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Research article

Identification of a de novo CACNA1B variant and a start-loss ADRA2B variant in paroxysmal kinesigenic dyskinesia

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ABSTRACT

Paroxysmal kinesigenic dyskinesia (PKD) represents the most prevalent form of paroxysmal dyskinesia, characterized by recurrent and transient attacks of involuntary movements triggered by a sudden voluntary action. In this study, whole-exome sequencing was conducted on a cohort of Chinese patients to identify causal mutations. In one young female case, a de novo *CACNA1B* variant (NM_000718.3:exon3:c.479C > T:p.S160F) was identified as the causative lesion. This finding may broaden the phenotypic spectrum of *CACNA1B* mutations and provide a prospective cause of primary PKD. Additionally, a novel start-loss variant (NM_000682.7:c.3G > A) within *ADRA2B* further denied its association with benign adult familial myoclonic epilepsy, and a *KCNQ2* E515D variant that was reported as a genetic susceptibility factor for seizures had no damaging effect in this family. In sum, this study established a correlation between *CACNA1B* and primary PKD, and found valid evidence that further negates the pathogenic role of *ADRA2B* in benign adult familial myoclonic epilepsy.

1. Introduction

Paroxysmal kinesigenic dyskinesia (PKD), the most prevalent form of paroxysmal dyskinesia, is a rare neurological disorder distinguished by recurrent and transient attacks of involuntary movements triggered by sudden voluntary movement [1]. PKD can be categorized into primary and secondary types depending on its etiology. Genetic abnormalities underlie the major cause of primary PKD, with the inheritance pattern being primarily autosomal dominant and exhibiting incomplete penetrance [2,3]. Mutations in the PRRT2 gene have been identified as the cause of PKD in some families, but the genetic basis remains unknown in many cases [4–6].

The symptoms typically begin in childhood or adolescence and may be precipitated by voluntary movement, such as walking, running, or hopping, or by a startling stimulus. Attacks are characterized by involuntary movements that may include dystonia, chorea, or both, and may last for seconds to minutes. Management typically involves avoidance of known triggers and treatment with medication such as anticonvulsants or benzodiazepines to reduce the frequency and severity of attacks [1,7]. PKD is a treatable condition and accurate diagnosis is important for optimal management and genetic counseling.

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CACNA1B gene (Voltage-dependent *N*-type calcium channel subunit alpha-1B) encodes a subunit of voltage-gated calcium channel that is widely expressed in the central nervous system and plays an important role in calcium ion influx and signal transduction. Mutations in this gene have been associated with a variety of neurological diseases, including developmental and epileptic encephalopathy with severe hyperkinetic movement disorder, myoclonus dystonia syndrome, epilepsy, and intellectual disability [8–11]. In addition, CACNA1B mutations have also been identified among patients with autism spectrum disorders, indicating its important role in brain function and development [12]. This gene, situated on chromosome 3q22.1, comprises 48 exons encoding a protein weighing approximately 200 kDa.

ADRA2B gene (Alpha-2B adrenergic receptor) encodes a subtype of adrenoreceptor, and was associated with benign adult familial myoclonic epilepsy (BAFME) [13]. BAFME is an autosomal dominant neurological disorder characterized by cortical tremor and epileptic seizures [14]. Fusco et al. identified the first and only mutation of *ADRA2B* (c.675_686delTGGTGGGGCTTTinsGTTTGGCAG; p.H225_L229delinsQ225_F_G_R228) in two Italian BAFME families [13]. And Corbett et al. identified the ATTTC repeat sequence as the genetic lesion in 22 pedigrees with BAFME, including the two Italian families mentioned above. Thus, they suggested that *ADRA2B* mutation is not causative [15].

