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Importance of targeted next-generation sequencing in pediatric patients with developmental epileptic encephalopathy

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SUMMARY

OBJECTIVE: Childhood epilepsy is a common neurological disorder with a prevalence of 300–600 cases per 100,000 people. It is associated with refractory epilepsies, global developmental delay, and epileptic encephalopathies, causing epileptic syndromes characterized by cognitive and behavioral disorders.

METHODS: In this retrospective cohort study, patients with refractory epilepsy and global developmental delay, defined as epileptic encephalopathy, who applied to the Aydın 7Maternity and Children's Hospital Genetic Diagnosis Center and were followed in the pediatric neurology clinic of our hospital, between July 2018 and July 2021, were included.

RESULTS: Targeted next-generation sequencing molecular genetics results were reviewed, and *3 ALDH7A1, 1 AARS, 3 CACNA1A, 1 CTNNB1, 1 DCX, 2 DBH, 2 DOCK7, 1 FOLR1, 2 GABRB3, 2 GCH1, 1 VGRIN2B, 1 GUF1, 3 KCNQ2, 2 KCNT1, 1 NECAP1, 1 PCDH19, 1 PNPO, 1 SCN8A, 1 SCN9A, 4 SCN1A, 2 SLC25A22, 1 SLC2A1, 2 SPTAN1, 2 SZT2, 4 TBC1D24, 2 TH, and 1 PCDH19* (X chromosome) mutations were detected in three of the patients using the next-generation sequencing method.

CONCLUSION: Although the development of gene panels aids in diagnosis, there are still unidentified disorders in this illness category, which is highly variable in genotype and phenotype. Understanding the genetic etiology is vital for genetic counseling and, maybe, the future development of remedies for the etiology.

KEYWORDS: Epilepsy. Neurological disorder. Pediatrics. Next-generation sequencing.

INTRODUCTION

Childhood epilepsy is a common neurological disorder with a prevalence of 300–600 cases per 100,000 people. Refractory epilepsy is characterized by the persistence of seizures despite treatment with two or more antiepileptic drugs, either separately or together, for an appropriate period of time and dose. It affects approximately 30% of children diagnosed with epilepsy[1,2].

It is associated with refractory epilepsies, global developmental delay (GDD), and epileptic encephalopathies (EE), causing epileptic syndromes characterized by cognitive and behavioral disorders. These diseases exhibit diversity in terms of etiology, age of onset, seizure types, electroencephalography (EEG) findings, and prognosis. Childhood epilepsy is a clinically heterogeneous neurological disorder. There is frequently a genetic etiology at play, which is defined as the reason for the cognitive dysfunction brought on by EE, refractory epilepsy, and ongoing epileptiform activity[3,4].

Knowing the etiology can help with treatment and prognosis for epilepsy patients. Due to the complexity of the genetic structure of epilepsy, there are many options for diagnostic research. The developments that have emerged in recent years to reveal the genomic etiology, particularly next-generation sequencing (NGS), have provided the opportunity to get rapid results. Patients can be genetically diagnosed in whole-exome studies using NGS platforms[5]. Recently, the importance of targeted next-generation sequencing panels (T-NGS) for epilepsy in revealing the genetic etiology has been increasing[2,4]. In studies, up to 265 monogenic epilepsy-associated genes have been investigated for being associated with a wide variety of epilepsy syndromes[6].

In this study, our aim was to determine the clinical features of patients followed up in our clinic whose genetic etiology was determined using the T-NGS method.

