Gene Mutation Analysis in 253 Chinese Children with Unexplained Epilepsy and Intellectual/Developmental Disabilities

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Abstract

Objective

Epilepsy and intellectual/developmental disabilities (ID/DD) have a high rate of co-occurrence. Here, we investigated gene mutations in Chinese children with unexplained epilepsy and ID/DD.

Methods

We used targeted next-generation sequencing to detect mutations within 300 genes related to epilepsy and ID/DD in 253 Chinese children with unexplained epilepsy and ID/DD. A series of filtering criteria was used to find the possible pathogenic variations. Validation and parental origin analyses were performed by Sanger sequencing. We reviewed the phenotypes of patients with each mutated gene.

Results

We identified 32 novel and 16 reported mutations within 24 genes in 46 patients. The detection rate was 18% (46/253) in the whole group and 26% (17/65) in the early-onset (before three months after birth) epilepsy group. To our knowledge, we are the first to report KCNAB1 is a disease-causing gene of epilepsy by identifying a novel de novo mutation (c.1062dupCA p.Leu355HisfsTer5) within this gene in one patient with early infantile epileptic encephalopathy (EIEE). Patients with an SCN7A mutation accounted for the largest proportion, 17% (8/46). A total of 38% (9/24) of the mutated genes re-occurred at least 2 times and 63% (15/24) occurred only one time. Ion channel genes are the most common (8/24) and genes related to synapse are the next most common to occur (5/24).
Significance

We have established genetic diagnosis for 46 patients of our cohort. Early-onset epilepsy had the highest detection rate. *KCNAB1* mutation was first identified in EIEE patient. We expanded the phenotype and mutation spectrum of the genes we identified. The mutated genes in this cohort are mostly isolated. This suggests that epilepsy and ID/DD phenotypes occur as a consequence of brain dysfunction caused by a highly diverse population of mutated genes. Ion channel genes and genes related to synapse were more common mutated in this patient cohort.

Introduction

Epilepsy and intellectual/developmental disabilities (ID/DD) are both common pediatric neurological disorders. ID/DD is one of the main comorbidities of epilepsy [1] with about a quarter of epileptic children having ID/DD. The prevalence is much higher in children presenting with early-onset (before three months after birth) epilepsy [2–4]. Although frequent and refractory seizures may cause cognitive and motor regression, common pathophysiological mechanisms may be responsible for the high rate of co-occurrence of epilepsy and ID/DD [5].

The etiologies of these two disorders are complex and diverse with the majority being unknown. Genetic factors play a major role in the etiologies of epilepsy and ID/DD, especially in pediatric patients, who are highly heterogeneous. Defects in many genes have been reported as shared underlying mechanisms of epilepsy and ID/DD [6–9]. These genes are involved in different pathways. Ion channel genes, which are particularly relevant to epilepsy, account for a significant proportion [10]. However, the phenotypes related to these genes are difficult to differentiate clinically, and the detection of mutations in suspected genes is always a challenge. Seizures were always intractable and indicated a poor prognosis when co-occurring with ID/DD. Therefore, knowing the genetic background and pathogenesis of epilepsy and ID/DD is valuable not only for diagnosis and prognosis, but also for genetic counseling and treatment.

Many genes related to epilepsy and ID/DD have been reported. However, for individual patients with non-syndromic epilepsy, it has been difficult to isolate the causative gene mutations from a large number of possible candidate genes using conventional Sanger sequencing. The rapidly developing targeted next-generation sequencing (NGS) has been proved to be a fast, economic and accurate approach for screening gene mutations in disorders with both genetic and phenotypic heterogeneity, including epilepsy and ID/DD [11]. In this study, we used targeted NGS to investigate 300 candidate genes related to epilepsy and ID/DD in 253 Chinese children with unexplained epilepsy and ID/DD. We aim to make genetic diagnosis for these patients and find clues to help us explain the common genetic background of epilepsy and ID/DD.

