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Exome sequencing identifies compound heterozygous *KCTD7* mutations in a girl with progressivemyoclonus epilepsy

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ABSTRACT

Progressive myoclonic epilepsies (PME) are a clinically and genetically heterogeneous group of rare diseases characterized by myoclonic seizures, tonic-clonic seizures, and neurological deterioration. Here, we genetically analyzed a Chinese patient affected by infantile-onset progressive myoclonic epilepsy. We applied next-generation whole exome capture sequencing with Sanger direct sequencing to the proband and her unaffected parents. Two compound heterozygous mutations were identified in the *KCTD7* gene. The first mutation [c. 434A > G(p.Q145R)] was inherited from her father, while the second [c.631C > T(p.R211X)] was inherited from her mother. The two were co-segregated with disease phenotype in the family. To our knowledge, this is the first report of *KCTD7* mutations causing PME in the Chinese population, with c. 434A > G in particular being a novel mutation. Our findings supported the important role of *KCTD7* in PME and broadened the gene's mutation spectrum. Thus, this study contributes to genetic diagnoses and counselling of families with PME.

1. Introduction

Progressive myoclonus epilepsies (PME) are a rare suite of conditions originating from multiple genetic disorders. The syndromes have an unfavorable course and poor prognosis, manifesting as therapy-resistant myoclonic and tonic-clonic seizures, together with progressive neurological decline [1]. The age of onset can vary from infancy to adulthood. A wide range of specific etiology contributes to PME [2], including neuronal ceroid lipofuscinoses (NCL), Unverricht-Lundborg disease (ULD), Lafora disease, myoclonic epilepsy with ragged red fiber (MERRF), Tay-Sachs, sialidosis, and dentatorubral-pallidoluysian atrophy (DRPLA). Molecular characterization found that most PME are autosomal recessively inherited, although a few rare cases exhibited autosomal dominant or mitochondrial inheritance [3,4]. Many PME-associated genes are associated with endosomal and lysosomal function [2,5], but exact disease mechanisms remain poorly understood. Indeed, the genetic heterogeneity of PME causes challenges in precise diagnosis, a problem compounded by phenotypic similarities and overlap of clinical symptoms with other epileptic and neurodegenerative diseases [4]. Fortunately, recent advances in whole-exome sequencing (WES) have offered a rapid and cost-effective method for PME diagnosis.

Here, we present the case study of a Chinese girl suffering from infantile-onset progressive myoclonic epilepsy, with symptoms including ataxia, inability to walk, intellectual disability, and treatment-resistant seizures. We employed whole-exome sequencing (WES) on the patient to identify potential causative genes and found two *KCTD7* compound heterozygous mutations. To the best of our knowledge, this is the first report associating a *KCTD7* mutation with PME in the Chinese population.

2. Materials and methods

2.1. Subjects

The study recruited a family from Fujian Province, China, presenting with refractory myoclonus (Fig. 1A). Clinical manifestation, physical examinations, laboratory tests, imaging examination, and electroencephalography (EEG) were performed. Study procedures adhered to the Declaration of Helsinki and the Ethics Committee of the Xiamen Maternal and Child Health Care Hospital (Xiamen, Fujian, China). Informed consent was obtained from all study subjects or their legal guardians.

