Phenotypic Spectrum and Long-term Outcome in Children With Genetic Causes of Early-onset Epileptic Encephalopathy

Chunhui Hu

Department of Neurology, Children's Hospital of Fudan University

Deying Liu

Wuhan Children's hospital, Tongji Medical college, Huazhong University of Science & Technology

Tian Luo

Department of Neurology, Children's Hospital of Fudan University

Yi Wang (✉ yiwangshmuedu@126.com)

Department of Neurology, Children's Hospital of Fudan University

Zhisheng Liu

Wuhan Children's hospital, Tongji Medical college, Huazhong University of Science & Technology

Research Article

Keywords: Phenotypic spectrum, Long-term outcome, Genetic, EOOE, Therapy

Posted Date: March 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-257334/v1>

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Abstract

Background To explore the clinical phenotype and long-term outcome in children with genetic causes of early-onset epileptic encephalopathies.

Methods The clinical data of 118 children between 2010 and 2020 was obtained and analyzed. The whole exome sequencing and copy number variation studies in family were used to find pathogenic mutations. The confirmed mutations were verified by Sanger sequencing.

Results Among 118 patients, 39 patients were diagnosed with DS, 18 were WS, 3 were OS, 3 were EME, 2 were MMFSI, 1 was GLUT1 deficiency syndrome, 1 was Pyridoxine dependent epilepsy and 51 were non-symptomatic EOEES. The initial EEG showed frequent multiple and multifocal sharp waves, spike waves, sharp slow waves or spike slow waves. In the later period, some transformed into infrequent discharging or normal EEG. 112 patients (112/118, 94.9%) showed normal brain MRI, and the remaining 6 had widened extracerebral space. In the later stage, 115 patients were re-examined with brain MRI 1 to 3 times, the widened gap became normal, only 2 had mild brain atrophy. After treatment, 42 patients (42/118, 35.6%) had seizure control. In EOEE-BS, 6 patients were found *KCNQ2* mutations and the remaining mutations were *SCN2A* (n=2), *STXBP1* (n=1). After treatment, only 2 patients had seizure control, 6 had uncontrolled seizures and 1 died. 7 patients with dyskinesia were found. 1 patient starting with a febrile convulsion was caused by *HNRNPU* mutation. *SCN1A* mutations were detected in 38 patients (38/118, 32.2%), representing the largest proportion. The second common mutations were *KCNQ2* mutations in 9 patients. The third one was *CDKL5* mutations in 8 patients. Genes associated with ionic channels represented the largest proportion (66/118, 55.9%), sodium channel potassium channel and calcium channel respectively. In WS, we detected *SCN3A*, *SCN2A*, *SCN8A*, *CACNA1H*, *DEPDC5*, *MECP2*, *DYNC1H1*, *CDKL5*, *ALG11*, *CCDC88C*, *GABAA1*, *IL1RAPL1*, *RNASEH2B*, *SLC19A3*, *STXBP1*, *QARS*, *COL4A2* mutations. In addition to common gene mutations, we reported rare possible pathogenic genes: *CCDC88C*, *IL1RAPL1*, *RNASEH2B* and *COL4A2* in WS. In non-syndromic genetic causes of EOEES, we detected rare possible pathogenic genes: *SETBP1*, *DPYD*, *CSNK2B* and *H3F3A*. As for genetic modes, denovo heterozygous mutations account for the largest proportion (104/118, 88.1%). 3 patients with *SMC1A* mutations response to KD add-on therapy. VPA added treatment showed good effects on *KCNB1* and *PACS2* encephalopathy. LEV showed good effects on *STXBP1*, and OXC showed good effects on *SCN8A* encephalopathy.

Conclusion The clinical manifestations of EOEE are variable, including dyskinesia. EOEE-BS usually response poorly to AEDS therapy. Although some patients achieve seizure-free, there is no remarkable improvement in their development. EOEEs starting with a febrile convulsion may be a special phenotype of *HNRNPU* related neurodevelopmental syndrome, similar to DS. We report rare possible pathogenic genes: *CCDC88C*, *IL1RAPL1*, *RNASEH2B*, *COL4A2* in WS and detect rare possible pathogenic genes: *SETBP1*, *DPYD*, *CSNK2B* and *H3F3A* in non-syndromic genetic causes of EOEEs. Although genetic causes of EOEEs response poorly to AEDS treatment, we find that some gene mutation related EOEEs receive good effects on specific AEDS.

1. Introduction

Epilepsy is a disease of brain defined by at least two unprovoked (or reflex) seizures occurring > 24 h apart or one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years, or diagnosis of an epilepsy syndrome[1]. A certain cluster of epilepsy syndromes is grouped as “early-onset epileptic encephalopathies” including early myoclonic encephalopathy(EME), Ohtahara syndrome(OS), West syndrome(WS), Dravet syndrome(DS), malignant migrating focal seizures of infancy (MMFSI) and non-syndromic epileptic encephalopathy[2]. Early-onset epileptic encephalopathies (EOEEs) or early infantile epileptic encephalopathies (EIEEs) are one of the most devastating early onset epilepsies that contributes to a progressive decline of cerebral function. The onset age of seizure is within 6 months. Most patients with EOEEs show three main features: refractory seizures, severe electroencephalography (EEG) abnormalities, and developmental delay or intellectual disability[3]. The etiology of EOEEs are classified as infectious, immune, structural, metabolic, genetic and unknown factors. Genetic etiology has attracted more attention with lots of gene mutations having been identified. At least 20–30% of EOEEs are caused by a single gene variation[4]. In recent years, an increasing number of novel genes are being identified in EOEEs. Many genes related to EOEEs have been detected, such as *SCN1A*, *SCN2A*, *SCN8A*, *STXBP1*, *CDKL5* and *KCNQ2*[5]. Many patients with genetic causes of EOEEs are sporadic, occurring in patients with no family history of seizures or epilepsy. Although genetic causes of EOEEs are increasingly being identified, there is considerable genetic heterogeneity as well as phenotypic heterogeneity. A relatively rare clinical symptom, dyskinesia, has been identified in EOEEs[6]. And some specific phenotype, genetic causes of EOEE with burst suppression (EOEE-BS), has also been characterized[7, 8]. However, the long-term outcome of genetic causes of EOEEs remains unknown. Hence, there is a need to develop a deeper understanding of the broader clinical spectrum, specific genotype–phenotype and long-term outcome of genetic causes of EOEEs. In this study we aimed to describe the clinical features and long-term outcome of genetic causes of EOEEs in a cohort of patients and followed for a period of up to ten years.

2. Materials And Methods

2.1. Patients

The retrospective study included children with genetic causes of EOEEs at the Department of Neurology, Children's Hospital of Fudan University.

The project ethics were approved by Ethic Committees of Children's Hospital of Fudan University.

All the experiment protocol for involving humans was in accordance to guidelines of national/international/institutional or Declaration of Helsinki. Informed consent was obtained from all subjects.

The inclusion criteria are as follows: (1) seizure within 6 months after birth, (2) frequent seizures, (3) developmental retardation, stagnation or regression.

The exclusion criteria are as follows: (1) perinatal brain injury, (2) metabolic disease, (3) intrauterine infection, (4) neonatal and infantile seizures caused by brain structural abnormalities. The clinical data of 470 affected patients between January 2010 and January 2020 was obtained.

