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The landscape of early infantile epileptic encephalopathy in a consanguineous population

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ABSTRACT

Purpose: Epileptic encephalopathies (EE), are a group of age-related disorders characterized by intractable seizures and electroencephalogram (EEG) abnormalities that may result in cognitive and motor delay. Early infantile epileptic encephalopathies (EIEE) manifest in the first year of life. EIEE are highly heterogeneous genetically but a genetic etiology is only identified in half of the cases, typically in the form of de novo dominant mutations.

Method: This is a descriptive retrospective study of a consecutive series of patients diagnosed with EIEE from the participating hospitals. A chart review was performed for all patients. The diagnosis of epileptic encephalopathy was confirmed by molecular investigations in commercial labs. *In silico* study was done for all novel mutations. A systematic search was done for all the types of EIEE and their correlated genes in the literature using the Online Mendelian Inheritance In Man and PubMed databases.

Results: In this case series, we report 72 molecularly characterized EIEE from a highly consanguineous population, and review their clinical course. We identified 50 variants, 26 of which are novel, causing 26 different types of EIEE. Unlike outbred populations, autosomal recessive EIEE accounted for half the cases. The phenotypes ranged from self-limiting and drug-responsive to severe refractory seizures or even death.

Conclusions: We reported the largest EIEE case series in the region with confirmed molecular testing and detailed clinical phenotyping. The number autosomal recessive predominance could be explained by the society's high consanguinity. We reviewed all the EIEE registered causative genes in the literature and proposed a functional classification.

1. Introduction

Epileptic encephalopathies (EE), are disorders of the developing brain characterized by intractable seizures and electroencephalogram (EEG) abnormalities, that typically result in cognitive and motor delay, regression, and sometimes death. [1,2] Forty percent of seizures in children aged three years or less can be classified as EE [3]. Early infantile epileptic encephalopathy (EIEE) have their onset during infancy and are highly variable in etiology and natural history. While seizure is the core symptom for all EIEE syndromes often accompanied by

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progressive cognitive delay, these disorders are highly variable in the age of onset, severity, type of the seizures, EEG patterns, other associated symptoms and outcome [4].

The classification of epilepsy, in general, is challenging. Many intersecting classification models were proposed based on the type of seizures, the electrical and imaging features, and the underlying etiology [1]. Historically, EIEE were classified into five main syndromes: Ohtahara syndrome, West syndrome, Lennox-Gastaut syndrome, Dravet syndrome, and Landau-Kleffner syndrome [5].

EIEE are genetically heterogeneous, but despite the recent advances in molecular diagnostics, only around 50% of the cases have a recognizable underlying genetic cause [4,6,7], The identification of the genetic etiology of EIEE has greatly improved our understanding of the disease pathophysiology at the molecular level, however, the genotype/phenotype correlation remains poorly understood. Some epileptic syndromes have been correlated with certain genes like Dravet syndrome, in which around 80% of the cases are due to *SCN1A* gene mutations. Otahara syndrome was attributed to mutations in *STXBP1* and *ARX* genes. The outcomes may range from self-limited and drug-responsive to severe debilitating syndromes [1,4].

In this study, we provide an extensive clinical and genetic characterization of 72 molecularly characterized EIEE patients from Saudi Arabia. Additionally, we reviewed all the reported genes causing EIEE and proposed a functional classification.

2. Methods

2.1. Patients

This is a descriptive retrospective study of a consecutive series of patients diagnosed with EIEE from the participating hospitals. A chart review was performed for all patients to record the following variables: perinatal history, developmental history, family history, magnetic resonance imaging (MRI), EEG findings, genetic testing, antiepileptic medications, and clinical outcome. Only patients with a genetic confirmatory test of EIEE were included.

2.2. Mutation identification

The diagnosis of epileptic encephalopathy was confirmed by whole exome sequencing (WES) of genomic DNA for most of the patients, while a few were diagnosed by relevant gene panels. All the molecular genetic studies were performed by accredited commercial labs. Cases are negative to customized gene panels were subjected to WES and performed as described elsewhere [8]. We strictly followed a criterion in reporting pathogenic variants from whole exome sequencing. For variants minor allele frequency < 0.001 based on our internal database (2379 exomes), gnomad database and fully segregated within available family members. Whereas for dominant disease-causing variants considered *de novo* variants confirmed paternally and novel in our internal database and gnomad database. Loss of function variants (indels, nonsense and canonical splicing mutations) are considered as pathogenic. All the discovered variants were confirmed by Sanger sequencing. For missense mutation, *in silico* study analysis tools were used to predict the genetic damaging of the novel variants including Polyphen2 (http://www.genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.bii.a-star.edu.sg/), and MutationTaster (http://www.mutationtaster.org/) for variants in coding regions. For intronic variants, Human Splicing Finder (HSF) (http://www.umd.be/HSF3/index.html) was used. To determine the novelty of the variant, we checked for the presence of the variants in HGMD and ClinVar databases.

2.3. EIEE literature review

We conducted a systematic search for all the types of EIEE and their correlated genes in the literature using the Online Mendelian Inheritance In Man (OMIM) and PubMed databases. A thorough literature review was conducted to summarize the functions of all the identified genes.

2.4. Ethical approval

The study was approved by the Institutional Review Board at King Abdullah International Medical Research Centre (KAIMRC) (Ref. RC 16/113R).

3. Results

A total of 72 patients from 59 unrelated families were identified as eligible and included in the study. Tables 1 and 2 provide a summary of the clinical and mutation information for all cases. The male to female ratio was almost 1:1. The age at onset of seizures ranged from one day to 2 years with average at 9.8 months. The current age ranges from 1 year to 22 years, with a mean of 6.8 years. Consanguinity was positive in 75% of the cases but was always noted in those with an autosomal recessive etiology.

The predominant type of seizure was generalized tonic-clonic (84.2%), one-third of them were accompanied by other types of seizures like focal seizures. The vast majority of patients (97.2%) were delayed in their developmental milestones. Microcephaly was noted in 22.2% of the patients, while 20.8% were hypotonic and 15.3% had ophthalmologic involvement. The most prevalent EEG pattern was the epileptic encephalopathy (29.1%) followed by Lennox-Gastaut Syndrome (LGS) and multifocal patterns (13.9% each). The brain MRI was normal in more than half of the patients (51.4%). The most commonly identified structural brain anomaly was brain atrophy (16.7%) followed by anomalies in the corpus callosum (8.3%), cerebellar hypoplasia (5.5%), and white matter hyperintensity (5.5%). The treatment with antiepileptic medications ranged from one medication (36.1%) to more than three medications (6.9%) with variable response to treatment. The majority of patients (61.1%) had poor seizure control, while 34.7% were well controlled. Most of the controlled patients (69.2%) required only one medication. Two patients had their seizures resolved without treatment. On the other hand, two patients died early at two months of age.

All the patients underwent molecular confirmatory investigations. In most of the patients, the diagnostic tool was the whole exome sequencing (WES). We identified 26 different types of EIEE including types 1–4, types 6–9, types 11–14, types 17,21,23, types 25–28, type 32, types 37–39, types 48, 49 and 52. The identified genes were *ARX, CDKL, SLC25A22, STXBP1, SCN1A, KCNQ2, ARHGEF9, PCDH19, SCN2A, PLCB1, SCN8A, KCNT1, GNAO1, NECAP1, DOCK7, SLC13A5, KCNB1, GRIN2B, WWOX, KCNA2, FRRS1L, ARV1, SLC25A12, AP3B2, DENND5A,* and *SCN1B* respectively. The age at diagnosis confirmation ranged from 3 months to 18 years with average at 4 years and 7 months. The most prevalent type in this cohort was EIEE type 25 caused by *SLC13A5* mutation, which was found in 11 patients (15.2%). This was followed by type 11 and type 37, each of which was confirmed in seven patients (9.7%). We identified 50 variants, 26 of them were novel. All the identified novel variants were subjected to *in silico* prediction (Table 2). The types of mutations were as follows, 61.1% of the patients had missense mutations, 13.9% nonsense, 9.7% had deletion mutations, 6.9% had insertion mutations, 6.9% had intronic splice site mutations, and one patient (1.4%) had a synonymous mutation. The mode of inheritance was autosomal recessive (AR) in 50% of the patients, while 45.8% of the patients had an autosomal dominant (AD) mode of inheritance. Three patients had an X-linked mode of inheritance, one of them was dominant, the other was recessive and the third could not be determined (*PCDH19* gene). In all the autosomal recessive cases, the parents were carriers. On the other hand, dominant cases were *de novo*, except in four cases where the mutation was inherited from an affected parent.

Table 1
Summary of the clinical features.

List	Patient Number	Gene and Mutation	Gender	Age at onset of seizures	Current age	Seizure pattern	AED	Consang-uinity	Developme-nt	Clinical features			MRI	EEG	Outcome and seizure control
										Hypoton-ia	Microce-phaly	Ophthal-mologic involve-ment			
Genes responsible for the synapsis, neurotransmitters, and receptors:															
AP3B2															
1	KF27-C	*AP3B2* NM_001278512.1 c.1837del (p.Glu613Serfs*182)	F	6 M	10 y	GTC	2 AED	Y	GDD	No	No	No	cerebellar hypoplasia, WM nonspecific changes	LGS	Refractory to medications, still having seizure
2	KF28-C	*AP3B2* NM_001278512.1 c.1837del (p.Glu613Serfs*182)	M	6 M	12 y	GTC	2AED	Y	GDD	No	No	No	cerebellar hypoplasia	LGS	Refractory to medications, still having seizure
3	KF29	*AP3B2* NM_001278512.1 c.1837del (p.Glu613Serfs*182)	F	6 M	4 y	GTC	2AED	Y	GDD	No	No	No	cerebellar hypoplasia	LGS	Refractory to medications, still having seizure
FRRS1L															
4	KF35-E	*FRRS1L* NM_014334.2 c.961C > T (p.Gln321*)	M	9 m	10 y	GTC	AED	Y	regression mainly speech	No		No	unremarkable	Infantile Epileptic Encephalopathy. Landau-Kleffner Syndrome (LKS)	Controlled
5	KF36-E	*FRRS1L* NM_014334.2 c.961C > T (p.Gln321*)	M	12 M	17 y	GTC	AED	Y	regression mainly speech	No	No	No	unremarkable	LKS	Controlled
6	KF37-E	*FRRS1L* NM_014334.2 c.961C > T (p.Gln321*)	F	14 m	16 y	GTC	AED	Y	regression mainly speech	No	No	No	unremarkable	LKS	Controlled
7	KF38-E	*FRRS1L* NM_014334.2 c.961C > T (p.Gln321*)	F	18 m	14 y	GTC	AED	Y	regression mainly speech	No	No	No	unremarkable	LKS	Controlled
8	KF39-F	*FRRS1L* NM_014334.2 c.961C > T (p.Gln321*)	M	12 m	12 y	GTC	AED	Y	regression mainly speech	No	No	No	unremarkable	Infantile Epileptic Encephalopathy. LKS	Controlled
9	KF40-F	*FRRS1L* NM_014334.2 c.961C > T (p.Gln321*)	F	14 m	4 y	GTC	AED	Y	regression mainly speech	No	No	No	unremarkable	Infantile Epileptic Encephalopathy. LKS	Controlled
10	KF41-F	*FRRS1L* NM_014334.2 c.961C > T (p.Gln321*)	F	18 m	5 y	GTC	AED	Y	regression mainly speech	No	No	No	unremarkable	Infantile Epileptic Encephalopathy. LKS	Controlled
GRIN2B															
11	KA13	*GRIN2B* NM_000834 c.2429 G > A (p.Ser810Asn)	F	4 m	6 y	GTC	1 AED	Y	Delayed	yes	yes	Poor vision, and possible	Microcephaly with a diffuse pattern of polymicrogyria, less prominent in occipital lobes	Bitemporal epileptiform discharges left more than right. Multifocal epileptic	No seizure for more than a year Spastic quadriplegic

