Original Article

Gene mutation analysis of 175 Chinese patients with early-onset epileptic encephalopathy

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The aim of the study is to investigate the genetic characteristics and clinical features of a cohort of Chinese patients with early-onset epileptic encephalopathies (EOEEs). Targeted next-generation sequencing (NGS), focusing on 17 genes, was performed on 175 Chinese patients with EOEEs to screen gene mutations. The mutation rate was 32% (56/175). All mutations were de novo and heterozygous, including 41 novel and 15 reported mutations. Patients with cyclin-dependent kinase-like 5 (CDKL5) gene mutation accounted for the largest proportion, 13.1% (23/175). All patients with CDKL5 mutation presented severe psychomotor developmental delay and refractory seizures. The female patients presented obvious Rett-like features, which were not observed in male patients. Potassium channel, voltage-gated QQT-like subfamily Q member 2 (KCNQ2) gene mutations were detected in 13 patients. Patients with this mutation presented with early seizure onset within the first week after birth. Valproate (VPA), levetiracetam (LEV) and topiramate (TPM) were effective in most patients. Patients with specific gene mutations presented some unique clinical features, but not always. Many genes are involved in EOEEs. Targeted NGS showed a high diagnostic yield in patients with EOEEs. These findings provide useful insights for recommending treatment of gene-associated EOEEs using antiepileptic drugs.

Conflict of interest

None of the authors has any conflict of interest to disclose.

Early-onset epileptic encephalopathies (EOEEs) or early infantile epileptic encephalopathies (EIEEs) include a series of epileptic encephalopathies, which is characterized by seizures onset before 6 months of age. EOEEs have three main features: refractory seizures, severe electroencephalography (EEG) abnormalities, and developmental delay (DD) or intellectual disability (ID) (1). EOEEs include some syndromes such as Ohtahara syndrome (OS), West syndrome (WS), early myoclonic encephalopathy, malignant migrating focal seizures of infancy (MMFSI), and Dravet syndrome (DS) as well as non-specific epileptic encephalopathy (2). The etiologies of this disease are complex and diverse. Beside metabolic and structural abnormalities, genetic factors play an important role in the pathogenesis of epilepsy (3). Many genes related to epilepsy have been detected, such as SCN1A, CDKL5, and ALDH7A1 (4). However, it is very time-consuming and laborious to perform conventional polymerase chain reaction (PCR)-Sanger sequencing of the gene mutation responsible for the disease. Next-generation sequencing (NGS) and whole exome sequencing allow the analysis of a variety of genes...
simultaneously, which is very useful in large sample analysis or multi-gene analysis. In this study, targeted NGS was performed to investigate 17 candidate genes (Table 1) related to epilepsy in 175 Chinese children with EOEEs, to identify the genetic characteristics and clinical features of patients with EOEEs.

**Methods**

**Patients**

A total of 175 Chinese patients with EOEEs, including 96 females and 79 males, referred to Peking university first hospital between January 2012 and December 2015, were recruited after informed consent was obtained from their parents. All patients fulfilled the following criteria: seizure onset within 6 months of life; severe EEG abnormalities; DD or ID; and no identifiable immediate or remote cause; no metabolic or mitochondrial disorders. This cohort of patients included 85 patients with WS, 19 patients with Hanefeld variant of Rett syndrome (Han-RTT), 17 patients with OS, 6 patients with MMFSI, and 48 patients with unknown epileptic syndrome. All patients met the diagnosis criteria of EOEEs. *MECP2* mutations were negative in all patients with Han-RTT. Clinical information, including clinical manifestation, EEG, magnetic resonance imaging (MRI), and family history were collected. Patients were followed up by telephone or outpatient visits.

**Targeted NGS**

Pathogenic genes have been identified in 18 subtypes of EOEEs according to the genetic classification updated in 2012. ‘EIEE’ was used as the search keyword to search the ‘Online Mendelian Inheritance in Man, OMIM’ website to find the genetic information of EOEEs. Using ‘EIEE’ and ‘EOEE’ as keywords, ‘GENETICS EIEE’, and ‘AND GENETICS EOEE’ as the search type to search all related documents in the PubMed database, 17 genes (Table 1) were selected as candidate genes that may lead to the pathogenesis of early-onset epilepsy. Patients diagnosed with DS were not recruited in our study because its clinical manifestation is easily identified and *SCN1A* is the main pathogenic gene of DS.