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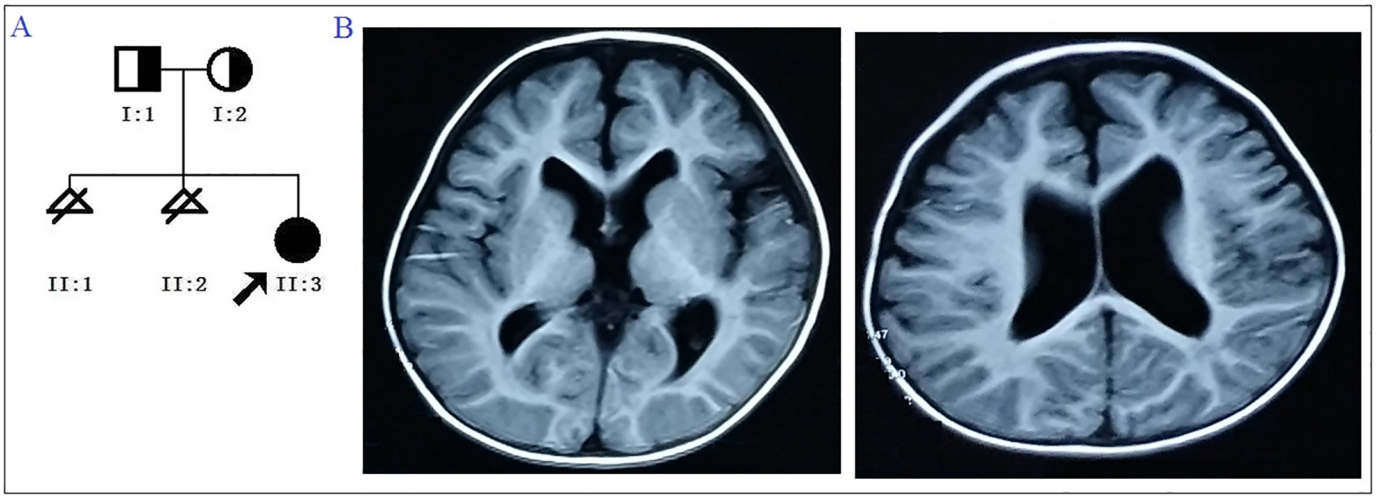

Fig. 1. A. Pedigree with autosomal recessive progressive myoclonus epilepsy. The black arrow indicates proband (patient II 3). B. Cerebral morphological magnetic resonance imaging of proband at 1.5 years of age, showing T2-imaging, bilateral frontotemporal atrophy, bilateral ventricular third ventricle dilatation.

2.2. Genomic DNA extraction and exome sequencing

Genomic DNA was extracted from peripheral blood leucocytes of the proband and her parents and then purified using the QIAamp blood kit (QIAGEN, Hilden, Germany) following manufacturer protocol. Next, DNA concentration was quantified with Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA).

Exons of DNA samples were captured via AgilentSureSelect Human All Exon V6 kits System (Agilent Technologies, Inc., Santa Clara, CA, USA) followed by a paired-end high-throughput sequencing using Illumina HiSeq 2500 platform (Illumine Inc., San Diego, CA, USA). In brief, a minimum of 3 μg Genomic DNA was sheared into fragments of 350–400 base pairs using Covaris S2 (Covaris, Woburn, MA, USA). After adaptor ligation, the library was PCR-amplified following standard Illumina protocols, and amplicons were validated using the Agilent Bioanalyzer (Santa Clara, CA, USA). Exon-enriched DNA libraries were then prepared for high-throughput sequencing on Illumina HiSeq2500. Data analysis was performed using the Illumina Bioinformatics analysis pipeline.

2.3. Bioinformatics analysis

Post-sequencing primary data were processed in SolexaQA and cutadapt (http://code.google.com/p/cutadapt/). High-quality sequenced reads were aligned to the NCBI human reference genome (gh19/NCBI37.1) in SOAPaligner. Subsequently, dataset files (including removed PCR duplicates and single nucleotide variants [SNVs]) were analyzed in Picard and SOAPsnp, respectively. Small insertions/deletions (Indels) were identified in GATK. Annotations of SNVs and indels were performed in the following databases: including dbSNP137, 1000 Genome Project, HapMap, Genome Aggregation Database, and ExAC Browser. All suspected pathogenic variants were verified in HGMD (http://www.hgmd.cf.ac.uk) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar). In addition, SIFT, PolyPhen, PROVEAN, and MutationTaster were applied to predict how variants affected protein function.

2.4. Sanger sequencing

Forward and reverse Sanger sequencing was performed to confirm WES-identified mutations. Segregation analyses were then performed within the family members. Primers were designed in Primer 5 (Premier Biosoft International, Palo Alto, CA). Candidate regions were PCR-amplified and directly sequenced using ABI PRISM 3730 gene analyzer (Applied Biosystems, California, USA). Sequencing results were then analyzed using DNASTAR package (DNASTAR, Madison, WI).