METHODS

In this retrospective cohort study, patients with refractory epilepsy and GDD, defined as epileptic encephalopathy, who

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applied to the Maternity and Children's Hospital Genetic Diagnosis Center and were followed in the pediatric neurology clinic of our hospital between July 2018 and July 2021, were included. The reason for the admission of the patients was to identify the underlying genetic causes. The patients' age, gender, clinical features, electroencephalography examinations (EEG), neuroimaging findings, biochemical and metabolic tests, and T-NGS molecular genetic research results for epileptic encephalopathy were reviewed by screening electronic patient files. All epilepsy diagnoses, seizure types, and epilepsy syndromes were determined and classified according to the ILAE (International League Against Epilepsy) 2017 Exclusion criteria, which included seizures caused by non-genetic factors such as an acquired or structural brain injury (including traumatic brain injury, encephalitis, vasculitis, hypoxia, abscess, neoplasm, metabolic disturbance, and toxicity). Brain magnetic resonance imaging (MRI) was performed in all patients, and the genes identified through T-NGS are presented in Table 1.

Genomic DNA was extracted from the peripheral blood, and NGS was performed by capturing the coding regions and splice sites of targeted genes using a commercial NGS kit (Celemics, South Korea). The list of targeted genes in the panels is provided in Table 1. NGS was performed on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). The sequencing reads were aligned to the human genome reference (GRCh37: Genome Reference Consortium human build 37) using Burrows-Wheeler Alignment Tool (BWA). Subsequently, BAM files were sorted, indexed, and de-duplicated using SAMtools and Picard tools. For the filtering process, exonic and splicing variants, including mis-/nonsense variants, and indels were selected. Rare variants with minor allele frequency less than 0.001 were filtered. Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines[6]. All variants identified by NGS were confirmed through Sanger sequencing. Patients and parents were tested to determine whether the pathogenic variations were *de novo* or inherited.

A custom target enrichment panel was designed to capture 54 genes related to epileptic encephalopathies (Table 1). All exons, the 25 base pairs of the intronic flanking region, and 5′ and 3′ untranslated region of each gene were sequenced. After library enrichment and quality control, the samples were sequenced using the MiSeq platform (Illumina, San Diego, CA, United States). Raw reads were trimmed with Trimmomatic and mapped to the reference human genome (hg19) using BWA. Duplicates were removed using SAMTools, and realignment across indels and base quality recalibration were performed using GATK. Annotation of detected variants was performed using Illumina BaseSpace Variant Interpreter, InterVar, Franklin, VarSome, ClinVar, OMIM, and Pubmed. Variants with a frequency higher than 0.5% were filtered out. dbNSFP (containing SIFT, PolyPhen-2, LRT, and Mutation Taster) was used to predict the pathogenicity and deleteriousness of variants. Rare variants were classified according to the ACMG/AMP variant interpretation framework[7].

RESULTS

A total of 198 patients were included in this retrospective cohort study. The mean age of the patients was 7.4±5.8 standard deviation (SD) (age range of 8 months to 16 years). An identifiable underlying genetic cause was identified in 48 (25%) out of 198 patients. The F/M ratio of patients with genetic mutations was found to be 6:10. The *3 ALDH7A1, 1 AARS, 3 CACNA1A, 1 CTNNB1, 1 DCX, 2 DBH, 2 DOCK7, 1 FOLR1, 2 GABRB3, 2 GCH1, 1 VGRIN2B, 1 GUF1, 3 KCNQ2, 2 KCNT1, 1 NECAP1, 1 PCDH19, 1 PNPO, 1 SCN8A, 1 SCN9A, 4 SCN1A, 2 SLC25A22, 1 SLC2A1, 2 SPTAN1, 2 SZT2, 4 TBC1D24, 2 TH*, and *1 PCDH19* (chromosome X) mutations were detected in three patients. All mutation distributions are shown in Table 2. The age of seizure onset in our patients was 14.8 months. Four

Table 1. Genes identified for targeted next-generation sequencing that are thought to be related to magnetic resonance imaging disorders in the literature.

