**Novel and de novo point and large microdeletion mutation in PRRT2-related epilepsy**

Li Yang1,2 | Cuiping You3 | Shiyan Qiu2 | Xiaofan Yang1 | Yufen Li2 | Feng Liu4 | Dongqing Zhang1 | Yue Niu1 | Liyun Xu2,5 | Na Xu2 | Xia Li2 | Fang Luo6 | Junli Yang1 | Baomin Li1

1Department of Pediatrics, Qilu Hospital of Shandong University, Jinan, China
2Department of Pediatrics, Linyi People’s Hospital Affiliated to Shandong University, Linyi, China
3Central Laboratory, Linyi People’s Hospital Affiliated to Shandong University, Linyi, China
4Department of Neurology, Zibo Zhangdian Hospital of Traditional Chinese Medicine, Zibo, China
5Department of Pediatrics, Shandong medical college, Linyi, China
6MyGenostics Inc, Beijing, China

**Correspondence**
Junli Yang and Baomin Li, Department of Pediatrics, Qilu Hospital of Shandong University, No.107 Cultural West Road, Jinan, Shandong 250012, China.
Email: yangjunli2003@163.com (JY); 198962000693@sdu.edu.cn (BL)

**Funding information**
Talent training foundation of Natural Science Foundation of Shandong, Grant/Award Number: ZR2015PH014; General program of Qilu Hospital Affiliated to Shandong University, Grant/Award Number: 2015QLM508; National Natural Science Foundation of China, Grant/Award Number: 816601195; National Key Research and Development Program of China, Grant/Award Number: 2016YFC1306202; Science and Technology Development Plan of Shandong Province, Grant/Award Number: 2018WS399; Natural Science Foundation of Shandong Province, Grant/Award Number: ZR2016HB38; Science and Technology Development Plan of Shandong Province, Grant/Award Number: ZR2015PH014; General program of Qilu Hospital Affiliated to Shandong University, Grant/Award Number: 2015QLM508; National Natural Science Foundation of China, Grant/Award Number: 816601195; National Key Research and Development Program of China, Grant/Award Number: 2016YFC1306202; Science and Technology Development Plan of Shandong Province, Grant/Award Number: 2018WS399; Natural Science Foundation of Shandong Province, Grant/Award Number: ZR2016HB38; Science and Technology Development Plan of Shandong Province, Grant/Award Number: ZR2015PH014.

**Abstract**

**Background:** Point and copy number variant mutations in the PRRT2 gene have been identified in a variety of paroxysmal disorders and different types of epilepsy. In this study, we analyzed the phenotypes and PRRT2-related mutations in Chinese epilepsy children.

**Methods:** A total of 492 children with epilepsy were analyzed by whole exome sequencing (WES) and low-coverage massively parallel CNV sequencing (CNV-seq) to find the single nucleotide variants and copy number variations (CNVs). And quantitative polymerase chain reaction was utilized to verify the CNVs. Their clinical information was followed up.

**Results:** We found PRRT2-related mutations in 19 patients (10 males and nine females, six sporadic cases and 13 family cases). Twelve point mutations, four whole gene deletion, and three 16p11.2 deletions were detected. The clinical features of 39 patients in 19 families included one early childhood myoclonic epilepsy (ECME), one febrile seizure (FS), two infantile convulsions with paroxysmal choreoathetosis (ICCA), six paroxysmal kinesigenic dyskinesias (PKD), 12 benign infantile epilepsy (BIE), and 17 benign familial infantile epilepsy (BFIE). All patients had normal brain MRI. Interictal EEG showed only one patient had generalized polyspike wave and five patients had focal transient discharges. Focal seizures originating in the frontal region were recorded in one patient, two from the temporal region, and two from the occipital region. Most patients were treated effectively with VPA or OXC, and the child with myoclonic seizures was not sensitive to antiepileptic drugs.

**Conclusion:** PRRT2 mutations can be inherited or de novo, mainly inherited. The clinical spectrum of PRRT2 mutation includes BIE, BFIE, ICCA, PKD, FS, and ECME. The PRRT2-related mutations contained point mutation, whole gene deletion and 16p11.2 deletions, and large microdeletion mutations mostly de novo. It is the first report of...
1 | INTRODUCTION

Mutations in the proline-rich transmembrane protein 2 (PRRT2) gene on chromosome 16 were first identified in the paroxysmal kinesigenic dyskinesias (PKD) family and subsequently in benign familial infantile epilepsy (BFIE) and infantile convulsions with paroxysmal choreoathetosis (ICCA) (Mathot, Lederer, Gerard, Gueulette, & Deprez, 2017; Okumura et al., 2019; Zhao et al., 2019) and were also reported to be associated with benign infants myoclonic (Maini et al., 2016), west syndrome (Djemie et al., 2014), febrile convulsions (FS) (Zheng et al., 2016), hemiplegic migraines (Cloarec et al., 2012), intermittent ataxia, and other differential movement disorders (Castelnovo et al., 2016; Delcourt et al., 2015; Ebrahimi-Fakhari et al., 2014; Legris et al., 2019). Homozygous PRRT2 mutations give rise to the more severe clinical disease of mental retardation and other phenotypes, such as 16p11.2 deletion being a hot spot mutation (Fabbri et al., 2013; Zhang, Li, Chen, Gan, & Liu, 2017). Copy number deletions of this gene such as 16p11.2 deletion are known to cause PRRT2-related diseases (Dale, Grattan-Smith, Nicholson, & Peters, 2012; Silveira-Moriyama et al., 2018; Termsarasab et al., 2014; Weber, Kohler, Hahn, Neubauer, & Muller, 2013). 16p11.2 microdeletion syndrome is a kind of congenital gene deletion disease, with the clinical manifestations as autism, developmental delay, mental retardation, spinal deformity, and a series of neuropsychiatric developmental diseases (Al-Jawahiri, Jones, & Milne, 2019; Castelein, Steyaert, Peeters, & van Buggenhout, 2019; Hinkley et al., 2019; Li et al., 2018a; Siu et al., 2019). Phenotypic heterogeneity between patients is obvious, and its pathogenic mechanism is not yet clear.

Therefore, in this study, we conducted whole exome sequencing and copy number variation sequencing (CNV-seq) on 492 epilepsy children who had benign or refractory epilepsy, in order to find new epileptic phenotypes related to PRRT2 and verify the positive prediction results of PRRT2-related CNV by qPCR, so as to further clarify the role of 16p11.2 deletion and further understand the clinical and mutational features of PRRT2-related epilepsy.