2.2. Next generation sequencing and copy number variation studies

The peripheral blood samples of these children and their parents were collected. The whole exome sequencing and copy number variation studies in family were used to find pathogenic mutations.

The inclusion criteria of sequencing are as follows: (1) insertion or deletion mutations, (2) mutations in coding amino acids or termination codons, (3) mutations in splicing sites, (4) non-synonymous mutations may destroy protein function predicted by PolyPhen-2 HVAR.

The exclusion criteria of sequencing are as follows: (1) copy number variations, microdeletions or microduplications, (2) nucleotide variations in all normal controls, (3) synonymous mutations, (4) single nucleotide polymorphisms (SNPs) annotated in human gene mutation database (HGMD), thousand human genome database, PubMed database and UCSC database.

Sanger sequencing was performed on verifying mutations. PolyPhen-2 analysis was carried out to predict variant effects. All patients were followed up for 1 year to 10 years.

3. Results

3.1 Patients' demographics and clinical features

118 patients diagnosed with genetic causes of EOEes were analyzed excluding 10 patients with copy number variations. The gene mutation rate was 27.2% (128/470). Among 118 patients, 62 (62/118, 52.5%) were males, 56 (56/118, 47.5%) were females. The seizure onset age ranged from 1 day to 6 months (3.5 $\pm$ 1.5 months). Their parents were not close relatives, and these children were not related except patients 73 and 74.

39 patients (39/118, 33.1%) were diagnosed with DS, 18 (18/118, 15.3%) were WS, 3 (3/118, 2.5%) were OS, 3 (3/118, 2.5%) were EME, 2 (2/118, 1.7%) were MMFSI, 1 (3/52, 0.8%) was GLUT1 deficiency syndrome, 1 (1/118, 0.8%) was Pyridoxine dependent epilepsy, 51 (51/118, 43.3%) were non-symptomatic EOEes. The initial EEG showed frequent multiple and multifocal sharp waves, spike waves, sharp slow waves or spike slow waves. In the later period, some transformed into infrequent discharging or normal EEG. 112 patients (112/118, 94.9%) showed normal brain MRI, and the remaining 6 had widened extracerebral space. In the later stage, 115 patients were re-examined with brain MRI 1 to 3 times, the widened gap became normal, only 2 had mild brain atrophy. After treatment, 42 patients (42/118, 35.6%) had seizure control. 16 patients (16/118, 13.6%) had seizure control for more than 1 year, 3 (3/118, 2.5%) had seizure control for more than 2 years, 9 patients had seizure control for more than 3 years (9/118, 7.6%), 8 had seizure control for more than 4 years (8/118, 6.8%), 4 had seizure control for more than 5 years (4/118, 3.4%), 2 had seizure control for more than 6 years (2/118, 1.7%). 2 patients died from SE (2/118, 1.7%). At the final follow-up, those patients remained seizure-free but no remarkable improvement in their development. 38 patients diagnosed with DS caused by *SCN7A* are not listed in Table 1 because their clinical features are easily identified. All remaining 80 patients' features were summarized in Table 1.

Table 1
Summary of the phenotypic features in our 80 patients

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
1	SCN2A	10y, F	3d	FS, GTCS, T	No	Severe → Severe	EOEE	Frequent → Infrequent	N → N	B6 TPM LEV VPA LTG OXC PB KD	No	Unc
2	SCN2A	10y, F	5m	FS, GTCS, T, SE	No	Severe → Severe	EOEE	BS → Frequent → Infrequent	N → Atrophy	TPM VPA LTG OXC LEV PB Mexitil KD VNS	No	Unc
3	SCN2A	5y, M	4m	S	No	Severe → Moderate	WS	BS → H → Infrequent	N → N	VPA MP KD	MP KD	Cor Frei
4	SCN3A	8y, M	6m	S	No	Severe → Moderate	WS	H → Infrequent	N → N	VPA P KD	P KD	Cor Frei
5	SCN8A	10y, M	6m	S	No	Severe → Moderate	WS	H → Infrequent	N → N	ACTH P TPM	ACTH P TPM	Cor Frei
6	SCN8A	3y, M	6m	FS, T, S	No	Severe → Severe	EOEE	Frequent → Infrequent	WG → N	LEV CZP TPM OXC	OXC	Cor Frei
7	SCN8A	3y, F	6m	T, FS	No	Severe → Severe	EOEE	Frequent → Infrequent	N → N	LEV PB VPA TPM OXC KD	No	Unc

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
8	SCN8A	5y, F	6m	S, FS, T, AA	No	Severe → Severe	EOEE	Frequent → Frequent	N → N	ACTH P OXC TPM LCM KD	No	Unc
9	SCN8A	2y, M	6m	FS	No	Severe → Severe	EOEE	Infrequent → Infrequent	N → N	PB OXC	OXC	Cor Frei
10	KCNQ2	6m, M	6d	GTCS, SE	No	Severe	EOEE	Frequent, Low voltage, BS	N	B6 PB VPA LEV	No	Die at 6
11	KCNQ2	4y, M	1d	T, FS, S	No	Severe → Severe	OS	Frequent, BS → Infrequent	N → N	B6 LEV OXC TPM	No	Unc
12	KCNQ2	4y, F	1d	FS,T	No	Severe → Severe	EOEE	BS → Frequent → Frequent	N → N	B6 TPM OXC VPA LCM KD	No	Unc
13	KCNQ2	6y, M	3m	T, GTCS	No	Severe → Severe	EOEE	Frequent → Frequent	N → N	TPM OXC LEV VPA LTG KD	No	Unc
14	KCNQ2	7y, M	3d	FS,S, GTCS	No	Severe → Severe	EOEE	BS → Frequent → Frequent	N → N	B6 NZP LEV ACTH P OXC TPM KD	TPM KD	Cor Frei

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
15	KCNQ2	5y, M	1d	FS,T, GTCS	No	Severe → Severe	EOEE	BS → Frequent → Frequent	N → N	B6 PB LEV OXC TPM VPA KD	No	Unc
16	KCNQ2	2y, M	10d	FS,T, GTCS	No	Severe → Severe	EOEE	Frequent → Frequent	N → N	B6 PB LEV TPM	PB LEV TPM	Cor Fre
17	KCNQ2	2y, M	3d	FS,T, S,GTCS	No	Severe → Severe	EOEE	Frequent → Frequent	N → N	B6 PB LEV ACTH TPM VPA	No	Unc
18	KCNQ2	2y, M	4d	FS,T, S,GTCS	No	Severe → Severe	OS → WS	BS → Frequent → H → Frequent	N → N	B6 PB LEV ACTH P TPM VPA OXC KD	No	Unc
19	KCNQ3	2y, M	6m	S,FS,T, GTCS, M	No	Severe → Severe	WS	H → Frequent → Frequent	N → N	VPA LEV TPM ACTH P VGB OXC	No	Unc
20	KCTD7	10y, M	5m	M,FS,T	No	Severe → Severe	EOEE	Frequent → Infrequent → N	N → N	LEV VPA TPM	VPA TPM	Cor Fre