The AmpliSeq library was prepared following the modified Ion AmpliSeq library preparation protocol (Pub No. MAN0006735). The Illumina adapters were used instead of standard Ion adapters for higher throughput and lower cost. Two rounds of enrich PCR instead of one were performed to increase the efficiency of the elongation step during the PCR when adding the barcode on the library. Detailed steps are described in the Supporting information. The libraries were quantified by qPCR and pooled together according to the molecular concentration. The pooled library was sequenced on Illumina HiSeq 2500 (CA, USA), generating approximately 1M 100-bp pair-end reads for each sample.

Fast QC v0.10.1 was used to check the quality of the reads and bwa0.7.12-r1039 to align the reads to the hg19 genome, producing a file in BAM format sorted by coordinates. Local realignment around indels and base quality score recombination by GATK v3.2 were performed on the BAM file for pre-processing. We used Unified Genotyper and Haplotype Caller in GATK v3.2 to call variants. Rare mutations, whose population frequency were less than 1%, were filtered according to the 1000 Genomes data, ESP6500 population data, and ExAC data. Rare variants were annotated to gene and protein change by Annovar July 2015 with the RefSeq Gene dataset. Reported pathogenic mutations in HGMD Professional database and PubMed were marked, while the pathogenicity of other rare mutations was annotated by Mutation Taster. We performed validation and parental origin analyses for these variations by PCR-Sanger sequencing and confirmed causative mutations according to parental origin of the variations and clinical features of the patients.

**Results**

Heterozygous mutations in seven genes were detected in 56 patients, including 41 novel mutations and 15 reported mutations. All mutations were predicted to be ‘disease causing’ by Mutation Taster. The gene mutation rate was 33% (56/175). *CDKL5* mutations were detected in 23 patients, representing the largest proportion (13.1%; 23/175). The second most detected mutations were *KCNQ2* mutations, detected in 13 cases. The third one was *KCNJ1* mutation, identified in six cases. Other detected gene mutations included those in the *STXBP1* gene in five patients, *SCN2A* in four patients, *SCN8A* in three patients, and *SLC2A1* in two patients. Each patient had a unique mutation, except for twin sisters presenting with the same *CDKL5* mutation. The clinical features and mutations of the patients are summarized in Table 2.

*CDKL5* mutations included 6 missense mutations, 3 splicing site mutations, and 14 mutations, which lead to a premature termination codon. All mutations were de novo mutations. None of the parents carried the same mutation. Among the 23 patients with *CDKL5* mutations, 20 were females accounting for 20.8% (20/96) of female patients with EIEE and only 3 were males (3.8%, 3/79). Out of the 23 patients who carried a *CDKL5* mutation, 11 females (57.9%, 11/19) were diagnosed with Han-RTT, followed by 3 males and 7 females (8.5%, 10/85) with WS, and 2 females (4.2%, 2/48) with non-syndromic epilepsy. All 23 patients suffered early-onset seizures before 4 months of age (from 10 to 100 days after birth). Various seizure types presented in the course of the disease, including epileptic spasms, partial seizures, myoclonic seizures, and tonic seizures. The initial seizure types of the patients were partial seizures in 20 cases and tonic seizures in 3 cases. Epileptic spasms developed in 22 patients later in life, the rest one only presented with partial spasms and tonic seizures. The age of epileptic spasm onset ranged from 10 to 100 days after birth. Hypsarrhythmia was identified on EEG records in 14 patients. The epilepsy in patients with *CDKL5* gene mutations was intractable and resistant to antiepileptic drugs (AEDs). Eleven female patients showed some Rett-like features, such as microcephaly, limited hand
skills, and stereotypic hand movements, which were not detected in patients with WS and non-syndromic epilepsy, and the three male patients. One patient died of severe pulmonary infection at 2 years of age.