2 | METHODS

2.1 | Subjects

We analyzed 492 epilepsy children with the onset age of 0–14 years between 2016 and 2019 in the Pediatrics Department of Qilu Hospital Affiliated to Shandong University and Linyi people’s Hospital Affiliated to Shandong University, China. Their clinical information was retrospectively collected and followed up, including seizure types, onset age, treatment process, growth and development history, previous disease history, family history, intellectual test, cranial magnetic resonance imaging (MRI), and video-EEG, antiepileptic drugs (AEDs), and age of epilepsy remission. The patients were followed up by phone or visit clinic every three months. Exclusion criteria included seizures caused by nongenetic factors, such as an acquired brain injury, metabolic disease, and clinically phenotypically defined monogenic diseases (e.g., tuberous sclerosis complex). The study protocol was approved by the ethical committee of the Qilu Hospital Affiliated to Shandong University (No. 2016(027)) and Linyi people’s hospital Affiliated to Shandong University (No.13003). All guardians signed informed consent forms.

2.2 | Next-generation sequencing (NGS) and DNA sequence analysis

Informed consent and blood samples were obtained from all the participants in the families. Genomic DNA was extracted using QIAamp DNA Blood Mini Kit (Qiagen), according to the manufacturer’s protocol. Each DNA sample is quantified by agarose gel electrophoresis and Nanodrop 2000 (Thermo). Libraries were prepared using Illumina standard protocol. The amplified DNA was captured with whole exome sequencing. The capture experiment was conducted according to the manufacturer’s protocol. The junction sequences were trimmed, and the contamination or low-quality reads were filtered for the raw data. Then, the clean data were aligned to the human reference genome sequence (hg19) by Burrows–Wheeler Alignment. Single nucleotide variation (SNV) and insertion deletion mutation (InDel) were called by Genome Analysis Toolkit. Then, all SNVs and InDels were annotated by ANNOVAR (RRID: SCR_012821). The mutation sites with frequencies less than 0.05 in the normal population database were screened out, including the 1,000 genome project, Exome Variant Server, and Exome Aggregation Consortium. Mutations were predicted by Mutation Taster (MT), Sorting Intolerant From Tolerant (SIFT, RRID: SCR_012813), PolyPhen-2
(PP2, RRID: SCR_013189), Genomic Evolutionary Rate Profiling (GERP++, RRID: SCR_000563), and Clustal-W (RRID: SCR_017277). The selected mutation sites were verified by Sanger sequencing. The analysis of deletions or duplications was performed using low-coverage massively parallel CNV sequencing (CNV-seq). After sequencing, the raw data were saved as a FASTQ format, then followed the bioinformatics analysis: First, Illumina sequencing adapters and low-quality reads (<60bp) were filtered by Cutadapt (1.16) software (RRID: SCR_011841). After quality control, the clean reads were mapped to the UCSC hg19 human reference genome using BWA (0.7.12) software (RRID: SCR_010910). Only uniquely mapped reads were selected. Then, we use GATK (4.0.8.1) (RRID: SCR_001876) Mark Duplicates to remove duplicated reads. Mapped reads were classified into adjustable sliding windows, which were 50 kb in length with 5 kb increments. The coverage of each window was calculated by the read amount and underwent two-step bias correction (GC correction and population-scale normalization). After correction, we use the binary segmentation algorithm to localize the segment breakpoints to identify the candidate CNV regions and determine CNV genotype. Then, we use U test and Parallelism test to estimate the genotype and significance of each segment. All the obtained suspected missing repetitive regions were compared with OMIM (RRID: SCR_006437), GeneReviews (RRID: SCR_006560), Decipher (RRID: SCR_006552), ClinVar (RRID: SCR_006169), Database of Genomic Variants (DGV, RRID: SCR_007000) and other databases. CNV-related genes will also be searched in the Human Phenotype Ontology (HPO, RRID: SCR_006016) database to match similar phenotypes. After the analysis, the data were analyzed for advanced manual analysis, and the suspicious mutation fragments that were highly similar to the clinical phenotype of the proband were selected. Then, real-time quantitative PCR detecting system (qPCR) experiment was conducted to verify this section, so as to exclude false positive of second-generation sequencing and ensure the accuracy of the results.

3 | RESULTS

3.1 | Genetic analyses

We screened a cohort of 492 children with epilepsy for mutations in the PRRT2 gene using whole exome sequencing (WES) and low-coverage massively parallel CNV sequencing (CNV-seq) (281 male and 211 female). We found heterozygous PRRT2-related mutations in 19 patients (19/492, 3.86%), six sporadic cases and 13 family cases. Four point mutations were found in 12 patients (12/19, 63.16%), nine of them were c.649dupC mutation (9/12, 75%), four whole gene deletion (4/19, 21.05%) and three 16p11.2 deletions (3/19, 15.79%) (Table 1 and Figure 1). Three mutations were located in exon 2 and one in exon 3. Two missense mutations (c.640G > C/p.Ala214Pro; c.950G > A/p.Ser317Asn), one nonsense mutation (c.718C > T/p.Arg240*), one frameshift mutation (c.649dupC/p.Arg217Profs*8). Missense mutations all affected amino acids of the cytoplasmic domain of proline-rich transmembrane protein 2 (PRRT2), which are highly conserved in orthologs and in paralogs of PRRT2, and were predicted to be pathogenic by Mutation Taster, Polyphen2, and SIFT (Figure 2, Table 2). Four cases were deletion of whole PRRT2 gene (Table 1, Figure 1 and Figure 3), three were de novo (3/4, 75%), and all the three 16p11.2 deletions were de novo (3/3, 100%) (Figure 4). Six of the 19 probands for PRRT2 mutations were de novo (6/19, 31.58%). The inheritance of PRRT2 mutations in 13 families, family No.2 was four-generation pedigrees, family No.3,5,7 were three-generation pedigrees, and the others were two-generation pedigrees (Figure 5). Five probands inherited the mutation from asymptomatic parents. Six families exhibit incomplete penetrance phenomenon (family No.1: I-1; family No.2: III-1; family No.4: I-1; family No.8: I-1; family No.12: I-2; family No.14: I-1).