Methods

Ethics statement

Written informed consent was obtained from the parents of all the patients. This study was approved by the Institutional Review Boards of Peking University First Hospital. All data of this study were analyzed anonymously.
Patients

A total of 253 Chinese children with unexplained epilepsy and ID/DD were recruited from the Department of Pediatrics, Peking University First Hospital from 2006 to 2014. All patients were clinically diagnosed as having epilepsy and ID/DD of unknown origin, including 65 patients diagnosed as early-onset (before three months after birth) epilepsy. Nevertheless, it was strongly suspected that the etiology of these patients’ diseases was genetic, owing to the following evidences: (1) no definite perinatal brain injury; (2) no hypoxia, ischemia, infection of the central nervous system or cranial trauma; (3) no evidence of typical inherited metabolic disorders or specific neurodegenerative disorders based on clinical features, neuroimaging or blood/urinary metabolic diseases screening; (4) normal routine karyotyping and (5) the detection of chromosome sub-telomeric rearrangements with multiplex ligation-dependent probe amplification (MLPA) showing no abnormalities [12]. 253 cases of our cohort included cases from 246 trios, of which father or mother of four trios (1649, 5165, 5168, and 5237) had epilepsy history, and parents of all the other 242 trios had no epilepsy and any related history. Other seven cases were probands of seven families with more than one patient, of which six families (3604, 5750, 6047, 6364, 6526, and 6636) consisted of two affected children and their unaffected parents, and one family (5240) with five affected patients (proband, father, uncle, aunt and grandfather) accorded with autosomal dominant inheritance. All genomic DNA used in experiments were extracted from peripheral leukocytes.

Targeted next-generation sequencing

In accordance with the literatures searched within online databases, a total of 300 candidate genes associated with epilepsy and ID/DD were selected as the genes of interest. We used a custom-designed gene panel, synthesized using the Agilent Sure-Select Target Enrichment technique (Zhongguancun Huakang Gene Institute, China), to capture the coding regions from the 300 genes, including their exons and exon-intron boundaries (1.285M bp in total). The following NGS was performed on an Illumina GAIIx platform (Illumina, San Diego, CA, U.S.A.) using paired-end sequencing of 110 bp. Bioinformatics analysis of the raw data included the following steps: (1) image analysis using RTA software (real-time analysis, Illumina); (2) base calling using CASAVA software v1.8.2 (Illumina); (3) filtered out duplicate and low base quality score reads using the Genome Analysis Tool kit (GATK); (4) aligned clean paired-end reads to the human reference genome build hg19 using BWA software (Pittsburgh Supercomputing Center, Pittsburgh, PA, U.S.A.); (5) identified insertion-deletions (indels) and single-nucleotide polymorphisms (SNPs) using the GATK and annotated using ANNOVAR; (6) performed in silico pathogenicity prediction of novel missense variations using Polyphen2 [12, 13].

The sequencing depth was more than 5X (range of 5X–185X; average of 136X), and the mean coverage was 98.56%. On average, 423 variations within the 300 genes were found in each patient. We then formulated the following filtering criteria to determine every possible pathogenic variation from the large amount of initial variations: (1) insertion/deletion variations; (2) premature/delayed termination codon variations; (3) splice site variations including substitution at nucleotide +1/-1 of intron; (4) missense variations predicted by polyphen2 as probably/possibly damaged or benign. The variation meeting any one of the above criteria was considered to be a candidate for pathogenic variations and was selected for further analysis [12].

According to the HGMD Professional database, the 1000 Genomes Browser, PubMed and the UCSC database, we marked the reported pathogenic mutations and excluded known polymorphisms. Finally, on average 17 possible pathogenic variations were identified in each patient (range 5–27). We chose variations which to validate according to the known
inheritance pattern of the involved genes. Heterozygous variations of genes with autosomal or X-linked dominant heredity, homozygous or compound heterozygous variations of genes with autosomal recessive heredity, and hemizygous variations of genes with X-linked recessive heredity were regarded as likely causative variations. We performed validation and parental origin analyses for these variations by conventional Sanger sequencing, and confirmed causative mutations according to parental origin of the variations and clinical features of the patients [12].

