

Identification of Mutation in SCN4A, Hot-Spot for Periodic Paralysis, in a Large Chinese Family with a Typical Normokalemic PP Using the Whole-Exome Sequencing



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ABSTRACT

Background: Normokalemic periodic paralysis (Normo KPP) of skeletal muscle is an autosomal dominant disorder caused by mutations in the calcium voltage-gated channel subunit (*CACNA1S*) or sodium voltage-gated channel subunit 4 (*SCN4A*) genes, which lead to ion-channel dysfunction. Little is known about the relationship between mutation genotypes and the clinical symptoms of Normo KPP.

Methods: To examine the mutation status of *CACNA1S* and *SCN4A*, in a Chinese family segregating NormoKPP with atypical clinical symptoms, we performed a series of clinical examinations and genetic analyses with whole-exome sequencing.

Result: Whole-exome sequencing revealed a substitution of c.2111C>T in *SCN4A*, resulting in the amino acid substitution p.T704M in all affected family members.

Conclusion: The present study supports a causative role for this mutation in *SCN4A* in NormoKPP and provides information about the relationship between genotype and atypical clinical symptoms.

Keywords: Normokalemic; Periodic Paralysis; Ion Channel; Mutation; SCN4A; CACNA1S

Introduction

Periodic paralysis (PP) is a group of ion-channel dysfunction diseases [1]. Based on serum potassium level, PP can be classified into hypokalemic periodic paralysis (Hypo KPP), hyperkalemic periodic paralysis (Hyper KPP), and normokalemic periodic paralysis (Normo KPP). The term Normo KPP was originally used in the 1960s [2] to describe a very rare disease characterized by repeated attacks of flaccid muscle weakness or paralysis, despite normal serum potassium levels. Trigger factors for these episodes include rest after acute exercise, cold, hunger, emotional tension, carbohydrate-rich meals, and potassium supplements. Concerns

episodes of paralysis is most in the early morning or during breaks from vigorous activities [3,4]. Several familial and sporadic cases of this disease have been reported [5,6]. Pathogenic mutations in exons 12, 13, 18, and 24 of *SCN4A*, leading to the amino acid changes T704M, R675G, R675W, R675H, R675Q, R1129Q, and M1592V, have been reported to cause Normo KPP in patients from China and other countries [5-8]. Compared with other diseases causing PP, Normo KPP has no specific clinical features, with electromyography, electrocardiogram, blood sugar, cerebrospinal fluid, and routine biochemical laboratory and auxiliary examinations generating normal results during an attack.

In this study, we adopted the Ion Torrent Proton sequencing platform for whole-exome scanning, which has advantages over Sanger sequencing in terms of read throughput, cost, operation time, and potential for use as a standardized procedure in hospital. Given the diverse clinical symptoms of Normo KPP, the scope of the investigation was expanded to the whole-exome scale, to obtain comprehensive information on the genetics underlying the pathogenic features of the condition. We characterized a Chinese family with atypical Normo KPP features and identified a classical c.2111C>T (p.T704M) mutation in *SCN4A* that is predicted to be responsible for the disease.