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
21	KCNB1	10y, F	6m	FS,T	No	Severe	EOEE	Frequent	N	P	P	Cor
						→		→	→	VPA	VPA	Fren
						Severe		ESES	N			
22	KCNB1	4y, M	6m	FS,T	No	Severe	EOEE	Frequent	N	LEV	LEV	Cor
						→		→	→	VPA	VPA	Fren
						Severe		Infrequent	N			
23	KCNT1	4y, F	1.5m	FS,T	No	Severe	MMFSI	Migrating	N	PB	No	Unc
						→		Frequent	→	LEV		
						Severe		→	N	VPA		
24	KCNT1	2y, M	3d	FS,T	No	Severe	MMFSI	Migrating	N	B6	No	Unc
						→		Frequent	→	OXC		
						Severe		→	N	LEV		
25	HCN1	8y, M	6m	FS,T	No	Severe	EOEE	Frequent	N	OXC	VPA	Cor
						→		→	→	NZP		Fren
						Moderate		Infrequent	N	VPA		
26	CACNB4	6y, F	1m	FS,T	No	Severe	EOEE	Frequent	N	B6	OXC	Cor
						→		→	→	PB		Fren
						Moderate		Infrequent	N	OXC		
27	CACNA1H	6y, M	6m	S	No	Severe	WS	H	N	MP	No	Unc
						→		→	→	VPA		
						Severe		Frequent	N	TPM		
								→		OXC		
								Frequent		LTG		
										KD		

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
28	<i>CACNA1E</i>	2y, M	20d	FS,T, GTCS	No	Severe → Severe	EOEE	Frequent → Infrequent	N → N	OXC LEV VPA TPM	VPA TPM	Cor Frei
29	<i>CDKL5</i>	8y, F	3m	FS,T, GTCS, S	No	Severe → Severe	WS	H → Frequent → Frequent	N → N	VPA TPM OXC LEV PB LTG KD	No	Unc
30	<i>CDKL5</i>	8y, F	2m	FS,T,S GTCS	No	Severe → Severe	EOEE	Frequent → Frequent	N → N	VPA TPM OXC LEV PB LTG KD	No	Unc
31	<i>CDKL5</i>	8y, F	2m	FS,T,S	No	Severe → Severe	EOEE	Frequent → Frequent	N → N	VPA TPM OXC LEV PB LTG KD	No	Unc
32	<i>CDKL5</i>	6y, F	1m	FS,T,S	No	Severe → Severe	EOEE	Frequent → Frequent	N → N	VPA TPM OXC LEV PB LTG KD	No	Unc
33	<i>CDKL5</i>	8y, F	2m	FS,T, GTCS	Chorea	Severe → Severe	EOEE	Frequent → Infrequent	N → N	VPA TPM OXC LEV PB LTG KD	No	Unc

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
34	CDKL5	5y, F	6m	S, M, FS	Chorea	Severe → Severe	EOEE	Frequent → Infrequent	WG → N	VPA TPM OXC LEV PB LTG KD	KD	Cor Frei
35	CDKL5	4y, F	6m	GTCS,T, S, FS	No	Severe → Severe	EOEE	Frequent → Infrequent → N	N → N	ACTH P VPA TPM OXC LEV PB LTG KD	KD	Cor Frei
36	CDKL5	4y, F	1m	GTCS,T, S, FS	No	Severe → Severe	EOEE	Frequent → Infrequent	N → N	ACTH P VPA TPM OXC LEV PB LTG KD	KD	Cor Frei
37	PCDH19	10y, F	5m	FS,GTCS, T	No	Severe → Severe	EOEE	Frequent → Infrequent	N → N	VPA LEV TPM	No	Unc
38	PCDH19	7y, F	5m	FS,T, GTCS, M	No	Severe → Severe	DS	Infrequent → Infrequent	N → N	VPA TPM OXC LEV PB LTG LCM KD	No	Unc
39	PCDH19	8y, F	6m	FS,T, GTCS	No	Severe → Severe	EOEE	Frequent → Infrequent	N → N	OXC VPA TPM PB LTG KD	No	Unc

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
40	<i>PCDH19</i>	4y, F	5m	FS,T, GTCS	No	Severe → Severe	EOEE	Frequent → Infrequent	N → N	OXC VPA TPM PB LTG LCM KD	No	Unc
41	<i>SLC2A1</i>	8y, M	2d	FS,T, GTCS	Dystonia	Severe → Severe	GLUT1	Frequent → Infrequent → N	WG → N	VPA LEV KD BAL	KD	Cor Fre
42	<i>STXBP1</i>	2y, M	1m	FS,T, GTCS	Dystonia	Severe → Severe	EOEE	Frequent → Infrequent → Infrequent	N → N	PB LEV BAL	LEV	Cor Fre
43	<i>STXBP1</i>	2y, M	2m	S, T, FS, GTCS	Dystonia	Severe → Severe	WS	H → Frequent → Infrequent	WG Arachnoid Cysts → Arachnoid Cysts	PB ACTH P VGB LEV BAL	LEV	Cor Fre
44	<i>STXBP1</i>	7y, M	1m	T, FS, S, GTCS	No	Severe → Severe	OS	BS → Frequent	N → N	B6 PB TPM LEV VPA OXC KD	No	Unc
45	<i>SETBP1</i>	9y, M	6m	T, FS, GTCS	No	Severe → Severe	EOEE	Frequent → Infrequent → N	N → N	PB TPM LEV VPA	VPA	Cor Fre
46	<i>ARHGEF9</i>	8y, M	4m	T, FS, GTCS	No	Severe → Moderate	EOEE	Frequent → Infrequent → N	N → N	LEV OXC	OXC	Cor Fre

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
47	GABRG2	8y, M	5m	T, FS, GTCS	No	Severe → Moderate	EOEE	Frequent → Infrequent → N	N → N	PB LEV VPA	LEV VPA	Cor Fre
48	GABAA1	3y, M	4m	S	No	Severe → Moderate	WS	H → Frequent → Infrequent	N → N	ACTH P KD TPM VGB	TPM VGB	Cor Fre
49	GABRA2	2y, M	6m	T, FS	No	Severe → Severe	EOEE	Frequent → Infrequent	N → N	LEV VPA	LEV VPA	Cor Fre
50	DEPDC5	5y, M	1m	S, FS	No	Severe → Severe	EOEE	Frequent → Infrequent	N → N	LEV TPM P OXC KD VGB	No	Unc
51	MECP2	8y, F	2m	S, FS, T	No	Severe→ Severe	WS	H → Frequent → Infrequent	N → N	P VPA CZP TPM	VPA TPM	Cor Fre
52	GRIN3B	10y, M	6m	FS,T, GTCS	No	Severe → Moderate	EOEE	Frequent → Infrequent → N	N → N	PB OXC VPA	OXC VPA	Cor Fre
53	GRIA4	3y, M	6m	FS,T, GTCS	Ataxia	Severe → Moderate	EOEE	Frequent → Infrequent → N	N → N	PB OXC VPA TPM	VPA TPM	Cor Fre
54	DYNC1H1	5y, M	2m	S, FS,T	No	Severe → Severe	WS	Frequent → Frequent	N → N	ACTH P VPA CZP TPM	No	Unc