3.2 | Clinical features

The onset age of 492 children ranged from one day after birth to eight years. Thirty-two (32/492, 6.5%) patients were diagnosed as BIE, 61 as Febrile convulsion plus, 59 as West syndrome, 41 as Dravet syndrome, 12 as Ohtahara syndrome, four as Lennox–Gastaut syndrome, three as West syndrome evolving to Lennox–Gastaut syndrome, four as Doose syndrome, three as childhood absence epilepsy, two as benign childhood epilepsy with central temporal spikes, and one as early childhood myoclonic epilepsy (ECME). Twenty-nine patients were diagnosed as unclassified epileptic encephalopathy, and 241 patients were diagnosed as unclassified epilepsy. We found PRRT2-related mutations in 19 patients. Among the 19 probands, 10 were males and nine were females. The onset age of the 19 probands ranged from 3 months to 3 years and 2 months. The mode of these 19 patients is six months old, and the median is six months old too. There were 39 patients in these 19 proband families with PRRT2 mutations. The common clinical features of the 39 patients included one ECME, one febrile seizure (FS), six paroxysmal kinesigenic dyskinesias (PKD), two infantile convulsions with paroxysmal choreoathetosis (ICCA), 12 benign infantile epilepsy (BIE), and 17 benign familial infantile epilepsy (BFIE). The clinical information of 39 patients with PRRT2 mutations in 19 proband’s families is summarized in Table 3. The proband with ECME was a boy aged four years and seven months old, the only child of nonconsanguineous parents. The child was born via spontaneous vaginal delivery at 37 weeks gestation, without asphyxia. The patient had normal cranial MRI and developmental quotient, no special family history or personal history, and no regression of development after onset. When the child was three years and two months old, one generalized tonic–clonic seizures (GTCS) occurred without obvious inducement. Six months after that, GTCS occurred when he had a fever. Then, another form of seizure occurred, characterized by a quick shake of the upper limbs or whole body. It occurred several times a day, without other associated symptoms. Interictal electroencephalography (EEG) showed normal background activity and high to very high amplitude spike or polyspike wave discharges at 3–4 Hz. Ictal EEG detected myoclonic
**TABLE 1**  Pathogenicity assessment of PRRT2 mutations

| Family | Mutation type | Position: Chr 16 | Exon | Amino acid changes | Consequence at the protein level | Parents’ analysis | ACMG scoring | ACMG pathogenicity |
|--------|---------------|------------------|------|--------------------|---------------------------------|-------------------|--------------|-------------------|
| 1      | Missense      | 29,825,015       | 2    | c.640G > C         | p.Ala214Pro                    | Paternal          | PS1 + PM2+PP3 | LP                |
| 2      | Frameshift    | 29,825,024       | 2    | c.649dupC          | p.Arg217Profs*8                | Paternal          | PVS1 + PS1+PM2 | P                 |
| 3      | Frameshift    | 29,825,024       | 2    | c.649dupC          | p.Arg217Profs*8                | Maternal          | PVS1 + PS1+PM2 | P                 |
| 4      | Frameshift    | 29,825,024       | 2    | c.649dupC          | p.Arg217Profs*8                | Paternal          | PVS1 + PS1+PM2 | P                 |
| 5      | Frameshift    | 29,825,024       | 2    | c.649dupC          | p.Arg217Profs*8                | Paternal          | PVS1 + PS1+PM2 | P                 |
| 6      | Frameshift    | 29,825,024       | 2    | c.649dupC          | p.Arg217Profs*8                | Paternal          | PVS1 + PS1+PM2 | P                 |
| 7      | Frameshift    | 29,825,024       | 2    | c.649dupC          | p.Arg217Profs*8                | Maternal          | PVS1 + PS1+PM2 | P                 |
| 8      | Frameshift    | 29,825,024       | 2    | c.649dupC          | p.Arg217Profs*8                | Paternal          | PVS1 + PS1+PM2 | P                 |
| 9      | Frameshift    | 29,825,024       | 2    | c.649dupC          | p.Arg217Profs*8                | Maternal          | PVS1 + PS1+PM2 | P                 |
| 10     | Frameshift    | 29,825,024       | 2    | c.649dupC          | p.Arg217Profs*8                | Paternal          | PVS1 + PS1+PM2 | P                 |
| 11     | Nonsense      | 29,825,093       | 2    | c.718C > T         | p.Arg240*                      | Maternal          | PVS1 + PS1+PM2 | P                 |
| 12     | Missense      | 29,825,724       | 3    | c.950G > A         | p.Ser317Asn                    | Maternal          | PS1 + PM2+PP3 | LP                |
| 13     | Large deletion|                   |      | Whole gene del     | Absence of protein synthesis   | De novo           | P             |                   |
| 14     | Large deletion|                   |      | Whole gene del     | Absence of protein synthesis   | Paternal          | P             |                   |
| 15     | Large deletion|                   |      | Whole gene del     | Absence of protein synthesis   | De novo           | P             |                   |
| 16     | Large deletion|                   |      | Whole gene del     | Absence of protein synthesis   | De novo           | P             |                   |
| 17     | Large deletion| 29571922–30211921 |    |                   |                                 | De novo           | P             |                   |
| 18     | Large deletion| 29580565–30199596 |    |                   |                                 | De novo           | P             |                   |
| 19     | Large deletion| 29453235–30318412 |    |                   |                                 | De novo           | P             |                   |

Abbreviations: P, pathogenic; LP, likely pathogenic.
seizures as generalized polyspike wave with a time-locked relation to muscle activation (Figure 6). The whole gene sequencing revealed a heterozygous mutation (c.640G > C), resulting in protein truncation (p.Ala214Pro). Three other variants of likely pathogenic or pathogenic were identified in this patient as well (VWF, SYNGAP1, and ABCB4). SYNGAP1 (c.3964_3965insCCCCCC/p.P1326Tfs*38) and ABCB4 (c.1015dupT/p.S339Ffs*16) variations were not found after one generation verification. VWF (c.5014G > A/p.G1672R) mutation originated from the father of the child. However, neither the child nor the father has hemophilia phenotype, which is inconsistent with the clinical manifestations, so it is not considered as a pathogenic gene mutation. The patient was insensitive to valproate sodium (VPA), levetiracetam (LEV), topiramate (TPM), and clonazepam (CZP).