Protein structure modelling

The homology modelling server SWISS-MODEL [14–16] was used to predict the tertiary structure of KCNAB1 protein. We chose the protein crystal structure 3EAU [17] of KCNAB2 from Protein Data Bank (PDB) [18] as the template. For the homology of KCNAB1 and KCNAB2, the structure from 89 to 413 amino acids of KCNAB1 protein (UniProtKB ID: Q14722) were predicted.

Results

We identified causative mutations within 24 genes in 46 patients of our cohort, including two likely pathogenic mutated genes in two patients. The total detection rate of our study was 18% (46/253) in the whole group and 26% (17/65) in the early-onset epilepsy group. The detected mutations included 32 novel and 16 reported mutations. Nineteen of the mutations were severe, including eight premature/delayed termination codon mutations, ten insertion/deletion mutations and one splicing site mutation. The remaining 29 mutations were non-synonymous, including 27 mutations predicted to be “probably damaging” and two mutations (AFF2 p. Gly547Asp and RELN p.Val3426Ile) that were predicted to be “possibly damaging” by Polyphen2. In two patients (5240 and 6189) with distinct epilepsy and ID/DD phenotypes, two mutated genes were regarded as likely pathogenic due to the unmatched phenotype or the unavailability of segregation analysis in the family. An overview of the clinical features of patients and their mutations is described in Table 1.

Among the 46 cases with a causative mutation, patients with an SCN1A mutation accounted for the largest proportion of 17% (8/46), followed by SCN8A, KCNQ2 and IQSEC2 of 13% (6/46), 9% (4/46) and 7% (3/46) respectively. A total of 38% (9/24) of the mutated genes (SCN1A, SCN8A, KCNQ2, IQSEC2, CDKL5, DYRK1A, GABRB3, PCDH19, and STXBP1) reoccurred at least two times in this study, and 63% (15/24) of the mutated genes (ALDH7A1, AFF2, ATP1A2, CASK, FOXG1, GRIN2A, GRIN2B, KCNAB1, PRRT2, RELN, SHANK3, SLC2A1, SYNGAP1, UPF3B and ZEB2) occurred only one time. All mutated genes detected in this study along with the number of patients in whom each gene was detected were shown in Fig 1.

