INTRODUCTION

PRRT2 was recently identified as a causative gene of paroxysmal kinesigenic dyskinesia (PKD) and other paroxysmal disorders, including benign familial infantile seizures (BFIS), infantile convulsions with choreoathetosis (ICCA), and paroxysmal hypnogenic dyskinesia (PHD). Among these PRRT2-related disorders, PKD is the most common phenotype, which is a dominantly hereditary disorder characterized by short and recurrent attacks triggered by a sudden initiation or alteration of voluntary movement. Age at onset (AAO) is usually during childhood or early adulthood. The primary manifestations are chorea or dystonia with duration <1 minute and...
a variable frequency ranging from one per month to hundreds per day. Incomplete penetrance is usually observed in PRRT2 variant carriers.\(^6,7\)

The potential mechanisms of PRRT2 variants in PRRT2-related disorders remain largely unclear. PRRT2 consists of four exons and encodes a 340-amino-acid protein with two predicted transmembrane (TM) domains in the C-terminal and one proline-rich domain in the N-terminal. Enriched in cerebral cortex, cerebellum, substantia nigra, and hippocampus, PRRT2 protein was found to involve in synaptic transmission by modulating soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) complex.\(^8\) To date, more than 90 variants in PRRT2 have been included in human gene mutation database (HGMD) and most of them are nonsense or frameshift variants, causing the truncation of the protein. Among them, c.649dupC (p.R217Pfs*8) is the most frequent variant, accounting for 78.5% of mutation carriers.\(^9\) Truncated variants leading to conspicuous reduced protein level have been reported in various \textit{in vitro} studies.\(^3,8,10\) Besides the truncated variants, about 29 missense variants of PRRT2 are documented in PRRT2-related disorders. However, only three of them has been functionally studied.\(^11,13\) Therefore, the clinical significance of these alleles of PRRT2 in paroxysmal disorders is difficult to evaluate. It is of great value to perform the functional studies to address the pathogenicity of PRRT2 missense variants.

In this study, we summarized the reported missense variants in PRRT2 and performed functional experiments to investigate the alternation of subcellular location and protein expression of PRRT2 missense variants. We further assigned the pathogenicity of the missense variants according to the guidelines of American College of Medical Genetics and Genomics (ACMG).\(^14\)

## 2 | MATERIALS AND METHODS

### 2.1 | Missense variants in PRRT2

To identify all the reported missense variants within PRRT2, we searched the HGMD (http://www.hgmd.org) that provided the most up-to-date version of PRRT2 mutation and the PubMed (https://www.ncbi.nlm.nih.gov/pubmed) from November 2011 when PRRT2 was first reported as a disease-causing gene and up to December 2017. The genotype-phenotype correlation analysis was carried out after a comprehensive literature review. Three in silico tools, SIFT (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and MutationTaster (http://www.mutationtaster.org/), were applied to predict the functional impact of the missense variants. Population databases including 1000 Genomes Project (1000G) (http://browser.1000genomes.org) and Exome Aggregation Consortium (http://exac.broadinstitute.org/) were used to obtain the frequency of variants in populations. The frequency was also evaluated in 2800 healthy individuals of Chinese ancestry (Data from Novogene Company). After that, the pathogenicity of PRRT2 missense variants was preliminarily evaluated according to the ACMG guidelines.

### 2.2 | Plasmid constructs

GenBank sequence NG_032039.1 was used as reference for the PRRT2 gene, while reference sequences NM_145239.2 and NP_660282.2 were used for PRRT2 cDNA and protein, respectively. Based on homologous recombination technology, the 1023-bp PRRT2 open reading frame was cloned into HindIII/KpnI site of pEGFP-C2 vector using ClonExpress II One Step Cloning Kit (Vazyme). After that, the variants were introduced by PCR-mediated mutagenesis with KOD DNA polymerase (Toyobo), followed by confirmation using Sanger sequencing. The primers for mutagenesis plasmids were provided in Table S1.

### 2.3 | Cell culture and transient transfection

HEK293T cells and HeLa cells were cultured in DMEM (HyClone) supplemented with 10% fetal bovine serum (Gibco) in a 5% CO\(_2\) incubator at 37°C. Cells were seeded in suitable wells the day before transfection. Transient transfection was performed using the Lipofectamine 3000 according to the manufacturer’s protocol (Invitrogen). Forty-eight hours of cultivation was needed for the protein expression after transfection.