The onset age of the thirty-one patients with BIE or BFIE ranged from three months to one year and three months, both the mode and median age were six months. Among them, there were 16 patients with onset age of <6 months (16/31, 51.61%), 13 patients with onset of disease at 6–12 months (13/31, 41.94%), and one patient with onset after 12 months (1/31, 3.22%). Remission age ranged from six months to two years and one month. The type of seizures in one patient was unknown, two patients only had generalized tonic–clonic seizures (GTCS) (2/30, 6.67%), 25 patients had focal motor seizures (25/30, 83.33%), three patients had both types of seizures (10%), and 20 patients had seizure clusters (20/29, 68.97%). All patients had normal intelligence, except one patient with 863kb deletions of 16p11.2 who had a mild language delay. All the PKD patients were triggered by sudden movement. All patients had a normal brain MRI. Only one patient had generalized polyspike wave, and five patients had focal transient discharges in interictal EEG. Focal seizures originating in the frontal region were recorded in one patient, two from the temporal region, and two from the occipital region. Most patients were treated effectively with sodium valproate (VPA) or oxcarbazepine (OXC); the child with myoclonic seizures was not sensitive to antiepileptic drugs.

4 | DISCUSSION

PRRT2 is localized to chromosome 16p11.2, with a total length of 3,794 bp. It contains four exons and codes 340 amino acids and is highly expressed in the cerebral cortex, basal ganglia, and cerebellum. PRRT2 is composed of n-terminal sequence rich in proline (n-glycosylation site), two transmembrane domains and c-terminal sequence (Rossi et al., 2016), and its transmembrane
region is highly conserved, suggesting important physiological functions. Studies have shown that PRRT2 has analogues in vertebrates such as humans, gorillas, macaques, and mice, while no homologous products have been found in invertebrates such as nematodes. In humans and rodents, PRRT2 is a neural protein and is most expressed in the cerebellum, basal ganglia, and neocortex. PRRT2 serves as a regulator of the SNARE complex and provides a circuit mechanism underlying the PRRT2-related diseases (Tan et al., 2018). The mutant PRRT2 may affect glutamate signal transduction and glutamate receptor activity through its weak interaction with synaptic proteins SNAP-25, leading to an increase in glutamate release, which in turn leads to overexcitation of neurons (M. Li et al., 2015). PRRT2 is closely related to Ca\(^{2+}\) sensing mechanisms and plays an important role in the final phase of neurotransmitter release by interacting with SNAP-25 and synaptotagmin (Tan et al., 2018; Valente et al., 2016).

**Table 2** Pathogenicity assessment and conservative analysis of 2 missense mutations

| Family | Amino acid changes | Consequence at the protein level | Parents’ analysis | SIFT         | PolyPhen 2   | Mutation Taster | GERP++ |
|--------|-------------------|----------------------------------|-------------------|--------------|--------------|-----------------|--------|
| 1      | c.640G>C          | p.Ala214Pro                      | paternal          | Damaging     | Probably damaging | Polymorphism    | 3.9 (Conserved) |
| 12     | c.950G>A          | p.Ser317Asn                      | maternal          | Damaging     | Probably damaging | Disease causing | 3.75 (Conserved) |
Currently, the clinical phenotypes caused by PRRT2 gene mutation are mainly BFIE, PKD, and ICCA. Other rare phenotypes include hemiplegic migraine (HM) and sporadic benign infant epilepsy (BIE), suggesting phenotypic heterogeneity in PRRT2 mutations. So far, most of the mutations associated with the PRRT2 have been labeled “benign.” However, west syndrome and FS were identified in some PRRT2 families (Djemie et al., 2014; Igarashi et al., 2016), suggesting that the spectrum of PRRT2-related diseases may be broader. To explore the phenotypic boundaries of PPRT2-related mutations, we screened a wide range of benign and severe infantile epilepsy patients. In this study, we found one ECME, one FS, six PKD, two ICCA, 12 BIE, and 17 BFIE in 39 patients of 19 probands’ families. This is the first time PRRT2 mutation has been discovered in ECME. The proband with the onset age of three years and two months had myoclonic seizures and GTCS, and febrile or afebrile GTCS appeared before myoclonic seizures, normal cranial MRI and developmental quotient, 3-4 Hz generalized spike-wave, and polyspike wave on interictal EEG. According to the characteristics of ECME summarized by Yang et al. (2017), we diagnosed the child as ECME. This is the first report of PRRT2

**Figure 3** Quantitative PCR validation of whole PRRT2 gene deletion. Y-axes represent Log R ratio; the X-axis indicates the exon on PRRT2

**Figure 4** Genomic positions of the deletions and phenotypes of the PKD patients with 16p11.2 deletions. Genomic positions of the 16p11.2 deletions in patients with PKD are shown using red bars
mutation in ECME and extends the spectrum of diseases associated with PRRT2.

In our study, five probands of BIE or BFIE families had their first seizure episodes caused by diarrhea (family 5, 8, 11, 12, 16), and some of them had clusters of seizures. We could not figure out whether these patients manifested convulsions with mild gastroenteritis (CwG) or the symptoms were the presentation of BFIE itself at first. CwG is characterized by the following clinical features: infants aged 6 months to 3 years having febrile generalized convulsions induced by mild gastroenteritis; clustering seizures; normal laboratory examination results including electrolytes, blood glucose and cerebrospinal fluid; normal interictal EEG and brain MRI; and good prognosis. As the disease progressed, the children developed unprovoked seizures a few months later that set them apart from the CwG. Moreover, PRRT2 mutations have not been reported in such patients with CwG, which is one of the distinguishing points with PRRT2-related diseases (Ishii et al., 2013). Therefore, when we encounter the first cluster or single seizure induced by mild gastroenteritis in clinical, we should pay attention to the family history and the follow-up prognosis. The patient with family history could choose genetic test to assist diagnosis and prognosis.

