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

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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 ion channel genes represented the largest proportion (66/118, 55.9%), sodium channel potassium channel and calcium channel respectively. In WS, we detected SCN3A, SCN2A, SCN8A, CACNA1H, DEPD5, MECP2, Dyn1C1H1, CDKL5, ALG11, CDC88C, GABAA1, IL1RAPL1, RNASEH2B, SLC19A3, STXBP1, QARS, COL4A2 mutations. In addition to common gene mutations, we reported rare possible pathogenic genes: CDC88C, 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 SMCA1 mutations response to KD add-on therapy. VPA added treatment showed good effects on KCN2B 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: CDC88C, 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 is 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[3]. 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 ± 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 (3/118, 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 SCN1A are not listed in Table 1 because their clinical features are easily identified. All remaining 80 patients’ features were summarized in Table 1.
| P | Gene | Current age, Sex | Seizure onset age | Seizure semiology | Other Phenotype | Development retardation | Epilepsy syndrome | EEG | MRI | AEDS tried | Effective AEDS | Seiz out |
|---|------|-----------------|------------------|------------------|-----------------|------------------------|-------------------|-----|-----|-----------|--------------|---------|
| 1 | SCN2A | 10y, F | 3d | FS, GTCS, T | No | Severe | EOEE | Frequent | N | B6 | No | Unc |
| 2 | SCN2A | 10y, F | 5m | FS, GTCS, T, SE | No | Severe | EOEE | BS | N | TPM | No | Unc |
| 3 | SCN2A | 5y, M | 4m | S | No | Severe | WS | BS | N | VPA | MP | Cor |
| 4 | SCN3A | 8y, M | 6m | S | No | Severe | WS | H | N | VPA | P | Cor |
| 5 | SCN8A | 10y, M | 6m | S | No | Severe | WS | H | N | ACTH | ACTH | Cor |
| 6 | SCN8A | 3y, M | 6m | FS, T, S | No | Severe | EOEE | Frequent | WG | LEV | OXC | Cor |
| 7 | SCN8A | 3y, F | 6m | T, FS | No | Severe | EOEE | Frequent | N | LEV | No | Unc |
| P | Gene | Current age, Sex | Seizure onset age | Seizure semiology | Other Phenotype | Development retardation | Epilepsy syndrome | EEG | MRI | AEDS tried | Effective AEDS | Seizure outcome |
|---|------|-----------------|------------------|------------------|----------------|------------------------|------------------|-----|-----|------------|----------------|-----------------|
| 8 | SCN8A| 5y, F           | 6m               | S, FS, T, AA     | No             | Severe                | EOEE             | Frequent | N   | ACTH       | No             | Unc             |
| 9  | SCN8A| 2y, M           | 6m               | FS               | No             | Severe                | EOEE             | Infrequent | N   | PB          | OXC             | Cor             |
| 10 | KCNQ2| 6m, M           | 6d               | GTCS, SE         | No             | Severe                | EOEE             | Frequent, Low voltage, BS | N   | B6          | No             | Died at 6       |
| 11 | KCNQ2| 4y, M           | 1d               | T, FS, S         | No             | Severe                | OS               | Frequent, BS | N   | LEV         | No             | Unc             |
| 12 | KCNQ2| 4y, F           | 1d               | FS, T            | No             | Severe                | EOEE             | BS             | N   | TPM         | No             | Unc             |
| 13 | KCNQ2| 6y, M           | 3m               | T, GTCS          | No             | Severe                | EOEE             | Frequent | N   | TPM         | No             | Unc             |
| 14 | KCNQ2| 7y, M           | 3d               | FS, S, GTCS      | No             | Severe                | EOEE             | BS             | N   | B6          | TPM             | Cor             |
| P | Gene | Current age, Sex | Seizure onset age | Seizure semiology | Other Phenotype | Development retardation | Epilepsy syndrome | EEG | MRI | AEDS tried | Effective AEDS | Seiz out |
|---|------|-----------------|------------------|------------------|-----------------|-----------------------|-------------------|-----|-----|------------|--------------|---------|
| 15 | KCNQ2 | 5y, M | 1d | FS, T, GTCS | No | Severe | EOEE | BS | N | B6 | No | Unc |
| 16 | KCNQ2 | 2y, M | 10d | FS, T, GTCS | No | Severe | EOEE | Frequent | N | B6 | PB | Cor |
| 17 | KCNQ2 | 2y, M | 3d | FS, T, GTCS | No | Severe | EOEE | Frequent | N | B6 | PB | Frer |
| 18 | KCNQ2 | 2y, M | 4d | FS, T, GTCS | No | Severe | OS | BS | N | B6 | No | Unc |
| 19 | KCNQ3 | 2y, M | 6m | S, FS, T, GTCS | No | Severe | WS | H | N | VPA | No | Unc |
| 20 | KCTD7 | 10y, M | 5m | M, FS, T | No | Severe | EOEE | Frequent | N | LEV | VPA | Cor |
| P  | Gene   | Current age, Sex | Seizure onset age | Seizure semiology | Other Phenotype | Development retardation | Epilepsy syndrome | EEG | MRI | AEDS tried | Effective AEDS | Seiz out |
|----|--------|------------------|-------------------|-------------------|----------------|------------------------|-------------------|-----|-----|------------|----------------|----------|
| 21 | KCNB1  | 10y, F           | 6m                | FS, T             | No             | Severe                 | EOEE              | Frequent | N   | P           | P               | Cor      |
|    |        |                  |                   |                   |                |                        |                   |       |     | VPA        | VPA             | Frei     |
| 22 | KCNB1  | 4y, M            | 6m                | FS, T             | No             | Severe                 | EOEE              | Frequent | N   | LEV         | LEV             | Cor      |
|    |        |                  |                   |                   |                |                        |                   |       |     | VPA        | VPA             | Frei     |
| 23 | KCNT1  | 4y, F            | 1.5m              | FS, T             | No             | Severe                 | MMFSI             | Frequent | N   | PB          | No              | Unc      |
|    |        |                  |                   |                   |                |                        |                   |       |     |             |                 |          |
| 24 | KCNT1  | 2y, M            | 3d                | FS, T             | No             | Severe                 | MMFSI             | Frequent | N   | B6          | No              | Unc      |