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
55	ALDH7A1	5y, F	2d	FS,T, GTCS	No	Severe → Moderate	Pyridoxine dependent epilepsy	Frequent → Infrequent → N	N → N	LEV PB B6	B6	Cor Fren
56	DPYD	5y, F	2m	FS,T, GTCS	No	Severe → Severe	EOEE	Frequent → Frequent	N → N	PB LEV TPM OXC VPA LTG KD	No	Unc
57	ALG11	2y, F	3m	S,FS,T	No	Severe → Severe	WS	H → Frequent → Frequent	N → N	ACTH P LEV TPM OXC VPA LTG KD	No	Unc
58	CCDC88C	2y, F	3m	S,FS,T	No	Severe → Severe	WS	H → Frequent → Frequent	N → N	ACTH P LEV TPM OXC VPA LTG KD	No	Unc
59	CSNK2B	2y, F	2m	FS,T, GTCS	No	Severe → Moderate	EOEE	Frequent → Infrequent	WG → N	LEV VPA	No	Cor Fren
60	CSNK2B	3y, M	3m	FS,T, GTCS	No	Severe → Moderate	EOEE	Frequent → Infrequent	N → N	LEV VPA	No	Cor Fren
61	IL1RAPL1	3y, M	6m	S,FS,T	No	Severe → Severe	WS	H → Frequent → Frequent	N → N	ACTH P TPM KD VGB	KD	Cor Fren

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
62	<i>IQSEC2</i>	2y, M	6m	S,FS,T	No	Severe → Severe	WS	H → Frequent → Frequent	N → N	ACTH P TPM KD VGB	No	Unc
63	<i>PACS2</i>	5y, M	10d	FS,T	Special face	Severe → Moderate	EOEE	Frequent → Infrequent	N → N	B6 LEV VPA	VPA	Cor Fre
64	<i>PACS2</i>	2y, F	1m	FS,T	Microcephaly Special face	Severe → Severe	EOEE	Frequent → Infrequent	N → N	B6 LEV TPM BP KD VPA	VPA	Cor Fre
65	<i>PACS2</i>	2y, F	1m	FS,T	Special face	Severe → Moderate	EOEE	Frequent → Infrequent	N → N	B6 LEV VPA	VPA	Cor Fre
66	<i>PIGA</i>	6y, M	6m	FS,T, SE	No	Severe → Severe	EOEE	Frequent → Infrequent	N → Atrophy	LEV PB VPA TPM KD	KD	Cor Fre
67	<i>QARS</i>	3y, F	2m	FS,T, S	No	Severe → Severe	WS	H → Frequent	N → N	LEV ACTH P TPM KD	No	Unc
68	<i>RNASEH2B</i>	3y, F	4m	FS,T, S	No	Severe → Severe	WS	H → Frequent	N → N	LEV ACTH P TPM VPA KD	No	Unc
69	<i>SMC1A</i>	4y, F	6m	FS, Cluster Seizures	No	Severe → Severe	EOEE	Frequent → Infrequent	N → N	LEV OXC KD	KD	Cor Fre
70	<i>SMC1A</i>	2y, F	2.5m	FS, Cluster seizures	No	Moderate → Moderate	EOEE	H → Frequent	N	LEV, TPM, PB OXC	KD	Cor Fre
71	<i>SMC1A</i>	1.5y, F	3m	FS, Cluster seizures	No	Moderate → Moderate	EOEE	Frequent → Infrequent	N	LEV OXC KD	KD	Cor Fre

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
72	TBC1D24	15y, F	6m	M, FS,T, EPC	Dystonia	Severe → Severe	EME	Frequent → Infrequent	N → N	LEV PB VPA LTG CLB KD	No	Unc
73	TBC1D24	8y, F	3m	M, EPC	No	Severe → Severe	EME	Frequent → Infrequent	N → N	LEV PB VPA LTG CLB OXC NZP	No	Unc
74	TBC1D24	5y, M	3m	M, EPC	No	Severe → Severe	EME	Frequent → Infrequent	N → N	LEV PB VPA LTG CLB OXC NZP	No	Unc
75	WWOX	5y, M	6m	FS,T	No	Severe → Moderate	EOEE	Frequent → Infrequent	N → N	PB LEV NZP	NZP	Cor Fre
76	COL4A2	2y, M	3m	S	No	Severe → Severe	WS	H → Frequent	N	ACTH P	ACTH P	Cor Fre
77	PTEN	5y, F	6m	S,FS	No	Severe → Severe	WS	H → Frequent	N → N	MP TPM VPA KD CLB LTG	No	Unc
78	H3F3A	2y, M	2m	FS,T	No	Severe → Severe	EOEE	Frequent → Frequent	WG	BP LEV	LEV	Cor Fre
79	CHD2	11y, M	6m	FS,T, GTCS, M	No	Severe → Moderate	EOEE	Frequent → Infrequent → N	N → N	BP LEV OXC VPA LTG KD	No	Unc

P	Gene	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	Epilepsy syndrome	EEG	MRI	AEDS tried	Effective AEDS	Sei: out
80	<i>HNRNPU</i>	6y, M	6m	FS,T, GTCS,AA	No	Moderate → Moderate	EOEE	Infrequent → Frequent	N → N	BP LEV VPA	VPA	Cor Frei

3.2 EOEE-BS

In our study, children with EOEE-BS had various clinical phenotypes. The common phenotypes were OS and non-syndromic EOEE. 7 patients with early EEG persistent BS from 1 day to 1 month, 1 of which was accompanied by double hemisphere intermittent low voltage. The persistent BS disappeared at 2 to 3 months. For the other 2 patients caused by *SCN2A* mutations, EEG was temporary BS during sleep at 4 months and 5 months, and disappeared at 7 months. After performing genetic tests, 6 patients were found *KCNQ2* mutations and the remaining mutations were *SCN2A* (n = 2), *STXBP1* (n = 1). After treatment, only 2 patients had seizure control, 6 had uncontrolled seizures and 1 had died from SE at 6 months.

3.3 EOEEs with dyskinesia

A total of 7 children diagnosed with EOEEs with dyskinesia were found. The dyskinesia onset age ranged from 1 month to 1 year. 2 patients with dyskinesia showed dancing-like movements, 4 patients showed dystonia, 1 patient showed ataxia. Genetic mutations included *CDKL5* (n = 2), *SLC2A1* (n = 1), *STXBP1* (n = 2), *TBC1D24* (n = 1), *GRIA4* (n = 1). Patients with *CDKL5* encephalopathy with dyskinesia were given Madopar but they continued to have chorea. 4 patients with dystonia received good effect while on Baclofen. Among them, 1 patient with *STXBP1* encephalopathy showed good responses while on LEV.

3.4 EOEEs limited to females with cluster seizures

Three patients with heterozygous de novo mutations in *SMC1A* gene were reviewed. All patients were females with moderate to severe developmental impairment. None of them had a clinical diagnosis of Cornelia de Lange syndrome. All three patients had prominent clinical features of cluster seizures. All the nonsense mutations were predicted damaging *SMC1A* protein by PolyPhen-2 HVAR. All the patients were treated with multiple antiepileptic drugs but their seizures remained refractory. When initiated with ketogenic diet, they became seizure free within 3 to 4 weeks.

