-7.

45. Rapaccini V, Esposito S, Strinati F, et al. A Child with a c.6923_6928dup (p.Arg2308_Met2309dup) SPTAN1 mutation associated with a severe early infantile epileptic encephalopathy. *Int J Mol Sci* 2018;19:1976.

46 Takai A, Yamaguchi M, Yoshida H, Chiyonobu T. Investigating developmental and epileptic encephalopathy using drosophila melanogaster. *Int J Mol Sci* 2020;21:6442.

47. Proposal for revised classification of epilepsies and epileptic syndromes. Commission on classification and terminology of the international league against epilepsy. *Epilepsia* 1989;30:389-99.

48. Avanzini G, Noebels J. *Genetics of epilepsy and genetic epilepsies*. Montrouge: John Libbey Eurotext, 2009.

49. Minassian BA. The progressive myoclonus epilepsies. *Prog Brain Res* 2014;213:113-22.

50. Zhang J, Yang Y, Niu X, et al. Clinical phenotype features and genetic etiologies of 38 children with progressive myoclonic epilepsy. *Acta Epileptologica* 2020;2;14.

51. Finsterer J. Leigh and Leigh-like syndrome in children and adults. *Pediatr Neurol* 2008;39:223-35.

52. Lee S, Na JH, Lee YM. Epilepsy in Leigh syndrome with mitochondrial DNA mutations. *Front Neurol* 2019;10:496.

53. Lee HN, Eom S, Kim SH, et al. Epilepsy characteristics and clinical outcome in patients with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). *Pediatr Neurol* 2016;64:59-65.

54. Gumus E. Extending the phenotype of Xia-Gibbs syndrome in a two-year-old patient with craniosynostosis with a novel de novo AHDC1 missense mutation. *Eur J Med Genet* 2020;63:103637.

55. Tucci A, Kara E, Schossig A, et al. Kohlschütter-Tönz syndrome: mutations in ROGDI and evidence of genetic heterogeneity. *Hum Mutat* 2013;34:296-300.

56. Taghdiri M, Dastsooz H, Fardaei M, et al. A novel mutation in ERCC8 gene causing Cockayne syndrome. *Front Pediatr* 2017;5:169.

57. Poirier K, Hubert L, Viot G, et al. CSNK2B splice site mutations in patients cause intellectual disability with or without myoclonic epilepsy. *Hum Mutat* 2017;38:932-41.

58. Selvam P, Jain A, Cheema A, Atwal H, Forghani I, Atwal PS. Poirier-Bienvenu neurodevelopmental syndrome: a report of a patient with a pathogenic variant in CSNK2B with abnormal linear growth. *Am J Med Genet A* 2021;185:539-43.

59. Rosenfeld JA, Leppig K, Ballif BC, et al. Genotype-phenotype analysis of TCF4 mutations causing Pitt-Hopkins syndrome shows increased seizure activity with missense mutations. *Genet Med* 2009;11:797-805.

60. Clark AJ, Rosenberg EH, Almeida LS, et al. X-linked creatine transporter (SLC6A8) mutations in about 1% of males with mental retardation of unknown etiology. *Hum Genet* 2006;119:604-10.

61. Helbig I, Ellis CA. Personalized medicine in genetic epilepsies - possibilities, challenges, and new frontiers. *Neuropharmacology* 2020;172:107970.

62. Boerma RS, Braun KP, van den Broek MP, et al. Remarkable phenytoin sensitivity in 4 children with SCN8A-related epilepsy: a molecular neuro-pharmacological approach. *Neurotherapeutics* 2016;13:192-7.

63. Lynch JM, Tate SK, Kinirons P, et al. No major role of common SV2A variation for predisposition or levetiracetam response in epilepsy. *Epilepsy Res* 2009;83:44-51.

64. Balciuniene J, DeChene ET, Akgumus G, et al. Use of a dynamic genetic testing approach for childhood-onset epilepsy. *JAMA Netw Open* 2019;2:e192129.

65. Noachtar S, Rémi J. The role of EEG in epilepsy: a critical review. *Epilepsy Behav* 2009;15:22-33.