|    |        |                  |                   |                   |                |                        |                   |       |     |             |                 |          |
| 25 | HCN1   | 8y, M            | 6m                | FS, T             | No             | Severe                 | EOEE              | Frequent | N   | OXC         | VPA             | Cor      |
|    |        |                  |                   |                   |                |                        |                   |       |     | NZP         |                 | Frei     |
| 26 | CACNB4 | 6y, F            | 1m                | FS, T             | No             | Severe                 | EOEE              | Frequent | N   | B6          | OXC             | Cor      |
|    |        |                  |                   |                   |                |                        |                   |       |     | PB          |                 | Frei     |
| 27 | CACNA1H| 6y, M            | 6m                | S                 | No             | Severe                 | WS                | H      | N   | MP          | VPA             | No       |
|    |        |                  |                   |                   |                |                        |                   |       |     |             |                 | Unc      |
|    |        |                  |                   |                   |                |                        |                   | Frequent | N   | VPA         |                 |          |
|    |        |                  |                   |                   |                |                        |                   |       |     | TPM         |                 |          |
|    |        |                  |                   |                   |                |                        |                   |       |     | OXC         |                 |          |
|    |        |                  |                   |                   |                |                        |                   |       |     | LTG         |                 |          |
|    |        |                  |                   |                   |                |                        |                   |       |     | KD          |                 |          |
| P | Gene | Current age, Sex | Seizure onset age | Seizure semiology | Other Phenotype | Development retardation | Epilepsy syndrome | EEG | MRI | AEDS tried | Effective AEDS | Seiz out |
|---|------|-----------------|------------------|------------------|----------------|------------------------|------------------|-----|-----|-------------|---------------|----------|
| 28 | CACNA1E | 2y, M | 20d | FS,T, GTCS | No | Severe → | EOEE | Frequent | N | OXC | VPA | Cor |
| 29 | CDKL5 | 8y, F | 3m | FS,T, GTCS, S | No | Severe → | WS | H | N | VPA | No | Unc |
| 30 | CDKL5 | 8y, F | 2m | FS,T, S | No | Severe → | EOEE | Frequent | N | VPA | TPM | Unc |
| 31 | CDKL5 | 8y, F | 2m | FS,T, S | No | Severe → | EOEE | Frequent | N | VPA | TPM | Unc |
| 32 | CDKL5 | 6y, F | 1m | FS,T, S | No | Severe → | EOEE | Frequent | N | VPA | TPM | Unc |
| 33 | CDKL5 | 8y, F | 2m | FS,T, GTCS | Chorea | Severe → | EOEE | Frequent | N | VPA | TPM | Unc |
| P  | Gene   | Current age, Sex | Seizure onset age | Seizure semiology | Other Phenotype | Development retardation | Epilepsy syndrome | EEG | MRI | AEDS tried | Effective AEDS | Seiz out  |
|----|--------|-----------------|-------------------|-------------------|-----------------|------------------------|------------------|-----|-----|------------|----------------|----------|
| 34 | CDKL5  | 5y, F           | 6m                | S, M, FS          | Chorea          | Severe                 | EOEE             | Frequent | WG | VPA        | KD            | Cor      |
|    |        |                 |                   |                   |                 |                        |                  |        |    | TPM        |               | Frer     |
| 35 | CDKL5  | 4y, F           | 6m                | GTCS, T, S, FS    | No             | Severe                 | EOEE             | Frequent | N  | ACTH       | KD            | Cor      |
|    |        |                 |                   |                   |                 |                        |                  |        |    | P          |               | Frer     |
| 36 | CDKL5  | 4y, F           | 1m                | GTCS, T, S, FS    | No             | Severe                 | EOEE             | Frequent | N  | ACTH       | KD            | Cor      |
|    |        |                 |                   |                   |                 |                        |                  |        |    | P          |               | Frer     |
| 37 | PCDH19 | 10y, F          | 5m                | FS, GTCS, T       | No             | Severe                 | EOEE             | Frequent | N  | VPA        | No            | Unc      |
|    |        |                 |                   |                   |                 |                        |                  |        |    | LEV        |               | Unc      |
| 38 | PCDH19 | 7y, F           | 5m                | FS, T, GTCS, M    | No             | Severe                 | DS               | Infrequent | N  | VPA        | No            | Unc      |
|    |        |                 |                   |                   |                 |                        |                  |        |    | TPM        |               | Unc      |
| 39 | PCDH19 | 8y, F           | 6m                | FS, T, GTCS       | No             | Severe                 | EOEE             | Frequent | N  | OXC        | No            | Unc      |
|    |        |                 |                   |                   |                 |                        |                  |        |    | VPA        |               | Unc      |
| P  | Gene | Current age, Sex | Seizure onset age | Seizure semiology | Other Phenotype | Development retardation | Epilepsy syndrome | EEG | MRI | AEDS tried | Effective AEDS | Seiz out |
|----|------|------------------|-------------------|------------------|----------------|------------------------|-------------------|-----|-----|------------|-----------------|---------|
| 40 | PCDH19 | 4y, F | 5m | FS, T, GTCS | No | Severe → Severe | EOEE | Frequent → Infrequent | N | OXC | No | Unc |
| 41 | SLC2A1 | 8y, M | 2d | FS, T, GTCS | Dystonia | Severe → Infrequent | GLUT1 | Frequent → Infrequent | N | VPA | KD | Cor | Fre |