3.5 EOEEs starting with a febrile convulsion

The typical clinical features of DS is that the onset of a febrile convulsion often within 1-year-old, which is characterized by repeated generalized or hemiclonic seizures. Except for DS, we found another type of EOEE starting with a febrile convulsion caused by *HNRNPU* mutation. The index patient was a 6 years boy, being a first-born child from full term pregnancy and natural birth. Both the pregnancy and delivery history of this boy were unremarkable. In his family history, there was no similar disease. Developmental milestone showed moderate developmental retardation. She began having a febrile convulsion at 6 months of age, which occurred 5 times a day. Video EEG showed slow activity in the background and sharp slow waves in the left occipital and posterior temporal regions during the interictal period. A febrile convulsion occurred once in half a year on average. At the age of 4 years, he began to suffer seizures without any inducing factors. EEG showed a large number of multifocal sharp waves, spike waves, and spike slow waves. The effect of BP and LEV was poor, and the epileptic seizure was reduced associated with VPA. At the final follow-up, she remained seizure-free for 2 years with LEV and VPA treatment but no remarkable improvement in his development.

3.6 Genetic analysis

38 Patients diagnosed with DS caused by *SCN1A* are not listed in Table 1 because their clinical features are easily identified. *SCN1A* mutations were detected in these 38 patients (38/118, 32.2%), representing the largest proportion, including 27 missense, 7 frameshift and 4 nonsense mutations. Our other findings suggested that genetic causes of EOEEs involve pathogenic mutations (54 missense, 11 frameshift, 12 nonsense, 3 splicing mutations) (Fig. 1). All 80 patients' genetic findings were summarized in Table 2. All 80 patients' genetic findings were summarized in Table 2. We identified different specific types of EOEE. The identified genes were summarized in Table 3. *SCN1A* mutations were detected in 38 patients, representing the largest proportion (38/118, 32.2%). The second common mutations were *KCNQ2* mutations, detected in 9 patients. The third one was *CDKL5* mutations, identified in 8 patients. Genes associated with ionic channels represented the largest proportion (66/118, 55.9%), sodium channel potassium channel and calcium channel respectively. The number of identified genes were summarized in Table 4.

Table 2
Summary of the genetic findings in our 80 patients

P	Gene	Base change	Amino acid change	Predicted effect on protein	Zygosity	Inheritance
1	SCN2A	c.5635A > G	p.M1879V	Missense	Heterozygous	De novo
2	SCN2A	c.4384delT	p.F1462Sfs	Frameshift	Heterozygous	De novo
3	SCN2A	c.1159G > A	p.E387K	Missense	Heterozygous	De novo
4	SCN3A	c.716C > A	p.A239A	Missense	Heterozygous	De novo
5	SCN8A	c.641G > A	p.G214D	Missense	Heterozygous	De novo
6	SCN8A	c.2942G > C	p.S981T	Missense	Heterozygous	De novo
7	SCN8A	c.2879T > A	p.V960A	Missense	Heterozygous	De novo
8	SCN8A	c.641G > A	p.G214D	Missense	Heterozygous	De novo
9	SCN8A	c.5498A > T	p.A1833V	Missense	Heterozygous	De novo
10	KCNQ2	c.14G > T	p.5S > X	Nonsense	Heterozygous	De novo
11	KCNQ2	c.629G > A	p.A210H	Missense	Heterozygous	De novo
12	KCNQ2	c.740C > T	p.S247L	Missense	Heterozygous	De novo
13	KCNQ2	c.821C > T	p.T274M	Missense	Heterozygous	De novo
14	KCNQ2	c.1678C > T	p.A560T	Missense	Heterozygous	De novo
15	KCNQ2	c.821C > T	p.T274M	Missense	Heterozygous	De novo
16	KCNQ2	c.649A > C	p.T217P	Missense	Heterozygous	De novo
17	KCNQ2	c.1179del	p.Leu394T	Frameshift	Heterozygous	De novo
18	KCNQ2	c.2048_2051dup	p.C685AfsT181	Frameshift	Heterozygous	De novo
19	KCNQ3	c.1231A > T	p.L411T	Nonsense	Heterozygous	De novo
20	KCTD7	c.334C > G	p.A112G	Missense	Compound	Father
		c.686A > T	p.A229V	Missense	heterozygous	Mother
21	KCNB1	c.916C > T	p.A306C	Missense	Heterozygous	De novo
22	KCNB1	c.635C > A	p.P212H	Missense	Heterozygous	De novo
23	KCNT1	c.1421G > A	p.A474H	Missense	Heterozygous	De novo
24	KCNT1	c.1420C > T	p.A474C	Missense	Heterozygous	De novo
25	HCN1	c.1679G > A	p.R560H	Missense	Heterozygous	De novo
26	CACNB4	c.668C > T	p.T223M	Missense	Heterozygous	De novo
27	CACNA1H	c.2491G > A	p.V831M	Missense	Heterozygous	De novo
28	CACNA1E	c.2767C > T	p.H923T	Missense	Heterozygous	De novo
29	CDKL5	c.1326_1327ins A	p.443,N > Kfs	Frameshift	Heterozygous	De novo
30	CDKL5	c.1794_1795ins A	p.332,N > Kfs	Frameshift	Heterozygous	De novo
31	CDKL5	IVS9-1G > A	Splice	Splicing	Heterozygous	De novo
32	CDKL5	c.2774_c.2775: del TG	p.925M > Ifs	Frameshift	Heterozygous	De novo
33	CDKL5	c.1245_c.1246: del AG	p.T415Tfs	Frameshift	Heterozygous	De novo
34	CDKL5	c.1700C > T	p.T567M	Missense	Heterozygous	De novo
35	CDKL5	c.238C > T	p.R80C	Missense	Heterozygous	De novo
36	CDKL5	c.428T > A	p.I143A	Missense	Heterozygous	De novo
37	PCDH19	c.471C > G	p.A157G	Missense	Heterozygous	De novo
38	PCDH19	c.2341delA	p.I781Sfs	Frameshift	Heterozygous	De novo
39	PCDH19	c.2113C > T	p.A705T	Nonsense	Heterozygous	De novo