3. Results

3.1. Clinical findings

The proband is a 2-year-old girl born to healthy non-consanguineous parents using assisted reproductive technology. Her height is 84.5 cm; weight, 9.5 kg; and head circumference, 45 cm. Pregnancy, delivery, and early development were normal. However, shortly after birth, she presented with mental retardation, as well as psychomotor and developmental delay. Recurrent febrile convulsions (palpebral myoclonus and nystagmus) manifested at age 1. At 2 years old, myoclonus and ankylosis-clonic seizures occurred, accompanied by motor regression, specifically sitting instability, weakness of the right lower extremity, as well as instability and dragging the limbs while walking. Analysis with EEG showed generalized polyspike waves or spike-slow complex waves, especially in the left parietal, occipital, and temporal lobes. Next, MRI revealed bilateral frontotemporal atrophy and bilateral ventricular third ventricle dilatation(Fig. 1B). We did not observe clear improvement after the antiepileptic drug oxcarbazepine was administered. Electromyogram and MRI results were normal, as were hepatic and renal function, blood glucose, and electrolyte levels. Light stimulation frequently caused photosensitivity. Finally, CNV-seq analysis of the proband revealed a normal karyotype.

3.2. Mutation identification by targeted NGS and sanger sequencing

Average read depth of targeted regions and sequencing depth distribution indicated high WES quality. Average sequencing depth was 165. Coverage was > 98.9%, and average coverage across each targeted exome was > 75×. Furthermore, > 95.8% of all known SNPs were detected. Whole-exome sequencing yielded 42,375 variants in the proband.

We used an autosomal recessive inheritance model and clinical information to evaluate variants. Two compound heterozygous *KCTD7* mutations (c.434A > G and c.631C > T) (Fig. 2) were consistent with the recessive inheritance model (Table 1). Sanger sequencing confirmed that c.434A > G was heterozygous paternally, whereas c.631C > T was heterozygous maternally, indicating complete co-segregation with the disease phenotype (Fig. 3).

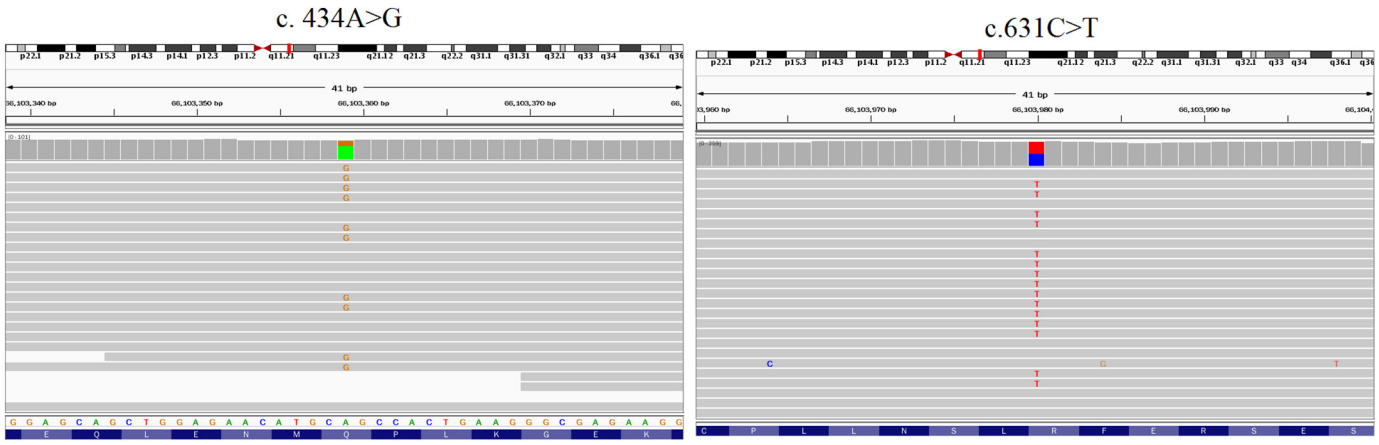

Fig. 2. Deep sequencing data for compound heterozygous mutations in *KCTD7*. The left and right panels show sequencing alignment data for mutations c.434A > G and c.631C > T, respectively.