| 42 | STXB1 | 2y, M | 1m | FS, T, GTCS | Dystonia | Severe → Infrequent | EOEE | Frequent → Infrequent | N | PB | LEV | Cor | Fre |
| 43 | STXB1 | 2y, M | 2m | S, T, FS, GTCS | Dystonia | Severe → Infrequent | WS | H → Arachnoid Cysts | Frequent → Infrequent | B6 | No | Unc |
| 44 | STXB1 | 7y, M | 1m | T, FS, S, GTCS | No | Severe → Frequent | BS | N | PB | VPA | Cor | Fre |
| 45 | SETBP1 | 9y, M | 6m | T, FS, GTCS | No | Severe → Infrequent | EOEE | Frequent → Infrequent | N | PB | VPA | Cor | Fre |
| 46 | ARHGEF9 | 8y, M | 4m | T, FS, GTCS | No | Severe → Infrequent | EOEE | Frequent → Infrequent | N | LEV | OXC | Cor | Fre |
| P  | Gene   | Current age, Sex | Seizure onset age | Seizure semionlogy | Other Phenotype | Development retardation | Epilepsy syndrome | EEG | MRI | AEDS tried | Effective AEDS | Seiz out |
|----|--------|------------------|-------------------|--------------------|-----------------|-----------------------|-------------------|-----|-----|------------|----------------|----------|
| 47 | GABRG2 | 8y, M            | 5m                | T, FS, GTCS        | No              | Severe                | EOEE              | Frequent | N   | PB         | LEV            | VPA      |
|    |        |                  |                   |                    |                 | → Moderate            |                   | → Infrequent | N   |            |                |          |
| 48 | GABAA1 | 3y, M            | 4m                | S                  | No              | Severe                | WS                | H     | N   | ACTH       | TPM            | Cor      |
|    |        |                  |                   |                    |                 | → Moderate            |                   | → Frequent | N   | P           | VGB            | Frer     |
|    |        |                  |                   |                    |                 |                       |                   | → Infrequent | N   |            |                |          |
| 49 | GABRA2 | 2y, M            | 6m                | T, FS              | No              | Severe                | EOEE              | Frequent | N   | LEV         | LEV            | Cor      |
|    |        |                  |                   |                    |                 | → Severe              |                   | → Infrequent | N   | VPA         | VPA            | Frer     |
| 50 | DEPDC5 | 5y, M            | 1m                | S, FS              | No              | Severe                | EOEE              | Frequent | N   | LEV         | TPM            | Unc      |
|    |        |                  |                   |                    |                 | → Severe              |                   | → Infrequent | N   | P           | OXC            | Freer    |
|    |        |                  |                   |                    |                 |                       |                   |                   |     |             |                |          |
| 51 | MECP2  | 8y, F            | 2m                | S, FS, T           | No              | Severe                | WS                | H     | N   | P           | VPA            | Cor      |
|    |        |                  |                   |                    |                 | → Severe              |                   | → Frequent | N   | VPA         | TPM            | Freer    |
|    |        |                  |                   |                    |                 |                       |                   | → Infrequent | N   | CZP         | TPM            |          |
| 52 | GRIN3B | 10y, M           | 6m                | FS, T, GTCS        | No              | Severe                | EOEE              | Frequent | N   | PB          | OXC            | Cor      |
|    |        |                  |                   |                    |                 | → Moderate            |                   | → Infrequent | N   | OXC         | VPA            | Freer    |
|    |        |                  |                   |                    |                 |                       |                   |                   |     | VPA         | TPM            |          |
| 53 | GRIA4  | 3y, M            | 6m                | FS, T, GTCS        | Ataxia          | Severe                | EOEE              | Frequent | N   | PB          | VPA            | Cor      |
|    |        |                  |                   |                    |                 | → Moderate            |                   | → Infrequent | N   | VPA         | TPM            | Freer    |
|    |        |                  |                   |                    |                 |                       |                   |                   |     |             |                |          |
| 54 | DYNC1H1| 5y, M            | 2m                | S, FS, T           | No              | Severe                | WS                | Frequent | N   | ACTH       | No             | Unc      |
|    |        |                  |                   |                    |                 | → Severe              |                   | → Frequent | N   | P           | VPA            |          |
|    |        |                  |                   |                    |                 |                       |                   |                   |     |             | CZP            |          |
|    |        |                  |                   |                    |                 |                       |                   |                   |     |             | TPM            |          |
| P | Gene   | Current age, Sex | Seizure onset age | Seizure semiology | Other Phenotype | Development retardation | Epilepsy syndrome | EEG | MRI | AEDs tried | Effective AEDs | Seiz. outcome |
|---|--------|------------------|-------------------|-------------------|-----------------|------------------------|------------------|-----|-----|------------|----------------|-------------|
| 55 | ALDH7A1 | 5y, F            | 2d                | FS,T, GTCS        | No              | Severe                | Severe           | N   |     | B6         | Cor           | Free        |
|    |        |                  |                   |                   |                 | → Moderate            | No               |     |     |            |                |             |
|    |        |                  |                   |                   |                 |                        | Infrequent       |     |     |            |                |             |