P	Gene	Base change	Amino acid change	Predicted effect on protein	Zygosity	Inheritance
40	<i>PCDH19</i>	c.798C > G	p.A266G	Missense	Heterozygous	De novo
41	<i>SLC2A1</i>	c.1278 + 30_1278 + 31insATTCTCACC	Splice	Splicing	Homozygosis	De novo
42	<i>STXBP1</i>	c.69_c.70insA	p.L24Lfs	Frameshift	Heterozygous	De novo
43	<i>STXBP1</i>	c.364C→T	p.A122T	Nonsense	Heterozygous	De novo
44	<i>STXBP1</i>	c.364C > T	p122R > X	Nonsense	Heterozygous	De novo
45	<i>SETBP1</i>	c.2339C > G	PS780T	Nonsense	Heterozygous	De novo
46	<i>ARHGEF9</i>	c.1364A > C	p.G455P	Missense	Heterozygous	De novo
47	<i>GABRG2</i>	c.929C > T	p.T310I	Missense	Heterozygous	De novo
48	<i>GABAA1</i>	c.779C > T	p.P260L	Missense	Heterozygous	De novo
49	<i>GABRA2</i>	c.995C > T	p.A332V	Missense	Heterozygous	De novo
50	<i>DEPDC5</i>	c.280-1G > A	Splicing	Splicing	Heterozygous	De novo
51	<i>MECP2</i>	c.158G > T	p.G53V	Missense	Heterozygous	De novo
52	<i>GRIN3B</i>	c.1829G > A	p.A610H	Missense	Heterozygous	De novo
53	<i>GRIA4</i>	c.1378A > G	p.I460V	Missense	Heterozygous	De novo
54	<i>DYNC1H1</i>	c.1682A > G	p.G561G	Missense	Heterozygous	De novo
55	<i>ALDH7A1</i>	c.961G > A	p.A321T	Missense	Homozygosis	De novo
56		c.1774C > T	p.R592W	Missense	Compound	Father
		c.2897C > T	p.S966F	Missense	heterozygous	Mother
57	<i>ALG11</i>	c.1192G > A	p.G398L	Missense	Compound	Mother
		c.1403G > A	p.A468H	Missense	heterozygous	Father
58	<i>CCDC88C</i>	c.1158G→C	p.E386D	Missense	Compound	Mother
		c.5635C→T	p.R1879W	Missense	heterozygous	Father
59	<i>CSNK2B</i>	c.508_509del	p.V170Afs	Frameshift	Heterozygous	De novo
60	<i>CSNK2B</i>	c.142C > T	p.G48T	Nonsense	Heterozygous	De novo
61	<i>IL1RAPL1</i>	c.2062G > C	p.G688G	Missense	Heterozygous	De novo
62	<i>IQSEC2</i>	c.2776C > T	p.A926T	Nonsense	Hemizygous	De novo
63	<i>PACS2</i>	c.625G > A	p.G209L	Missense	Heterozygous	De novo
64	<i>PACS2</i>	c.625G > A	p.G209L	Missense	Heterozygous	De novo
65	<i>PACS2</i>	c.625G > A	p.G209L	Missense	Heterozygous	De novo
66	<i>PIGA</i>	c.241C > T	p.A81C	Missense	Hemizygous	De novo
67	<i>QARS</i>	c.1852G > A	p.A618A	Missense	Compound	Mother
		c.2068C > T	p.A690C	Missense	heterozygous	Father
68	<i>RNASEH2B</i>	c.629G > A	p.A210H	Missense	Compound	Mother
		c.905G > A	p.G302G	Missense	heterozygous	Father
69	<i>SMC1A</i>	c.1495C > T	p.A499T	Nonsense	Heterozygous	De novo
70	<i>SMC1A</i>	c.1489C > T	p.G497T	Nonsense	Heterozygous	De novo
71	<i>SMC1A</i>	c.3463C > T	p.G1155T	Nonsense	Heterozygous	De novo
72	<i>TBC1D24</i>	c.1571G > C	p.A524P	Missense	Compound	Mother
		c.680G > A	p.A227G	Missense	heterozygous	Father
73	<i>TBC1D24</i>	c.1207G > T	p.V403L	Missense	Compound	Father
		c.1499C > T	p.A500V	Missense	heterozygous	Mother

P	Gene	Base change	Amino acid change	Predicted effect on protein	Zygoty	Inheritance
74	<i>TBC1D24</i>	c.1207G > T	p.V403L	Missense	Compound	Father
		c.1499C > T	p.A500V	Missense	heterozygous	Mother
75	<i>WWOX</i>	c.468G > T	p.A156S	Missense	Homozygosis	De novo
76	<i>COL4A2</i>	c.1148C > T	p.P383L	Missense	Heterozygous	De novo
77	<i>PTEN</i>	c.1034T > C	p.L345P	Missense	Heterozygous	De novo
78	<i>H3F3A</i>	c.377A > G	p.G126A	Missense	Heterozygous	De novo
79	<i>CHD2</i>	c.4909C > T	p.A1637T	Nonsense	Heterozygous	De novo
80	<i>HNRNPU</i>	c.1341dup	p.V448Cfs	Frameshift	Heterozygous	De novo

Table 3
Summary of the identified genes in specific EOEES

Specific classifications of EOE	Associated gene
Dravet syndrome	<i>SCN1A</i>
Ohtahara syndrome	<i>KCNQ2, STXBP1</i>
West syndrome	<i>SCN3A, SCN2A, SCN8A, CACNA1H, DEPDC5, MECP2, DYNC1H1, CDKL5, ALG11, CCDC88C, GABAA1, IL1RAPL1, RNASEH2B, SLC19A3, STXBP1, QARS, COL4A2</i>
Early myoclonic epileptic encephalopathy	<i>TBC1D24</i>
GLUT1 deficiency syndrome	<i>SLC2A1</i>
Malignant migrating focal seizures of infancy	<i>KCNT1</i>
EOEE-BS	<i>KCNQ2</i> ∥ <i>STXBP1</i> ∥ <i>SCN2A</i> ∥ <i>PIGA</i>
EOEEs with dyskinesia	<i>STXBP1, CDKL5, SLC2A1</i>
EOEEs limited to females with cluster seizures	<i>SMC1A</i>
EOEEs starting with febrile convulsion	<i>SCN1A, PCDH19</i> ∥ <i>HNRNPU</i>

Table 4
Summary of the number of identified genes in EOEES

Gene function	Mutated gene	Corresponding total cases
Sodium channel	<i>SCN1A, SCN2A, SCN3A, SCN8A</i>	38, 3, 1, 5
Potassium channel	<i>KCNQ2, KCNQ3, KCTD7, KCNB1, KCNT1, HCN1</i>	9, 1, 1, 2, 2, 1
Calcium channel	<i>CACNB4, CACNA1H, CACNA1E</i>	1, 1, 1
Cyclin-dependent kinase-like	<i>CDKL5</i>	8
Protocadherin	<i>PCDH19</i>	4
Solute carrier family	<i>SLC2A1</i>	1
Syntaxin-binding protein	<i>STXBP1</i>	3
SET binding protein	<i>SETBP1</i>	1
CDC42 guanine nucleotide exchange factor	<i>ARHGEF9</i>	1
Gamma-aminobutyric acid receptor	<i>GABRG2, GABAA1, GABRA2</i>	1, 1, 1
DEP domain containing 5, GATOR1 subcomplex subunit	<i>DEPDC5</i>	1
Methyl-CpG binding protein	<i>MECP2</i>	1
Glutamate ionotropic receptor	<i>GRIN3B, GRIA4</i>	1, 1
Dynein cytoplasmic 1 heavy chain	<i>DYNC1H1</i>	1
Aldehyde dehydrogenase 7 family member	<i>ALDH7A1</i>	1
Dihydropyrimidine dehydrogenase	<i>DPYD</i>	1
ALG11 alpha-1,2-mannosyltransferase	<i>ALG11</i>	1
Coiled-coil domain containing	<i>CCDC88C</i>	1
Casein kinase 2	<i>CSNK2B</i>	2
Interleukin 1 receptor accessory protein like	<i>IL1RAPL1</i>	1
IQ motif and Sec7 domain ArfGEF	<i>IQSEC2</i>	1
Phosphofurin acidic cluster sorting protein	<i>PACS2</i>	3
Phosphatidylinositol glycan anchor biosynthesis class	<i>PIGA</i>	1
Glutaminyl-tRNA synthetase	<i>QARS</i>	1
Ribonuclease H2 subunit	<i>RNASEH2B</i>	1
Structural maintenance of chromosomes	<i>SMC1A</i>	3
TBC1 domain family member	<i>TBC1D24</i>	3
WW domain containing oxidoreductase	<i>WWOX</i>	1
Collagen type IV chain	<i>COL4A2</i>	1
Phosphatase and tensin homolog	<i>PTEN</i>	1
H3.3 histone	<i>H3F3A</i>	1
Chromodomain helicase DNA binding protein	<i>CHD2</i>	1
Heterogeneous nuclear ribonucleoprotein	<i>HNRNPU</i>	1