The reported mutation types summarized by Darius (Ebrahimi-Fakhari, Saffari, Westenberger, & Klein, 2015) include missense, nonsense, insertion or deletion of bases, splicing, deletion of fragments or whole genes, and deletion of other adjacent genes. In our study, 63.16% patients had reported point mutations inherited from symptomatic or asymptomatic parents, two missense mutations (Heron et al., 2012; Liu et al., 2016), one nonsense (Lee et al., 2012), one frameshift mutation (Chen et al., 2011), and c.649dupC mutation is also the hot spot mutation. Since the majority of PRRT2 mutations are truncating mutations that lead to loss of function or haploinsufficiency of PRRT2, copy number deletions of this gene are suspected of causing PRRT2-related diseases. There were still 21.05% of patients who had whole gene deletion, and 15.79% had 16p11.2 deletions, most of them were de novo. On the contrary, all the point mutations in this study were inherited. It indicates that screening for PRRT2 CNVs is necessary in these patients, especially in sporadic cases. Only one patient with the whole gene deletion inherited from asymptomatic father, PRRT2 point mutations had incomplete extraneous dominance in previous studies, but the whole gene heterozygous deletion had no clinical phenotype, which has not been reported. Chromosome 16p11.2 deletion syndrome is a genetic disorder associated with multiple system abnormalities, including intellectual impairment, language developmental disorders, seizures, psychical and psychological disease, PKD, obesity, hearing loss, and cardiac defects (Li et al., 2018b; Yang et al., 2015). In our study, all three BIE patients with 16p11.2 deletion had focal motor seizures, only one with the largest 16p11.2 deletion of 863kb
| Family | Patient | Gender | Age at exam (y.m) | Type of seizures | Seizures in cluster | Onset age of epilepsy (y.m) | Seizure-free age (y.m) | Onset age of PKD (y.m) | Seizure-free age of PKD (y.m) | Language delay | Onset area of focal seizures | Brain MRI | Interictal EEG | Onset age of focal seizures | The last follow-up | Present age (y.m) | Current AEDs |
|--------|---------|--------|------------------|------------------|---------------------|---------------------------|------------------------|------------------------|---------------------------|---------------|--------------------------|-----------|--------------|-----------------------------|----------------|----------------|-------------|
| 1      | II1     | M      | 4.7              | ECME             | GTCS, ME            | N                         | 3.2                    | -                      | -                         | N             | normal                   | normal     | normal       | -                           | 5.3            | TPM/ VPA/ CEPZ                  |
| 2      | II1     | M      | NA               | BFIE             | GTCS               | NA                        | 0.6                    | 2                      | -                         | N             | NA                       | normal     | normal       | -                           | -              | -                           | N           |
| 2      | III1    | M      | 31               | BFIE             | GTCS Focal         | Y                         | 0.3                    | 1.8                    | -                         | N             | normal                   | normal     | normal       | NA                          | 32             | N                           |
| 2      | IV1     | F      | 3.5              | BFIE             | GTCS Focal         | Y                         | 0.3                    | 1.11                   | -                         | N             | normal                   | normal     | normal       | NA                          | 4.1            | N                           |
| 2      | IV3     | M      | 2.1              | BFIE             | GTCS Focal         | Y                         | 1.5                    | 2.1                    | -                         | N             | normal                   | normal     | normal       | NA                          | 2.9            | OXC                         |
| 3      | I1      | M      | 65               | PKD              | -                   | -                         | -                      | 12                     | SM                       | N             | normal                   | normal     | normal       | -                           | 66             | N                           |
| 3      | II2     | F      | 27               | PKD              | -                   | -                         | -                      | 10                     | SM                       | N             | normal                   | normal     | normal       | -                           | 27             | N                           |
| 3      | III1    | M      | 2.1              | BIE Focal        | N                   | 0.4                       | 0.8                    | -                      | -                         | N             | normal                   | normal     | normal       | NA                          | 2.8            | VPA                         |
| 4      | II2     | F      | 0.6              | BIE Focal        | Y                   | 0.5                       | 0.6                    | -                      | -                         | N             | normal                   | normal     | normal       | Right temporal              | 1.2            | OXC                         |
| 5      | I1      | M      | 35               | FS               | GTCS               | N                         | 1                      | 3                      | -                         | -             | normal                   | normal     | normal       | NA                          | 36             | N                           |
| 5      | II2     | F      | 2.9              | PKD              | -                   | -                         | -                      | 15                     | SM                       | N             | normal                   | normal     | normal       | -                           | 30             | N                           |
| 5      | II2     | F      | 2.9              | BIE Focal        | Y                   | 0.5                       | 0.9                    | -                      | -                         | N             | normal                   | normal     | normal       | -                           | 30             | N                           |
| 6      | I1      | M      | 36               | PKD              | -                   | -                         | -                      | 8                      | SM                       | N             | normal                   | normal     | normal       | -                           | 38             | N                           |
| 6      | II1     | M      | 7.4              | ICCA Focal       | N                   | 0.6                       | 1                      | 7                      | SM                       | N             | normal                   | normal     | normal       | NA                          | 8.10           | OXC                         |
| 7      | II2     | F      | 72               | BFIE NA          | NA                 | 0.6                       | 1                      | -                      | -                         | N             | normal                   | normal     | normal       | NA                          | 73             | N                           |
| 7      | II2     | F      | 33               | BFIE Focal       | Y                   | 0.6                       | 1                      | -                      | -                         | N             | normal                   | normal     | normal       | NA                          | 34             | N                           |
| 7      | II4     | F      | 31               | BFIE Focal       | Y                   | 0.5                       | 1.3                    | -                      | -                         | N             | normal                   | normal     | normal       | NA                          | 32             | N                           |
| 7      | II5     | M      | 28               | ICCA Focal       | Y                   | 0.7                       | 1                      | 6                      | SM                       | N             | normal                   | normal     | normal       | NA                          | 29             | N                           |
| 7      | III1    | F      | 8                | BFIE Focal       | Y                   | 0.5                       | 1.7                    | -                      | -                         | N             | normal                   | normal     | normal       | NA                          | 9.6             | N                           |
| 7      | III2    | M      | 0.5              | BFIE Focal       | Y                   | 0.4                       | 1.2                    | -                      | -                         | N             | normal                   | normal     | normal       | NA                          | 1.11           | OXC                         |
| 8      | II1     | M      | 2.4              | BIE Focal        | Y                   | 0.8                       | 2                      | -                      | -                         | N             | normal                   | normal     | normal       | NA                          | 3.8            | OXC                         |
| 9      | II1     | F      | 32               | BFIE Focal       | N                   | 0.6                       | 1                      | -                      | -                         | N             | normal                   | normal     | normal       | NA                          | 32             | N                           |
| 9      | II1     | M      | 1                | BFIE Focal       | N                   | 0.4                       | 0.8                    | -                      | -                         | N             | normal                   | normal     | normal       | NA                          | 1.4            | LEV                         |
| 10     | II1     | M      | 25               | PKD              | -                   | -                         | -                      | 17                     | SM                       | N             | normal                   | normal     | normal       | -                           | 25             | CBZ                         |