|    |        |                  |                   |                   |                 |                        | No               |     |     |            |                |             |
| 56 | DPYD   | 5y, F            | 2m                | FS,T, GTCS        | No              | Severe                | EOEE             | N   |     | PB         | No            | Unc        |
|    |        |                  |                   |                   |                 |                        | Frequent         |     |     |            |                |             |
|    |        |                  |                   |                   |                 |                        | N               |     |     |            |                |             |
|    |        |                  |                   |                   |                 |                        |                 |     |     |            |                |             |
| 57 | ALG11  | 2y, F            | 3m                | S,FS,T            | No              | Severe                | WS               | H   | N   | ACTH       | No            | Unc        |
|    |        |                  |                   |                   |                 |                        | Frequent         |     |     |            |                |             |
|    |        |                  |                   |                   |                 |                        | N               |     |     |            |                |             |
|    |        |                  |                   |                   |                 |                        |                 |     |     |            |                |             |
| 58 | CCDC88C | 2y, F           | 3m                | S,FS,T            | No              | Severe                | WS               | H   | N   | ACTH       | No            | Unc        |
|    |        |                  |                   |                   |                 |                        | Frequent         |     |     |            |                |             |
|    |        |                  |                   |                   |                 |                        | N               |     |     |            |                |             |
|    |        |                  |                   |                   |                 |                        |                 |     |     |            |                |             |
| 59 | CSNK2B | 2y, F            | 2m                | FS,T, GTCS        | No              | Severe                | EOEE             | Frequent | WG | LEV        | No            | Cor        |
|    |        |                  |                   |                   |                 |                        | Infrequent       |     |     |            |                | Free       |
|    |        |                  |                   |                   |                 |                        | No               |     |     |            |                |             |
| 60 | CSNK2B | 3y, M            | 3m                | FS,T, GTCS        | No              | Severe                | EOEE             | Frequent | N  | LEV        | No            | Cor        |
|    |        |                  |                   |                   |                 |                        | Infrequent       |     |     |            |                | Free       |
|    |        |                  |                   |                   |                 |                        | No               |     |     |            |                |             |
| 61 | IL1RAPL1 | 3y, M          | 6m                | S,FS,T            | No              | Severe                | WS               | H   | N   | ACTH       | KD            | Cor        |
|    |        |                  |                   |                   |                 |                        | Frequent         |     |     |            |                |             |
|    |        |                  |                   |                   |                 |                        | N               |     |     |            |                |             |
|    |        |                  |                   |                   |                 |                        |                 |     |     |            |                |             |
| P  | Gene   | Current age, Sex | Seizure onset age | Seizure semiology | Other Phenotype | Development retardation | Epilepsy syndrome | EEG | MRI | AEDS tried | Effective AEDS | Seiz out |
|----|--------|------------------|-------------------|------------------|----------------|------------------------|------------------|-----|-----|------------|----------------|----------|
| 62 | IQSEC2 | 2y, M            | 6m                | S,FS,T           | No             | Severe                 | WS               | H   | N   | ACTH       | No             | Unc     |
|    |        |                  |                   |                  |                |                        |                  |      |     | P           |                |          |
|    |        |                  |                   |                  |                |                        |                  |      |     | TPM         |                |          |
|    |        |                  |                   |                  |                |                        |                  |      |     | KD          |                |          |
|    |        |                  |                   |                  |                |                        |                  |      |     | VGB         |                |          |
| 63 | PACS2  | 5y, M            | 10d               | FS,T             | Special face   | Severe                 | EOEE             | Frequent | N | B6          | VPA            | Cor     |
|    |        |                  |                   |                  |                |                        |                  |      |     | VPA         |                | Freer    |
|    |        |                  |                   |                  |                |                        |                  |      |     | VPA         |                | Freer    |
| 64 | PACS2  | 2y, F            | 1m                | FS,T             | Special face   | Microcephaly          | EOEE             | Frequent | N | B6          | VPA            | Cor     |
|    |        |                  |                   |                  |                |                        |                  |      |     | VPA         |                | Freer    |