EEF1A2	PNPO	DOCK7	ARHGEF9	AARS1	GNAO1
ST3GAL3	SLC1A2	GUF1	TBC1D24	HCN1	SCN1A
ARV1	GABRB3	SLC25A1	PNKP	PIGA	PLCB1
DCX	MAOA	NECAP1	SCN8A	KCNQ2	CACNA1A
KCNB1	KCNT1	SPTAN1	DNM1	PCDH19	STXBP1
SCN2A	ARX	CDKL5	ITPA	TH	KCNQ3
DBH	FOLR1	ALG13	ALDH7A1	SCN9A	SLC25A12
SLC2A1	GABRA1	SLC13A5	SLC35A2	SLC12A5	KCNA2
GCH1	FRRS1L	SZT2	WWOX	GRIN2B	GLRA1

patients were diagnosed with mental retardation (2 with moderate mental retardation and 2 with severe mental retardation). Notably, 12 patients were being followed up by the child psychiatry clinic due to autism spectrum disorder. A total of 198 patients were included in this retrospective cohort study. The mean age of the patients was 7.4±5.8 standard deviation (SD) (age range of 8 months to 16 years) (Table 2).

Metabolic examination tests were performed in all patients, and no specific findings indicative of a disease were detected. Cranial imaging was performed in all patients. There was no structural anomaly that could cause a seizure. In contrast, hippocampal sclerosis was detected in a patient with Dravet syndrome, which is secondary to frequently recurring long-term febrile seizures. Notably, 6 patients were diagnosed with West syndrome, 2 patients developed Lennox-Gastaut syndrome, and 12 patients had a history of febrile convulsions. Ataxia was observed during the neurological evaluation of the patient with the *CACNA1A* mutation.

Table 2. Distribution and location of mutations detected in patients.

Number of patients	Gene	Mutation location	ACMG pathogenicity
3	*ALDH7A1*	c.1597delG (p.Ala533ProfsTer18) c.1597delG (p.Ala533Profs*18) c.328C>T .p.Arg110Ter	Pathogenic (PP5, PVS1, PM2)
1	*AARS*	c.601G>A (p.Ala201Thr)	VUS (PM2)
3	*CACNA1A*	c.4687G>A (p.Val1563Met), c.6409G>C (p.Asp2137His)	Likely pathogenic (PM2, PP3, PP2), VUS (PM2, PP2)
2	*CTNNB1*	c.1320A>C p.(Gln440His)	VUS (PM2, PP2)
1	*DCX*	c.1120A>T (p.Thr374Ser)	VUS (PM2, PP2)
2	*DBH*	c.1627C>A (p.Pro543Thr), c.1025-6T>A	VUS (PM2), VUS (PM2, BP4)
2	*DOCK7*	c.4073G>A p.(Arg1358Gln), c.1724A>G p.(Asn575Ser)	VUS (PM2, PP2), VUS (PM2, PP2, BP4)
1	*FOLR1*	c.493+2T>C	VUS (PVS1, PP5, BS2)
2	*GABRB3*	c.4073G>A p.(Arg1358Gln), c.56G>A (p.Gly19Glu)	VUS (PM2, PP2, PP3)
2	*GCH1*	c.333G>A (p.Glu111=)	VUS (PM2, BP7)
1	*GRIN2B*	c.2642A>G (p.Gln881Arg)	VUS (PM2, PP2, BP4)
2	*GUF1*	c.1402_1403delGA (p.Glu468Ilefs*15)	Likely pathogenic (PVS1, PM2)
3	*KCNQ2*	c.1741C>T (p.Arg581*), c.88G>A (p.Gly30Ser), c.2173C>T (p.Arg725Cys)	Pathogenic (PVS1, PM2, PP5), VUS (PM2, PP2)
2	*KCNT1*	c.2594+7C>T, c.1210G>A (p.Val404Met)	VUS (PP3, BP6), VUS (PM2)
1	*NECAP1*	c.812A>G (pAsn271Ser)	VUS (PM2, BP4)
1	*PCDH19*	c.2838G>A (p.Pro543Thr)	VUS (PP2, PM2, BP4)
1	*PNPO*	c.20G>A (p.LysY7Asp)	VUS (PM2, PP2, BP4)
2	*SCN9A*	c.1828C>A p.(Pro610Thr), c.3267C>A (p.Asn1089Lys)	VUS (PM2, BP4, BP7),VUS (PM2, BP7)
4	*SCN1A*	c.812A>G (pAsn271Ser), c.1625G>A (p.Arg542Gln), c.4324T>A (p.Tyr1442Asn), c.3840_3843delTGTT (p.Ile1280Metfs*8), c.80G>C (p.Arg27Thr)	Pathogenic (PM2, PVS1), VUS (PP2, BP4)
2	*SLC25A12*	c.784G>A (p.Glu262Lys), c.279G>C (p.Gln93His)	Likely pathogenic (PM2, PP3) VUS (PM2, PP2)
1	*SLC25A1*	c.376C>T (p.Arg126Cys)	VUS (PM2, BP7)
2	*SPTAN1*	c.2119G>A (p.Gly707Ser), c.1143A>C (p.Lys381Asn)	Likely pathogenic (PM2, PP3, PP2), VUS (PM2, PP2, BP4)
2	*SZT2*	c.9458G>A (p.Arg3153His), c.7342C>T (p.Arg2448Cys)	Likely Benign (PM2, BP4), VUS (PM2)
4	*TBC1D24*	c.418C>G (p.Leu140Val), c.1015A>G (p.Asn339Asp), c.1020C>G (p.Phe340Leu), c.871G>A (p.Ala291Thr)	VUS (PM2), VUS (PM2), VUS (PM2), VUS (PM2)
2	*TH*	c.1144A>G (p.Ile382Val), c.440G>A (p.Arg147Gln)	VUS (PM2, PP2), VUS (PM2, PP2)
1	*PCDH19*	c.1238G>T (p.Arg413Leu)	VUS (PP2, PM2, BP4)