3.3. Mutation detection and analysis

Variant c.434A > G was a missense mutation in exon 3 of *KCTD7* that resulted in glutamine substituted for arginine at amino acid 145 (p.Q145R). ClustalX sequence alignment revealed that the region containing p.Gln145Arg was highly conserved across various species (Fig. 4B). Pathogenesis prediction programs indicated that c.434A > G was "Probably Damaging" (PolyPhen-2; score: 0.998, sensitivity: 0.63, specificity: 0.92), "Deleterious" (PROVEAN), and "Affect Protein Function" (SIFT). This mutation has not been previously reported and was not found in the HGMD, ClinVar, ESP6500, ExAC, dbSNP, or in any other single-nucleotide polymorphism database. Variant c.631C > T is a nonsense mutation in exon 3 of *KCTD7* and predicted to introduce a premature stop codon (p.R211X). MutationTaster predicts this variant to be disease-causing through protein truncation and nonsense-mediated decay. Collectively, these results strongly support the causative nature of these *KCTD7* mutations in PME etiology.

4. Discussion

Whole exome sequencing, Sanger validation, and co-segregation analysis identified two compound heterozygous *KCTD7* mutations in a young female PME patient. Clinical characteristics associated with pathogenic mutations of *KCTD7* include myoclonus, tonic-clonic seizures, and progressive neurological deterioration, the latter causing psychomotor decline, cerebellar ataxia, and potentially early death [2,6]. Patients with *KCTD7* mutations have been diagnosed separately with PME [7,8], opsoclonus-myoclonus syndrome [9], and neuronal ceroid lipofuscinosis 14 [10]. Early childhood onset is the primary phenotype that distinguishes PME patients (presenting the *KCTD7* mutation) from related disorders, and proband age in this study was consistent with previously published case reports [6,11].

Previous research has identified KCTD7-related PME in 41 patients from 32 families with autosomal recessive inheritance [7–15]. These individuals had variable ethnic origin and a high rate of consanguinity. Using WES, we identified two compound heterozygous *KCTD7* mutations in our patient. First, a damaging missense mutation in exon 3 is located within a highly conserved region across multiple species. Its deleterious effects likely occur through disturbing appropriate gene function. Second, a nonsense mutation in exon 4 initiates a premature stop codon in KCTD7. Such a truncation may result in nonsense-mediated mRNA decay or encode a nonfunctional protein. While the first mutation is novel, the second mutation had been previously identified in a Caucasian British family presenting PME with compound heterozygous mutations (T64A,R211X) in *KCTD7* [11]. Here, Sanger sequencing revealed that c.434A > G was paternally inherited, while c.631C > T was maternally inherited.

The *KCTD7* gene is located at 7q11.21 and encodes a member of the potassium channel tetramerization domain-containing protein 7 family [12]. Additionally, the gene is known to be part of cullin 3 ubiquitin ligase complexes [16,17]. Mouse immunohistochemistry studies demonstrated that *KCTD7* is widely expressed in post-mitotic neurons throughout the brain [8]. All KCTD members share an N-terminal BTB/POZ (Pox virus Zinc/Bric-a-brack, Tram-track, Broad complex) domain, a region homologous to the T1 domain of the voltage-gated potassium channel [18,19]. In wild-type *KCTD7*, the BTB domain is in N-terminal amino acids 51–150, and the novel mutation we identified here occurred within this domain (Fig. 4A). Previous studies have confirmed that BTB/POZ mutations in full-length *KCTD7* (R70W, L108 M, and likely R84Q) impair the interaction between KCTD7 and cullin 3. In turn, endosomal and autophagosomal maturation processes are negatively affected, causing abnormal storage of ceroid lipofuscinosis-type material [11]. However, we do not have much data regarding the cellular and molecular outcomes of *KCTD7* deficiency. In vitro experiments may help elucidate how the identified mutations influence KCTD7 protein interactions.