Notably, we detected a novel de novo heterozygous mutation (c.1062dupCA p.Leu355HisfsTer5) within KCNAB1 in one patient with EIEE. This mutation led to a premature termination codon. The patient with the mutation was a four-year-old girl with early-onset seizures (onset at ten days after birth). The frequent partial seizures were followed by intractable generalized tonic-clonic seizures (GTCS). Until now, she has used seven antiepileptic drugs (AEDs). Sodium Valproate, Levetiracetam, Topiramate and Clonazepam had no obvious effect, while Oxcarbazepine, Lamotrigine and Zonisamide reduced the seizures; among them Zonisamide add-on had the best effect with a 75% reduction of the seizures. Her parents are planning to consent for her to receive vagal nerve stimulation (VNS) to treat her intractable epilepsy. She also has severe ID/DD now (non-verbal, limited interaction with her parents, but can walk independent from 2 years 3 months), with occasional panic attacks. Her prenatal history was normal and neurological examination was unremarkable. One of her electroencephalograph
| Gene    | Case | Sex | Study age (y) | Seizures (onset age) | ID/DD | Nucleotide substitution | Amino acid substitution | Parental origin | Novel/reported | Final diagnosis |
|---------|------|-----|---------------|----------------------|-------|------------------------|------------------------|-----------------|---------------|----------------|
| SCN1A   | 152  | M   | 8             | GTCS, FS (6 m)       | Severe| c.4547C>T              | p.Ser1516Ter           | De novo         | Reported      | DS             |
|         | 2038 | M   | 10            | GTCS, PS (4 m)       | Severe| c.2134C>T              | p.Arg712Ter            | De novo         | Reported      | DS             |
|         | 5791 | M   | 3             | PS, GTCS (3 m)       | Severe| c.4942C>T              | p.Arg1648Cys           | De novo         | Reported      | DS             |
|         | 6047 | M   | 3             | PS, FS, Absences (8 m) | Severe| c.2589+3A>T          | -                      | De novo         | Reported      | DS             |
|         | 6207 | F   | 15            | PS, GTCS, FS (3 m)   | Severe| c.3733C>T              | p.Arg1245Ter           | De novo         | Reported      | DS             |
|         | 6222 | F   | 0.5           | PS, SE (16 d)        | Severe| c.659T>A               | p.Val220Asp            | De novo         | Novel         | MMPSI          |
|         | 6300 | F   | 3             | PS, Myoclonus, GTCS, FS (6 m) | Severe| c.3372delT          | p. Phe1124LeufsTer4    | De novo         | Novel         | DS             |
|         | 6492 | M   | 4             | PS, FS (7 m)         | Severe| c.2488G>T              | p.Glu830Ter            | De novo         | Novel         | DS             |
| SCN8A   | 3129*| M   | 9.5           | GTCS, FS (11 m)      | Severe| c.2668G>A              | p.Ala890Thr            | De novo         | Novel         |                |
|         | 5487*| M   | 3.5           | GS (6 m)             | Severe| c.4850G>A              | p.Arg1617Gin           | De novo         | Reported      | EE             |
|         | 6219*| F   | 1.5           | GTCS, Myoclonus, IS (3 d) | Severe| c.1221G>C              | p.Leu407Phe            | De novo         | Novel         | EIEE           |
|         | 6325*| F   | 1             | PS, GTCS (2.5 m)     | Moderate to severe | c.2549G>A            | p.Arg850Gln           | De novo         | Novel         | EIEE           |
|         | 6908*| M   | 1.5           | GTCS (4 m)           | Moderate to severe | c.4787C>G            | p.Ser1596Cys          | De novo         | Novel         | EE             |
| SCN8A   |      |     |               |                      |       |                        |                        |                 |               |                |
| KCNQ2   | YL   | F   | 3             | PS (6 m)             | Severe| c.4935G>A              | p.Met1645Ile           | De novo         | Novel         |                |
|         | 597  | M   | 3             | GS (3 d)             | Severe| c.365C>T               | p.Ser122Leu            | De novo         | Reported      | EIEE           |
|         | 2202 | M   | 2             | PS, GTCS, FS (3 d)   | Severe| c.956A>C               | p.Lys319Thr            | De novo         | Novel         | EIEE           |
|         | 2312 | M   | 1.5           | PS, FS (4 h)         | Severe| c.830C>T               | p.Thr277Ile            | De novo         | Novel         | EIEE           |
|         | 5630 | M   | 1             | PS, IS (3 d)         | Severe| c.1655A>C              | p.Lys552Thr            | De novo         | Reported      | EIEE           |
| IQSEC2  | 3481 | M   | 9             | PS (3 y 6 m)         | Severe| c.88_90delATC           | p.Ile30del             | De novo         | Novel         |                |
|         | 3604 | M   | 7             | PS, FS (6 m)         | Severe| c.1049C>A              | p.Ala350Asp            | Mother          | Novel         |                |
|         | 5292 | M   | 2             | PS, IS, Myoclonus (1 y 4 m) | Severe| c.2846_2852delCCCAGGT | p.Ser949CysfsTer7      | De novo         | Novel         |                |
| CDKL5   | 1613 | F   | 1             | PS (40 d)            | Severe| c.2314delA             | p.Lys772ArgfsTer12     | De novo         | Novel         | EIEE           |
|         | 5057 | F   | 1.5           | PS (1 m)             | Severe| c.528G>A               | p.Trp176Ter            | De novo         | Novel         | EIEE           |
| DYRK1A  | 2091 | M   | 7             | GTCS, FS, SE (1 y 6 m) | Severe| c.859G>T              | p.Asp287Tyr            | De novo         | Novel         |                |
|         | 2959 | F   | 3             | GS (2 y)             | Severe| c.946C>T              | p.Gln316Ter            | De novo         | Novel         |                |