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Table 1 (*continued*)

List	Patient Number	Gene and Mutation	Gender	Age at onset of seizures	Current age	Seizure pattern	AED	Consang-uinity	Developme-nt	Clinical features			MRI	EEG	Outcome and seizure control
										Hypoton-ia	Microce-phaly	Ophthal-mologic involve-ment			
												kerato-conus		discharges positive on the right	GERD On GT feeding
NECAP1															
12	KF68	*NECAP1* NM_015509.3 c.142C > T (p.Arg48*)	F	6 m	9 y	GTC	2 AED	Y	GDD	No	No	No	brain atrophy	Epileptic Encephalopathy	Refractory to medications, still having seizure
STXBP1															
13	KA10	*STXBP1* The deletion (9q33.3 to 9q34.11) involves eight genes including *STXBP1*	M	N/A	22 y	N/A	N/A 2 AED	N	Delayed	yes	NA	N/A	Prominence of cerebral sulci of the right parietal lobe with enlargement of adjacent subarachnoid space Spine	Slowing of background, and epileptiform discharges over both frontal head regions	Controlled seizures Bedridden, on tracheostomy, home oxygen, and PEG tube feeding
14	KF52	*STXBP1* NM_001032221.3 c.874C > T (p.Arg292Cys)	M	2 m	4 y	GTC	3 AED	N	severe GDD	No	No	No	scattered subcortical high-intensity changes	LGS	Refractory to medications, still having seizure
Genes responsible for signal transduction:															
ARHGEF9															
15	KF128	*ARHGEF9* NM_015185.2 c.1476 T > G (p.Phe492Leu)	M	2 y	10 y	GTC	AED	N	GDD + ID	No	No	No	unremarkable	normal	Controlled Myopathy
DENND5A															
16	KF31	*DENND5A* NM_015213.3 c. 1622 A > G (p.Asp541Gly)	F	2 w	3 y	spasm	> 3 AED	Y	GDD	No	yes	No	"Diffuse white matter abnormality in T2/FLAIR periventricular & subcortical white matter. Brain atrophy & dysgenesis of the corpus callosum	LGS	Controlled
GNAO1															
17	KA3	*GNAO1* NM_020988.2 c.683 T > C (p.Leu228Pro)	F	1 m	5 y	NA	No AED	N	Delayed	yes	Yes (ac-quired)	No	Mild microcephaly	Normal	No seizures since neonatal period
PLCB1															
18	KF112-Q	*PLCB1* NM_015192.3 c.550C > T (p.Arg184*)	M	2 m	2 y	focal + GTC	AED	Y	regression	No	No	No	brain atrophy	spike and sharp waves	Controlled
19	KF113-Q	*PLCB1* NM_015192.3 c.550C > T (p.Arg184*)	M	1 y	4 y	focal + GTC	AED	Y	regression	No	No	No	brain atrophy	LGS	Controlled
Genes responsible for ion channels:															

(*continued on next page*)

Table 1 (*continued*)

List	Patient Number	Gene and Mutation	Gender	Age at onset of seizures	Current age	Seizure pattern	AED	Consang-uinity	Developme-nt	Clinical features			MRI	EEG	Outcome and seizure control
										Hypoton-ia	Microce-phaly	Ophthal-mologic involve-ment			
KCNA2															
20	KF108	*KCNA2* NM_004974.3 c.1120 A > G (p.Thr374Ala)	M	18 m	5 y	GTC	2 AED	N	GDD	No	No	No	brain atrophy	Epileptic Encephalopathy	Refractory to medications, still having seizure
21	KF109	*KCNA2* NM_004974.3 c.890 G > A (p.Arg297Gln)	M	20 m	8 y	GTC	2AED	Y	GDD	No	No	No	brain atrophy + cerebellar hypoplasia	Epileptic Encephalopathy	Refractory to medications, still having seizure Ataxia
22	KF151	*KCNA2* NM_004974.3 c.1265_1266del (p.Glu422Glyfs*21)	M	9 m	8 years	FTC and myo-clonic	AED	N	ID, ADHD	No	No	No	Vermis hypoplasia	Generalized poly spikes & wave	Controlled
KCNB1															
23	KA4	*KCNB1* NM_004975 c.1222C > T (p.Pro408Ser)	F	11 m	5 y	GTC, eye myo-clonus	2 AED	N	Delayed	yes	No	No	Normal	Very active right sided centrotemporal epileptiform discharges. Other EEG showed epileptic discharges over both sides mainly over both frontal head regions	Still has eye myoclonus and GTC Unsteady gait, toe walking, became weaker
KCNQ2															
24	KA1	*KCNQ2* NM_172107.2 c.1464C > G (p.Asp488Glu)	F	2 m	16 y	GTC, tonic	No AED	N	Delayed	yes	No	No	Normal	Normal	No seizures for more than 4 years
25	KF110	*KCNQ2* NM_172107.2 c.1744 A > T (p.Ile582Phe)	M	neonatal	3 y	focal + GTC	2 AED	Y	GDD	No	No	No	brain atrophy + delayed myelination	Epileptic Encephalopathy	Refractory to medications, still having seizure
26	KF140	*KCNQ2* NM_172107.2 c.793 G > A (p.Ala265Thr)	F	birth	1 Y	focal + GTC	2 AED	N	GDD	Yes	No	No	high glycerin peak	Epileptic Encephalopathy	Refractory to medications, still having seizure
KCNT1															
27	KA6	*KCNT1* NM_020822.2 c.1130 G > C (p.Cys377Ser)	F	Day 20	2 y & 9 m	GTC, eye blinking, facial twitching	5 AED	N	Delayed	yes	yes	Poor eye contact	Delayed myelination	Diffuse slowing, multifocal spike, and wave discharges	Intractable seizures GERD Oropharyngeal dysphagia Esophagitis pseudoachalasia On GT feeding Mild ventricular dilatation and

(*continued on next page*)

Table 1 (*continued*)

List	Patient Number	Gene and Mutation	Gender	Age at onset of seizures	Current age	Seizure pattern	AED	Consang-uinity	Developme-nt	Clinical features			MRI	EEG	Outcome and seizure control
										Hypoton-ia	Microce-phaly	Ophthal-mologic involve-ment			
															mild mitral regurgitation
28	KF138	*KCNT1* NM_020822.2 c.862 G > A (p.Gly288Ser)	F	4 m	2 y	focal + GTC	2 AED	Y	ID, regression	No	No	Yes	abnormal shape of corpus callosum with verticalization of the splenium	multifocal	Refractory to medications, still having seizure
29	KF147	*KCNT1* NM_020822.2 c.2800 G > A (p.Ala934Thr)	F	4 m	5 y	GTC, focal	2 AED	N	ID	No	Yes	No	Thin CC, prominent CDF Spaces in the frontal and parietal region.	Focal discharges	Refractory to medications, still having seizure
30	KF155	*KCNT1* NM_020822.2 c.862 G > A (p.Gly288Ser)	F	4 m	2 years	focal, GTC	2 AED	Y	ID	No	No	Yes	thin CC, brain atrophy	multifocal	Refractory to medications, still having seizure
SCN1A															
31	KA14	*SCN1A* NM_001165963.1 c.1244 T > C (p.Ile415Thr)	M	2 years	11 y	GTC	1 AED	N	Normal	No	No	No	Normal	generalized spike-wave	Partially controlled seizures Normal development.
32	KA17	*SCN1A* NM_001165963.1 c.1625 G > A (p.Arg542Gln)	F	3 m	16 m	GTC	1 AED	Y	Delay	Yes	Yes	No	Delayed myelination, Posterior fossa arachnoid cyst, and microcephaly.	Normal	Controlled seizures Developmental delay Microcephaly
33	KF133	*SCN1A* NM_001165963.1 c.1498C > T (p.Arg500Trp)	M	18 m	8 y	focal + GTC + f-ebrile	3 AED	Y	regression	No	No	No	thick CC	LGS	Refractory to medications, still having seizure
34	KF150	*SCN1A* NM_001202435.1 c.671 T > G (p.Leu224Trp)	F	2 y	6 years	absence	AED	Y	Normal	No	No	No	unremarkable	generalized spike slow wave 3 Hz or less	Controlled
35	KF157	*SCN1A* NM_001202435.1 c.3714 A > C (p.Glu1238Asp)	M	18 m	4 years	GTC	3 AED	Y	regression	No	No	No	unremarkable	Epileptic Encephalopathy	Refractory to medications, still having seizure
SCN1B															
36	KF159	*SCN1B* NM_001037.4 c.449-2A > G	F	3 m	14 months	focal, GTC, myo-clonic	3 AED	Y	N/A	N/A	N/A	N/A	Unremarkable	Epileptic Encephalopathy	Refractory to medications, still having seizure
SCN2A															
37	KA12	*SCN2A* NM_021007.2 c.4886 G > A (p.Arg1629His)	M	Day 14	5 y	NA	1 AED	N	Delayed	yes	yes	History of nys-tagmus, poor eye contact	Delayed myelination	EEG in the first year of life showed hypsarrhythmia	Resolved infantile spasm Severe spasticity Sleep disturbance GERD