(Continues)
| Family | Patient | Gender | Age at exam (y.m) | Phenotype | Type of seizures | Seizures in cluster | Onset age of epilepsy (y.m) | Seizure-free age (y.m) | Onset age of PKD (y.m) | Trigger | Language delay | Brain MRI | Interictal EEG | Onset area of focal seizures | The last follow-up |
|--------|---------|--------|------------------|-----------|-----------------|---------------------|--------------------------|------------------------|----------------------|---------|--------------|----------|--------------|----------------------------|------------------|
| 10     | II1     | F      | 0.5              | BIE       | Focal           | Y                   | 0.5                      | NA                     | -                    | -       | N            | normal   | N            | normal                      | 0.6              |
| 11     | II2     | F      | 31               | BFIE      | Focal           | N                   | 0.6                      | 1.6                    | -                    | -       | N            | normal   | normal       | NA                          | 32               |
| 11     | II1     | F      | 7                | BFIE      | Focal           | Y                   | 0.4                      | 1                      | -                    | -       | N            | normal   | normal       | NA                          | 8                |
| 11     | II1     | M      | 0.4              | BFIE      | Focal           | Y                   | 0.3                      | 0.6                    | -                    | -       | N            | normal   | normal       | Right frontal              | 1.2              |
| 12     | II1     | F      | 2                | BFIE      | Focal           | N                   | 0.6                      | 0.11                   | -                    | -       | N            | normal   | normal       | NA                          | 2.2              |
| 12     | II2     | M      | 0.6              | BFIE      | Focal           | N                   | 0.5                      | 0.6                    | -                    | -       | N            | normal   | normal       | NA                          | 0.8              |
| 13     | II1     | M      | 0.8              | BIE       | Focal           | Y                   | 0.5                      | 0.11                   | -                    | -       | N            | normal   | normal       | Left occipital              | 3.4              |
| 14     | II1     | F      | 1.2              | BIE       | Focal           | N                   | 1                        | 1.5                    | -                    | -       | N            | normal   | normal       | NA                          | 4.5              |
| 15     | II1     | F      | 2.5              | BIE       | Focal           | N                   | 0.8                      | 1.8                    | -                    | -       | N            | normal   | normal       | NA                          | 3.6              |
| 16     | II1     | F      | 25               | PKD       | -               | -                   | -                        | 18                     | SM                   | N       | normal       | normal   | -            | 25                          | N                |
| 16     | II1     | M      | 0.6              | BIE       | Focal           | 0.5                 | NA                       | -                      | -                    | N       | normal       | FD        | Left occipital | 0.7                        | OXC              |
| 17     | II1     | M      | 0.6              | BIE       | Focal           | Y                   | 0.5                      | 1                      | -                    | -       | N            | normal   | FD           | NA                          | 2.7              |
| 18     | II1     | F      | 1.2              | BIE       | Focal           | Y                   | 0.6                      | 1.3                    | -                    | -       | N            | normal   | normal       | NA                          | 1.6              |
| 19     | II1     | F      | 1.6              | BIE       | Focal           | Y                   | 0.7                      | 1                      | -                    | mild    | normal       | FD        | normal       | NA                          | 1.9              |

Abbreviations: AEDs, antiepileptic drugs; BFIE, benign familial infantile epilepsy; BIE, benign infantile epilepsy; CBZ, carbamazepine; CDP, clonazepam; F, female; FD, focal discharge; Focal, focal motor seizures; FS, febrile seizures; GSW, generalized polyspike wave; GSW, generalized spike-wave; GTCs, generalized tonic-clonic seizures; ICCA, infantile convulsions with paroxysmal choreoathetosis; LEV, levetiracetam; M, male; ME, myoclonic seizures; N, none; NA, Not available; OXC, oxcarbazepine; PKD, paroxysmal kinesigenic dyskinesias; SM, sudden movement; VPA, sodium valproate; Y, yes; y.m, years.months.
had mild language delay, which indicates that pure BIE or with mild language delay can be a phenotype of 16p11.2 deletion syndrome that extending the phenotype of 16p11.2 deletions to typical BIE/BCIE. Screening for 16p11.2 deletions should be prescribed for patients with BIE, particularly in sporadic cases, although it is not common. A case of BIE and normal neurodevelopment in a child with a loss of 1.064 MB 16p11.2 was reported (Milone, Valetto, Bertini, & Sicca, 2017), with regression leading to autism, intelligence retardation, and language impairment at 18 months of age. We had not found the autism or intellect regression in the other two children with 16p11.2 deletions of our study who had excellent seizure-free and developmental outcomes as followed up at 18 months to 31 months old. But, the patients with 16p11.2 deletions still need long-term clinical follow-up.

5 | CONCLUSIONS

In conclusion, PRRT2 mutations can be inherited or de novo. PRRT2 mutation-related epilepsy has incomplete penetration rate and phenotypic heterogeneity. The clinical spectrum of PRRT2 mutation includes BIE, BFIE, ICCA, PKD, FS, and ECME. The PRRT2-related mutations contained point mutations, whole gene deletion, and 16p11.2 deletions. Point mutations were mainly inherited, and large microdeletion mutations were mostly de novo. Screening for 16p11.2 deletions should be prescribed for patients with BIE, particularly in sporadic cases. Our report expands the mutation and clinical spectrum of PRRT2-related epilepsy.

ACKNOWLEDGEMENTS

This study was supported by the foundation of National Key Research and Development Program of China (NO. 2016YFC1306202), Key Research and Development Plan in Shandong Province (NO. 2016GSF201073), General program of Qilu Hospital Affiliated to Shandong University (NO.2015QLM508), Science and Technology Development Plan of Shandong Province (NO.2018WS399), Science and Technology Development Plan of Linyi (NO.201818009), China Postdoctoral Science Foundation (NO.2016M592198), Talent training foundation of National Science Foundation of Shandong, China (NO.ZR2015PH014), National Natural Science Foundation of China (NO. 81601195), and Shandong Provincial Natural Science Foundation (NO. ZR2016HB38). We thank the patients and their family members for taking part in this study. Thanks to Rowan Coates for his guidance on English grammar.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