DISCUSSION

Epileptic encephalopathies with severe electroencephalography findings that may result in severe neurological deficits and drug-resistant seizures that occur in the first years of life have several differences according to etiology, phenotype, and prognosis. While the etiology is mostly dependent on symptomatic causes (e.g., Ohtahara syndrome and West syndrome), in some syndromes (e.g., Dravet syndrome), genetic etiology plays a significant role[1,2,4]. In this study, out of 16 patients, 4 were followed up for West syndrome, 2 for Lennox-Gastaut syndrome, and 1 for Dravet syndrome, while a definitive diagnosis could not be made in 9 patients due to the absence of findings that would indicate a specific syndrome. An identifiable underlying genetic cause was identified in 48 (25%) out of 198 patients. In a study conducted by Ara K. et al., the rate was found to be 37.1%, which is higher than this study. However, in previous studies, this rate ranged between 20% and +2% and these results are in agreement with this study[8-11]. While the mutation found in 10 (5.5%) patients was considered definitively pathogenic, the changes found in 11 (5.5%) patients were determined as likely pathogenic. In the studies conducted by Ara k Parrini E and Trump N, this rate was found to be between 19.5 and 26.6%[9-11]. The high number of patients observed in these studies is thought to be effective in obtaining these results. Among them, 23 (11.1%) patients with other mutations were evaluated as VUS. This probably explained the etiology of cases with VUS. In this study, the diagnostic rate for epilepsy was 25% using whole-exome sequencing. With NGS systems, faster epilepsy genes will be detected. In other studies, these rates varied between 14.5 and 41.6%[8-10].

West syndrome developed in four patients (25%), and LGS developed after West syndrome in two patients. In the literature, the rate of LGS developing after infantile spasms is reported to be 20–40%. In the study, in which 98 patients with West syndrome were followed up for 3 years, LGS developed in 48% of patients. In this study, in which no relationship was found between the development of LGS and the age of onset of West syndrome or the etiology of West syndrome, the risk of developing LGS was lower in patients who received a ketogenic diet, prednisone, or ACTH[12,13]. Six of our patients had a history of febrile convulsions before the onset of seizures. The mean age at which febrile seizures were seen was 1.4 years (with an age range of 1–3 years). In a study conducted on 38 LGS patients, febrile seizures were observed in 3 patients (7.9% of patients with LGS) before seizures started, and the mean age at which febrile seizures were observed was reported to be 6 months[14]. The rate of febrile seizures (12/16) among the patients included in this study was higher than that of the healthy population.