|    |        |                  |                   |                  |                |                        |                  |      |     | VPA         |                | Freer    |
| 65 | PACS2  | 2y, F            | 1m                | FS,T             | Special face   | Severe                 | EOEE             | Frequent | N | B6          | VPA            | Cor     |
|    |        |                  |                   |                  |                |                        |                  |      |     | VPA         |                | Freer    |
|    |        |                  |                   |                  |                |                        |                  |      |     | VPA         |                | Freer    |
| 66 | PIGA   | 6y, M            | 6m                | FS,T, SE         | No             | Severe                 | EOEE             | Frequent | N | LEV         | KD             | Cor     |
|    |        |                  |                   |                  |                |                        |                  |      |     | VPA         |                | Freer    |
|    |        |                  |                   |                  |                |                        |                  |      |     | TPM         |                | Freer    |
|    |        |                  |                   |                  |                |                        |                  |      |     | KD          |                | Freer    |
| 67 | QARS   | 3y, F            | 2m                | FS,T,S           | No             | Severe                 | WS               | H   | N   | ACTH       | No             | Unc     |
|    |        |                  |                   |                  |                |                        |                  |      |     | P           |                |          |
|    |        |                  |                   |                  |                |                        |                  |      |     | TPM         |                |          |
|    |        |                  |                   |                  |                |                        |                  |      |     | KD          |                |          |
| 68 | RNASEH2B| 3y, F           | 4m                | FS,T,S           | No             | Severe                 | WS               | H   | N   | ACTH       | No             | Unc     |
|    |        |                  |                   |                  |                |                        |                  |      |     | P           |                |          |
|    |        |                  |                   |                  |                |                        |                  |      |     | TPM         |                |          |
|    |        |                  |                   |                  |                |                        |                  |      |     | VPA         |                |          |
|    |        |                  |                   |                  |                |                        |                  |      |     | KD          |                |          |
| 69 | SMC1A  | 4y, F            | 6m                | FS, Cluster Seizures | No             | Severe                 | EOEE             | Frequent | N | LEV         | KD             | Cor     |
|    |        |                  |                   |                  |                |                        |                  |      |     | OXC         |                | Freer    |
|    |        |                  |                   |                  |                |                        |                  |      |     | KD          |                | Freer    |
| 70 | SMC1A  | 2y, F            | 2.5m              | FS, Cluster seizures | No             | Moderate               | EOEE             | H   | N   | LEV, TPM, PB, VPC | KD             | Cor     |
|    |        |                  |                   |                  |                |                        |                  |      |     | OXC         |                | Freer    |
| 71 | SMC1A  | 1.5y, F          | 3m                | FS, Cluster seizures | No             | Moderate               | EOEE             | Frequent | N | LEV         | KD             | Cor     |
|    |        |                  |                   |                  |                |                        |                  |      |     | OXC         |                | Freer    |
| P  | Gene   | Current age, Sex | Seizure onset age | Seizure semiology | Other Phenotype | Development retardation | Epilepsy syndrome | EEG | MRI | AEDS tried | Effective AEDS | Seiz out |
|----|--------|------------------|-------------------|------------------|----------------|------------------------|-------------------|-----|-----|------------|----------------|----------|
| 72 | TBC1D24 | 15y, F           | 6m                | M, FS,T, EPC     | Dystonia       | Severe                | EME               | Frequent | N   | LEV        | No             | Unc      |
| 73 | TBC1D24 | 8y, F            | 3m                | M, EPC           | No             | Severe                | EME               | Frequent | N   | LEV        | No             | Unc      |
| 74 | TBC1D24 | 5y, M            | 3m                | M, EPC           | No             | Severe                | EME               | Frequent | N   | LEV        | No             | Unc      |
| 75 | WWOX   | 5y, M            | 6m                | FS,T             | No             | Severe                | EOEE              | Frequent | N   | PB         | NZP            | Cor Fre |
| 76 | COL4A2 | 2y, M            | 3m                | S                | No             | Severe                | WS                | H     | N   | ACTH       | ACTH           | Cor Fre |
| 77 | PTEN   | 5y, F            | 6m                | S,FS             | No             | Severe                | WS                | H     | N   | MP         | No             | Unc      |
| 78 | H3F3A  | 2y, M            | 2m                | FS,T             | No             | Severe                | EOEE              | Frequent | WG  | BP         | LEV            | Cor Fre |
| 79 | CHD2   | 11y, M           | 6m                | FS,T, GTCS, M    | No             | Severe                | EOEE              | Frequent | N   | BP         | No             | Unc      |
### Table 3.3: Phenotype and Treatment Details