(*continued on next page*)

Table 1 (*continued*)

List	Patient Number	Gene and Mutation	Gender	Age at onset of seizures	Current age	Seizure pattern	AED	Consang-uinity	Developme-nt	Clinical features			MRI	EEG	Outcome and seizure control
										Hypoton-ia	Microce-phaly	Ophthal-mologic involve-ment			
38	KA15	*SCN2A* NM_021007.2 c.4390 A > G (p.Thr1464Ala)	M	3 days	8 m	GTC	2 AED	N	Delayed	Yes	No	No	No structural abnormalities	Focal sharp wave activity was noted in the left and right parietal temporal areas.	Scoliosis On NGT feeding No seizure since the last five months
39	KF59	*SCN2A* NM_021007.2 c.3956 G > T (p.Arg1319Leu)	F	14 m	6 y	GTC	2 AED	Y	GDD	No	No	No	unremarkable	Epileptic Encephalopathy	Refractory to medications, still having seizure
40	KF62	*SCN2A* NM_021007.2 c.3956 G > T (p.Arg1319Leu)	F	12 m	3 y	N/A	N/A	Y	N/A	No	No	No	N/A		N/A
41	KF126	*SCN2A* NM_001040143.1 c.638 T > C (p.Val213Ala)	F	3 m	7 y	GTC	2 AED	N	GDD	No	No	No	diffuse white matter	Epileptic Encephalopathy	Refractory to medications, still having seizure
42	KF142	*SCN2A* NM_001040142.1 c.2995 G > A (p.Glu999Lys)	M	12 m	12 y	focal + GTC	2 AED	N	GDD, ID	No	No	No	multifocal hyperintensities in frontal and parietal and restricted diffusion	Epileptic Encephalopathy	Refractory to medications, still having seizure
43	KF154	*SCN2A* NM_001040142.1 c.788C > T (p.Ala263Val)	M	neonatal	4 years	GTC	AED	Y	GDD	No	No	No	unremarkable	Occipital sharp waves	Controlled
SCN8A															
44	KF114	*SCN8A* NM_014191.3 c.82C > T (p.Arg28Cys)	M	1 y	5 y	GTC	AED	Y	GDD	No	No	No	unremarkable	LGS	Controlled
45	KF115	*SCN8A* NM_014191.3 c.82C > T (p.Arg28Cys)	F	neonatal	4 y	focal + GTC	2 AED	Y	GDD	No	No	No	brain atrophy	LGS	Refractory to medications, still having seizure
46	KF127	*SCN8A* NM_014191.3 c.82C > T (p.Arg28Cys)	F	1 y	12 y	GTC	AED	N	GDD+	No	No	No	unremarkable	slow spike and waves	obesity + hirsu-tism + ID
47	KF139	*SCN8A* NM_014191.3 c.4398C > G (p.Asn1466Lys)	F	birth	2 y	focal + GTC	AED	N	GDD+	No	No	No	unremarkable	focal with secondary generalization	Controlled Spasticity
Genes responsible for the organelles and cell membrane:															
AP3B2 (mentioned above)															
ARV1															
48	KF46-H		M	Early	12 y	GTC	AED	Y		No	No	No	unremarkable	Epileptic Encephalopathy	Refractory to medications,

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Table 1 (*continued*)

List	Patient Number	Gene and Mutation	Gender	Age at onset of seizures	Current age	Seizure pattern	AED	Consang-uinity	Developme-nt	Clinical features			MRI	EEG	Outcome and seizure control
										Hypoton-ia	Microce-phaly	Ophthal-mologic involve-ment			
		ARV1 NM_022786 c.565 G > A (p.Gln189Arg)							GDD + Pro-found ID + Ataxia						still having seizure
49	KF47-H	*ARV1* NM_022786 c.565 G > A (p.Gln189Arg)	F	Early	10 y	GTC	AED	Y	GDD + Pro-found ID + Ataxia	No	No	No	unremarkable	Epileptic Encephalopathy	Refractory to medications, still having seizure
50	KF48-H	*ARV1* NM_022786 c.565 G > A (p.Gln189Arg)	M	Neonatal	2 y	GTC	AED	Y	GDD + Pro-found ID + Ataxia	No	No	No	unremarkable	Epileptic Encephalopathy	Refractory to medications, still having seizure
PCDH19															
51	KA2	*PCDH19* NM_001105243.1 c.3263_3264delAA (p.Lys1088Argfs-X28)	M	18 m	10 y	GTC, myo-clonic	3 AED	Y	Delayed	no	Yes	No	Normal	Slowing of background, high amplitude delta activity, and bifrontal epileptiform discharges	Intractable seizures
SLC13A5															
52	KA11	*SLC13A5* NM_177550.4 c.1227dupC (p.Ile410Hisfs*13)	F	Day 2	7 y	NA	3 AED	Y	Delayed	Yes (mild)	yes	Normal	Delayed myelination for age, nonspecific spectral pattern, and microcephaly	Excessive beta activity (may be secondary to medications) Others normal, no epileptic discharges	Controlled, no seizures for 2 years
53	KA16	*SLC13A5* NM_177550.4 c.655 G > A (p.Gly219Arg)	M	2 days	5 y	GTC	N/A	Y	Delayed	Yes	No	No	Arachnoid cyst in the right temporal lobe,	consistent with Lennox Gastaut syndrome (LGS).	Still having seizure
54	KF55	*SLC13A5* NM_177550.4 c.231 + 2T > G	M	neonatal	7 y	focal + GTC	4 AED	Y	profound GDD, ID	No	Yes	No	unremarkable	multifocal	Refractory to medications, still having seizure
55	KF117-S	*SLC13A5* NM_177550.4 c.785 T > C (p.Leu262Pro)	F	neonatal	12 y	GTC	2 AED	Y	GDD, ID	No	No	No	unremarkable	multifocal	Refractory to medications, still having seizure
56	KF118	*SLC13A5* NM_177550.4 c.1227dupC (p.Ile410Hisfs*13)	M	neonatal	4 y	GTC	Refracto-ry > 3AED	Y	GDD, ID	No	No	No	unremarkable	multifocal	Refractory to medications, still having seizure
57	KF119-T	*SLC13A5* NM_177550.4 c.1227_1228insC (p.Ile410Hisfs*13)	M	neonatal	10 y	GTC	3 AED	Y	GDD, ID	No	No	No	unremarkable	multifocal	Refractory to medications, still having seizure
58	KF120-T	*SLC13A5* NM_177550.4 c.1227_1228insC (p.Ile410Hisfs*13)	M	neonatal	5 y	GTC	3 AED	Y	GDD, ID	No	No	No	unremarkable	multifocal	Refractory to medications, still having seizure

(*continued on next page*)

Table 1 (*continued*)

List	Patient Number	Gene and Mutation	Gender	Age at onset of seizures	Current age	Seizure pattern	AED	Consang-uinity	Developme-nt	Clinical features			MRI	EEG	Outcome and seizure control
										Hypoton-ia	Microce-phaly	Ophthal-mologic involve-ment			
59	KF121-T	*SLC13A5* NM_177550.4 c.1227_1228insC (p.Ile410Hisfs*13)	M	neonatal	11 y	GTC	Refracto-ry > 3AED	Y	GDD, ID	No	No	No	unremarkable	multifocal	Refractory to medications, still having seizure
60	KF122-S	*SLC13A5* NM_177550.4 c.785 T > C (p.Leu262Pro)	F	1 y	8 y	GTC	3 AED	Y	GDD, ID	No	No	No	unremarkable	multifocal	Refractory to medications, still having seizure
61	KF152	*SLC13A5* NM_177550.4 c.785 T > C (p.Leu262Pro)	F	neonatal	1 year	focal, GTC	3 AED	Y	Delayed	No	Yes	No	unremarkable	Epileptic Encephalopathy	Refractory to medications, still having seizure
62	KF156	*SLC13A5* NM_177550.3 c.1654 T > A (p.Phe552Ile)	F	neonatal	2 years	focal, GTC	2 AED	Y	N/A	N/A	N/A	N/A	unremarkable	Multifocal	Refractory to medications, still having seizure
SLC25A12															
63	KF143	*SLC25A12* NM_003705.4 c.1385C > T (p.Thr462Met)	F	12 m	8 y	focal + GTC	2 AED	Y	GDD +	No	No	Yes	unremarkable	Epileptic Encephalopathy	Refractory to medications, still having seizure
SLC25A22															
64	KA5	*SLC25A22* NM_001191060.1 c.754C > T (p.Arg252Trp)	M	Day 3	4 y	GTC	No AED (family discon-tinued the medica-tion)	Y	Delayed	yes	Yes (ac-quired)	Poor eye contact	Generalized widening of cerebral sulci with prominent lateral and third ventricle and basal cistern	Diffuse slowing, burst suppression pattern during sleep, and multifocal epileptiform discharges	Intractable seizures
65	KF129	*SLC25A22* NM_001191060.1 c.55 G > A (p.Gly19Arg)	M	18 m	7 y	GTC	3 AED	Y	GDD	No	No	No	neurodegenerative disease	LGS	Controlled
Genes responsible for the development and growth of the neurons:															
ARX															
66	KF160	*ARX* NM_139058.2 c.1019 T > C (p.Leu340Pro)	F	neonatal	6 years	GTC	3 AED	Y	ID	No	Yes	No	unremarkable	burst suppression	Refractory to medications, still having seizure
CDKL5															
67	KA7	*CDKL5* NM_003159.2 c.119C > A (p.Ala40Glu)	F	Day 29	2y & 10 m	Tonic with head deviation and staring	3 AED	Y	Delayed	yes	no	Optokin-etic nys-tagmus	N/A	Encephalopathy	Intractable seizures
68	KF102	*CDKL5* NM_003159.2 c.291C > T (p.leu97leu)	F	neonatal	7 y	GTC	2 AED	Y	GDD	No	No	No	Cystic changes & subdural hematoma	Epileptic Encephalopathy	Refractory to medications, still having seizure
DENND5A (mentioned above)															

(*continued on next page*)

Table 1 (*continued*)

List	Patient Number	Gene and Mutation	Gender	Age at onset of seizures	Current age	Seizure pattern	AED	Consang-uinity	Developme-nt	Clinical features			MRI	EEG	Outcome and seizure control
										Hypoton-ia	Microce-phaly	Ophthal-mologic involve-ment			
DOCK7															
69	KF161	*DOCK7* NM_001271999.1 c.884del (p.Lys295Argfs*15)	F	neonatal	9 months	GTC	AED	Y	Normal	No	Yes	No	unremarkable	Epileptic Encephalopathy	Controlled
WWOX															
70	KA8	*WWOX* NM_016373.3 c.409 + 1G > T	M	2 m	2.5 y	NA	N/A	Y	Delayed	yes	NA	Poor eye contact	Brain atrophy Increased white signal in the cerebellar area	N/A	Still has seizures Hepatomegaly
71	KF92-O	*WWOX* NM_016373.3 c.606-1G > A	M	2 m	Deceased	GTC	AED	Y	GDD	No	Yes	Yes	thin corpus callosum, hypomyelination.	Normal	Arthrogryposis, Deceased
72	KF93-O	*WWOX* NM_016373.3 c.606-1G > A	F	2 m	Deceased	N/A	AED	Y	GDD	No	Yes	Yes	thin corpus callosum, hypomyelination.	Normal	Arthrogryposis, Deceased

ADHD: Attention deficit hyperactivity disorder; AED: Antiepileptic Drug; CC: Corpus callosum; F: Female; GDD: Global developmental delay; GTC: Generalized tonic-clonic; ID: Intellectual disability; LGS: Lennox-Gastaut syndrome; LKS: Landau-Kleffner syndrome; M: Male; mo: months; N/A: Not available.

