Dear Editor,

Paroxysmal kinesigenic dyskinesia (PKD) (MIM 128200) is an autosomal dominant movement disorder characterized by involuntary movements which are usually triggered by sudden movements. We previously identified PRRT2 as the first causative gene of PKD\(^1\), which was widely verified in different populations\(^2\)–\(^4\). It is estimated that PRRT2 variants account for 77%–93% familial PKD and 21%–45% isolated PKD\(^5\). A significant percentage of PRRT2-negative PKD patients indicate that other genes are implicated in PKD. Here, we performed whole-exome sequencing (WES) in 5 PKD pedigrees and 31 isolated PKD patients without PRRT2 variants. Moreover, 1000 unrelated healthy individuals of matched ethnicity were included as control subjects. This study was approved by the local Ethics Committee. All participants or their guardians provided written informed consents.

To systematically identify the causative genes for these PRRT2-negative PKD patients, we first analyzed three PKD families with multiple patients and unaffected individuals. Based on the autosomal dominant inheritance pattern in these families, we prioritized the heterozygous non-synonymous variants in coding regions and splicing sites, with a minor allele frequency (MAF) <0.01% in the genome Aggregation Database (gnomAD), and absent in the 1000 Genomes Project (1000G), Exome Sequencing Project v.6500 (ESP6500), Exome Aggregation Consortium (ExAC). Besides, variants should be shared by all affected individuals but not by the unaffected parent of the proband in each family. After filtering, we found 11 candidate genes in Family 1, 29 in Family 2, and 52 in Family 3 (Supplementary Table S1). The comparison of these candidate genes revealed that TMEM151A (NM_153266) was the only gene implicated in these families. Three TMEM151A variants including c.1275dupG (p. P426Afsl9), c.375 C > A (p.C125X), and c.758 T > C (p. L253P) were confirmed by Sanger sequencing and co-segregation analysis (Fig. 1a). We did not find any TMEM151A variant in the other two PKD families. The detailed WES data are shown in Supplementary Table S2. We then screened TMEM151A variants in the WES data of 31 isolated PKD patients. We found four truncated variants (c.7 G > T [p.E3X], c.623_624insA [p. L210Asfs*136], c.739 G > T [p.E247X], and c.897_912del [p. L300Pfs*118]), three missense variants (c.140 T > C [p. L253P], c.683 T > C [p.F288S], and c.889 T > A [p.S297T]), and a non-frameshift deletion (c.142_153del [p.48_51delTLLL]) in 8 index patients (Fig. 1b and Supplementary Table S3).

To evaluate the frequency of identified TMEM151A variants in the general population, we performed Sanger sequencing in 1000 control individuals. Totally, we found four missense variants and three synonymous variants, which were predicted to be benign by bioinformatic software (Supplementary Fig. S1 and Table S4). None of the TMEM151A variants identified in PKD patients was found in controls. We then screened TMEM151A rare variants (MAF < 1%) in gnomAD and found 115 damaging missense variants, 6 truncated variants, and 1 in-frame deletion in 792 individuals. There was a significant enrichment of potentially pathogenic TMEM151A variants in PKD patients, compared with the gnomAD database (11/36 vs 792/76,000, \(P = 8.05 \times 10^{-14}\), Supplementary Table S5).

Li et al. Cell Discovery (2021) 7:83
https://doi.org/10.1038/s41421-021-00322-w

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total, we identified 11 TMEM151A variants in 3 PKD pedigrees and 8 isolated patients, whose detailed clinical features are summarized in Supplementary Tables S6 and S7. TMEM151A is a poorly characterized gene whose function is largely unknown. It is highly conserved among species (Supplementary Fig. S2). To explore its expression pattern, we measured the level of mice Tmem151a mRNA...
by real-time PCR. We found Tmem151a was highly expressed in the central nervous system (CNS), including the brain and spinal cord, followed by the stomach (Fig. 1c). Tmem151a was relatively low during the embryonic period, markedly increased during postnatal stages, peaked at postnatal day 14 (P14), and remarkably declined in adulthood (Fig. 1d). Real-time PCR and in situ hybridization analyses in P14 mice revealed that Tmem151a was ubiquitously expressed in the CNS, with a high level in the cerebral cortex, hippocampus, spinal cord, brainstem, and thalamus (Fig. 1e–g). On a more granular level, Tmem151a was enriched in cortical layers of the cerebral cortex and CA1 of the hippocampus (Fig. 1h). In the cerebellum, Tmem151a was mainly expressed in white matter, not in granule cells or Purkinje cell layers (Fig. 1h).