| P | Gene | Current Age, Sex | Seizure Onset Age | Seizure Semiology | Other Phenotype | Development Retardation | Epilepsy Syndrome | EEG | MRI | AEDS Tried | Effective AEDs | Seiz out |
|---|------|------------------|-----------------|------------------|-----------------|----------------------|------------------|-----|-----|------------|--------------|---------|
| 80 | HNRNPU | 6y, M | 6m | FS, T, GTCS, AA | No | Moderate | EOE | Infrequent | N | BP | VPA | VPA | Cor |
|    |       |    |    |                  |        |          |     |           |    |    |     |      |     |

#### 3.2 EOEES with dyskinesia

In our study, children with EOEES with dyskinesia had various clinical phenotypes. The common phenotypes were OS and non-syndromic EOEES. 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 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.

#### 3.4 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 EOEES 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 convolution 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.

### 3.5 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 EOEES. 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 | SCN2A | 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| SCN8A | 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, c.686A>T| p.A112G, p.A229V  | Missense, Compound          | Father, Mother| De novo     |
| 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_1327insA   | p.443N>Kfs        | Frameshift                  | Heterozygous | De novo     |
| 30| CDKL5 | c.1794_1795insA   | p.332N>Kfs        | Frameshift                  | Heterozygous | De novo     |
| 31| CDKL5 | IVS9-1G>         | Splice            | Splicing                    | Heterozygous | De novo     |
| 32| CDKL5 | c.2774_c.2775>delTG| p.925M>lfs       | Frameshift                  | Heterozygous | De novo     |
| 33| CDKL5 | c.1245_c.1246>delAG| 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.I781fs          | 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 | PCDH19    | c.798C > G                                                                  | p.A266G           | Missense                   | Heterozygous | De novo     |
| 41 | SLC2A1    | c.1278 + 30_1278 + 31insATTTCTCACC                                          |                   |                            |            |             |
| 42 | STXBP1    | c.69_c.70insA                                                                | p.L24Lfs          | Frameshift                 | Heterozygous | De novo     |
| 43 | STXB1     | c.364C>T                                                                    | p.A122T           | Nonsense                   | Heterozygous | De novo     |
| 44 | STXBP1    | c.364C>T                                                                    | p.122R > X        | Nonsense                   | Heterozygous | De novo     |
| 45 | SETBP1    | c.2339C>G                                                                   | PS780T            | Nonsense                   | Heterozygous | De novo     |
| 46 | STXBP1    | c.69_c.70insA                                                                |                   |                            |            |             |
| 47 | GABRG2    | c.929C>T                                                                    | p.T310I           | Missense                   | Heterozygous | De novo     |
| 48 | GABAEE    | c.779C > T                                                                   |                   |                            |            |             |
| 49 | GABRA2    | c.995C > T                                                                   |                   |                            |            |             |
| 50 | DEPDC5    | c.280-1G > A                                                                 |                   |                            |            |             |
| 51 | MECP2     | c.158G > T                                                                   | p.G53V            | Missense                   | Heterozygous | De novo     |
| 52 | GRIN3B    | c.1829G > A                                                                  | p.A610H           | Missense                   | Heterozygous | De novo     |
| 53 | GRIA4     | c.1378A>G                                                                   | p.I460V           | Missense                   | Heterozygous | De novo     |