3.7 Genetic causes of EOEES with a good therapeutic effect

In general, the effect of KD is sure on the treatment of genetic causes of EOEES. 3 patients with *SMC1A* mutations response to KD add-on therapy. VPA added treatment showed a good effect on *KCNB1* (n = 2) and *PACS2* (n = 3) encephalopathy. LEV added treatment showed a good effect on *STXBP1* (n = 2) encephalopathy. OXC added treatment showed a good effect on *SCN8A* (n = 2) encephalopathy.

4. Discussion

We report a series of individuals with genetic causes of EOEES, delineating the phenotypic spectrum and long-term outcome. In the unknown causes of EOEES, detection of the gene mutation rate was 27.2% (128/470). In the genetic causes of EOEES, the non-symptomatic EOEES represent the largest proportion, which is 43.3% (51/118). We find the initial EEG of most patients showing frequent multiple and multifocal discharging. With seizure controlled, EEG discharging

gradually decreases. But only a minority of patients' EEG transform into infrequent discharging or normal EEG. Despite performing several brain MRI, there is no significant change in the later brain MRI. In the long outcome, we find the seizure control rate in the genetic causes of EOEES is 35.6% (42/118). The death rate is 1.7% (2/118). And we don't find sudden unexpected death in the genetic causes of EOEES. Although some patients achieve seizure-free, there is no remarkable improvement in their development.

BS is a common EEG phenomenon in EOEES, which usually occurs during OS sleep and wakefulness, EME sleep period. There are two different types of BS patterns, namely early BS and late BS[7, 9]. As for the definition of early BS and late BS, it is not very clear at present. Yoshitomi thought that it should be divided according to the age of one month[9]. It is believed that the appearance in the early infancy is related to asymmetric BS pattern, but the appearance in late infancy is related to symmetric BS characteristics. This study did find that BS is not only found in OS, but also in other non-syndromic EOEES. This study provides an in-depth understanding of the genetic factors of EOEES-BS and explains the important role of genetic factors in addition to common causes such as cortical malformations. In this study, pathogenic mutations were identified, accounting for 7.6% (9/118). This study found that the largest genetic subgroup of EOEES-BS is the subgroup with *KCNQ2* mutations, accounting for 66.7% (6/9). These 9 patients in this group had various types of seizures. The treatment effect and prognosis were poor. For the early onset of persistent tonic, spasm seizures and other types of intractable seizures, seizures with early EEG-BS performance may suggest the possibility of *KCNQ2* pathogenic mutations. However, EOEES-BS is highly heterogeneous in terms of genetic etiology. Except for the largest genetic *KCNQ2* subgroup, the second is *SCN2A* subgroup. For these 2 patients in this study, EEG was temporarily suppressed during sleep at 4 months and 5 months, and disappeared at 7 months. The reason for transient BS in *SCN2A* subgroup is unknown, which may be related to the immaturity of the central nervous system or gene mutation leading to brain dysfunction at this stage. 1 patient of *STXBP1* subgroup was found in the third genetic subgroup. EOEES related to *STXBP1* gene mutation has been mostly reported, and the common phenotype is OS. Mutations in the *STXBP1* gene can cause abnormal neurotransmitter release, and cause brain stem cell apoptosis and dysfunction, change the excitability of neurons, and cause seizures[10]. This study find that EOEES-BS usually response poorly to AEDS.

Symptoms of dyskinesia include dystonia, chorea, paroxysmal dyskinesia, Parkinson's syndrome, ataxia, tremor and so on. At first, EOEES with dyskinesia were focused on by Guerrini[11], who first reported *ARX* gene mutation associated with dyskinesia. And then *STXBP1*, *FOXG1*, *CDKL5* related dyskinesia were gradually reported[12–14]. Kobayashi reported 11 cases of infantile dyskinesia associated with EOEES. 9 cases were definitely diagnosed with epilepsy syndrome including WS[6]. In this study, 7 cases of EOEES with dyskinesia were found. The onset age of dyskinesia ranged from 1 month to 1 year. 3 patients were diagnosed as epilepsy syndrome, namely WS, GLUT1 deficiency syndrome, EME. In this study, the main clinical symptoms of dyskinesia were dystonia, chorea and ataxia. Genetic mutations included *CDKL5*, *SLC2A1*, *STXBP1*, *TBC1D24* and *GRIA4*. Our findings indicate 4 patients with dystonia received a good effect with Baclofen. 1 patient of *STXBP1* encephalopathy with dystonia showed good response with LEV. This study find that with the control of epileptic seizures, the symptoms of dyskinesia in a few patients were also relieved.

SMC1A mutations can cause early onset epilepsy only in females with cluster seizures. At present, a spectrum of *SMC1A* gene have been related with Cornelia de Lange syndrome (CdLS), *SMC1A*-related encephalopathy only with female patients, colorectal carcinomas, bladder cancer and leukemia[15–22]. Consistent with previous clinical reports, our 3 patients have moderate to severe neurological impairment and epilepsy. The seizures usually start in infancy. The presence of cluster seizures is an obvious characteristic. Although a minority of variants have also been found pathogenic, there is no clear relationship between severity of clinical phenotype and mutation types of truncation and missense variants[23]. However, the therapy strategy is still challenging. Among them, most patients show drug-resistant. All our 3 patients became seizure-free when KD was used as add-on therapy. There is evidence to show a relationship between *SMC1A*-mutated CdLS cell lines and oxidative stress[24]. KD in children with refractory epilepsy has also been demonstrated to improve mitochondrial function and decrease oxidative stress[25, 26]. Therefore, we speculate that KD add-on therapy reduces seizures by down-regulating the level of oxidative stress when combined with AEDS.

HNRNPUL locating at chromosome 1, encodes heteronuclear ribonucleoprotein u. It is expressed in adult brain, heart, kidney and liver, especially in cerebellum. Firstly, it was reported related to 1q43-q44 deletion syndrome[27]. Later, a variety of clinical phenotypes related to *HNRNPUL* mutation were reported, mainly including early-onset epilepsy with severe mental retardation, WS, EOEES, Lennox Gastaut syndrome and craniofacial deformity[28–30]. Durkin thought that *HNRNPUL* gene mutation related disease is more likely to be a kind of neurodevelopmental syndrome[30]. Durkin reported 21 cases of children, of which 3 cases were onset with febrile convulsion. Combined with 1 case in this study, we think that EOEES onset with febrile convulsion is a special phenotype of *HNRNPUL* related neurodevelopmental syndrome, similar to DS.