(Continued)
| Gene   | Case | Sex | Study age (y) | Seizures (onset age) | ID/DD | Nucleotide substitution | Amino acid substitution | Parental origin | Novel reported | Final diagnosis |
|--------|------|-----|---------------|----------------------|-------|-----------------------|------------------------|-----------------|---------------|----------------|
| GABRB3 | 1538 | M   | 1             | PS, GTCS (6 m)       | Severe | c.914C>T               | p.Ala305Val            | De novo         | Novel         |                |
| SYH    | 1.5  | F   | 1             | PS (9 m)             | Severe | c.509T>G               | p.Leu170Arg            | De novo         | Novel         |                |
| PCDH19 | 6526 | F   | 9             | PS (5 m)             | Severe | c.1091delC             | p.Pro364ArgfsTer4      | Father          | Reported      |                |
| LXX    | 3    | F   | 3             | PS, FS (9 m)         | Moderate | c.370G>A               | p.Asp124Asn           | Father          | Novel         |                |
| STXB1  | 527  | M   | 2             | IS (7 d)             | Severe | c.568C>T               | p.Arg190Trp           | De novo         | Reported      | EIEE           |
| MYS    | 2    | IS (3 d) | Severe | c.568C>T           | p.Arg190Trp | De novo         | Reported      | Novel         |                |
| AFF2   | 6636 | M   | 11            | PS (6 y)             | Moderate | c.1640G>A             | p.Gly547Asp           | Mother          | Novel         |                |
| ALDH7A1| 5921 | M   | 4             | PS (3 m)             | Moderate | c.1553G>C             | p.Arg518Thr p. Tyr354Cys | Father/Mother | Novel         | PDE            |
| ATP1A2 | 5871 | M   | 1.5           | PS, FS (1 m)         | Mild    | c.2563G>A             | p.Gly855Arg          | De novo         | Reported      |                |
| CASK   | 2584 | F   | 6             | IS (1 y)             | Severe | c.2141delC             | p.Ala714GlufsTer13    | De novo         | Novel         | MICPCH         |
| FOXG1  | 2539 | M   | 2             | GTCS, PS, Laugh attack (6 m) | Severe | c.738C>A             | p.Tyr246Ter           | De novo         | Novel         |                |
| GRIN2A | 6245 | F   | 6             | PS, ESES (5 y)       | Severe | c.2191G>A             | p.Asp731Asn           | De novo         | Reported      |                |
| GRIN2B | 1503 | M   | 2             | PS, IS, Myoclonus, Tonic, Startle (6 m) | Severe | c.1985A>C             | p.Gln662Pro           | De novo         | Novel         |                |
| KCNA1  | HY   | F   | 4             | PS, GTCS (10 d)      | Severe | c.1062dupCA           | p.Leu355HisfsTer5     | De novo         | Novel         | EIEE           |
| PRRT2  | 5240 | F   | 1             | GS, IS (3 m)         | Severe | c.649dupC             | p.Arg217ProfsTer8     | Father          | Reported      |                |
| RELN   | 6235 | F   | 7             | Myoclonus (3 y)      | Severe | c.10276G>A            | p.Val3426lle p. Lys751Thr | Father/Mother | Novel         |                |
| SHANK3 | ZXT  | M   | 3             | IS (1 y 10 m)        | Severe | c.3598G>C             | p.Ala1200Pro          | De novo         | Novel         |                |
| SLC2A1 | 1649 | M   | 5.5           | GTCS (4 y 8 m)       | Mild    | c.1477T>C             | p.Ter493ArgexTer56    | Mother          | Novel         |                |
| SYNGAP1| 5828 | F   | 5             | Atypical absences, Myoclonus, Atonic, GTCS, FS (10 m) | Severe | c.829dupC             | p.Pro277ProfsTer7    | De novo         | Novel         |                |
| UPF3B  | 6189 | M   | 2             | IS (8 m)             | Severe | c.883T>A              | p.Leu295Met           | Mother          | Novel         |                |
| ZEB2   | 4620 | M   | 3             | PS (?)               | Severe | c.1426_1427insA       | p.Met476AsnfsTer6     | De novo         | Reported      |                |