### 2.4 | Western blot analysis

To get the protein lysate, cells were rinsed with phosphate buffer saline (PBS) and harvested in lysis buffer. After centrifuging, the supernatants were collected. Western blot was performed as previously described.\(^15\) The GFP (1:5000) and β-actin (1:5000; Sigma-Aldrich) antibodies were used. The blots were semiquantified by gel densitometry using the Photoshop.

### 2.5 | Live cell imaging

Growing in 35 mm glass-bottomed dishes (Shengyou Biotechnology), HeLa cells were transfected with wild-type or mutant PRRT2 expression plasmids. Forty-eight hours after transfection, NucBlue Live Reagent (Thermo Fisher Scientific) and Alexa Fluor 594 wheat germ agglutinin (WGA; Invitrogen) were added and incubated for 20 and 10 minutes, respectively. After washing, cells were directly visualized under a confocal microscope (Leica TCS SP8; Leica Microsystems). The green-fluorescent IOD (integrated optic density) of the whole cell and the cytoplasm was measured by ImageJ.

### 2.6 | Statistical analyses

All the experiments were repeated at least three times independently. For the Western blot, proteins were normalized to β-actin. Differences in the mean values between wild-type or mutant PRRT2 were analyzed by one-way ANOVA using GraphPad Prism software. \(P\) value <0.05 was considered statistically significant.
| Variant | Phenotype | ExAC | 1000G | Polyphen-2 | SIFT | MutationTaster | Final Classification |
|---------|-----------|------|-------|------------|------|----------------|---------------------|
| c.412C>G (p.P138A) | PKD, FS+, DS, GEFS+, BECTS | 0.03 | 0.0009088 | 0.071277 | Tolerated | Benign Polymorphism | Benign Normal Benign |
| c.439G>C (p.D147H) | PKD, GEFS+, BECTS | 0.003 | 0.000536 | 0.0008798 | Tolerated | Benign Polymorphism | Benign Normal Benign |
| c.529G>A (p.E177K) | ID | 0 | 0 | 0 | Deleterious | Disease causing | US Normal US |
| c.623C>A (p.S208Y) | DS | 0.0002 | 9.61E-05 | 0.000354 | Tolerated | Benign Polymorphism | US Normal Likely benign |
| c.640G>C (p.A214P) | PKD, PHD | 0 | 0.001515 | 0.003715 | Tolerated | Probably damaging Polymorphism | US Normal Likely benign |
| c.644C>G (p.P215R) | PKD | 0.001 | 0.0007764 | 0 | Tolerated | Benign Polymorphism | US Normal Likely benign |
| c.647C>T (p.P216L) | PKD | 0.002 | 0.0007705 | 0 | Tolerated | Possibly damaging | Disease causing US Normal |
| c.647C>G (p.P216R) | PKD | 0 | 0.0003953 | 0 | Deleterious | Disease causing | US Normal |
| c.647C>A (p.P216H) | PKD | 0 | 0.000536 | 0 | Deleterious | Disease causing | US Normal |
| c.796C>T (p.R266W) | PKD | 0 | 8.28E-06 | 0 | Deleterious | Disease causing | US Normal |
| c.797G>A (p.R266Q) | PKD | 0 | 1.66E-05 | 0 | Deleterious | Disease causing | US Normal |
| c.824C>T (p.S275F) | PKD, BFIS | 0 | 0 | 0 | Deleterious | Disease causing | US Abnormal Likely pathogenic |
| c.836C>T (p.P279L) | BS, movement disorder | 0 | 0 | 0 | Deleterious | Disease causing | US Abnormal Likely pathogenic |
| c.841T>C (p.W281R) | PKD, PNS, BIECA, PNS | 0 | 0 | 0 | Deleterious | Disease causing | US Normal |
| c.859G>A (p.A287T) | PKD, EA | 0 | 0.0003953 | 0 | Deleterious | Disease causing | US Normal |
| c.872C>T (p.A291V) | PKD | 0 | 0 | 0 | Deleterious | Disease causing | US Abnormal Likely pathogenic |
| c.884G>A (p.R295Q) | PKD, PNKD, Migraine | 0 | 0 | 0 | Deleterious | Disease causing | US Abnormal Likely pathogenic |
| c.913G>A (p.G305R) | PKD, EA, IC, EA | 0 | 0.0001024 | 0 | Deleterious | Disease causing | US Normal |
| c.916G>A (p.A306T) | BFIS | 0 | 0 | 0 | Deleterious | Disease causing | US Abnormal Likely pathogenic |
| c.917G>A (p.A306D) | PKD | 0 | 0 | 0 | Deleterious | Disease causing | US Abnormal Likely pathogenic |
| c.922C>T (p.R308C) | PKD | 0 | 0 | 0 | Deleterious | Disease causing | US Abnormal Likely pathogenic |
| c.931C>T (p.R311W) | PKD, EA | 0 | 0 | 0 | Deleterious | Disease causing | US Normal |
| c.950G>A (p.S317N) | ICCA | 0 | 0 | 0.000177 | Deleterious | Disease causing | US Normal |
| c.967G>A (p.G323R) | PKD | 0 | 0 | 0 | Deleterious | Disease causing | US Abnormal Likely pathogenic |
| c.968G>A (p.G323E) | BFIS | 0 | 0 | 0 | Deleterious | Disease causing | US Abnormal Likely pathogenic |
| c.970G>A (p.G324R) | ICCA | 0 | 0 | 0 | Deleterious | Disease causing | US Abnormal Likely pathogenic |
| c.971G>A (p.G324E) | BFIS | 0 | 0 | 0 | Deleterious | Disease causing | US Abnormal Likely pathogenic |
| c.981C>G (p.I327M) | PKD, BFIS | 0 | 0 | 0.000177 | Deleterious | Disease causing | US Normal |