| 54 | DYN1H1    | c.1682A > G                                                                  | p.G561G           | Missense                   | Heterozygous | De novo     |
| 55 | ALDH7A1   | c.961G > A                                                                   |                   |                            |            |             |
| 56 | DPYD      | c.1774C > T                                                                  | p.R592W           | Missense                   | Compound    | Father      |
| 57 | ALG11     | c.1192G > A                                                                  | p.G398L           | Missense                   | Compound    | Mother      |
| 58 | CCDC88C   | c.5635C>T                                                                   | p.R1879W          | Missense                   | Compound    | Father      |
| 59 | CSNK2B    | c.508_509del                                                                 | p.V170Af          | Frameshift                 | Heterozygous | De novo     |
| 60 | CSNK2B    | c.142C > T                                                                   | p.G48T            | Nonsense                   | Heterozygous | De novo     |
| 61 | IL1RAP1   | c.2062G > C                                                                  | p.G688G           | Missense                   | Heterozygous | De novo     |
| 62 | IQSEC2    | c.2776C > T                                                                  | p.A926T           | Nonsense                   | Heterozygous | De novo     |
| 63 | PACS2     | c.625G > A                                                                   | p.G209L           | Missense                   | Heterozygous | De novo     |
| 64 | PACS2     | c.625G > A                                                                   | p.G209L           | Missense                   | Heterozygous | De novo     |
| 65 | PACS2     | c.625G > A                                                                   | p.G209L           | Missense                   | Heterozygous | De novo     |
| 66 | PIGA      | c.241C > T                                                                   | p.A81C            | Missense                   | Hemizygous  | De novo     |
| 67 | QARS      | c.1852G > A                                                                  | p.A618A           | Missense                   | Compound    | Mother      |
| 68 | RNASEH2B  | c.629G > A                                                                   | p.A210H           | Missense                   | Compound    | Mother      |
| 69 | SM1A      | c.1495C>T                                                                   | p.A499T           | Nonsense                   | Heterozygous | De novo     |
| 70 | SM1A      | c.1489C>T                                                                   | p.G497T           | Nonsense                   | Heterozygous | De novo     |
| 71 | SM1A      | c.3463C > T                                                                  | p.G1155T          | Nonsense                   | Heterozygous | De novo     |
| 72 | TBC1D24   | c.1571G > C                                                                  | p.A524P           | Missense                   | Compound    | Mother      |
| 73 | TBC1D24   | c.1207G > T                                                                  | p.V403L           | Missense                   | Compound    | Father      |
| P  | Gene      | Base change | Amino acid change | Predicted effect on protein | Zygosity    | Inheritance |
|----|-----------|-------------|-------------------|-----------------------------|-------------|-------------|
| 74 | TBC1D24   | c.1207G > T | p.V403L           | Missense                    | Compound    | Father      |
|    |           | c.1499C > T | p.A500V           | Missense                    | Heterozygous| Mother      |
| 75 | WWOX      | c.468G > T  | p.A156S           | Missense                    | homozygosis | De novo     |
| 76 | COL4A2    | c.1148C > T | p.P383L           | Missense                    | Heterozygous| De novo     |
| 77 | PTEN      | c.1034T > C | p.L345P           | Missense                    | Heterozygous| De novo     |
| 78 | H3F3A     | c.377A > G  | p.G126A           | Missense                    | Heterozygous| De novo     |
| 79 | CHD2      | c.4909C > T | p.A1637T          | Nonsense                     | Heterozygous| De novo     |
| 80 | HNRNU     | c.1341C > T | p.V448Cfs         | Frameshift                  | Heterozygous| De novo     |