In this study, we recruited one young female patient with pure PKD, and multiple variants may relate to neurological diseases were identified through whole exome sequencing. A de novo *CACNA1B* variant (NM_000718.3:exon3:c.479C > T:p.S160F) was finally identified as the genetic cause. In addition, a novel start-loss mutation in *ADRA2B* (NM_000682.7:c.3G > A), a *SGCE* splicing variant (NM_001099401.1:c.1254-1G > C), and a *KCNQ2* variant (NM_172107.3:c.1545G > C:p.E515D) were identified in the patient, and these may be used as supportive evidence to negate their association with certain diseases.

2. Materials and methods

2.1. Participants

All patients were recruited exclusively from the Department of Neurology at the Affiliated Hospital of Yangzhou University and Xiangya Hospital, the participants of control group were recruited from healthy volunteers and a few patients without neurological diseases. All the patients signed informed consent. The study obtained approval from the Ethics Committee of the Affiliated Hospital of Yangzhou University, adhering strictly to the principles outlined in the Declaration of Helsinki.

2.2. Genetic analysis

Whole peripheral blood samples were obtained from the family trios and stored in ethylenediaminetetraacetic acid tubes. We extracted genomic DNA using a DNA Blood Mini Kit from Qiagen. Whole-Exome Sequencing (WES) was completed in Novogene Institute. The Agilent SureSelect Human All Exon V6 kits were utilized to capture the exomes, which were then sequenced on the Illumina NovaSeq6000 platform manufactured by Illumina Inc. in San Diego, USA. To filter the WES data, three criteria were applied: Firstly, variants occurring outside the coding regions, such as intronic, intergenic, and untranslated regions, as well as synonymous variants, were excluded. Secondly, variants with a high allele frequency (>0.01%) were disregarded. Lastly, variants predicted to be deleterious by bioinformatics, particularly loss-of-function and damaging variants, were retained [16].

2.3. Minigene splicing assay

The insert fragment was amplified and purified from the patient's peripheral blood DNA sample, and then inserted into a minigene carrier (D7010FT, Beyotime). Wild-type and mutated SGCE minigene plasmids were separated by single colony isolation and sequencing. Subsequently, the two plasmids were transfected into the 293 cell line, respectively. After 24 h of incubation, the mRNA was extracted and reverse transcribed. The cDNA was amplified using universal primers and then subjected to agarose gel electrophoresis. Sanger sequencing was conducted for validation.

2.4. Western blot analysis

Proteins were extracted from peripheral blood. Further details about Western blot analysis have been described elsewhere [17].

3. Results

The proband is a 16-year-old female who developed paroxysmal dyskinesia four years ago, mostly upon sudden movement, manifested as limb spasm, torsion towards the lateral side, and a feeling of limb tightness. Each episode lasted several seconds and resolved spontaneously. Episodes alternate between bilateral limbs, with the right limb being more commonly affected. The patient also felt weakness in the ipsilateral limb without numbness, pain, or consciousness disturbance, and there were no episodes of incontinence and electroencephalogram change. After taking carbamazepine, symptoms were well controlled but severe adverse reactions of somnolence occurred. Switching to oxcarbazepine resulted in good control of symptoms. According to the modified clinical diagnostic criteria for PKD, the proband was diagnosed as primary PKD without doubt [7].

We conducted whole-exome sequencing on the proband to identify the pathogenic factor, assist in clinical diagnosis and treatment. Through data filtration, fifteen variants within thirteen genes were retained, and nine genes were associated with neurological

disorders among them. Variants in *GBA*, *KCNQ2*, *PROC*, *HMBS*, *GJB2*, and *SKI* were further excluded due to inconsistent genetic pattern or clinical manifestations.

A de novo *CACNA1B* variant (NM_000718.3:exon3:c.479C > T:p.S160F) was identified, and the amino acid and surrounding sequences at the mutation site are highly conserved in mammals (Fig. 1A–C). Bioinformatic analysis (MutationTaster, PolyPhen-2, SIFT, and CADD, among others) indicated that this variant is deleterious. Prediction of transmembrane domain suggests that amino acids 158–163 may be intracellular (Fig. 1D). The protein 3D model was downloaded from the AlphaFold Protein Structure Database. The model with specific variant was generated using PyMOL, and a minor structural change was observed (Fig. 1E). Electrostatic potential maps showed altered surface potential (Fig. 1F).