4. Discussion

The latest definition of epileptic encephalopathy by the International League Against Epilepsy (ILAE) stated that "the epileptic activity itself may contribute to severe cognitive and behavioral impairments above and beyond what might be expected from the underlying pathology alone (e.g., cortical malformation), and that these can worsen over time" [9]. The definition correlates the seizure activity with the patient's condition, which is known to have a considerable impact [10]. But it also highlights the underlying condition as an essential factor to determine the outcome and prognosis of the disease. In 2013, Bender, A. et al conducted a study on the *Scn1a* knocked-down murine model. Interestingly, they found that the gene defect per se was responsible for the animal cognitive impairment even without seizures [11]. This and other similar findings highlighted the essential role of the underlying genetic defect and its effect on the brain's normal development and function with or without seizure activity.

The classification of the epileptic encephalopathy has been changing over time [12]. It was initially based particularly on the clinical features and the EEG patterns. Recently, in 2017, ILAE published a new classification for the epilepsies in general. The new classification emphasized the importance of the underlying genetic cause, if any, for the proper management and prognosis of the disease [1]. The recent advances in the molecular genetics field and the ability to overcome many genetic diagnostic hurdles impelled to redefine and reclassify many of the well-known diseases back again based on the identified underlying causes. Wang et al. and Zhou et al. presented functional classifications for multiple epilepsy-related genes, reported in their studies [13,14], In this study, we collected the clinical and molecular data for 72 patients diagnosed to have 26 out of 59 types of EIEE registered in OMIM database (from type 1 to type 61, except type 20 and 22, which do not exist). All the reported genes in the current study, including the ones with novel variants, are well known to cause EIEE if they harbored a pathogenic mutation. The genetic damaging effect of all the discovered novel variants was positively predicted using multiple *in silico* prediction tools. Furthermore, the vast majority of these novel variants were not found in general population databases like Exome Aggregation Consortium (ExAC) and 1000 Genome Project databases. The allele frequencies for the variants found in ExAC or 1000 G were extremely low, which goes with the predicted damaging effect on the genes' functions.

To facilitate our review, we opted to classify our patients and all the remaining types of EIEE based on the responsible genes and their described functions (Fig. 1, Table S1, Figure S1). The genes were classified into six groups:

1 Genes responsible for the synapsis, neurotransmitters, and receptors: this group includes genes encoding for variable receptors like γ-Aminobutyric acid (GABA) and *N*-methyl-D-aspartate (NMDA) and other receptors. It also includes the genes responsible for the neurotransmitters dynamics, like the release by vesicles and reuptake from the synaptic cleft. Finally, it includes the genes regulating the synapses.
2 Genes responsible for signal transduction: this group of genes control various types of intracellular signaling and signal transduction processes.
3 Genes responsible for ion channels: these genes encode for the different types of sodium, potassium, and calcium ion channels.
4 Genes regulating DNA and RNA: this group includes the genes responsible for DNA repair, regulation of the DNA and RNA (including tRNA), DNA synthesis and protection. None of our patients belongs to this group.
5 Genes responsible for the organelles and cell membrane: these genes exhibit variable structural and functional roles in the cellular organelles like Golgi apparatus, endoplasmic reticulum, and mitochondria. They also play a role in cell membrane function and

Table 2
Molecular results.

List	Patient number	EIEE type	Gene	Zygosity	Mutation type	Nucleotide change	Amino acid change	Novel or reported / *in silico* prediction	Diagnostic tool	Inheritance	Age at confirmed diagnosis
1	KA1	7	*KCNQ2* NM_172107.2	Heterozygous	Missense	c.1464C > G	p.Asp488Glu	Novel, MT: disease-causing	WES	AD, Mother is a carrier	13 years
2	KA2	9	*PCDH19* NM_001105243.1	Hemizygous	Deletion	c.3263_3264delAA	p.Lys1088ArgfsX28	Novel, MT: disease-causing	WES	XLD-Linked *De novo*	8 years
3	KA3	17	*GNAO1* NM_020988.2	Heterozygous	Missense	c.683 T > C	p.Leu228Pro	Novel, MT: disease-causing Polyphen2: probably damaging SIFT: Damaging	WES	AD *De novo*	3 years
4	KA4	26	*KCNB1* NM_004975	Heterozygous	Missense	c.1222C > T	p.Pro408Ser	Novel, MT: disease-causing Polyphen2: probably damaging SIFT: Damaging	WGS	AD *De novo*	4 years
5	KA5	3	*SLC25A22* NM_001191060.1	Homozygous	Missense	c.754C > T	p.Arg252Trp	Novel, MT: disease-causing Polyphen2: probably damaging SIFT: Damaging	WES	AR, both parents are carriers	2 years
6	KA6	14	*KCNT1* NM_020822.2	Heterozygous	Missense	c.1130 G > C	p.Cys377Ser	Novel, MT: disease-causing SIFT: Damaging	WES	AD *De novo*	11 months
7	KA7	2	*CDKL5* NM_003159.2	Heterozygous	Missense	c.119C > A	p.Ala40Glu	Novel The variant p.Ala40Val was previously reported [45]	WES	AD *De novo*	11 months
8	KA8	14	*WWOX* NM_016373.3	Homozygous	Intronic	c.409 + 1G > T	NA	Novel, Found in ClinVar as likely pathogenic HSF: Alteration of the WT donor site, most probably affecting splicing.	WES	AR, both parents are carriers	1 year
9	KA10	4	*STXBP1*	Heterozygous	Deletion	The deletion (9q33.3 to 9q34.11) involves eight genes including *STXBP1*	NA	Reported [19]	CGH	AD *De novo*	18 years
10	KA11	25	*SLC13A5* NM_177550.4	Homozygous	Insertion	c.1227dupC	p.Ile410Hisfs*13	Novel, Found in ClinVar as Pathogenic variant. MT: disease-causing	WES	AR, Parents are carriers of both variant	6 years
11	KA12	11	*SCN2A* NM_021007.2	Heterozygous	Missense	c.4886 G > A	p.Arg1629His	Reported [32]	WES	AD *De novo*	4 years
12	KA13	27	*GRIN2B* NM_000834	Heterozygous	Missense	c.2429 G > A	p.Ser810Asn	Reported [18]	WES	AD *De novo*	5 years
13	KA14	6	*SCN1A* NM_001165963.1	Heterozygous	Missense	c.1244 T > C	p.Ile415Thr	Novel, MT: disease-causing Polyphen2: Possibly damaging SIFT: Damaging	WES	AD, inherited from father	10 years
14	KA15	11	*SCN2A* NM_021007.2	Heterozygous	Missense	c.4390 A > G	p.Thr1464Ala	Novel, MT: disease-causing Polyphen2: Possibly	Infantile Epilepsy panel (75 genes)	AD, inherited from father	3 months

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Table 2 (*continued*)