**KCNQ2** gene mutations were identified in 13 patients, including 12 missense mutations and 1 micro-deletion mutation. All mutations were de novo mutations. Four mutations were located on exon 5, and six mutations were located on exon 6. The other three mutations were located on exons 4, 12, and 13, respectively. In our cohort, **KCNQ2** mutation rate was 7.4% (13/175). Among the 13 cases, 6 were males and 7 were females. Eight cases (9.4%, 8/85) were diagnosed with WS, two cases (11.8%, 2/17) with OS, and three cases (6.25%, 3/48) with non-syndromic epileptic encephalopathy. All patients presented with severe DD. Twelve patients had seizure onset in the first week of life, the rest one had seizure onset at 9 days of life. The semiology of 11 patients was characterized by tonic or asymmetric tonic seizures, accompanied by cyanosis. The other seizure types were tonic seizures, epileptic spasms, partial seizures, and atypical absence seizures. EEG showed hypsarrhythmia in 11 patients and frequent multifocal epileptic activity in the other 2 patients. Seven patients were completely seizure free after taking AEDs at 3–22 months of age. One patient had two periods of seizure freedom. Brain MRI, blood and urine amino acid and organic acid were normal in all patients.

Clinical seizure freedom was achieved in seven patients after AEDs therapy (detail in Fig. 1). The age of seizure freedom ranged from 3 to 22 months of age. Two patients were treated with sodium valproate (VPA) and levetiracetam (LEV). One patient was treated with VPA, LEV, and topiramate (TPM). One patient was treated with TPM, LEV, and clonazepam (CZP). One was treated with VPA, TPM, and CZP. One patient was treated with LEV and TPM. The last one was treated with carbamazepine (CBZ). Epilepsy in the other six patients was intractable, but seizure frequency was reduced in four patients to 1–4 times per month. Only one patient had no significant improvement after multiple AED treatments. However, one case among them had two periods of clinically seizure freedom: for 11 months by treatment with LEV and vigabatrin (VGB) and for 5 months with VPA and TPM treatment. Hypsarrhythmia was ever presented in EEG among 11 patients, then changed to multifocal epileptic activity after effective treatment, especially treated with ACTH. All six patients with intractable seizure ever had hypsarrhythmia. MRI showed myelination delay in the cerebral white matter in two patients and thinning of the corpus callosum in two of them.

Missense mutations of **KCNT1** were detected in six patients, including two males and four females. The age of final follow-up was ranging from 5 to 31 months. Seizure onset was from 2 to 32 days after birth. All of them were diagnosed with MMFSI. All patients with **KCNT1** gene mutation had intractable epilepsy, which was resistant to AEDs. Seizure frequency in one case was significantly reduced after taking quinidine. The seizure attacks decreased from more than 100 to 10 times per day. Hypsarrhythmia was identified on EEG records in four patients. All patients had severe developmental delay and refractory seizures. None of these patients could speech or walk. In addition, frequent upper respiratory tract infections occurred in these patients. One patient died of severe pulmonary infection at 1 year and 4 months of age.

**STXBP1** gene mutations, including four missense and one nonsense mutation, were detected in five patients, one male and four females. All patients were diagnosed with OS (29.4%, 5/17) with severe developmental delay. They had seizures onset within the neonatal period (from 2 to 15 days), which was refractory to AEDs. Missense mutations of **SCN2A** gene were detected in four patients, one female and three males. Three patients were diagnosed with non-syndromic epileptic encephalopathy, one with WS. The seizures onset in