Patients and Methods

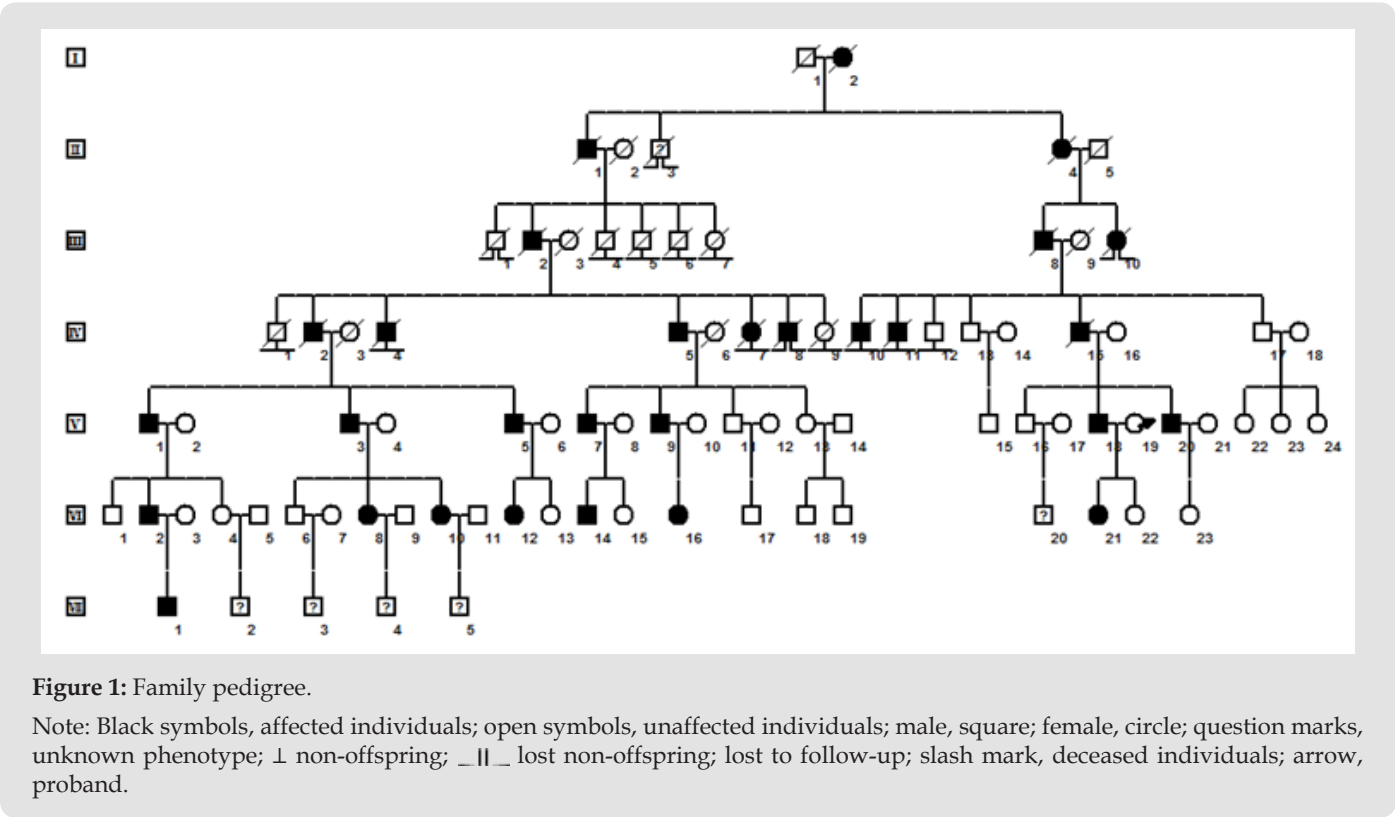
Patients and Families

A Chinese family of 29 members with Normo KPP was recruited from the city of XX, China (Figure 1). The proband was a 32-year-old man with a history of recurrent muscle weakness and gait disturbance, who was hospitalized in 2015. The age of initial onset was 8 years. The common clinical features affecting living 16 family affected members were as follows (Table 1): episodic

paralysis of the limbs with concomitant normal serum potassium concentration; onset at 7–10 years old; patients could stand but not walk, and could lift their upper limbs, but found it difficult to hold them; no dyspnea or dysphagia and no episode of painful cramps and stiffness in limbs; examination of electrocardiogram , thyroid function, and muscle enzymes generated normal results; performance of electromyogram showed no myotonic discharge or increased insertion activity. The patients awake paralyzed the morning following vigorous exercise and cold temperatures. Significantly atypical feature from other Normo KPP families [6-9] was that treatment with large doses of physiological saline had no effect, whereas calcium gluconate was effective. In addition, acetazolamide could reduce the number of attacks and alleviate attack symptoms.