List	Patient number	EIEE type	Gene	Zygosity	Mutation type	Nucleotide change	Amino acid change	Novel or reported / *in silico* prediction	Diagnostic tool	Inheritance	Age at confirmed diagnosis
								damaging SIFT: Damaging			
15	KA 16	25	*SLC13A5* NM_177550.4	Homozygous	Missense	c.655 G > A	p.Gly219Arg	Reported [54]	WES	AR, Parents are carriers.	4 years
16	KA17	6	*SCN1A* NM_001165963.1	Heterozygous	Missense	c.1625 G > A	p.Arg542Gln	Reported [55]	Early infantile epileptic encephalopathy gene panel (43 genes)	AD, mother carrier	5 months
17	KF27-C	48	*AP3B2* NM_ 001278512.1	Homozygous	Deletion	c.1837del	p. Glu613Serfs*182	Reported [8]	WES	AR, Parents are carriers	7 years
18	KF28-C	48	*AP3B2* NM_001278512.1	Homozygous	Deletion	c.1837del	p. Glu613Serfs*182	Reported [8]	WES	AR, Parents are carriers	9 years
19	KF29	48	*AP3B2* NM_ 001278512.1	Homozygous	Deletion	c.1837del	p. Glu613Serfs*182	Reported [8]	WES	AR, Parents are carriers	2 years
20	KF31	49	*DENND5A* NM_ 015213.3	Homozygous	Missense	c.1622 A > G	p.Asp541Gly	Reported [8]	WES	AR, Parents are carriers	1 year
21	KF35-E	37	*FRRS1L* NM_014334.2	Homozygous	Nonsense	c.961C > T	p.Gln321*	Reported [16] [17],	WES	AR, Parents are carriers	6 years
22	KF36-E	37	*FRRS1L* NM_014334.2	Homozygous	Nonsense	c.961C > T	p.Gln321*	Reported [16] [17],	WES	AR, Parents are carriers	13 years
23	KF37-E	37	*FRRS1L* NM_014334.2	Homozygous	Nonsense	c.961C > T	p.Gln321*	Reported [16] [17],	WES	AR, Parents are carriers	12 years
24	KF38-E	37	*FRRS1L* NM_014334.2	Homozygous	Nonsense	c.961C > T	p.Gln321*	Reported [16] [17],	WES	AR, Parents are carriers	10 years
25	KF39-F	37	*FRRS1L* NM_014334.2	Homozygous	Nonsense	c.961C > T	p.Gln321*	Reported [16] [17],	Autozygome guided direct sequencing	AR, Parents are carriers	9 years
26	KF40-F	37	*FRRS1L* NM_014334.2	Homozygous	Nonsense	c.961C > T	p.Gln321*	Reported [16] [17],	Autozygome guided direct sequencing	AR, Parents are carriers	1 year
27	KF41-F	37	*FRRS1L* NM_014334.2	Homozygous	Nonsense	c.961C > T	p. Gln321*	Reported [16] [17],	Autozygome guided direct sequencing	AR, Parents are carriers	2 years
28	KF46-H	38	*ARV1* NM_022786	Homozygous	Missense	c.565 G > A	p.Gln189Arg	Reported [34]	WES	AR, Parents are carriers	10 years
29	KF47-H	38	*ARV1* NM_022786	Homozygous	Missense	c.565 G > A	p.Gln189Arg	Reported [34]	WES	AR, Parents are carriers	8 years
30	KF48-H	38	*ARV1* NM_022786	Homozygous	Missense	c.565 G > A	p.Gln189Arg	Reported [34]	WES	AR, Parents are carriers	1 year
31	KF52	4	*STXBP1* NM_001032221.3	Heterozygous	Missense	c.874C > T	p.Arg292Cys	Reported [56]	WES	AD, *De Novo*	3 years
32	KF55	25	*SLC13A5* NM_177550.3	Homozygous	Intronic, splice site	c.231 + 2T > G		Reported [34] [8],	WES	AR, Parents are carriers	4 years
33	KF59	11	*SCN2A* NM_021007.2	Heterozygous	Missense	c.3956 G > T	p. Arg1319Leu	Reported [57]	WES	AD, *De Novo*	4 years
34	KF62	11	*SCN2A* NM_021007.2	Heterozygous	Missense	c.3956 G > T	p. Arg1319Leu	Reported [8]	WES	AD, De Novo	*2 years*
35	KF68	21	*NECAP1* NM_015509.3	Homozygous	Nonsense	c.142C > T	p.Arg48*	Reported, [58]	WES	AR, Parents are carriers	6 years
36	KF92-O	28	*WWOX* NM_016373.3	Homozygous	Intronic	c.606-1G > A		Reported [34]	WES	AR, Parents are carriers	2 years
37	KF93-O	28	*WWOX* NM_016373.3	Homozygous	Intronic	c.606-1G > A		Reported [34]	WES	AR, Parents are carriers	1 year
38	KF102	2	*CDKL5* NM_003159.2	Heterozygous	Synonymous	c.291C > T	p.leu97leu	Novel, MT: disease-causing	WES	XLD, *De Novo*	3 years
39	KF108	32	*KCNA2* NM_004974.3	Heterozygous	Missense	c.1120 A > G	p.Thr374Ala	Reported [59]	WES	AD, De Novo	2 years
40	KF109	32	*KCNA2* NM_004974.3	Heterozygous	Missense	c.890 G > A	p.Arg297Gln	Reported [60]	WES	AD, *De Novo*	5 years
41	KF110	7	*KCNQ2* NM_172107.2	Heterozygous	Missense	c.1744 A > T	p.Ile582Phe	Novel, MT: disease-causing	WES	AD, *De Novo*	1 year

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Table 2 (*continued*)

List	Patient number	EIEE type	Gene	Zygosity	Mutation type	Nucleotide change	Amino acid change	Novel or reported / *in silico* prediction	Diagnostic tool	Inheritance	Age at confirmed diagnosis
								Polyphen2: possibly damaging SIFT: Damaging			
42	KF112-Q	12	*PLCB1* NM_015192.3	Homozygous	Nonsense	c.550C > T	p.Arg184*	Novel, MT: disease-causing	WES	AR, Parents are carriers	5 months
43	KF113-Q	12	*PLCB1* NM_015192.3	Homozygous	Nonsense	c.550C > T	p.Arg184*	Novel, MT: disease-causing	WES	AR, Parents are carriers	2 years
44	KF114	13	*SCN8A* NM_014191.3	Heterozygous	Missense	c.82C > T	p.Arg28Cys	Novel, MT: disease-causing Polyphen2: probably damaging SIFT: Damaging	WES	AD, *De novo*	2 years
45	KF115	13	*SCN8A* NM_014191.3	Heterozygous	Missense	c.82C > T	p.Arg28Cys	Novel, MT: disease-causing Polyphen2: probably damaging SIFT: Damaging	WES	AD, *De novo*	1 year
46	KF117-S	25	*SLC13A5* NM_177550.4	Homozygous	Missense	c.785 T > C	p.Leu262Pro	Novel, MT: Polymorphism Polyphen2: possibly damaging SIFT: Damaging	WES	AR, Parents are carriers	10 years
47	KF118	25	*SLC13A5* NM_177550.4	Homozygous	insertion	c.1227dupC	p.Ile410Hisfs*13	Novel, Found in ClinVar as Pathogenic variant. MT: disease-causing	WES	AR, Parents are carriers	1 year
48	KF119-T	25	*SLC13A5* NM_177550.4	Homozygous	Insertion	c.1227_1228insC	p.Ile410Hisfs*13	Novel, Found in ClinVar as Pathogenic variant. MT: disease-causing	WES	AR, Parents are carriers	8 years
49	KF120-T	25	*SLC13A5* NM_177550.4	Homozygous	Insertion	c.1227_1228insC	p.Ile410Hisfs*13	Novel, Found in ClinVar as Pathogenic variant. MT: disease-causing	WES	AR, Parents are carriers	3 years
50	KF121-T	25	*SLC13A5* NM_177550.4	Homozygous	Insertion	c.1227_1228insC	p.Ile410Hisfs*13	Novel, Found in ClinVar as Pathogenic variant. MT: disease-causing	WES	AR, Parents are carriers	9 years
51	KF122-S	25	*SLC13A5* NM_177550.4	Homozygous	Missense	c.785 T > C	p.Leu262Pro	Novel, MT: Polymorphism Polyphen2: possibly damaging SIFT: Damaging	WES	AR, Parents are carriers	6 years
52	KF126	11	*SCN2A* NM_001040143.1	Heterozygous	Missense	c.638 T > C	p.Val213Ala	Novel, MT: disease-causing Polyphen2: possibly damaging SIFT: Damaging	WES	AD, *De Novo*	4 years
53	KF127	13	*SCN8A* NM_014191.3	Heterozygous	Missense	c.82C > T	p.Arg28Cys	Novel, MT: disease-causing Polyphen2: probably damaging SIFT: Damaging	WES	AD, *De Novo*	8 years

(*continued on next page*)

Table 2 (*continued*)

List	Patient number	EIEE type	Gene	Zygosity	Mutation type	Nucleotide change	Amino acid change	Novel or reported / *in silico* prediction	Diagnostic tool	Inheritance	Age at confirmed diagnosis
54	KF128	8	*ARHGEF9* NM_015185.2	Hemizygous	Missense	c.1476 T > G	p.Phe492Leu	Novel, MT: disease-causing	WES	XLR, *De Novo*	8 years
55	KF129	3	*SLC25A22* NM_001191060.1	Homozygous	Missense	c.55 G > A	p.Gly19Arg	Novel, MT: disease-causing Polyphen2: probably damaging SIFT: Damaging	WES	AR, Parents are carriers	5 years
56	KF133	6	*SCN1A* NM_001165963.1	Heterozygous	Missense	c.1498C > T	p.Arg500Trp	Novel, Found in ClinVar as a variant of uncertain significance MT: disease-causing Polyphen2: probably damaging SIFT: Damaging	WES	AD, *De Novo*	5 years
57	KF138	14	*KCNT1* NM_020822.2	Heterozygous	Missense	c.862 G > A	p.Gly288Ser	Reported [61]	WES	AD, *De Novo*	7 months
58	KF139	13	*SCN8A* NM_014191.3	Heterozygous	Missense	c.4398C > G	p.Asn1466Lys	Reported [62]	WES	AD, *De Novo*	6 months
59	KF140	7	*KCNQ2* NM_172107.2	Heterozygous	Missense	c.793 G > A	p.Ala265Thr	Reported [63]	WES	AD, *De Novo*	2 months
60	KF142	11	*SCN2A* NM_001040142.1	Heterozygous	Missense	c.2995 G > A	p.Glu999Lys	Reported [33]	WES	AD, *De Novo*	10 years
61	KF143	39	*SLC25A12* NM_003705.4	Homozygous	Missense	c.1385C > T	p.Thr462Met	Novel, Found in ClinVar as a variant of uncertain significance MT: disease-causing Polyphen2: probably damaging	WES	AR, Parents are carriers	5 years
62	KF147	14	*KCNT1* NM_020822.2	Heterozygous	Missense	c.2800 G > A	p.Ala934Thr	Reported [64]	WES	AD, *De Novo*	4 years
63	KF150	6	*SCN1A* NM_001202435.1	Heterozygous	Missense	c.671 T > G	p.Leu224Trp	Novel, Found in ClinVar as likely pathogenic. MT: disease-causing Polyphen2: probably damaging SIFT: Damaging	WES	AD, *De Novo*	4 years
64	KF151	32	*KCNA2* NM_004974.3	Heterozygous	Deletion	c.1265_1266del	p.Glu422Glyfs*21	Novel, Found in ClinVar as likely pathogenic. MT: disease-causing.	WES	AD, *De Novo*	7 years
65	KF152	25	*SLC13A5* NM_177550.4	Homozygous	Missense	c.785 T > C	p.Leu262Pro	Novel, Polyphen2: possibly damaging SIFT: Damaging	WES	AR, Parents are carriers	3 months
66	KF154	11	*SCN2A* NM_001040142.1	Heterozygous	Missense	c.788C > T	p.Ala263Val	Reported [65]	WES	AD, *De Novo*	3 years
67	KF155	14	*KCNT1* NM_020822.2	Heterozygous	Missense	c.862 G > A	p.Gly288Ser	Reported [61]	WES	AD, *De Novo*	9 months
68	KF156	25	*SLC13A5* NM_177550.3	Homozygous	Missense	c.1654 T > A	p.Phe552Ile	Novel, MT: disease-causing, Polyphen2: probably Damaging SIFT: Damaging	WES	AR, Parents are carriers	1 year
69	KF157	6	*SCN1A* NM_001202435.1	Heterozygous	Missense	c.3714 A > C	p.Glu1238Asp	Reported [28]	WES	AD, *De Novo*	3 years

(*continued on next page*)

Table 2 (*continued*)

List	Patient number	EIEE type	Gene	Zygosity	Mutation type	Nucleotide change	Amino acid change	Novel or reported / *in silico* prediction	Diagnostic tool	Inheritance	Age at confirmed diagnosis
70	KF159	52	*SCN1B* NM_001037.4	Homozygous	Intronic Splice site	c.449-2A > G		Reported [30]	WES	AR, Parents are carriers	1 year
71	KF160	1	*ARX* NM_139058.2	Heterozygous	Missense	c.1019 T > C	p.Leu340Pro	Novel, MT: disease-causing, Polyphen2: probably Damaging SIFT: Damaging	WES	AD, *De Novo*	4 years
72	KF161	23	*DOCK7* NM_001271999.1	Homozygous	Deletion	c.884del	p.Lys295Argfs*15	Novel, MT: disease-causing.	WES	AR, Parents are carriers	6 months

Abbreviations: ADAutosomal Dominant; ARautosomal recessive; MTMutation Taster; WESwhole exome sequencing; XLDX-linked dominant; XLRX-linked recessive.

characteristics like glycosylation.