These responsible genes and their functions are mainly classified as: genes responsible for ion channels, genes responsible for the synapsis, neurotransmitters, and receptors, genes responsible for signal transduction, genes regulating DNA and RNA, genes responsible for the organelles and cell membrane, genes responsible for the development and growth of the neurons[31]. In this study, we find the ion channel gene mutations are the most common, representing the largest proportion (66/118, 55.9%). Among them, sodium channel gene mutations represent the largest proportion (47/66, 71.2%). In WS, we detect *SCN3A*, *SCN2A*, *SCN8A*, *CACNA1H*, *DEPDC5*, *MECP2*, *DYNC1H1*, *CDKL5*, *ALG11*, *CCDC88C*, *GABAA1*, *IL1RAPL1*, *RNASEH2B*, *SLC19A3*, *STXBP1*, *RARS2*, *COL4A2* mutations. In addition to common gene mutations, we report rare possible pathogenic genes: *CCDC88C*, *IL1RAPL1*, *RNASEH2B* and *COL4A2* in WS. In non-syndromic genetic causes of EOEES, we detect rare possible pathogenic genes: *SETBP1*, *DPYD*, *CSNK2B* and *H3F3A*. As for genetic modes, denovo heterozygous mutations account for the largest proportion, 88.1% (104/118). Among these types of mutations, missense mutations represent the largest proportion, 68.6%(81/118). As expected, some of the genes are included in more than one group of the classification, as they have multiple functions.

Generally, genetic causes of EOEES response poorly to AEDS treatment. However, we find that some gene mutation related EOEES receive a good effect on specific AEDS. Besides the effect of KD is sure on the treatment of *SMC1A* encephalopathy. We also find that VPA added treatment shows a good effect on *KCNB1* and *PACS2* encephalopathy, LEV added treatment shows a good effect on *STXBP1* encephalopathy, OXC added treatment shows a good effect on *SCN8A* encephalopathy.

VPA is a broad-spectrum antiepileptic drug, which exerts its anticonvulsant effect through a variety of mechanisms. VPA promotes the synthesis and release of gamma aminobutyric acid (GABA) through presynaptic and postsynaptic mechanisms, thereby increasing GABA mediated inhibition[32, 33]. VPA can regulate the expression of endoplasmic reticulum stress proteins (GRP78, GRP94 and calreticulin). It proves that VPA can inhibit excessive endoplasmic reticulum stress, reduce neuronal apoptosis and play a neuroprotective role in acute epileptic seizures[34–36]. VPA can also regulate the level of intracellular Ca^{2+} by increasing the expression of endoplasmic reticulum stress protein, improve the calcium binding ability of endoplasmic reticulum, and enable cells to adapt to the cellular stress caused by the imbalance of intracellular Ca^{2+} homeostasis[36]. *PACS2* plays an important role in controlling endoplasmic reticulum (ER) - mitochondrial communication, including the connection between mitochondria and ER and the homeostasis of ER. *PACS2* is necessary for effective Ca^{2+} transfer between endoplasmic reticulum and mitochondria, while GRP78 is involved in Ca^{2+} transport from endoplasmic reticulum to mitochondria[37–39]. Both of them play an important role in maintaining endoplasmic reticulum mitochondrial Ca^{2+} homeostasis. Therefore, we speculate that VPA may not only increase the concentration of GABA neurotransmitter and inhibit the voltage-gated Na^+ channel, but also play a role by enhancing Akt phosphorylation, inhibiting endoplasmic reticulum stress and regulating intracellular Ca^{2+} level in children with *PACS2* encephalopathy.

Certainly, knowing the pathophysiology of the underlying gene defect will help to pave the way for possible future individualized treatments. The limitations of our study are the small number of rare genes. Further research should include a larger cohort to validate our observations. We will continue to study and explore the detailed mechanism between rare gene mutation and seizure outcome.

5. Conclusion

We describe the clinical features and long-term outcome of genetic causes of EOEES. The clinical manifestations of EOEES are variable, including dyskinesia. EOEES usually responds poorly to AEDS therapy. Although some patients achieve seizure-free, there is no remarkable improvement in their development. EOEES starting with febrile convulsion may be a special phenotype of *HNRNPU* related neurodevelopmental syndrome, similar to DS. We find the ion channel gene mutations are the most common. We report rare possible pathogenic genes: *CCDC88C*, *IL1RAPL1*, *RNASEH2B* and *COL4A2* in WS and detect rare possible pathogenic genes: *SETBP1*, *DPYD*, *CSNK2B* and *H3F3A* in non-syndromic genetic causes of EOEES. Although genetic causes of EOEES response poorly to AEDS treatment, we find that some gene mutation related EOEES receive good effects on specific AEDS.

Abbreviations

P=Patient; y=Year/Years; d=Day; m=Month/Months; F=Female; M=Male; FS=focal seizures; GTCS=generalized tonic-clonic seizures; T=Tonic seizures; SE=Status epilepticus; S=Spasm; AA=Atypical absence; M=Myoclonic; EPC= Epilepsia partialis continua

N=Normal; AH=Atypical hypsarrhythmia; H=Hypsarrhythmia; Frequent=Frequent multiple and multifocal sharp waves, spike waves, sharp slow waves or spike slow waves; Infrequent=Infrequent sharp waves, spike waves, sharp slow waves or spike slow waves; BS=Burst suppression; LEV=levetiracetam; TPM=topiramate; VPA=valproate; LTG=lamotrigine; OXC=oxcarbazepine; KD=Ketogenic diet; PB=Phenobarbital; VNS=Vagus nerve stimulation; P=Prednisone; LCM=Lacosamide; MP=Methylprednisolone; CZP=Clonazepam; B6=Vitamin B6; NZP=Nitrazepam; VGB=Vigabatrin; QD=Quinidine; CLB=Clobazam; BAL=Baclofen; WG=Widen gap in extracerebral space.

Declarations

Acknowledgement

We appreciate all the patients and their guardians participating in this study. And I express my heartfelt gratitude for the hard work of all team members.

Ethics approval and consent to participate

The project ethics were approved by Ethic Committees of Children's Hospital of Fudan University. All the experiment protocol for involving humans was in accordance to guidelines of national/international/institutional or Declaration of Helsinki.

Authors' contributions

Chunhui Hu wrote the main manuscript text. Deying Liu and Tian Luo prepared figures and tables. Zhisheng Liu drafted the manuscript. Yi Wang revised the manuscript. All authors reviewed the manuscript.

Funding

The project was supported by Shanghai Municipal Science and Technology Major Project (Grant No. 2017SHZDZX01).

Availability of data and materials

The data that support the findings of this study are available from the authors upon reasonable request. The results/data/figures in this manuscript have not been published elsewhere, nor are they under consideration by another publisher.

Consent for publication

Written informed consent was obtained from all the patient's parent for the publication.

Competing interests

None of all authors have any disclosures to make in relation to this manuscript.

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Figures

Figure 1

Summary of gene mutation types in 118 patients, including 81 missense, 18 frameshift, 16 nonsense and 3 splicing mutations.