Additionally, a novel splicing variant in *SGCE* (NM_001099401.1:c.1254-1G > C) that is inherited from her father was identified (Figs. 1A and 2A). Considering the incomplete penetrance of PKD, this variant is still considered likely pathogenic. The minigene splicing assay was used to demonstrate that this variant does not cause splicing abnormalities (Fig. 2B and C). Although a novel start-loss variant in *ADRA2B* (NM_000682.7:c.3G > A) significantly attenuates protein expression, it was ruled out due to phenotypic differences, and the patient's mother also carries this variant but does not have neurological disorder (Figs. 1A, 2D and 2E). It is worth noting that this *ADRA2B* variant further disproves the association between *ADRA2B* and BAFME. The identification of the *KCNQ2* E515D variant in this family also questions its relationship to benign familial neonatal seizures and intellectual disability [18].

Finally, to explore the adverse reactions caused by carbamazepine, we screened the WES data and only identified the HLA-B I76V and S48A variants.

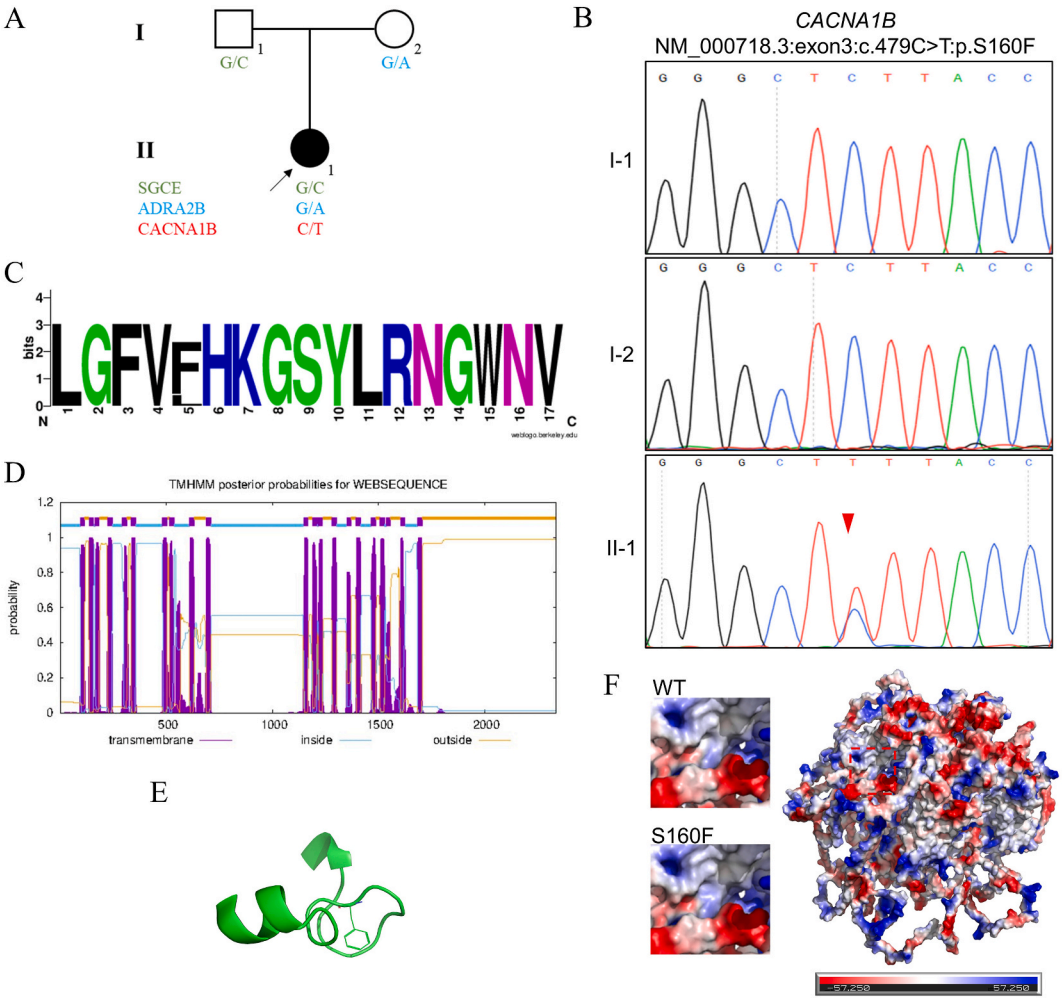