To conclude, we successfully used WES to identify one novel and one known *KCTD7* mutation in a family exhibiting PME. These results highlight the possibility that *KCTD7* is important to PME etiology in Chinese patients. Moreover, we demonstrated that WES is a valuable approach for genomic diagnosis of disorders with high degrees of genomic heterogeneity.

Conflict of interest

There is no conflict of interest.

Table 1
Candidate variants identified in the proband.

Gene symbol	Transcript	Position	Exon	Nucleotide change	Amino acid change	Mode
KCTD7	NM_153033	Chr7:66103359	exon3	c.A434G	p.Q145R	Het
KCTD7	NM_153033	Chr7:66103980	exon4	c.C631T	p.R211X	Het

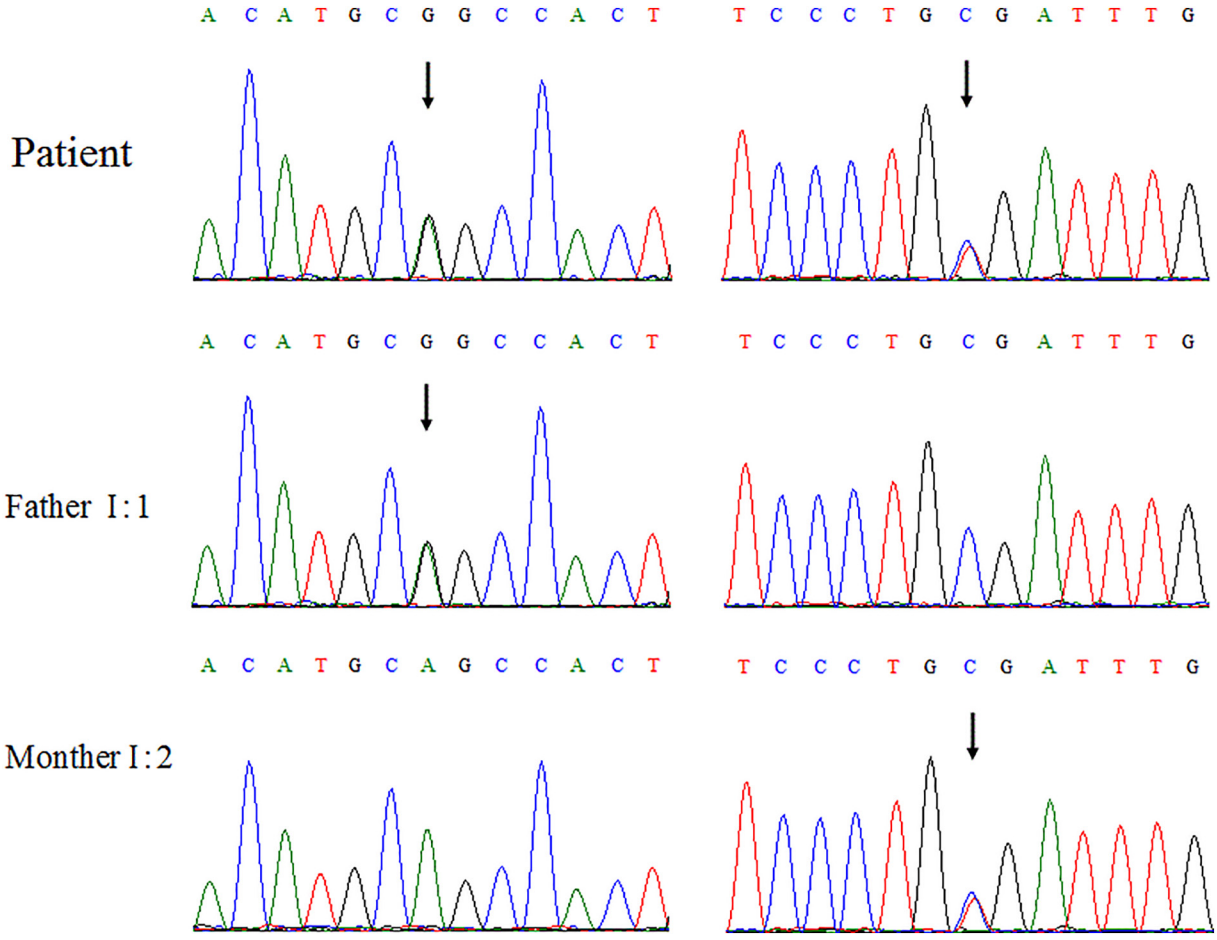

Fig. 3. Identifying mutations in *KCTD7*. Electropherogram analysis of *KCTD7 in proband showing compound heterozygous c.434A > G and c.631C > T mutations of KCTD7*. The father (I 1) carried c. 434A > G, while the mother (I 2) carried c.631C > T.

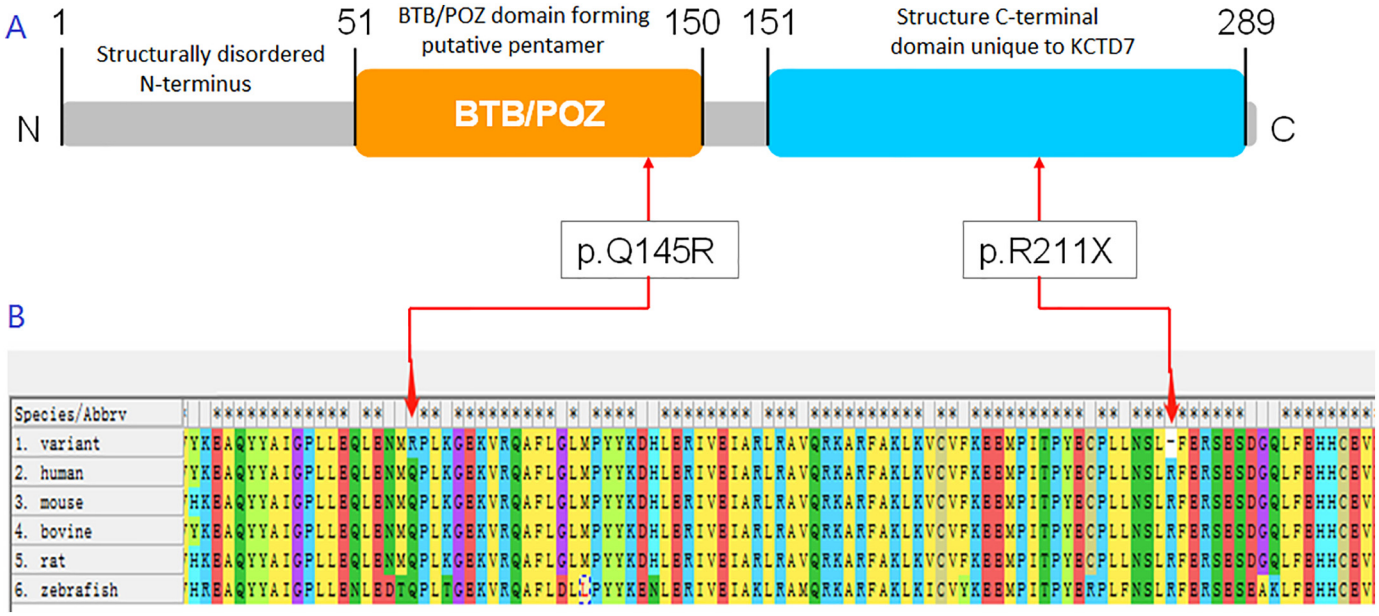

Fig. 4. A. Diagram of KCTD7 with predicted locations of protein sequence changes. B. Protein alignment showed that *KCTD7* p.Q145R and p.R211X residues were conserved across multiple species. Thus, the two mutations occurred at an evolutionarily conserved amino acid.

Acknowledgments

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