M, male; F, female; y, years; m, months; d, days; h, hours; PS, partial seizures; FS, febrile seizures; SE, status epilepticus; DS, Dravet syndrome; EIEE, early infantile epileptic encephalopathy; EE, epileptic encephalopathy; IS, infantile spasms; GS, generalized seizures; PDE, pyridoxine-dependent epilepsy; MMPSI, malignant migrating partial seizures in infancy; ESES, electrical status epilepticus in sleep; MICPCH, mental retardation and microcephaly with pontine and cerebellar hypoplasia

*have been reported in [12].

doi:10.1371/journal.pone.0141782.t001
(EEG) recordings showed a slow spike-wave in the left frontotemporal region, while another EEG recorded frequent sharp waves in the left temporocipital region. Her cranial magnetic resonance imaging (MRI) was normal.

*KCNAB1* encodes the beta-1 member of the shaker-related family of voltage-gated potassium channels. This member includes three isoforms (Kvβ1.1 - Kvβ1.3) of the *KCNAB1* gene \[19–23\]. Kvβ1.1, the longest isoform, is restricted expressed in brain \[24\]. The shaker-related voltage-gated K+ (Kv) channels consist of alpha and beta subunits \[25\]. The beta subunits modulate the gating properties of the alpha-subunit potassium channels. Voltage-dependent potassium channel proteins are responsible for the electrical properties of excitable cells and play physiological roles in non-excitable cells \[26\]. To further study the pathogenicity of p. Leu355HisfsTer5 mutation, protein tertiary structures of wild type and p.Leu355HisfsTer5 mutation of *KCNAB1* were predicted using the SWISS-MODEL. Because the template 3EAU was a homotetramers crystal structure, we predicted both monomer and tetramer of *KCNAB1* protein. According to the monomer prediction (Fig 2A), the mutated *KCNAB1* protein lost C-terminal helices when compared to wild type. According to the tetramer prediction (Fig 2B), the wild type was able to bind to NADP⁺ by sharing the same binding domains of *KCNAB2* protein (data from: http://www.uniprot.org/uniprot/Q14722). But the mutated *KCNAB1* protein was not able to bind to NADP⁺ for losing a NADP⁺ binding domain (375–381 amino acids), though might still be able to form a tetramer. Previous study of experimental point mutation within NADP⁺ binding domain of *KCNAB1* protein showed significant effects on Kv1 channel trafficking and axonal targeting \[27\]. Therefore, the p.Leu355HisfsTer5 mutation that we identified is probably pathogenic.

To understand a possible common genetic mechanism of epilepsy and ID/DD, we classified 24 mutated genes identified in our study into groups according to gene function. We found that ion channel genes had the largest percentage of occurrence, 33% (8/24) with genes related to synapse coming second at 21% (5/24). Other genes also identified were classified as having

![Fig 1. Pathogenic (red) and likely pathogenic (green) mutated genes identified in 253 patients with unexplained epilepsy and intellectual/developmental disabilities.](#)
functions in transcriptional regulation, protein kinase modulation, cell metabolism and cell-cell interaction. The classification and the number of genes of each group were shown in Table 2.