6 Genes responsible for the development and growth of the neurons: this group includes the genes that control the cellular growth and cell cycle. Some of these genes play a pivotal role in neuronal development, like the myelination, and axons and dendrite development.

4.1. Genes responsible for the synapsis, neurotransmitters, and receptors

Fifteen patients in our study were diagnosed to have mutations in genes that belong to this group. Three patients from two unrelated families had mutations in *AP3B2* gene. All of them had refractory seizures and demonstrated cerebellar atrophy in brain MRI. Assoum et al. reported 12 patients with *AP3B2* gene mutations. The majority of them were resistant to antiepileptic medications. Four out of six had structural brain findings in the brain MRI. However, none of the current patients had optic atrophy, which was reported previously [15]. All our patients had the same mutation, c.1837del (p.Glu613Serfs*182), that was described previously in Saudi population, which might reflect a founder effect.

In our study, we found seven patients from two unrelated families with mutations in *FRRS1L* gene. All of them have the same mutation, c.961C > T (p.Gln321*), which was reported before in other Saudi patients [16,17]. All of them share the previously reported phenotype including the developmental regression and poor response to antiepileptic medications, however, none of them had chorea [17].

The *GRIN2B* mutation, c.2429 G > A (p.Ser810Asn), was reported previously by Platzer et al. Although the current patient's seizures were controlled, she manifested the previously reported features of global developmental delay, microcephaly, and polymicrogyria in the brain MRI [18].

The deletion (9q33.3 to 9q34.11), which involves eight genes including *STXBP1* gene was reported twice in the literature to be associated with epileptic encephalopathy and intellectual disability [19].

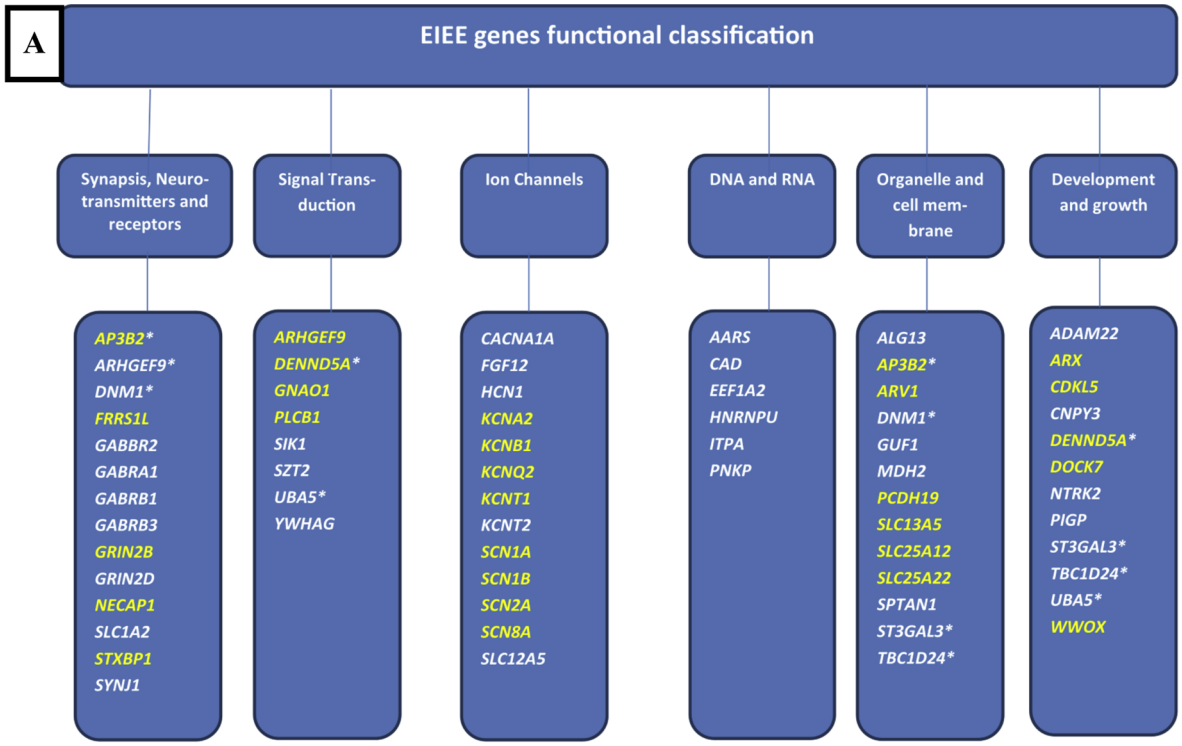
4.2. Genes responsible for signal transduction

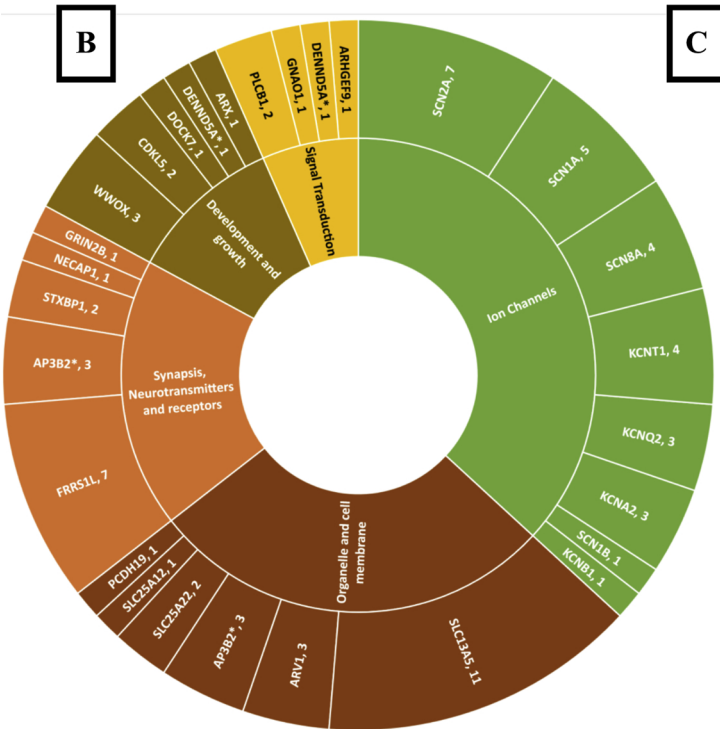
Regarding the current patient with *ARHGEF9* mutation, his presentation is consistent with the genotype/phenotype correlation proposed by Wang et al. The patient's seizures were well controlled, which could be because the mutation c.1476T > G (p.Phe492Leu), is located outside the three domains of the protein. Seizures improved in eight out of the twelve cases reported in Wang et al. review [14].

Han et al. reported three patients from two unrelated families with *DENND5A* gene mutations, two of the patients were from Saudi Arabia [20]. Although the three patients had the same brain MRI findings, brain atrophy and dysgenesis of the corpus callosum, as the patient presented here, however, they had severe intractable seizures while our patient's seizures were well controlled with three antiepileptic medications.

Regarding the *GNAO1* gene mutations, there is a wide variation in the clinical presentations and outcomes in the literature ranging from well controlled to intractable seizures. The genotype/phenotype may not be correlated for this gene [21]. The current patient's presentation goes with the previously reported case by Okumura et al. with mild phenotype and no seizures [22]. The presence of developmental delay, hypotonia, and microcephaly in patients with well-controlled epilepsy, may reflect the considerable effect of the underlying genetic mutation on the patients' phenotype.

The previously reported cases with *PLCB1* gene mutation had truncating deletion mutations involving multiple exons leading to drug-resistant epilepsy. The fourth case reported in the literature was responding to medication, however, ended up with severe developmental delay [23]. Herein we reported the first nonsense mutation in *PLCB1*, c.550C > T (p.Arg184*), which causes a very early termination and loss of all the protein domains. The patients were found to have brain





C

Semiology of Seizures	Associated genes
Infantile spasm	*AP3B2, ARV1, ARHGEF9*
LGS	*AP3B2, PLCB1, SCN8A*
Early onset without clear phenotype	*SLC13A5, PLCB1, WWOX*
Burst-suppression	*ARX, ARV1*
During sleep / LKS	*FRRS1L*
Malignant migratory	*KCNA2, PLCB1*

Fig. 1. A: EIEE genes functional classification scheme. Genes annotated with "*" have multiple functions and can fit more than one group. Genes reported in the current study were highlighted in yellow. **B**: Scheme showing the distribution of EIEE among the proposed functional groups. **C**: Seizure semiology associated genes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

atrophy, which was a common feature in most of the reported cases [23].

4.3. Genes responsible for ion channels

The *KCNA2* mutations of the patients KF108 and KF109 (p.Thr374Ala and p.Arg297Gln) are located in the intramembrane domains, however, the patient KF151 mutation (p.Glu422Glyfs*21) is located in the intracellular domain near to the C terminal, which could explain the milder phenotype and better control of the seizures. The same was reported previously regarding the variant p.Pro405Leu [24]. The gain of function mutation, p.Arg297Gln and p.Thr374Ala are among the most commonly reported mutations. Although two of the previously reported seven patients with p.Arg297Gln mutation had a favorable outcome, our patient was resistant to all AED and is still seizing [24].

Like the patient KA4, none of the previously reported patients with *KCNB1* mutations had a seizure control. The patient's mutation (p.Pro408Ser) is novel and involves the S6 domain of the protein, which could affect the function of the protein significantly. Normal brain MRI was reported previously in two patients [25].