**Abbreviations:** 1000G, 1000 Genomes; BECTS, Benign epilepsy with centrotemporal spikes; BS, Benign familial idiopathic seizures; Control, 2800 healthy individuals of Chinese ancestry; DS, Dravet syndrome; EA, Epileptic ataxia; ExAC, Exome Aggregation Consortium; FS+, Febrile seizures plus; GEFS+, Generalized epilepsy with febrile seizures plus; ICCA, Infantile convulsions and choreoathetosis; ID, Intellectual Disability; PNKD, Paroxysmal nonkinesiogenic dyskinesia; PNS, Paroxysmal kinesiogenic dyskinesia; PKD, Paroxysmal kinesiogenic dyskinesia; US, Uncertain significance.

**Note:** Missense variants were preliminarily classified without the functional data according to ACMG guidelines. The decreased or abnormally localized mutant protein was considered functionally impaired.

**Compound heterozygous missense variant in PRRT2 had been observed.**

**Homzygous missense variant in PRRT2 had been observed.**

**Uncertain significance.**
3 | RESULTS

3.1 Overview of the missense variants in PRRT2

After a systematic review of PRRT2-related disorders, we found a total of 29 missense variants in the literature (Table 1). Of which, 26 were heterozygous, two (p.P279L and p.R311W) were homozygous, and one (p.G305R) was described in both homozygous and heterozygous condition.16 The predominant phenotype of these missense variants was PKD, while BFIS and ICCA were also documented.

Two variants (p.P138A and p.D147H) had a minor allele frequency (MAF) ≥0.05 in a population database17 and were predicted to be benign by functional software (Table 1). Another variant (p.P216L) was found to possess 5.2% of 115 controls in an Australian study,18 although it was predicted to be deleterious (Table 1). Of note, these three variants were not co-segregated with the disease in the family pedigrees reported previously.17 In addition, eight variants (p.R266W, p.S275F, p.A291V, p.G305W, p.A306T, p.G324R, p.G324E, and p.I327M) were reported to co-segregate with PKD or BFIS in multiple affected family members.18-24 They were predicted to be deleterious by SIFT, Polyphen-2, and MutationTaster. All the eight variants but one (p.G305R) were absent in population database and our control. However, they were classified as uncertain significance variants for lack of sufficient evidence. Other 18 variants were also classified as uncertain significance variants combining the population MAF and prediction data.

3.2 Missense variants decreased the protein level

For these variants with uncertain significance, functional experiments were required. To determine the consequence of amino acid change in PRRT2, the expression level of mutant PRRT2 was evaluated in mammalian cells. HEK293T cells were transiently transfected with N-terminal EGFP tagged wild-type or mutant PRRT2. The protein expression levels were examined by Western blot. The data revealed that 12 variants (p.P279L, p.W281R, p.A287T, p.R295Q, p.G305W, p.A306T, p.R308C, p.S317N, p.G323R, p.G323E, p.G324R, and p.G324E) had a dramatic reduced expression or hardly detectable protein level compared to wild-type PRRT2 (Figure 1). The remaining 17 variants, including the benign ones, had undifferentiated expression of PRRT2 protein as wild-type (Figure 1).