The term "epileptic encephalopathy" has been used since the late 1970s to refer to certain devastating epilepsies seen early in life. Epileptic encephalopathies include epileptic syndromes characterized by cognitive and behavioral disorders. These diseases exhibit diversity in terms of etiology, age of onset, seizure types, electroencephalography (EEG) findings, and prognosis. Epileptic encephalopathies are severe syndromes characterized by drug-resistant, generalized or focal seizures, cognitive dysfunction, decline, and severe electroencephalographic findings that occur early in life. The International League Against Epilepsy (ILAE) defines epileptic encephalopathies as conditions where "epileptiform abnormalities are considered to cause progressive impairment of cerebral function." Ictal and interictal epileptic discharges are age-related and constitute the main cause of cognitive decline. In some cases, clinical and EEG abnormalities continue as the child grows, and transformation from one type to another may be observed[15,16]. All the patients in this study were diagnosed with epilepsy after seizures, and with the progression of the disease, the diagnosis of epileptic encephalopathy was done at the center.

Two of our patients had moderate and two had severe intellectual disabilities. All other patients were followed up with a diagnosis of autism spectrum disorder. Jansen et al. reported these rates as 45.45% (5 out of 11 patients) and 54.55% (6 out of 11 patients) in their series[17]. Akiyama et al. reported that 22.58% of the patients did not have word output (7 out of 31 patients) in their study[18]. While a patient with moderate intellectual disability was partially dependent on the help of their relatives, a patient with severe intellectual disability was completely dependent on the help of their relatives in their daily life activities. In other studies, these rates were 33.3% (8 out of 24) and 54.2% (13 out of 24), 14.28% (2 out of 14), and 71.42% (10 out of 14)[17,18]. Cranial imaging could be performed in all patients with autism and epilepsy comorbidity. These patients' MRIs revealed no structural abnormalities.

The MRI of our patient with Dravet syndrome showed hippocampal sclerosis. Additionally, the patient had findings of autism spectrum disorder. One study reported that 22.4% (8 out of 58 patients) with Dravet syndrome had abnormal cranial MRI findings, 13.79% (8 out of 58 patients) had cortical atrophy, 1.72% (1 out of 58) had cerebellar atrophy, and 1.72% had hippocampal sclerosis[18,19]. Similar to other reported cases, our patient had a history of frequent febrile seizures, cognitive decline, and status epilepticus after phenytoin. Treatment options with proven efficacy in the treatment of Dravet syndrome include stiripentol, valproic acid, clobazam and other benzodiazepines, topiramate, levetiracetam, and a ketogenic diet. Increased body temperature,

lamotrigine, phenytoin, vigabatrin, oxcarbazepine, and carbamazepine may exacerbate seizures[20,21].

The limitations of this study are as follows. The number of patients was small in some syndromes, the collection of clinical data of patients from their childhood was found to be difficult, some patients missed follow-up examinations for a long time, and some patients could not be reached. The small number of participating centers and thus the patient number included in the genetic analyses are other limitations.

CONCLUSION

Epileptic encephalopathies are severe and refractory epilepsies requiring regular follow-up, clinical evaluations, and increased social support. Genetic diagnoses have been made in some cases, but uncertainty persists for most, posing a serious health problem. Gene panels aid diagnosis, but undiagnosed conditions remain in this genetically diverse disease group. Understanding the genetic etiology is crucial for counseling and future treatment development.

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AUTHORS' CONTRIBUTIONS

SB: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. **SK:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **ÖB:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft.

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