The patient (KA1) has a benign familial neonatal epilepsy presentation caused by *KCNQ2* mutation like what was reported by Soldovieri, MV. (2014), where 13 out of 16 patients had a favorable outcome and normal cognition and development [26]. Alternatively, some patients were found to have intractable seizures with a global developmental delay like the two other patients (KF110 and KF140). The inheritance from currently asymptomatic mother of our patient KA1 mimics the familial inheritance reported previously [26].

A recent cohort of 12 patient with *KCNT1* gene mutation delineated the phenotypic features, which goes with the clinical characteristics of the patients in the current study. Seven out of the twelve reported patients didn't respond to any antiepileptic medications similar to the current patients. The reported brain MRI features included mainly cerebral and cerebellar atrophy, however, the main MRI finding in the current cases was the thinning of the corpus callosum [27].

Five of the patients in our study harbored mutations in the *SCN1A* gene, encoding the voltage-gated sodium channel. These patients had a variable course of the disease; while two patients (KA14 and KF150) had a mild course and spontaneous resolution of the seizures, the other three (KA17, KF133, and KF157) had refractory seizures and cognitive impairment. Their described mutations (p.Ile415Thr, p.Leu224Trp, p.Arg542Gln, p.Arg500Trp, and p.Glu1238Asp) are expected to affect variable domains of the expressed protein, which might exhibit a variable impact on the protein function, and consequently a great variation of the phenotype [28,29].

The patient KF159 is the second reported case in the literature up to our knowledge with the *SCN1B* gene intronic mutation c.449-2A > G. He has a similar presentation like the case reported by Trujillano et al. [30].

The matched clinical presentation of the patients in the current study with *SCN2A* mutation and the previously reported cases supports the genotype/phenotype correlation proposed by Sanders et al [31]. Many associated symptoms have been reported with *SCN2A* gene mutations, including choreoathetosis, ataxia, and schizophrenia [31]. The patient KA12 had other associated symptoms like sleep disturbance, spasticity, scoliosis, and gastro-esophageal reflux disease. Scoliosis was reported previously in three patients in the literature and one patient had scoliosis with GERD [31–33].

All the current patients with *SCN8A* mutations had a global developmental delay. Although the patients KF114, KF115, and KF127 have the same mutation c.82C > T (p.Arg28Cys), they had marked differences in the phenotype, response to treatment and MRI findings. This could reflect the effect of other factors on the outcome of the disease.

4.4. Genes responsible for the organelles and cell membrane

The first reported patient with *ARV1* gene mutation had neurodegenerative disease and blindness [34], however, none of the patients in our study had any brain MRI findings nor ophthalmologic involvement. The second reported case in the literature had an early onset and very severe phenotype and died at the age of 12 months. The patient had a splice site mutation and demonstrated hyperintensity on brain MRI in the posterior pons [35]. Interestingly, all patients in the current study had ataxia, which was not reported previously.

Although the patient KA2 with the *PCDH19* mutation has a novel mutation that involves the most distal part of the intracellular domain of the protein, the patient shares the same clinical presentation with a recently reported series including the intractable seizure, and global developmental delay. This may reflect the critical role of the intracellular domain in the protein function [36]. *PCDH19* mutation was reported in the literature to have a unique type of inheritance, where the disease appeared to affect heterozygous females and mosaic hemizygous males [37–39]. It was called "female-limited epilepsy" as some of the affected females inherited the mutations from their healthy fathers. However, in some cases, the mutation was inherited from hemizygous father, who was reported to have neuropsychiatric symptoms without commenting on the presence of mosaicism, while some carrier mothers were asymptomatic [36].

The most common type of EIEE in our study was type 25 caused by *SLC13A5* gene mutation, which encodes for the sodium citrate transporter. Five out of the six mutations discovered in this study were novel. The most commonly found mutation was c.1227dupC (p.Ile410Hisfs*13), which was described in three unrelated families, raising the possibility of being a founder mutation in the Saudi population. All patients had a profound developmental delay and were resistant to medical treatment except one patient, who had partial control of her seizures. Teeth hypoplasia was reported previously in patients with *SLC13A5* mutations, [40] which was found in the patient KA11, who had hypodontia. As previously reported, the most common EEG pattern was the multifocal [41]. All the currently reported patients share a comparable course of the disease and outcome. Although one of them (KA11) did not develop any seizure in the last five years, she has a profound cognitive delay, which further support the effect of the underlying genetic mutation on the patients' outcome in addition to the seizure effect.

All the previously reported cases with *SLC25A22* gene mutation experienced their first seizure at a very early age of their neonatal life [42]. Alternatively, the patient KF129 presented later at 18 months of age. Although he has a global developmental delay, his seizures were controllable with the use of three antiepileptic medications. Hypotonia and microcephaly were found in patient KA5, which goes with the phenotype of the previously reported patients [42].

4.5. Genes responsible for the development and growth of the neurons

We reported one patient with a novel mutation in *ARX* gene. The patient's mutation is located in the DNA binding homeobox, the site at which most of the severe *ARX* mutations are clustered [43]. Although the patient had microcephaly and intellectual disability, she did not have any gross structural brain abnormality in the brain MRI.

The two variants in the *CDKL5* gene in our study were missense, novel, de novo and were located in the functional catalytic domain of the protein [44]. Although the p.Ala40Val mutation was reported before, the p.Ala40Glu mutation, which was found in the current patient was not reported. Both variants share the same phenotype of tonic seizures, refractory to treatment and developmental delay [45].

The *DOCK7* mutations were described for the first time in 2014 by Perrault, I. et al in three female patients from two unrelated families. These patients had refractory seizures, intellectual disability, and cortical blindness. All the reported mutations were truncating, two of the patients had compound heterozygous mutations and one had a stop codon mutation, (p.Asp837Alafs*48), (p.Arg1237*), and (p.Ser328*) respectively [46]. Although the patient in our study had a truncating mutation (p.Lys295Argfs*15) and microcephaly, she had a milder course with controllable seizures, normal development, and unremarkable ophthalmological examination and normal brain MRI.

In this report, we add another two patients with c.606-1G > A mutation in *WWOX* gene to the previously reported patients by our group [47]. The two patients had the same course of the disease with premature death. Which supports the severity of this intronic mutation. These two siblings presented with arthrogryposis, which was not reported in the previous cases [47]. A third patient with an intronic mutation in *WWOX* was presented here (KA8) has a global developmental delay, refractory seizures, hypotonia, and poor eye contact. His brain MRI showed cerebral atrophy, which goes with the previously reported cases of *WWOX* gene mutations [48].

Identifying the underlying genetic cause for all EIEE cases is of utmost importance not only for the personalized management and

prognostic prediction but also for proper genetic counseling. Four of our patients had inherited autosomal dominant mutations, one of them (KA14), had a similar phenotype of his affected father. The second and third patients (KA15 and KA17), had severe phenotypes in comparison to the affected fathers. Alternatively, the fourth patient (KA1), the carrier mother was completely asymptomatic. Incomplete penetrance and variable expressivity were reported in cases with *SCN1A* related seizures, which might be challenging for the counseling of these families [49]. Although the autosomal dominant epileptic encephalopathy is expected to be the most common,7 in our study the autosomal recessive cases were slightly more than the autosomal dominant, 50% and 45.8% respectively. This could be attributed to the high consanguinity rate in Saudi society, which explains the high incidence of AR diseases [50]. Of note, the discovered de novo mutations in autosomal dominant EIEE should be taken with extreme caution as some of the previously reported genes were found warrant further functional studies to proof their pathogenicity and correlation with EIEE phenotype [51].

As expected [52], most of the patients in this study (61.1%) were resistant to all the antiepileptic medications given. The clinical course of our cases, as well as the EEG patterns and brain MRI characteristics, were quite heterogeneous even for the same genes. However, intrafamilial homogeneity is noted. Some genes showed certain seizure semiology summarized in Fig. 1C.

The functional classification can be applied to other types of epilepsy syndromes. Additionally, it would be easy to add newly discovered genes either to the same proposed groups or to add new ones. As expected, some of the genes were included in more than one group of the classification, as they have multiple functions. It is important to bear in mind that this classification was based on the discovered functions of the genes, which could change or expand in the future. Certainly, knowing the pathophysiology of the underlying gene defect will help the clinicians in the personalized management for their patients and will pave the way for possible future treatments [53].

5. Conclusion

We reported the largest EIEE case series in the region with confirmed molecular testing and detailed clinical phenotyping. The AR outweighed the AD cases in this study, which could be expected in other societies with high consanguinity. The clinical manifestations of EIEE are widely variable. The breakthrough in molecular genetics led to the discovery of the underlying causes in a significant number of patients. A scrutinized look at the patient's genotype is of utmost importance for proper personalized management, prognostic prediction, and for genetic counseling. The functional classification of the disease might solve the dilemma of the existing classifications and would sort the patients easily and clearly. Although it is still a challenge to reach for the genetic diagnosis in all the EIEE patients, we are on the right way.

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Conflicts of interests

None declared.