3.3 Missense variants affected plasma membrane localization

To further characterized the subcellular distribution of mutant PRRT2 harboring missense variants, HeLa cells were transiently transfected with N-terminal EGFP tagged wild-type or mutant PRRT2 expression plasmids. Under live cell confocal microscopy, we found that wild-type PRRT2 was predominantly localized in the plasma membrane, consistent with previously studies.1 In contrast, 13 mutant PRRT2 (p.S275F, p.P279L, p.W281R, p.A287T, p.A291V, p.G305W, p.A306T, p.A306D, p.S317N, p.G323R, p.G323E, p.G324R, and p.G324E) lost membrane targeting and were located in the cytoplasm (Figure 2A,B), indicating the alternation of subcellular localization of these missense variants. The remaining 16 variants were still retained in plasma membrane (Figure 2A,B). The red-fluorescent labeling plasma membrane was shown in the Figure S1.

As expected, the three benign variants (p.P138A, p.D147H, and p.P216L) behaved normally in the in vitro functional analysis. Most of uncertain significance variants (15/26) had reduced protein expression or alteration of plasma membrane localization, suggesting functional impairment of these variants. Among them, 10 variants had abnormal both protein expression and intracellular localization, three variants only affected membrane localization, and two variants only affected the protein level.

**FIGURE 1** Protein levels of wild-type and mutant PRRT2. A, Western blot analysis of protein extracts obtained from HEK293 cells transfected with pEGFP-PRRT2 wild-type or mutant vectors. The anti-GFP antibody was used to detect the PRRT2 protein. B, Values represent mean ± SE, n = 3. *P < 0.05, **P < 0.01 vs wild-type
FIGURE 2 Localization of wild-type and mutant PRRT2. A, HeLa cells transfected with wild-type and mutant PRRT2 vectors were examined for green fluorescence 48 h after transfection by a confocal microscope. Cell nuclei were stained with NucBlue Live Reagent (blue). Scale bar, 5 μm. B. The relative IOD ratio of membrane to cytoplasm was calculated by five cells for each variant. Values represent mean ± SE. ***P < 0.001 vs wild-type
3.4 Classification of the missense variants in PRRT2

Decreased protein expression or alteration of subcellular localization was considered functionally impaired. We assigned the pathogenicity of the reported missense variants of PRRT2 with functional data. As a result (Table 1), 15 variants (p.S275F, p.P279L, p.W281R, p.A287T, p.A291V, p.R295Q, p.G305W, p.A306T, p.A306D, p.R308C, p.S317N, p.G323R, p.G323E, p.G324R, and p.G324E) were classified as "likely pathogenic variants", 3 (p.P138A, p.D147H, and p.P216L) as "benign variants", 3 (p.S208Y, p.A214P, and p.P215R) as "likely benign variants" and 8 (p.E177K, p.P216R, p.P216H, p.R266W, p.W281R, p.R311W, and p.I327M) as "uncertain significance".

A graphical representation of amino acid change in PRRT2 was shown in Figure 3. We found that missense variants were clustered in exon 2 and exon 3 of PRRT2. The likely pathogenic variants marked in red were concentrated in the C-terminal of PRRT2.

4 DISCUSSION

PRRT2 variants are associated with various paroxysmal disorders, implying a common pathway may be involved in these disorders. Thus, it is specifically necessary to elucidate the potential mechanisms of PRRT2 variants. In addition to frameshift and nonsense mutations, 29 missense variants were also documented in PRRT2-related disorders. Lack of functional experiments in majority of the missense variants makes their pathogenicity uncertain. In this study, we systematically reviewed the PRRT2-related disorders and summarized the reported PRRT2 missense variants in the literature. We performed functional experiments to investigate the subcellular localization and protein expression of 29 PRRT2 missense variants. Ten variants were found to affect both plasma membrane localization and protein level, three variants only affect membrane localization, and two variants only affect the protein level. Combining the population MAF, prediction, segregation data, and functional experiments, we classified these missense variants, respectively. To our knowledge, this is the first study to systematically evaluate the significance of PRRT2 missense variants according to the ACMG guidelines.