To examine the subcellular localization of Tmem151a protein, we generated EGFP-tagged wild-type (WT) Tmem151a plasmids and transfected them into COS-7 cells and cortical neurons. We found Tmem151a colocalized with endoplasmic reticulum (ER) marker Calnexin in COS-7 cells (Fig. 1i) and distributed in both axons and dendrites in primary cortical neurons (Fig. 1j). To elucidate the pathogenicity of TMEM151A non-truncated variants (p.L47P, p.48_51delTTL, p.L253P, p.F288S, and p.S297T), we investigated the alternation of subcellular localization and protein expression of mutant TMEM151A. Cos-7 cells were transfected with WT and mutant EGFP-TMEM151A plasmids. Immunostaining revealed that mutant TMEM151A protein still retained on ER (data not shown). We then quantitatively measured the protein expression level of these TMEM151A variants by transfecting WT and mutant pRES2-Flag-TMEM151A plasmids into HEK 293T cells. Western blot showed a significantly decreased protein expression of mutant TMEM151A compared to WT TMEM151A (Fig. 1k), suggesting a potential loss of function mechanism for these mutant TMEM151A.

We further generated Tmem151a knockout mice by CRISPR/Cas9-mediated genome editing according to our previous report and obtained Tmem151a+/− and Tmem151a−/− mice by breeding. Spontaneous dyskinesia was observed in both founder mice and F1 Tmem151a−/− mice. Within 48 h, eight times of dyskinesia attacks were observed in four out of eight 1-month-old Tmem151a−/− mice (Fig. 1l). No dyskinesia attacks were observed in Tmem151a+/− mice and WT mice. The duration of episodes ranged from 10 to 37 s (Fig. 1m). After dyskinesia attacks (Fig. 1n and Supplementary Movie S1), the mice recovered to normal locomotion.

PKD is a hereditary disorder with autosomal dominant inheritance. However, a significant proportion of PKD patients seem to be sporadic. Incomplete penetrance, de novo mutagenesis or autosomal recessive inheritance may account for this phenomenon. In this study, we found TMEM151A variants in three autosomal dominant families and eight isolated patients. Variants c.140 T > C, c.739 G > A, c.748 A > G, c.772 A > C, c.873 C > T, and c.623_624insA identified in three isolated patients were derived from one of their parents, who reported no obvious kinesigenic attacks. DNA samples were not available in the parents of the remaining five patients. We conjecture TMEM151A variants may have decreased penetrance, which has been observed in PRRT2 and genes responsible for dystonia. Besides, TMEM151A variants could also be de novo in isolated patients.

The potential mechanisms underlying PKD are not entirely clear. The identification of PRRT2 as the first causative gene of PKD has improved our understanding of the pathogenesis of the disease. Recent studies indicate that PRRT2 acts on the presynaptic terminal and plays an important role in regulating synaptic transmission and neuronal excitability. TMEM151A is predicted to be a...
transmembrane protein. We found TMEM151A was localized at ER in COS-7 cells. It is known that ER is the main intracellular Ca\(^{2+}\) store and plays a crucial role in intracellular Ca\(^{2+}\) mobilization and dynamics\(^{13}\). Considering the paroxysmal feature of PKD, we surmise TMEM151A may be an ER-associated Ca\(^{2+}\) channel. Alternatively, TMEM151A may interact with Ca\(^{2+}\) sensors and endow the SNARE complex, like the PRRT2 protein\(^{14}\). Given that patients with TMEM151A variants also obtain significant remission after carbamazepine treatment, it is possible that TMEM151A is an ion channel protein. Whether TMEM151A acts like PRRT2 in modulating Na\(^{+}\) channel is unclear\(^{13}\), which should be elucidated in the future. Loss of function might be the potential mechanism of mutant TMEM151A causing PKD. The decreased protein expression of non-truncated variants was in consistent with the mechanisms of haploinsufficiency. How amino acid residue changes affect TMEM151A protein functions requires further investigation.

In summary, we identified TMEM151A variants in both familial and isolated PKD patients, indicating that TMEM151A variants cause PKD. Although the function of TMEM151A remains elusive, we believe our findings will deepen the understanding of the mechanisms of PKD.

Acknowledgements
This work was supported by grants from the National Natural Science Foundation of China (81330025 to Z.-Y.W., 81500973 to H.-F.L.), Chinese Academy of Science (XDB20200007, QYZDJ-15SW-SMC010 to Z.-Q.X.), and the research foundation for the distinguished scholar of Zhejiang University (188020-193810101/089 to Z.-Y.W.). We thank XiangYin Biotechnology Co. Ltd and Genergy Biotechnology Inc. for assistance in WES analysis.

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Author contributions
Z.-Y.W. conceived and supervised the project. Z.-Q.X. supervised the project. H.-F.L. and Y.-L.C. collected clinical data, performed sequencing and experiments, and wrote the manuscript. L.Z. performed experiments and wrote the manuscript. D.-F.C. and H.-Z.K. performed sequencing. W.-J.L., G.-L.L., S.-N.W., and W.-H.Z. collected clinical data.

Data availability
The original data that support the findings are available from the corresponding author (Zhi-Ying Wu) on reasonable request.

Conflict of interest
The authors declare no competing interests.

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Supplementary information
The online version contains supplementary material available at https://doi.org/10.1038/s41421-021-00322-w.

Received: 10 March 2021 Accepted: 4 August 2021
Published online: 13 September 2021

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