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References

[1] Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: position paper of the ILAE commission for classification and terminology. Epilepsia 2017;58:512–21.
[2] Khan S, Al Baradie R. Epileptic encephalopathies: an overview. Epilepsy Res Treat 2012;2012:403592.
[3] Engel J. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. Epilepsia 2001;42:796–803.
[4] Noh GJ, Jane Tavyev Asher Y, Graham Jr JM. Clinical review of genetic epileptic encephalopathies. Eur J Med Genet 2012;55:281–98.
[5] Stafstrom CE, Kossoff EM. Epileptic encephalopathy in infants and children. Epilepsy Curr 2016;16:273–9.
[6] Pal DK, Pong AW, Chung WK. Genetic evaluation and counseling for epilepsy. Nat Rev Neurol 2010;6:445–53.
[7] McTague A, Howell KB, Cross JH, et al. The genetic landscape of the epileptic encephalopathies of infancy and childhood. Lancet Neurol 2016;15:304–16.
[8] Anazi S, Maddirevula S, Faqeih E, et al. Clinical genomics expands the morbid genome of intellectual disability and offers a high diagnostic yield. Mol Psychiatry 2017;22:615–24.
[9] 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.
[10] Holmes GL. Cognitive impairment in epilepsy: the role of network abnormalities. Epileptic Disord 2015;17:101–16.
[11] Bender AC, Natola H, Ndong C, et al. Focal Scn1a knockdown induces cognitive impairment without seizures. Neurobiol Dis 2013;54:297–307.
[12] Kalser J, Cross JH. The epileptic encephalopathy jungle - from Dr West to the concepts of aetiology-related and developmental encephalopathies. Curr Opin Neurol 2018;31:216–22.
[13] Wang J, Lin ZJ, Liu L, et al. Epilepsy-associated genes. Seizure 2017;44:11–20.
[14] Wang JY, Zhou P, Wang J, et al. ARHGEF9 mutations in epileptic encephalopathy/intellectual disability: toward understanding the mechanism underlying phenotypic variation. Neurogenetics 2018;19:9–16.
[15] Assoum M, Philippe C, Isidor B, et al. Autosomal-recessive mutations in AP3B2, adaptor-related protein complex 3 Beta 2 subunit, cause an early-onset epileptic encephalopathy with optic atrophy. Am J Hum Genet 2016;99:1368–76.
[16] Shaheen R, Al Tala S, Ewida N, et al. Epileptic encephalopathy with continuous spike-and-wave during sleep maps to a homozygous truncating mutation in AMPA receptor component FRRS1L. Clin Genet 2016;90:282–3.
[17] Madeo M, Stewart M, Sun Y, et al. Loss-of-Function mutations in FRRS1L lead to an epileptic-dyskinetic encephalopathy. Am J Hum Genet 2016;98:1249–55.
[18] Platzer K, Yuan H, Schutz H, et al. GRIN2B encephalopathy: novel findings on phenotype, variant clustering, functional consequences and treatment aspects. J Med Genet 2017;54:460–70.
[19] Aravindhan A, Shah K, Pak J, et al. Early-onset epileptic encephalopathy with myoclonic seizures related to 9q33.3-q34.11 deletion involving STXBP1 and SPTAN1 genes. Epileptic Disord 2018;20:214–8.
[20] Han C, Alkhater R, Froukh T, et al. Epileptic encephalopathy caused by mutations in the guanine nucleotide exchange factor DENND5A. Am J Hum Genet 2016;99:1359–67.
[21] Solis GP, Katanaev VL. Galphao (GNAO1) encephalopathies: plasma membrane vs. Golgi functions. Oncotarget 2018;9:23846–7.
[22] Okumura A, Maruyama K, Shibata M, et al. A patient with a GNAO1 mutation with decreased spontaneous movements, hypotonia, and dystonic features. Brain Dev 2018.
[23] Schoonjans AS, Meuwissen M, Reyniers E, et al. PLCB1 epileptic encephalopathies; Review and expansion of the phenotypic spectrum. Eur J Paediatr Neurol 2016;20:474–9.
[24] Syrbe S, Hedrich UBS, Riesch E, et al. De novo loss- or gain-of-function mutations in KCNA2 cause epileptic encephalopathy. Nat Genet 2015;47:393–9.
[25] Torkamani A, Bersell K, Jorge BS, et al. De novo KCNB1 mutations in epileptic encephalopathy. Ann Neurol 2014;76:529–40.
[26] Soldovieri MV, Boutry-Kryza N, Milh M, et al. Novel KCNQ2 and KCNQ3 mutations in a large cohort of families with benign neonatal epilepsy: first evidence for an altered channel regulation by syntaxin-1A. Hum Mutat 2014;35:356–67.
[27] McTague A, Nair U, Malhotra S, et al. Clinical and molecular characterization of KCNT1-related severe early-onset epilepsy. Neurology 2018;90:e55–66.
[28] Harkin LA, McMahon JM, Iona X, et al. The spectrum of SCN1A-related infantile epileptic encephalopathies. Brain 2007;130:843–52.
[29] Mantegazza M, Catterall WA, et al. Voltage-gated Na(+) channels: structure, function, and pathophysiology. In: th Noebels JL, Avoli M, editors. Jasper's basic mechanisms of the epilepsies: bethesda (MD). 2012.
[30] Trujillano D, Bertoli-Avella AM, Kumar Kandaswamy K, et al. Clinical exome sequencing: results from 2819 samples reflecting 1000 families. Eur J Hum Genet 2017;25:176–82.
[31] Sanders SJ, Campbell AJ, Cottrell JR, et al. Progress in understanding and treating SCN2A-Mediated disorders. Trends Neurosci 2018;41:442–56.
[32] Wolff M, Johannesen KM, Hedrich UBS, et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. Brain 2017;140:1316–36.
[33] Nakamura K, Kato M, Osaka H, et al. Clinical spectrum of SCN2A mutations expanding to Ohtahara syndrome. Neurology 2013;81:992–8.
[34] Alazami AM, Patel N, Shamseldin HE, et al. Accelerating novel candidate gene discovery in neurogenetic disorders via whole-exome sequencing of prescreened multiplex consanguineous families. Cell Rep 2015;10:148–61.
[35] Palmer EE, Jarrett KE, Sachdev RK, et al. Neuronal deficiency of ARV1 causes an autosomal recessive epileptic encephalopathy. Hum Mol Genet 2016;25:3042–54.
[36] Smith L, Singhal N, El Achkar CM, et al. PCDH19-related epilepsy is associated with a broad neurodevelopmental spectrum. Epilepsia 2018;59:679–89.

[37] Ryan SG, Chance PF, Zou CH, et al. Epilepsy and mental retardation limited to females: an X-linked dominant disorder with male sparing. Nat Genet 1997;17:92–5.
[38] Scheffer IE, Turner SJ, Dibbens LM, et al. Epilepsy and mental retardation limited to females: an under-recognized disorder. Brain 2008;131:918–27.
[39] de Lange IM, Rump P, Neuteboom RF, et al. Male patients affected by mosaic PCDH19 mutations: five new cases. Neurogenetics 2017;18:147–53.
[40] Schossig A, Bloch-Zupan A, Lussi A, et al. SLC13A5 is the second gene associated with Kohlschutter-Tonz syndrome. J Med Genet 2017;54:54–62.
[41] Hardies K, de Kovel CG, Weckhuysen S, et al. Recessive mutations in SLC13A5 result in a loss of citrate transport and cause neonatal epilepsy, developmental delay and teeth hypoplasia. Brain 2015;138:3238–50.
[42] Cohen R, Basel-Vanagaite L, Goldberg-Stern H, et al. Two siblings with early infantile myoclonic encephalopathy due to mutation in the gene encoding mitochondrial glutamate/H+ symporter SLC25A22. Eur J Paediatr Neurol 2014;18:801–5.
[43] Friocourt G, Parnavelas JG. Mutations in ARX result in several defects involving GABAergic neurons. Front Cell Neurosci 2010;4:4.
[44] Hector RD, Kalscheuer VM, Hennig F, et al. CDKL5 variants: improving our understanding of a rare neurologic disorder. Neurol Genet 2017;3:e200.
[45] Rosas-Vargas H, Bahi-Buisson N, Philippe C, et al. Impairment of CDKL5 nuclear localisation as a cause for severe infantile encephalopathy. J Med Genet 2008;45:172–8.
[46] 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.
[47] Tabarki B, AlHashem A, AlShahwan S, et al. Severe CNS involvement in WWOX mutations: description of five new cases. Am J Med Genet A 2015;167A:3209–13.
[48] Mignot C, Lambert L, Pasquier L, et al. WWOX-related encephalopathies: delineation of the phenotypical spectrum and emerging genotype-phenotype correlation. J Med Genet 2015;52:61–70.
[49] Miller IO, Sotero de Menezes MA, et al. Adam MP, Ardinger HH, Pagon RA, editors. SCN1A-related seizure disorders. Seattle (WA): GeneReviews((R)); 1993.
[50] Al-Owain M, Al-Zaidan H, Al-Hassnan Z. Map of autosomal recessive genetic disorders in Saudi Arabia: concepts and future directions. Am J Med Genet A 2012;158A:2629–40.
[51] He N, Lin ZJ, Wang J, et al. Evaluating the pathogenic potential of genes with de novo variants in epileptic encephalopathies. Genet Med 2019;21:17–27.
[52] Vigevano F, Arzimanoglou A, Plouin P, et al. Therapeutic approach to epileptic encephalopathies. Epilepsia 2013;54(Suppl 8):45–50.
[53] Nieh SE, Sherr EH. Epileptic encephalopathies: new genes and new pathways. Neurotherapeutics 2014;11:796–806.
[54] Thevenon J, Milh M, Feillet F, et al. Mutations in SLC13A5 cause autosomal-recessive epileptic encephalopathy with seizure onset in the first days of life. Am J Hum Genet 2014;95:113–20.
[55] Weiss LA, Escayg A, Kearney JA, et al. Sodium channels SCN1A, SCN2A and SCN3A in familial autism. Mol Psychiatry 2003;8:186–94.
[56] Saudi Mendeliome G. Comprehensive gene panels provide advantages over clinical exome sequencing for Mendelian diseases. Genome Biol 2015;16:134.
[57] Anazi S, Maddirevula S, Salpietro V, et al. Expanding the genetic heterogeneity of intellectual disability. Hum Genet 2017;136:1419–29.
[58] Alazami AM, Hijazi H, Kentab AY, et al. NECAP1 loss of function leads to a severe infantile epileptic encephalopathy. J Med Genet 2014;51:224–8.
[59] Hundallah K, Alenizi A, AlHashem A, et al. Severe early-onset epileptic encephalopathy due to mutations in the KCNA2 gene: expansion of the genotypic and phenotypic spectrum. Eur J Paediatr Neurol 2016;20:657–60.
[60] Pena SD, Coimbra RL. Ataxia and myoclonic epilepsy due to a heterozygous new mutation in KCNA2: proposal for a new channelopathy. Clin Genet 2015;87:e1–3.
[61] Ishii A, Shioda M, Okumura A, et al. A recurrent KCNT1 mutation in two sporadic cases with malignant migrating partial seizures in infancy. Gene 2013;531:467–71.
[62] Ohba C, Kato M, Takahashi S, et al. Early onset epileptic encephalopathy caused by de novo SCN8A mutations. Epilepsia 2014;55:994–1000.
[63] Milh M, Boutry-Kryza N, Sutera-Sardo J, et al. Similar early characteristics but variable neurological outcome of patients with a de novo mutation of KCNQ2. Orphanet J Rare Dis 2013;8:80.
[64] Barcia G, Fleming MR, Deligniere A, et al. De novo gain-of-function KCNT1 channel mutations cause malignant migrating partial seizures of infancy. Nat Genet 2012;44:1255–9.
[65] Liao Y, Anttonen AK, Liukkonen E, et al. SCN2A mutation associated with neonatal epilepsy, late-onset episodic ataxia, myoclonus, and pain. Neurology 2010;75:1454–8.