| Gene name | Abbreviation | Gene number | Gene location |
|-----------|--------------|-------------|---------------|
| Cyclin-dependent kinase-like 5 | CDKIL5 | NM_001037343 | chrX:18,442,188-18,653,629 |
| Aristless-related homeobox | ARX | NM_139058 | chrX:25,003,694-25,015,948 |
| Syntaxin-binding protein 1 | STXBP1 | NM_001032221 | chr9:127,612,283-127,692,716 |
| spectrin, alpha, non-erythrocytic 1 | SPTAN1 | NM_003127 | chr9:128,552,558-128,633,662 |
| solute carrier family 2 | SLC2A1 | NM_006516 | chr1:42,925,375-42,959,173 |
| solute carrier family 25 | SLC2A22 | NM_001191061 | chr1:790,475-798,333 |
| sodium channel, voltage gated, type I alpha subunit | SCN1A | NM_006920 | chr2:165,981,204-166,076,886 |
| potassium channel, voltage-gated KQT-like subfamily Q, member 2 | SCN2A2 | NM_001040142 | chr2:165,239,402-165,392,308 |
| sodium channel, voltage gated, type II alpha subunit | ARHGEF9 | NM_001173479 | chr3:63,636,331-63,758,524 |
| Cdc42 guanine nucleotide exchange factor (GEF) 9 | PCDH19 | NM_001105243 | chr10,291,646-100,408,597 |
| protocadherin 19 | PNKP | NM_007254 | chr19:49,861,204-49,867,886 |
| phospholipase C, beta 1 | PLCB1 | NM_015192 | chr20:8,122,266-8,884,903 |
| sodium channel, voltage gated, type VIII alpha subunit | SCN8A | NM_014191 | chr12:51,591,236-51,812,864 |
| potassium channel, subfamily T, member 1 | KCNT1 | NM_020822 | chr9:135,702,192-135,793,103 |
| ST3 beta-galactoside alpha-2,3-sialyltransferase 3 | ST3GAL3 | NM_062679 | chr1:43,707,547-43,931,159 |
| aldehyde dehydrogenase 7 family, member A1 | ALDH7A1 | NM_001182 | chr5:126,541,841-126,595,418 |
| Gene   | EOEE_ID | Gender | Mutation | AA change | Novel/reported | Exon | Age (month) | onset (day) | Diagnose | Seizure free |
|--------|---------|--------|----------|-----------|----------------|------|-------------|-------------|----------|--------------|
| CDKL5  | 13      | F      | 1111delC | L371fsX492 | N               | 12   | 24          | 22          | West     | –            |
| CDKL5  | 32      | F      | ISV134+1G>A |            | N               | 36   | 10          | 10          | Han-RTT  | –            |
| CDKL5  | 93      | F      | 1833_1834delITT | H611fsX617 | N               | 12   | 36          | 40          | Han-RTT  | –            |
| CDKL5  | 34      | F      | ISV6+1G>A | R178Q     | R               | 48   | 100         | –           | Han-RTT  | –            |
| CDKL5  | 39      | F      | 1375C>T | Q459X     | N               | 12   | 87          | 30          | Han-RTT  | –            |
| CDKL5  | 45-1    | F      | 891_892insTT | F298fsX349 | N               | 11   | 30          | 90          | Han-RTT  | –            |
| CDKL5  | 45-2    | M      | 533G>A   | R178Q     | R               | 8    | 5           | 60          | West     | –            |
| CDKL5  | 77      | F      | 2360delA | K787fsX502 | N               | 16   | 17          | 30          | Han-RTT  | –            |
| CDKL5  | 68      | F      | 234delA  | A78fsX112 | N               | 5    | 42          | 40          | Han-RTT  | –            |
| CDKL5  | 1       | F      | 1791_1792insG | G597fsX610 | N               | 12   | 36          | 40          | Non-syn  | –            |
| CDKL5  | 54      | F      | G100T    | E34X      | N               | 4    | 13          | 28          | West     | –            |
| CDKL5  | 106     | F      | 1785delA | G595fsX615 | N               | 12   | 12          | 21          | West     | –            |
| CDKL5  | 116     | M      | 215T>A   | I72N      | R               | 5    | 6           | 27          | West     | –            |
| CDKL5  | 124     | F      | 426-430insbpAAATCA | L142fsX145 | N               | 7    | 20          | 40          | Han-RTT  | –            |
| CDKL5  | 145     | F      | 1245-1246delAG | T415fsX417 | N               | 12   | 13          | 65          | Han-RTT  | –            |
| CDKL5  | 162     | F      | ISV9-1G>A |           | N               | 8.6  | 57          | –           | West     | –            |
| CDKL5  | 159     | F      | T260C    | L78S      | N               | 5    | 32          | 40          | Non-syn  | –            |