**Discussion**

In this study, we discovered 32 novel and 16 reported mutations within 24 genes in 46 patients of our cohort, including two likely pathogenic mutated genes in two patients. The total detection rate of our study was 18% (46/253) in the whole group and 26% (17/65) in the early-onset...
epilepsy group. Early-onset epilepsy had the relatively higher detection rate. We made genetic
diagnosis for these 46 patients. This was critical for them to improve further management and
genetic counseling for their epilepsy. We expanded the phenotype and mutation spectrum of
the 24 genes identified in our study. This provided more information for further understanding
of these disease-causing genes. Patients with an SCN1A mutation accounted for the largest pro-
portion, 17% (8/46), of which seven patients were diagnosed as Dravet syndrome and one
patient was diagnosed as MMPSI. MMPSI cases with a SCN1A mutation have been reported
and MMPSI is regarded as the most severe phenotype of SCN1A to date [28, 29]. Our MMPSI
patient with a novel SCN1A mutation provided further evidence that
SCN1A defects play an
important role in MMPSI. A total of 63% (15/24) of the mutated genes occurred only one time
in our study; therefore, it seems that epilepsy and ID/DD are phenotypes that occur as a conse-
quence of brain dysfunction caused by highly diverse mutated genes, most of which are isolated
and fit the rule of common disease rare variations. In addition, the spectrum of mutated genes
in our study is rather different from those reported in other similar studies [11, 30]. Population
diversity and different inclusion criteria (both for patients and candidate genes) may account
for this inconsistency.

In this study, we identified a novel de novo heterozygous mutation (c.1062dupCA p.Leu355-
HisfsTer5) within KCNAB1 in one patient with EIEE. KCNAB1 has been reported as a suscepti-
bility gene for epilepsy, particularly temporal lobe epilepsy (TLE), but no pathogenic mutation
has been reported. An association study of 2717 epileptic patients reported that numerous
SNPs located within KCNAB1 contributed to the susceptibility to epilepsy. These patients man-
ifested various forms of epilepsy [31]. Furthermore, KCNAB1 was regarded as a candidate gene
for lateral temporal epilepsy (LTE) because of its functional interaction with LGII [32], the
disease-causing gene of autosomal dominant LTE (ADLTE). However, sequencing of KCNAB1 in
ADLTE families without LGII mutations failed to identify any mutations. This suggested that
KCNAB1 does not act as a major disease-causing gene in ADLTE [33]. Nevertheless, another
association study of 142 LTE patients suggested that KCNAB1 may be a susceptibility gene of
LTE [34]. In addition; a genome-wide scan study was conducted on a TLE family. Linkage
analysis identified a locus on chromosome 3q25-q26. KCNAB1 was one of the highest priority
genes in this region, but sequencing of KCNAB1 was unable to identify any mutations [35]. In
summary, although several previous studies have supported the association of KCNAB1 with
epilepsy, no KCNAB1 mutations have been reported in patients with this disease previously.
However, a mouse model of KCNAB1 disruption showed significant alterations in hippocam-
pal learning and memory functions [36], supporting a possible relationship between KCNAB1
defects and brain dysfunction. Here we reported an epileptic patient with a KCNAB1 mutation,
which supports the relationship between KCNAB1 dysfunction and epilepsy, and interestingly, the epileptic discharges of this patient located mostly at temporal region.

We also found four patients with a KCNQ2 mutation. Because KCNQ2 [37] and KCNT1 [38] have already been reported to be common and important genes for epileptic encephalopathy, adding the recently reported KCNA2 [39], KCNH1 [40], KCNC1 [41] genes and now the KCNAB1 gene, this led us to pay more attention to the potassium channel genes as a group in epilepsy, especially epileptic encephalopathy.