**Table 3**

Summary of the identified genes in specific EOEEs

| Specific classifications of EOEE | Associated gene |
|--------------------------------|-----------------|
| Dravet syndrome                | SCN1A           |
| Ohtahara syndrome              | KCNQ2, STXBP1   |
| West syndrome                  | SCN3A, SCN2A, SCN8A, CACNA1H, DEPDC5, MECP2, DYNC1H1, CDKL5, ALG11, CCDC88C, GABA1, IL1RAPL1, RNASEH2B, SLC19A3, STXBP1, QARS, COL4A2 |
| Early myoclonic epileptic encephalopathy | TBC1D24     |
| GLUT1 deficiency syndrome      | SLC2A1          |
| Malignant migrating focal seizures of infancy | KCNT1        |
| EOEE-BS                        | KCNQ2:STXBP1:SCN2A:PIGA |
| EOEEs with dyskinesia           | STXBP1, CDKL5, SLC2A1 |
| EOEEs limited to females with cluster seizures | SMC1A        |
| EOEEs starting with febrile convulsion | SCN1A,PCDH19:HNRNU |
Table 4
Summary of the number of identified genes in EOEEs

| Gene function                      | Mutated gene                          | Corresponding total cases |
|------------------------------------|---------------------------------------|---------------------------|
| Sodium channel                     | SCN1A, SCN2A, SCN3A, SCN8A             | 38, 3, 1, 5               |
| Potassium channel                  | KCNQ2, KCNQ3, KCTD7, KCNB1, KCNT1, HCN1 | 9, 1, 1, 2, 2, 1          |
| Calcium channel                    | CACNB4, CACNA1H, CACNA1E              | 1, 1, 1                   |
| Cyclin-dependent kinase-like       | CDKL5                                 | 8                         |
| Protocadherin                      | PCDH19                                | 4                         |
| Solute carrier family              | SLC2A1                                | 1                         |
| Syntaxin-binding protein           | STXB1P1                               | 3                         |
| SET binding protein                | SETBP1                                | 1                         |
| CDC42 guanine nucleotide exchange factor | ARHGEF9                            | 1                         |
| Gamma-aminobutyric acid receptor   | GABRG2, GABA1A, GABRA2                | 1, 1, 1                   |
| DEP domain containing 5, GATOR1 subcomplex subunit | DEPDC5                            | 1                         |
| Methyl-CpG binding protein         | MECP2                                 | 1                         |
| Glutamate ionotropic receptor      | GRIN3B, GRIA4                         | 1, 1                      |
| Dynein cytoplasmic 1 heavy chain   | DYNC1H1                               | 1                         |
| Aldehyde dehydrogenase 7 family member | ALDH7A1                            | 1                         |
| Dihydropyrimidine dehydrogenase    | DPYD                                  | 1                         |
| ALG11 alpha-1,2-mannosyltransferase | ALG11                               | 1                         |
| Coiled-coil domain containing      | CCDC88C                               | 1                         |
| Casein kinase 2                    | CSNK2B                                | 2                         |
| Interleukin 1 receptor accessory protein like | IL1RAPL1                           | 1                         |
| IQ motif and Sec7 domain ArfGEF    | IQSEC2                                | 1                         |
| Phosphofurin acidic cluster sorting protein | PACS2                            | 3                         |
| Phosphatidylinositol glycan anchor biosynthesis class | PIGA                             | 1                         |
| Glutaminyl-tRNA synthetase         | QARS                                  | 1                         |
| Ribonuclease H2 subunit            | RNASEH2B                              | 1                         |
| Structural maintenance of chromosomes | SMC1A                              | 3                         |
| TBC1 domain family member          | TBC1D24                               | 3                         |
| WW domain containing oxidoreductase | WWOX                               | 1                         |
| Collagen type IV chain             | COL4A2                                | 1                         |
| Phosphatase and tensin homolog     | PTEN                                  | 1                         |
| H3.3 histone                       | H3F3A                                 | 1                         |
| Chromodomain helicase DNA binding protein | CHD2                             | 1                         |
| Heterogeneous nuclear ribonucleoprotein | HNRNPU                          | 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 EOEE. This study provides an in-depth understanding of the genetic factors of EOEE-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 EOEE-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 BS performance may suggest the possibility of KCNQ2 pathogenic mutations. However, EOEE-BS is highly heterogeneous in terms of genetic etiology. Except for the largest genetic KCNQ2 subgroup, the second is SCNA2 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 SCNA2 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. EOEE 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 EOEE-BS usually respond 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 Guerinii[11], who first reported ARX gene mutation associated with dyskinesia. And then STXBP1, FOXG1, CDKL5related dyskinesia were gradually reported[12–14]. Kobayashi reported 11 cases of infantile dyskinesia associated with EOEE. 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 majority 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.

HNRNPU 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 HNRNPU mutation were reported, mainly including early-onset epilepsy with severe mental retardation, WS, EOEE, Lennox Gastaut syndrome and craniofacial deformity[28–30]. Durkin thought that HNRNPU 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 HNRNPU 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, MECPC2, DYNCH1H1, CDKL5, ALG11, CCDC88C, GABA<A1, 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, DPDY, CNK28 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\cite{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\cite{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\cite{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\cite{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 EOEE are variable, including dyskinesia. EOEE-BS 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

\begin{itemize}
\item 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
\item 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.
\end{itemize}

Declarations

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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.

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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.
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