| CDKL5  | 14      | F      | 991delA  | K331fsX349 | N               | 12   | 9           | 30          | West     | –            |
| CDKL5  | 148     | F      | 683G>A   | G228E     | N               | 9    | 8.6         | 90          | Han-RTT  | –            |
| CDKL5  | 121     | F      | T182C    | L61P      | N               | 5    | 72          | 80          | West     | –            |
| CDKL5  | 111     | F      | C119T    | A40V      | R               | 5    | 4.5         | 46          | West     | –            |
| CDKL5  | 132     | M      | 1326-1327insT | N443fsX   | R               | 12   | 11.2        | 20          | West     | –            |
| KCNQ2  | 64      | F      | c.T850C  | Y284H     | N               | 6    | 4           | 1           | West     | –            |
| KCNQ2  | 69      | F      | c.A871G  | R291G     | N               | 6    | 16          | 1           | West     | –            |
| KCNQ2  | 70      | M      | c.A710T  | Y237F     | N               | 5    | 51          | 2           | West     | SF           |
| KCNQ2  | 97      | F      | c.G668A  | G290S     | N               | 6    | 24          | 3           | Non-syn  | SF           |
| KCNQ2  | 117     | F      | c.T883C  | Y280H     | N               | 6    | 26          | 9           | West     | –            |
| KCNQ2  | 134     | M      | c.G1452C | E484D     | R               | 13   | 12          | 1           | OS       | SF           |
| KCNQ2  | 135     | M      | c.1284delG | Q429Rfs*5  | N               | 12   | 3           | 2           | West     | SF           |
| KCNQ2  | 137     | F      | c.T913C  | F305L     | N               | 6    | 2.9         | 5           | West     | –            |
| KCNQ2  | 133     | M      | c.J365G>C | A246P     | N               | 5    | 19          | 1           | West     | –            |
| KCNQ2  | 25      | M      | c.739G>A | A265T     | R               | 5    | 28          | 1           | West     | SF           |
| KCNQ2  | 12      | F      | c.T182C  | L61P      | N               | 5    | 72          | 80          | West     | –            |
| KCNQ2  | 111     | F      | c.C119T  | A40V      | R               | 5    | 4.5         | 46          | West     | –            |
| KCNQ2  | ZH      | M      | 1326-1327insT | N443fsX   | R               | 12   | 11.2        | 20          | West     | –            |
| KCNQ2  | 64      | F      | c.T850C  | Y284H     | N               | 6    | 4           | 1           | West     | –            |
| KCNQ2  | 69      | F      | c.A871G  | R291G     | N               | 6    | 16          | 1           | West     | –            |
| KCNQ2  | 70      | M      | c.A710T  | Y237F     | N               | 5    | 51          | 2           | West     | SF           |
| KCNQ2  | 97      | F      | c.G668A  | G290S     | N               | 6    | 24          | 3           | Non-syn  | SF           |
| KCNQ2  | 117     | F      | c.T883C  | Y280H     | N               | 6    | 26          | 9           | West     | –            |
| KCNQ2  | 134     | M      | c.G1452C | E484D     | R               | 13   | 12          | 1           | OS       | SF           |
| KCNQ2  | 135     | M      | c.1284delG | Q429Rfs*5  | N               | 12   | 3           | 2           | West     | SF           |
| KCNQ2  | 137     | F      | c.T913C  | F305L     | N               | 6    | 2.9         | 5           | West     | –            |
| KCNQ2  | 133     | M      | c.J365G>C | A246P     | N               | 5    | 19          | 1           | West     | –            |
| KCNQ2  | 25      | M      | c.739G>A | A265T     | R               | 5    | 28          | 1           | West     | SF           |
| KCNQ2  | 12      | F      | c.T182C  | L61P      | N               | 5    | 72          | 80          | West     | –            |
| KCNQ2  | 111     | F      | c.C119T  | A40V      | R               | 5    | 4.5         | 46          | West     | –            |
| KCNQ2  | ZH      | M      | 1326-1327insT | N443fsX   | R               | 12   | 11.2        | 20          | West     | –            |