Previous studies revealed that PRRT2 variants modified the phenotype of PKD and caused earlier age at onset, longer duration, and higher frequency of complicated PKD attacks. Although the potential mechanism of PRRT2 variants in PKD was largely unknown, loss-of-function leading to haploinsufficiency was believed to play a crucial role in the pathogenesis. Recently, several studies uncovered the pathophysiological mechanism of PRRT2. It was demonstrated that PRRT2 was a transmembrane protein localizing primarily to the presynaptic terminals. PRRT2 involved in synaptic vesicle fusion and the release of neurotransmitters through interacting with the synaptic proteins SNAP25 and synaptotagmin 1/2. Moreover, electrophysiological study showed that PRRT2 controlled the excitability of excitatory neurons by interacting with Nav1.2/Nav1.6 channels.
It is widely known that the membrane localization of PRRT2 is crucial for its physiological function. We found 51.7% (15/29) of the PRRT2 missense variants affected subcellular localization and/or protein level, which was consistent with the hypothesis of loss-of-function in PRRT2-related diseases. The reduced protein level of mutant protein p.R308C and the cytoplasma location of mutant protein p.A287T has also been reported previously. The p.G305W has been reported to disrupt the SNARE-modulatory function. In our study, p.G305W was also functionally impaired by abnormal subcellular localization and protein level. 20.7% (6/29) of variants were benign or likely benign variants. 27.6% (8/29) of the variants were uncertain significance variants and their pathogenicity needs further investigation. Disease-causing genetic mutations are waiting to be discovered in these patients with benign PRRT2 variants or without PRRT2 variants.

The majority of reported PRRT2 variants are frameshift variants and cause truncation of PRRT2 protein, indicating the crucial role of the C-terminal. In this study, we found all the likely pathogenic missense variants were localized in the TM domains and loop domain of C-terminal, while the benign or likely benign missense variants were localized in the N-terminal. These findings further confirmed the importance of C-terminal of PRRT2 protein. PRRT2 mutant lacking the large N-terminal domain was localized correctly to the plasma membrane. In our study, only the C-terminal amino acid change could result in mislocalization. Possibly, membrane orientation of PRRT2 is imposed by the C-terminal, especially the TM domain.

In conclusion, a total of PRRT2 missense variants reported is firstly assessed by the ACMG guidelines. It will be of great value for its instructive and meaningful role in clinical molecular diagnosis. Missense variants could decrease the protein level and/or impair plasma membrane localization. C-terminal of PRRT2 is crucial for its physiological function. Functional study is vital for the classification and potential mechanisms associated with PRRT2 should be further explored.

ACKNOWLEDGMENTS

We sincerely thank the participants for their help and willingness to participate in this study. We also thank Novogene Company to share their whole exome sequencing data with us.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Zhi-Ying Wu https://orcid.org/0000-0003-2106-572X