Two mutated genes were regarded as likely having pathogenic mutation in our study. First, we detected a heterozygous mutation (p.Arg217ProfsTer8) within PRRT2 in one family (5240). The proband had severe ID/DD and infantile spasms, but other individuals with the same mutation in this family had benign epilepsy during infancy and normal intelligence during adulthood. We hypothesized that the PRRT2 mutation in the proband may only increase the risk of epilepsy, while another undiscovered mutated gene may instead contribute to the severe phenotype. In addition, we discovered a hemizygous mutation (p.Leu295Met) in UPF3B which have been reported as a causative gene of X-linked recessive mental retardation. The previously reported cases with mutation in UPF3B had no seizures. The mutation we found is novel, but probably damaging predicted by Polyphen2. Unfortunately, DNA samples of other male maternal family members were unavailable to make sure the pathogenicity of this mutation.

To understand a possible common genetic mechanism of epilepsy and ID/DD, we classified the mutated genes identified in our cohort according to gene function. We have found that ion channel genes had the largest percentage of occurrence. This suggests that ion channels play a vital role in the pathogenesis of epilepsy and ID/DD. Activation of neurotransmitter receptor ion channels at synapses promotes synaptic plasticity during brain development. Therefore, abnormal ion transport may affect neural excitability and brain development, resulting in epilepsy and ID/DD [42]. Further, synapse formation and normal function are essential in the signaling and the formation of neural networks. Genes related to synapse formation and function were also closely related to epilepsy and ID/DD. In addition, some factors in transcriptional regulation, protein kinase modulation, cell metabolism and cell-cell interaction may also participate in the common pathogenesis of epilepsy and ID/DD. However, relevant details remain unclear. We believe further study of the common pathogenesis of epilepsy and ID/DD are urgently needed.

In our study, with the detection rate of 18%, the targeted NGS is certainly supposed to be an efficient and precise approach to screen monogenic mutations in patients with highly heterogeneous disorders such as epilepsy and ID/DD. However, according to our experience, some limitations of this approach and tips for best performance should be discussed here. First, owing to false positive results, conventional Sanger sequencing is definitely required for the validation of the variations supposed to be significant, especially when the targeted regions have insufficient coverage. Second, on the other hand, false negative results may also occur and may lead to the loss of crucial data. This might be one of the reasons that our study failed to detect gene mutations in the other 207 patients. Third, DNA samples of the parents and other affected or even unaffected members of families are essential to analyze the pathogenicity of the variations. Availability of almost all parental DNA samples in our study played a significant role in data analysis. However, the unavailability of other members in a few families hampered further confirmation of their etiology. Finally, precise clinical data is a prerequisite, without which the genetic diagnosis cannot be made. For example, patient 5871 who carried a de novo mutation in ATP1A2 also had an inherited homozygous mutation (p.Ile105Val) in CLN3. Although the CLN3 nonsynonymous mutation was predicted to be "probably damaging", we still excluded its pathogenicity according to his phenotype, not like neuronal ceroid lipofuscinoses clinically.
In summary, we used targeted NGS to investigate causative gene mutations in Chinese children with unexplained epilepsy and ID/DD. We established genetic diagnosis for 46 patients of our cohort and expanded the phenotype and mutation spectrum of 24 genes associated with epilepsy and ID/DD. This study is the first to identify a KCNAB1 mutation in a patient with EIEE. More cases with mutations in this gene are needed to confirm and clarify its role in epilepsy.

**Acknowledgments**

We thank our patients for participating in this study. We thank Dr. Macdonald (Department of Neurology, Vanderbilt University Medical Center) for his kindly critical reading and valuable suggestion to this manuscript.

**Author Contributions**

Conceived and designed the experiments: YJ. Performed the experiments: YZ WK. Analyzed the data: YZ YJ WK YG KG HX. Contributed reagents/materials/analysis tools: YJ XL YW YZ JW FG XW. Wrote the paper: YJ YZ.

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