Glut1-DS: glucose transporter 1 deficiency syndrome; Han-RTT: Hanefeld variant of Rett syndrome; MMFSI: malignant migrating focal seizures of infancy; N (for novel); R (for reported); Non-syn: non-specific epileptic encephalopathy; OS: Ohtahara syndrome; SF: seizure free; West: west syndrome; –: negative.

*Patient died of died of severe pulmonary infection.
these patients was within the first week of life (from 2 to 5 days after birth). Seizure freedom was achieved in two patients after AED treatment. One patient was treated with VPA and TPM, another one with VPA and CBZ. Seizure frequency was remarkably reduced in one patient treated with VPA and CBZ. The other had intractable seizure. All patients presented with severe developmental delay.

Missense mutations of SCN8A were detected in three patients. All patients were males. The seizure onset was within 3 months of age (from 2 to 3 months after birth). Seizure types included partial seizures, myoclonic seizures, and partial seizure, secondarily generalized. Two patients were seizure free after treatment with AEDs, one with VPA and LTG and the other one with VPA, LEV and oxcarbazepine.

SLC2A1 gene mutations, including one missense mutation and one micro-deletion, were detected in two patients. One was a female and the other a male. Their phenotype included paroxysmal exercise-induced dyskinesia and epilepsy, non-epileptic allelic variants such as confusion, lethargy, or somnolence, and total body paralysis. These two patients had seizure onset at 33 days and 4 months of age. The seizure type in the two patients was partial seizures. Both were diagnosed with glucose transporter 1 deficiency syndrome (GLUT1-DS), as these neurologic signs could be influenced by factors such as fasting or fatigue. In addition, the glucose levels in the cerebrospinal fluid of these two patients were 1.74 and 2.01 mmol/L, respectively. These two patients presented with mental and development retardation. The male patient responded well to frequent meals with snacks. The ketogenic diet was introduced to the female patient, but her parents gave up this treatment shortly because of her intolerance to the food and the lack of response to the ketogenic diet.

Discussion

The detection rate of targeted NGS in our study was 32% (56/175). This study illustrates the diagnostic efficiency of complementary genetic approaches in children with EOEEs of unknown etiology. Our findings suggest that some unexplained sporadic EOEEs cases involve de novo pathogenic mutations (missense/nonsense/frameshift mutations).

CDKL5-related diseases include Han-RTT, X-linked infantile spasms, EIEE-2, autism spectrum disorders, Rett-like syndrome, and Angleman-like syndrome (5–7). In this study, the CDKL5 mutational rate was 13.1% (23/175), which is similar to that of previous studies (from 8% to 28%) (6, 8, 9). CDKL5 mutations were predominantly detected in females. Core symptoms of the patients’ CDKL5 mutations included epilepsy, severe psychomotor developmental delay, mental retardation, hypotonia, and slow growth in head circumference. Partial seizures and tonic seizures are the initial seizure types. Epileptic spasms are a common seizure type in the later course of the disease. Other seizure types included myoclonic seizures, tonic seizures, and atypical absence. Hypsarrhythmia or atypical hypsarrhythmia were detected in 14 patients. The epileptic events were resistant to all antiepileptic treatments. None of these children could talk or walk. Twelve patients could sit independently, and the acquired time was delayed from 10 to 24 months in seven patients. Although all patients showed some autistic symptoms such as poor eye contact and limited interest, only 11 female patients fulfilled the criteria of Han-RTT; showing some Rett-like features such as stereotypic hand movements and microcephaly. To date, more than 20 male patients with CDKL5 mutations have been reported (10, 11). Compared with female patients, males presented more severe phenotypes, manifested as severe mental and motor retardation. The limited hand-use and stereotyped movements were less significantly detected in male than in female patients (11). The age of the three male patients with CDKL5 mutations ranged from 5 to 11 months in our study. The seizure onset was from 20 to 60 days after birth. None of them could lift their heads or acquired sitting or walking skills. Rett-like features such as hand stereotypies were not obvious in the three male patients.