REFERENCES

1. Chen WJ, Lin Y, Xiong ZQ, et al. Exome sequencing identifies truncating mutations in PRRT2 that cause paroxysmal kinesigenic dyskinesia. Nat Genet. 2011;43(12):1252-1255.
2. de Vries B, Callenbach PM, Kamphorst JT, et al. PRRT2 mutation causes benign familial infantile convulsions. Neurology. 2012;79(21):2154-2155.
3. Lee HY, Huang Y, Bruneau N, et al. Mutations in the gene PRRT2 cause paroxysmal kinesigenic dyskinesia with infantile convulsions. Cell Rep. 2012;1(1):2-12.
4. Liu XR, Huang D, Wang J, et al. Paroxysmal hypnogenic dyskinesia is associated with mutations in the PRRT2 gene. Neurol Genet. 2016;2(2):e66.
5. Li HF, Wu ZY. PRRT2 Mutations and PRRT2 disorders. Human Genet Embryol. 2013;3:1-10.
6. Zhang LM, An Y, Pan G, et al. Reduced penetrance of PRRT2 mutation in a Chinese family with infantile convulsion and choreoathetosis syndrome. J Child Neurol. 2014;30(10):1263-1269.
7. Li HF, Ni W, Xiong ZQ, et al. PRRT2 c.649dupC mutation derived from de novo paroxysmal kinesigenic dyskinesia. CNS Neurosci Ther. 2013;19(1):61-65.
8. Tan GH, Liu YY, Wang L, et al. PRRT2 deficiency induces paroxysmal kinesigenic dyskinesia by regulating synaptic transmission in cerebellum. Cell Res. 2018;28(1):90-110.
9. Ebrahim-Fakhari D, Saffari A, Westenberger A, Klein C. The evolving spectrum of PRRT2-associated paroxysmal diseases. Brain. 2015;138(12):3476-3495.
10. Ji Z, Su Q, Hu L, et al. Novel loss-of-function PRRT2 mutation causes paroxysmal kinesigenic dyskinesia in a Han Chinese family. BMC Neurol. 2014;14:146.
11. Coleman J, Jouannot O, Ramakrishnan SK, et al. PRRT2 regulates synaptic fusion by directly modulating SNARE complex assembly. Cell Rep. 2018;22(3):820-831.
12. Liu YT, Nian FS, Chou WJ, et al. PRRT2 mutations lead to neuronal dysfunction and neurodevelopmental defects. Oncotarget. 2016;7(26):39184-39196.
13. Li M, Niu F, Zhu X, et al. PRRT2 mutant leads to dysfunction of glutamate signaling. Int J Mol Sci. 2015;16(5):9134-9151.
14. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-42412.
15. Li LX, Liu GL, Liu ZJ, Lu C, Wu ZY. Identification and functional characterization of two missense mutations in NDRG1 associated with Charcot-Marie-Tooth disease type 4D. Hum Mutat. 2017;38(11):1569-1578.
16. Delcourt M, Riant F, Mancini J, et al. Severe phenotypic spectrum of biallelic mutations in PRRT2 gene. J Neurol Neurosurg Psychiatry. 2015;86(7):782-785.
17. Jing XY, Li XH, Yuan P, Deng J, Hu B, Wang Y. A novel mutation and functional implications of 5 variants in the PRRT2 gene in 20 paroxysmal kinesigenic dyskinesia pedigrees. Parkinsonism Relat Disord. 2013;19(6):639-642.
18. Heron Sarah E, Grinton Bronwyn E, Kivity S, et al. PRRT2 Mutations cause benign familial infantile epilepsy and infantile convulsions with choreoathetosis syndrome. Am J Hum Genet. 2012;90(1):152-160.
19. Wang JL, Cao L, Li XH, et al. Identification of PRRT2 as the causative gene of paroxysmal kinesigenic dyskinesias. Brain. 2011;134(Pt 12):3493-3501.
20. van Vliet R, Breedveld G, de Rijk-van AJ, et al. PRRT2 phenotypes and penetrance of paroxysmal kinesigenic dyskinesia and infantile convulsions. Neurology. 2012;79(8):777-784.
21. Liu XR, Wu M, He N, et al. Novel PRRT2 mutations in paroxysmal dyskinesia patients with variant inheritance and phenotypes. Genes Brain Behav. 2013;12(2):234-240.
22. Friedman J, Olvera J, Silhavy JL, Gabriel SB, Gleeson JG. Mild paroxysmal kinesigenic dyskinesia caused by PRRT2 missense mutation with reduced penetrance. Neurology. 2012;79(9):946-948.
23. Marini C, Conti V, Mei D, et al. PRRT2 mutations in familial infantile seizures, paroxysmal dyskinesia, and hemiplegic migraine. Neurology. 2012;79(21):2109-2114.

24. Matsumoto N, Takahashi S, Okayama A, Araki A, Azuma H. Benign infantile convulsion as a diagnostic clue of paroxysmal kinesigenic dyskinesia: a case series. J Med Case Rep. 2014;8:174.

25. Mao CY, Shi CH, Song B, et al. Genotype-phenotype correlation in a cohort of paroxysmal kinesigenic dyskinesia cases. J Neurol Sci. 2014;340(1-2):91-93.

26. Michetti C, Castroflorio E, Marchionni I, et al. The PRRT2 knock-out mouse recapitulates the neurological diseases associated with PRRT2 mutations. Neurobiol Dis. 2017;99:66-83.

27. Fruscione F, Valente P, Sterlini B, et al. PRRT2 controls neuronal excitability by negatively modulating Na+ channel 1.2/1.6 activity. Brain. 2018;141(4):1000-1016.

28. Wang HX, Li HF, Liu GL, Wen XD, Wu ZY. Mutation analysis of MR-1, SLC2A1, and CLCN1 in 28 PRRT2-negative paroxysmal kinesigenic dyskinesia patients. Chin Med J (Engl). 2016;129(9):1017-1021.

29. Rossi P, Sterlini B, Castroflorio E, et al. A novel topology of proline-rich transmembrane protein 2 (PRRT2): Hints for an intracellular function at the synapse. J Biol Chem. 2016;291(12):6111-6123.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Zhao S-Y, Li L-X, Chen Y-L, et al. Functional study and pathogenicity classification of PRRT2 missense variants in PRRT2-related disorders. CNS Neurosci Ther. 2020;26:39–46. https://doi.org/10.1111/cns.13147