Fig. 1. (**A**) The pedigree of the subject. Three variants are indicated in different colors. (**B**) Sanger sequencing diagrams showing the de novo *CACNA1B* variant in the proband. (**C**) The amino acid and surrounding sequences at the mutation site are highly conserved. (**D**) Transmembrane prediction reveals that amino acids 158–163 are intracellular. (**E**) This variant generates a minor structural change compared to natural protein. (**F**) Electrostatic potential maps show altered surface potential. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

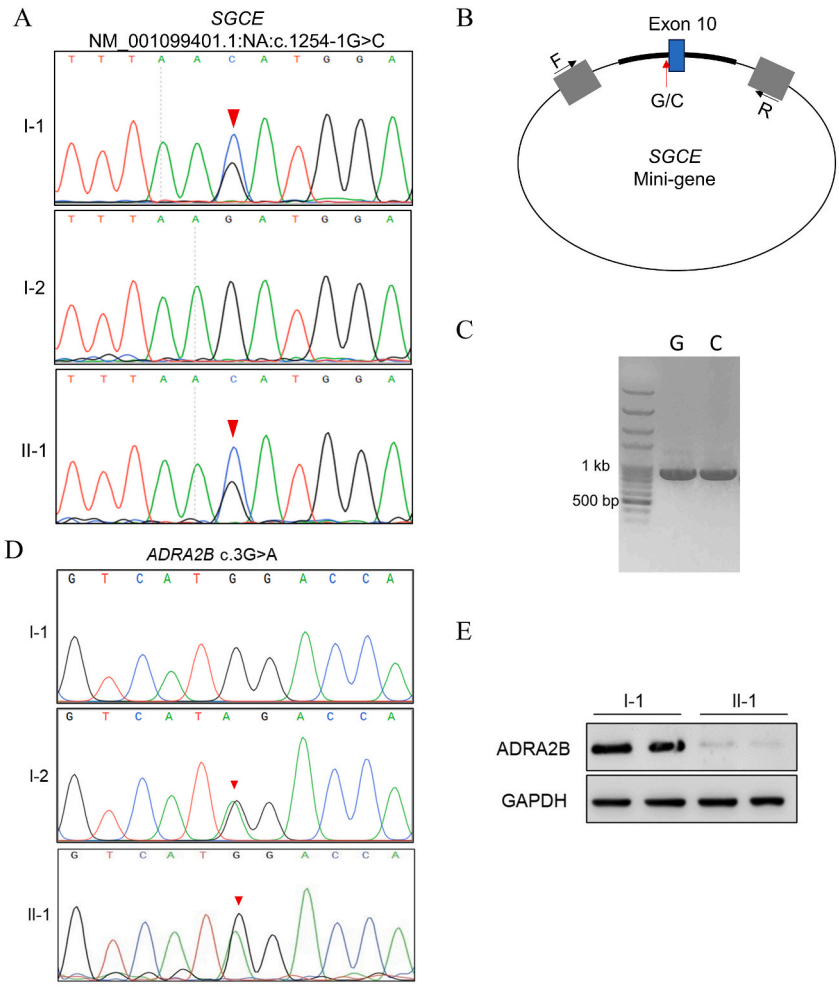

Fig. 2. (**A**) Sanger sequencing diagrams showing the *SGCE* splicing variant. (**B**) Schematic diagram of *SGCE* minigene (NM_001099401.1). (**C**) Agarose gel electrophoresis did not show splicing abnormality, and sequencing verified consistency. (**D**) Sanger sequencing diagrams showing the *ADRA2B* start-loss variant. (**E**) Western blot analysis of ADRA2B protein expression in peripheral blood. The original images of agarose gel electrophoresis (Figs1) and Western blot (Figs2) are available in Supplementary material.