LY and BML were responsible for the original concept and the overall design of the research. LY, SYQ, XFY, YFL, LYX, JLY, and BML analyzed the EEG results and diagnosed patients. XL, LY, NX, DQZ, and YN collected the clinical data and sample. LY, CPY, and FL carried the experiments and analyzed the sequencing data. LY, CPY, JLY, and BML wrote and revised the manuscript. All authors read and approved the final manuscript.
REFERENCES
Al-Jawahiri, R., Jones, M., & Milne, E. (2019). Atypical neural variability in carriers of 16p11.2 copy number variants. Autism Research, https://doi.org/10.1002/aur.2166
Castelein, L., Steyaert, J., Peeters, H., & van Buggenhout, G. (2019). Adolescent with a schizophreniaiform disorder and recurrent 16p11.2 duplication. Tijdschrift Psychiatie, 61(6), 421–425.
Castelgrovo, N., Renard, D., De Verdal, M., Luc, J., Thouvenot, E., & Riant, F. (2016). Progressive ataxia related to PRRT2 gene mutation. Journal of the Neurological Sciences, 367, 220–221. https://doi.org/10.1016/j.jns.2016.05.058
Chen, W.-J., Lin, Y. U., Xiong, Z.-Q., Wei, W., Ni, W., Tan, G.-H., ... Wu, Z.-Y. (2019). Microdeletions detected using chromosome microarray in children with suspected genetic movement disorders: A single-centre study. Developmental Medicine and Child Neurology, 54(7), 618–623. https://doi.org/10.1111/j.1469-8749.2012.04287.x
Delcourt, M., Riant, F., Mancini, J., Milh, M., Navarro, V., Roze, E., ... Roubertie, A. (2015). Severe phenotypic spectrum of biallelic mutations in PRRT2 gene. Journal of Neurology, Neurosurgery and Psychiatry, 86(7), 782–785. https://doi.org/10.1136/jnnp-2014-309025
Djemie, T., Weckhuysen, S., Holmgren, P., Hardies, K., Van Dyck, T., Hendrickx, R., ... Suls, A. (2014). PRRT2 mutations: Exploring the phenotypical boundaries. Journal of Neurology, Neurosurgery and Psychiatry, 85(4), 462–465. https://doi.org/10.1136/jnnp-2013-305122
Ebrahimi-Fakhari, D., Kang, K. S., Kotzaeridou, U., Kohlhase, J., Klein, C., & Assmann, B. E. (2014). Child Neurology: PRRT2-associated movement disorders and differential diagnoses. Neurology, 83(18), 1680–1683. https://doi.org/10.1212/wnl.0000000000000936
Ebrahimi-Fakhari, D., Saffari, A., Westenberger, A., & Klein, C. (2015). The evolving spectrum of PRRT2-associated paroxysmal diseases. Brain, 138(Pt 12), 3476–3495. https://doi.org/10.1093/brain/awv331
Fabbri, M., Marini, C., Bisulli, F., Di Vito, L., Elia, A., Guerrini, R., ... Tinuper, P. (2013). Clinical and polygraphic study of familial paroxysmal kinesigenic dyskinesia with PRRT2 mutation. Epileptic Disorders, 15(2), 123–127. https://doi.org/10.1684/epd.2013.0569
Heron, S. E., Grinton, B. E., Kivity, S., Aflawi, Z., Zuberi, S. M., Hughes, J. N., ... Dibbens, L. M. (2012). PRRT2 mutations cause benign familial infantile epilepsy and infantile convulsions with choreothetosis syndrome. American Journal of Human Genetics, 90(1), 152–160. https://doi.org/10.1016/j.ajhg.2011.12.003
Hinkley, L. B. N., Dale, C., Luks, T. L., Findlay, A. M., Bukshpun, P., Pojman, N., ... Nagarajan, S. S. (2019). Sensorimotor cortical oscillations during movement preparation in 16p11.2 deletion carriers. Clinical Epigenetics, https://doi.org/10.1186/s13480-019-0684-310.523/jneurosci.3001-17.2019
Igarashi, A., Okumura, A., Shimojima, K., Abe, S., Ikeno, M., Shimizu, T., & Yamamoto, T. (2016). Focal seizures and epileptic spasms in a child with Down syndrome from a family with a PRRT2 mutation. Brain and Development, 38(6), 597–600. https://doi.org/10.1016/j.braindev.2015.12.011
Ishi, A., Yasumoto, S., Ihara, Y., Inoue, T., Fujita, T., Nakamura, N., ... Hirose, S. (2013). Genetic analysis of PRRT2 for benign infantile epilepsy, infantile convulsions with choreothetosis syndrome, and benign convulsions with mild gastrointestinal. Brain and Development, 35(6), 524–530. https://doi.org/10.1016/j.braindev.2012.09.006
Labate, A., Tarantino, P., Viri, M., Mumoli, L., Gagliardi, M., Romeo, A., ... Gambardella, A. (2012). Homozygous c.649dupC mutation in PRRT2 worsens the BFIS/PKD phenotype with mental retardation, episodic ataxia, and absences. Epilepsia, 53(12), e196–199. https://doi.org/10.1111/j.epi.12009
Lee, H.-Y., Huang, Y., Bruneau, N., Rolli, P., Roberson, E. D. O., Hermann, M., ... Pratćek, L. J. (2012). Mutations in the gene PRRT2 cause paroxysmal kinesigenic dyskinesia with infantile convulsions. Cell Reports, 1(1), 2–12. https://doi.org/10.1016/j.celrep.2011.11.001
Legris, N., Chassin, O., Nasser, G., Riant, F., Tournier-Lasserve, E., & Denier, C. (2019). Acute-onset ataxia and transient cerebellar diffusion restriction associated with a PRRT2 mutation. Journal of Stroke and Cerebrovascular Diseases: the Official Journal of National Stroke Association, 28(2), e3–e4. https://doi.org/10.1016/j.jstrokecerebrovasdis.2018.10.021
Li, M., Niu, F., Zhu, X., Wu, X., Shen, N., Peng, X., & Liu, Y. (2015). PRRT2 mutant leads to dysfunction of glutamate signaling. International Journal of Molecular Sciences, 16(5), 9134–9151. https://doi.org/10.3390/ijms16059134
Li, W., Wang, Y., Li, B., Tang, B., Sun, H., Lai, J., ... Liu, X. (2018a). 16p11.2 deletion in patients with paroxysmal kinesigenic dyskinesia but without intellectual disability. Brain and Behavior, 8(11), e01134. https://doi.org/10.1002/brb3.1134
Li, W., Wang, Y., Li, B., Tang, B., Sun, H., Lai, J., ... Liu, X. (2018b). 16p11.2 deletion in patients with paroxysmal kinesigenic dyskinesia but without intellectual disability. Journal of Medical Genetics, 8(11), e01134. https://doi.org/10.1136/jmedgenet-2018-10547710.002/brb3.1134
Liu, X. R., Huang, D., Wang, J., Wang, Y. F., Sun, H., Tang, B., ... Liao, W. P. (2016). Paroxysmal hypnogenic dyskinesia is associated with mutations in the PRRT2 gene. Neurology Genetics, 2(2), e66. https://doi.org/10.1210/nneg.201600066
Maini, I., Iodice, A., Spagnoli, C., Salerno, G. G., Bertani, G., Frattini, D., & Fusco, C. (2016). Expanding phenotype of PRRT2 gene mutations: A new case with epilepsy and benign myoclonus of early infancy. European Journal of Paediatric Neurology, 20(3), 454–456. https://doi.org/10.1016/j.ejpn.2016.01.010
Mathot, M., Lederer, D., Gerard, S., Gueulette, E., & Deprez, M. (2017). PRRT2 mutation and infantile convulsions. Archives De Pediatrie, 24(10), 1010–1012. https://doi.org/10.1016/j.arcped.2017.08.004
Miline, R., Valetto, A., Bertini, V., & Sicca, F. (2017). Benign infantile seizures followed by autistic regression in a boy with 16p11.2 deletion. Epileptic Disorder, 19(2), 222–225. https://doi.org/10.1684/epd.2017.0909
Okumura, A., Shimojima, K., Kurahashi, H., Numoto, S., Shimada, S., Ishii, A., ... Yamamoto, T. (2019). PRRT2 mutations in Japanese patients with benign infantile epilepsy and paroxysmal kinesigenic dyskinesia. Seizure, 71, 1–5. https://doi.org/10.1016/j.seizure.2019.05.017
Rossi, P., Sterlini, B., Castroflorio, E., Marte, A., Onofri, F., Valtorta, F., ... Benfenati, F. (2016). A novel topology of proline-rich transmembrane protein 2 (PRRT2): Hints for an intracellular function at the synapse. Journal of Biological Chemistry, 291(12), 6111–6123. https://doi.org/10.1074/jbc.M115.683888
Silveira-Moriyama, L., Kovac, S., Kurian, M. A., Houlden, H., Lees, A. J., Walker, M. C., ... Warner, T. T. (2018). Phenotypes, genotypes, and the management of paroxysmal movement disorders. Developmental Medicine and Child Neurology, 60(6), 559–565. https://doi.org/10.1111/dmcn.13744