The KCNQ2 gene, encoding a voltage-gated potassium (K, 7.2) channel, is responsible for about 10% of EOEEs with neonatal onset (12, 13). K, 7 channels assemble as tetramers, with each subunit displaying a core domain formed by six transmembrane segments (S1–S6), and cytoplasmic N- and C-termini. Within each subunit, the S1–S4 segments form the voltage-sensing domain arranged symmetrically around the pore (Fig. 2). More than 50 KCNQ2 mutations associated with epileptic encephalopathy were reported (14–16). The hallmark of this disorder is the onset of refractory seizures within the first week of life. Motor and cognitive deficits are obvious from birth. The common seizure type is tonic seizures and is often asymmetric with ocular symptoms and apnea. Some patients become seizure free after several months to years (ranging from 2 months to adolescence) (13). At onset, EEG shows multifocal epileptic form of activity or a hypsarrhythmia pattern (17). Brain imaging may reveal hypoplasia of the corpus callosum, hyperintensity in the basal ganglia, and diffuse hypomyelination (18). In our study, KCNQ2 mutations were detected in 13 patients (7.4%, 13/175).
All patients with KCNQ2 mutations presented with neonatal seizures and severe developmental impairment. Seizures started within 9 days of age. The initial seizure types were tonic seizures or asymmetric tonic seizures. The effective AEDs were VPA, LEV, TPM and CBZ (Fig. 1). Pisano et al. reported that CBZ and phenytoin (PHT) were the first-line treatment in patients with KCNQ2 encephalopathy (17). CBZ and PHT were not recommended medication for 10 cases among these 13 patients presented with spasm seizures. However, LEV, TPM, VPA were effective in many patients in the study of Pisano et al. (17) VPA, LEV and TPM were recommended drugs for the treatment to patients with KCNQ2 mutations, especially when their seizure type was spasm.

An important phenomenon was observed in this study. Mutations in six patients with uncontrolled epilepsy were located in the pore-forming S5 and S6 regions of Kv7.2 channel (Fig. 2). Haploinsufficiency and dominant-negative effect are the main pathomechanism underpinning the severe phenotype of KCNQ2-related encephalopathy. KCNQ2 mutations decrease the inhibitory potassium current across cell membranes (19). All these KCNQ2 mutations can lead to haploinsufficiency. KCNQ2 mutations of the six patients with intractable seizure were all located in the area of the pore-loop between the S5 and S6 regions. The other patients with KCNQ2 mutations responded well to AEDs. Dominant-negative effect at subthreshold voltages may be the mechanism underlying the KCNQ2 refractory epilepsy, this need more test to confirm this phenomenon. Orhan et al. detected that two pore mutations induce a dominant-negative effect by a reduction of the overall current amplitude yielded larger currents (19). Amino acids in these regions may have a more important function, explaining the more severe phenotype. Another hypothesis is that the binding site of AEDs is probably located in the pore region, suggesting that the pore mutations may directly affect the binding site. Studies to identify a differential responsiveness of individual mutants to drug application may serve as a basis for further analysis of the disease mechanisms of Kv7.2-related EOEEs.

The six KCNT1 mutations were located in the KCNT1 protein (Fig. 3). KCNT1 encodes a sodium-activated potassium channel subunit that plays an important role in regulating neuron excitability. KCNT1 is not widely expressed in the cortex, but is highly expressed in neurons of the frontal cortex. (20) All pathogenic mutations in KCNT1 channels result in a strong gain-of-function phenotype. KCNT1 is identified as a cause of two forms of early-onset epileptic disorders, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (21) and MMFSI (22). ADNFLE usually occurs in individuals of normal intellect and its mean age of onset is 9 years (ranging from 1 to 15 years) (23). MMFSI is a severe EOEE and the age of epilepsy onset is before 6 months of age (22). Hyperactivation of K Na channels is the patho-mechanism of this disease. In this study, all six patients with KCNT1 mutations met the diagnostic criteria for MMFSI. The seizure onset ranged from 2 to 32 days after birth. Video-EEG recordings showed the specific migrating feature, presenting with ictal discharges on EEG occurring in one region and migrating to another in all patients. The prognosis of these patients is extremely poor with intractable seizures and severe psychomotor retardation. None of them had the ability of speak or walk. Researchers identified that quinidine could reverse KCNT1 gain-of-function mutation (24, 25). One of our patients had a nearly 90% decrease in seizure frequency after taking quinidine.