4. Discussion

Although primary PKD is a benign disease with a tendency for spontaneous remission and effective medicine, the psychological health issues caused by PKD cannot be ignored. Poor psychological state can worsen the frequency and severity of PKD attacks in turn [7]. Therefore, we recommend the psychotherapy is necessary, especially for young patients.

Until now, several genes associated with the development of PKD have been identified, including *PRRT2*, *TMEM151A*, *PNKD*, *SLC2A1*, *SCN8A*, *KCNMA1*, *KCNA1*, and *DEPDC5*, which characterize the genetic heterogeneity of PKD [4–6,19–28]. Although the specific mechanism remains unclear, it is evident that the occurrence of PKD is complex and involves numerous components. For instance, proteins encoded by *PRRT2* (a component of the SNARE complex) and *SCN8A* (a voltage-gated sodium channel) play crucial roles in PKD. The discovery of these causative genes through large-scale pedigree analysis not only highlights the critical roles of their encoded proteins in PKD but also provides valuable insights and directions for further basic research on this condition.

PRRT2 is the main pathogenic gene therein. The gene, situated on chromosome 16p11.2, comprises four exons encoding 340 amino acids. Over 80 mutations of PRRT2 have been documented, primarily nonsense and frameshift variants [29]. However, the precise function and pathogenic mechanism of PRRT2 remain unclear. PRRT2 is a component of the SNARE complex and interacts with SNAP-25, synaptotagmin 1/2, and synaptic vesicle protein 2, rendering the SNARE complex calcium-sensitive [30]. PRRT2 deficiency caused presynaptic dysfunction, abnormal neurotransmitter releases and a sharp decrease in Ca2+ sensitivity [31]. Notably, *CACNA1B* encodes a subunit of voltage-gated calcium channel that contributes to SNARE-mediated neurotransmission [9,32,33]. CACNA1B is also involved in various calcium-dependent processes, including muscle contraction, hormone or neurotransmitter release, gene expression, cell motility, cell division and cell death. Therefore, the pleiotropism and phenotypical heterogeneity of *CACNA1B* variants are expected, and several studies have demonstrated a correlation between mutations in *CACNA1B* and hyperkinetic movement

disorder, epilepsy, and dystonia [8–10]. Furthermore, incomplete penetrance is a common feature in *PRRT2*, *TMEM151A*, and genes responsible for dystonia [20]. We speculate that CACNA1B may exhibit this trait as well, which may be related to genetic background, epigenetics, and environmental factors.

In conclusion, this study proposes the association between a de novo *CACNA1B* variant with autosomal dominant trait and primary PKD, further negates the pathogenicity of *ADRA2B* mutations in BAFME, and suggests that the relationship between the *KCNQ2* E515D variant and benign familial neonatal seizures and intellectual disability needs more validation.

Ethics approval and consent to participate

The study obtained approval from the Ethics Committee of the Affiliated Hospital of Yangzhou University (2022-YKL02-G008), adhering strictly to the principles outlined in the Declaration of Helsinki. The written informed consent was obtained from all subjects and/or their legal guardian(s).

Consent for publication

Not Applicable.

Data availability statement

Data will be made available on request.

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CRediT authorship contribution statement

Zhuangzhuang Yuan: Writing – review & editing, Writing – original draft, Validation, Data curation. **Qian Wang:** Writing – review & editing, Writing – original draft, Validation, Data curation. **Chenyu Wang:** Writing – review & editing, Validation. **Yuxing Liu:** Writing – review & editing, Validation. **Liangliang Fan:** Writing – review & editing, Conceptualization. **Yihui Liu:** Writing – review & editing, Funding acquisition, Data curation. **Hao Huang:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28674.

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