Siu, M. T., Butcher, D. T., Turinsky, A. L., Cytrynbaum, C., Stavropoulos, D. J., Walker, S., ... Weksberg, R. (2019). Functional DNA methylation signatures for autism spectrum disorder genomic risk loci: 16p11.2 deletions and CHD8 variants. Clinical Epigenetics, 11(1), 103. https://doi.org/10.1186/s13148-019-0684-3

Tan, G.-H., Liu, Y.-Y., Wang, L. U., Li, K., Zhang, Z.-Q., Li, H.-F., ... Xiong, Z.-Q. (2018). PRRT2 deficiency induces paroxysmal kinesigenic dyskinesia by regulating synaptic transmission in cerebellum. Cell Research, 28(1), 90–110. https://doi.org/10.1038/cr.2017.128

Termsarasab, P., Yang, A. C., Reiner, J., Mei, H., Scott, S. A., & Frucht, S. J. (2014). Paroxysmal kinesigenic dyskinesia caused by 16p11.2 microdeletion. Tremor and Other Hyperkinet Movements (NY), 4, 274. https://doi.org/10.7916/d8n58k0q

Valente, P., Castroflorio, E., Rossi, P., Fadda, M., Sterlini, B., Cervigni, R. I., ... Benfenati, F. (2016). PRRT2 is a key component of the Ca(2+)-dependent neurotransmitter release machinery. Cell Reports, 15(1), 117–131. https://doi.org/10.1016/j.celrep.2016.03.005

Weber, A., Kohler, A., Hahn, A., Neubauer, B., & Muller, U. (2013). Benign infantile convulsions (IC) and subsequent paroxysmal kinesigenic dyskinesia (PKD) in a patient with 16p11.2 microdeletion syndrome. Neurogenetics, 14(3–4), 251–253. https://doi.org/10.1007/s10048-013-0376-7

Yang, M. U., Mahrt, E. J., Lewis, F., Foley, G., Portmann, T., Dolmetsch, R. E., ... Crawley, J. N. (2015). 16p11.2 deletion syndrome mice display sensory and ultrasonic vocalization deficits during social interactions. Autism Research, 8(5), 507–521. https://doi.org/10.1002/aur.1465

Yang, Z., Xue, J., Li, H., Qian, P., Liu, X., Jiang, Y., & Zhang, Y. (2017). Early childhood myoclonic epilepsy: An independent genetic generalized epilepsy with myoclonic seizures as the main seizure type. Clinical Neurophysiology, 128(9), 1656–1663. https://doi.org/10.1016/j.clinph.2017.06.244

Zhang, Y., Li, L., Chen, W., Gan, J., & Liu, Z. G. (2017). Clinical characteristics and PRRT2 gene mutation analysis of sporadic patients with paroxysmal kinesigenic dyskinesia in China. Clinical Neurology and Neurosurgery, 159, 25–28. https://doi.org/10.1016/j.clineuro.2017.05.004

Zhao, S. Y., Li, L. X., Chen, Y. L., Chen, Y. J., Liu, G. L., Dong, H. L., ... Wu, Z. Y. (2019). Functional study and pathogenicity classification of PRRT2 missense variants in PRRT2-related disorders. https://doi.org/10.1016/j.j.seizure.2019.05.01710.1111/cns.13147

Zheng, W., Zhang, J., Deng, X., Xiao, J., Yuan, L., Yang, Y., ... Deng, H. (2016). Identification of a premature termination mutation in the proline-rich transmembrane protein 2 gene in a chinese family with febrile seizures. Molecular Neurobiology, 53(2), 835–841. https://doi.org/10.1007/s12035-014-9047-4

How to cite this article: Yang L, You C, Qiu S, et al. Novel and de novo point and large microdeletion mutation in PRRT2-related epilepsy. Brain Behav. 2020;10:e01597. https://doi.org/10.1002/brb3.1597