SCN8A and SCN2A have already been reported as important genes in epileptic encephalopathy. STXBP1 is a main pathogenic gene of OS, while SLC2A1 is associated with GLUT1-DS. The ketogenic diet is effective at controlling seizures in most patients with GLUT1-DS (26). The follow-up of the two patients with SLC2A1 mutations was about 1 year. Hypoglycorrhachia was detected in these two patients. They all fulfilled the criteria of GLUT1-DS. One patient responded well to a frequent meal supplemented with snacks, while the other one could not tolerate the ketogenic diet and did not respond to this treatment.

To understand the possible underlying pathology of EOEEs, the mutated genes identified in our cohort were divided into four groups according to gene function (Table 3). The largest portion included genes encoding ion channels (46.4%, 26/56), suggesting that ion channels play important roles in the pathogenesis of EOEEs. The abnormal ion transport may affect various processes such as nerve excitation, cell proliferation, sensory transduction, learning, and memory, resulting in EOEEs (27, 28). Furthermore, some factors in protein kinase modulation, synapse, transcriptional regulation, cell metabolism, and cell–cell interaction are also involved in the pathogenesis of EOEEs. CDKL5 is a protein kinase and cyclin-dependent kinase. CDKL5 defect may affect the early steps of differentiation by altering molecular processes, resulting in a delayed refinement of cortical architecture, besides impacting cytoskeletal modifications (29). Synapse formation and normal function are important for developmental processes that contribute to circuit refinement in the nervous system (30). Moreover, genes related to synapse elimination and maturation are closely related to EOEEs. However, the understanding of the mechanisms of EOEEs is currently limited. Further studies of the neurological dysfunctions underlying the etiology of EOEEs are urgently needed.

Seizures, especially early-onset epilepsy, are usually intractable and co-occurring with a poor motor and cognitive prognosis. Therefore, it is very important to determine the cause of EOEEs and explore treatment options.
A rapid and efficient system of target capture sequencing can be applied to the comprehensive genetic analysis of EOEEs. In this study, targeted NGS was used to investigate the causative gene mutations in 175 Chinese children with unexplained EOEEs. The causing genes were established in 56 patients. It expanded the phenotype and mutation spectrum of seven genes associated with EOEEs. With a gene detection rate of 32%, targeted NGS is certainly considered an efficient and precise approach to screen monogenic mutations in patients with EOEEs. However, according to our experience, some limitations of this approach and tips for better detection should be discussed. First, to eliminate false positive results, conventional Sanger sequencing is required for the validation of the variations considered significant by targeted NGS. Secondly, targeted NGS resulted in false negative results. In our study, the majority of cases (68%, 119/175) remained unexplained. This observation indicates that additional candidate pathogenic genes need to be detected in the future. We will also add more pathogenic genes to our panel in the future. Third, copy number variations (CNVs) were not detected in
our study. Thus, array comparative genomic hybridization and multiplex ligation-dependent probe amplification were needed to detect the CNVs. In this regard, we did not summarize the characteristics of the gene mutation in patients with DS because these patients were not recruited for our study.

In this study, we summarized the clinical features of EOEEs patients with CDKL5, KCNQ2, and KCNT1 mutations, and determined that LEV, VPA and TPM are the recommended drugs for patients with KCNQ2 mutations, and quinidine is a recommended drug for patients with KCNT1 mutations. Our study indicates that gene mutations are highly pleiotropic and can cause a wide spectrum of seizure disorders, and the genetic study may guide the treatment of gene-associated-epilepsy in the future. Our study provides useful insights that can help improve the routine diagnosis of EOEEs and understanding of the genetic architecture of epilepsy as well as the clinical diagnosis, treatment, and the clinical and genetic counseling to families of patients.

**Supporting Information**

Additional supporting information may be found in the online version of this article at the publisher’s web-site.

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**Ethics approval**

The study was approved by Clinical Research Ethics Committee, Peking University (NM: IRB00001052-11087). We confirm that we have read the journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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