Table 1: PCR primers used for SCN4A exon 13 screening.

Gene	Primer Name	Primer Sequence
SCN4A	E13F	TCCTAAGGCTGGGGCTGCCT
	E13R	GGCCGGGGATCTATGTTTTA



Detailed records of the patients’ medical histories, including physical examinations, blood potassium levels and results of electromyogram were obtained during attacks. The diagnosis of Normo KPP was made based on symptoms, physical signs, and normal blood potassium levels range during attacks. All participants in this study were enrolled and evaluated at the First Affiliated Hospital of Bengbu Medical College. Informed consent was obtained from participants under the research protocols approved by the Bengbu Medical College ethics review board (BYFFY-2012KY04).

DNA Extraction, Ion Torrent Proton Library Preparation, and Sequencing

Peripheral anticoagulated whole blood samples were collected from four family members, including a father (V-18; affected), mother (V-19; unaffected), and two daughters (one affected and one unaffected; VI-21 and VI-22, respectively), and genomic DNA was extracted using a commercial Blood Genomic DNA Miniprep kit (Axygen, Union City, CA, USA). The Qubit dsDNA HS (High Sensitivity

ty) Assay kit was used to quantify DNA with the Qubit® Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's instructions. Ion Torrent Proton adapter-ligated libraries were generated for each of the four family members using the Ion AmpliSeq Library Kit 2.0 (Termofisher, Cat.no.4476610), following the manufacturer's protocol. Following AMPureXP bead purification (Beckman Coulter), the concentrations of the libraries were determined by qPCR using an Ion Library Taqman Quantitation Kit (Termofisher, Cat. No. 4468802). Emulsion PCR and sample enrichment were performed using the Ion PI HI-Q Template OT2 200 Kit v2 (Termofisher, Cat.no.A26434), according to the manufacturer's instructions. Isolation of templated on the Ion Sphere Particles (ISPs) was performed using Ion OneTouch ES (Termofisher, Guilford, CT, United States). Template-positive of ISPs were enriched and sequencing was performed using an Ion PI HI-Q Sequencing 200 Kit v2 (Termofisher, Cat.no.A26433) on the Ion Torrent Proton instrument (Termofisher, Guilford, CT, United States).

Bioinformatics Analyses

Raw data from Ion Torrent Proton runs were processed using the Ion Torrent platform-specific pipeline software, Torrent Suite v4.6 (Life Technologies) to generate sequence reads, for sequence alignment, and to extract single nucleotide variants and indels. Initial variant calling was performed using the Ion Torrent platform-specific pipeline software with the plug-in "variant caller"

program. Two annotation steps were used to obtain information about gene mutations associated with disease. Torrent Suite Variant Caller (TSVC; version 4.6) plug-in generated files were filtered and annotated using ANNOVAR software [10], then the variant caller format (VCF) files generated by ANNOVAR were further filtered and annotated using WANNVAR software [11]. LOVD database and PubMed literature queries were performed to determine whether variants had been reported as pathogenic and identify disease loci; suspected pathogenic mutations were verified by Sanger sequencing. *SCN4A* sequences of five non-human species for homology comparisons were obtained using NCBI BLAST and alignments were carried out using Clustal W to check conservation.

Sanger Sequencing

Mutation in candidate gene *SCN4A* exon 13 (*SCN4A*, NM_000334.4) were confirmed by Sanger sequencing. Primers were designed using Primer Premier 5.0 (*SCN4A* E13F- TCCTAAGGCTGGGGCTGCCT, *SCN4A* E13R- GGCCGGGGATCTATGTTT). Amplified fragments were sequenced on an ABI Prism 3730XL DNA analyzer (Applied Biosystems) using a Big Dye Terminator Cycle Sequencing Kit v2 (Applied Biosystems). Chromatograms illustrating the Sanger sequencing results were analyzed using the SeqMan Pro application in DNASTAR's Lasergene Molecular Biology Suite software.

Result

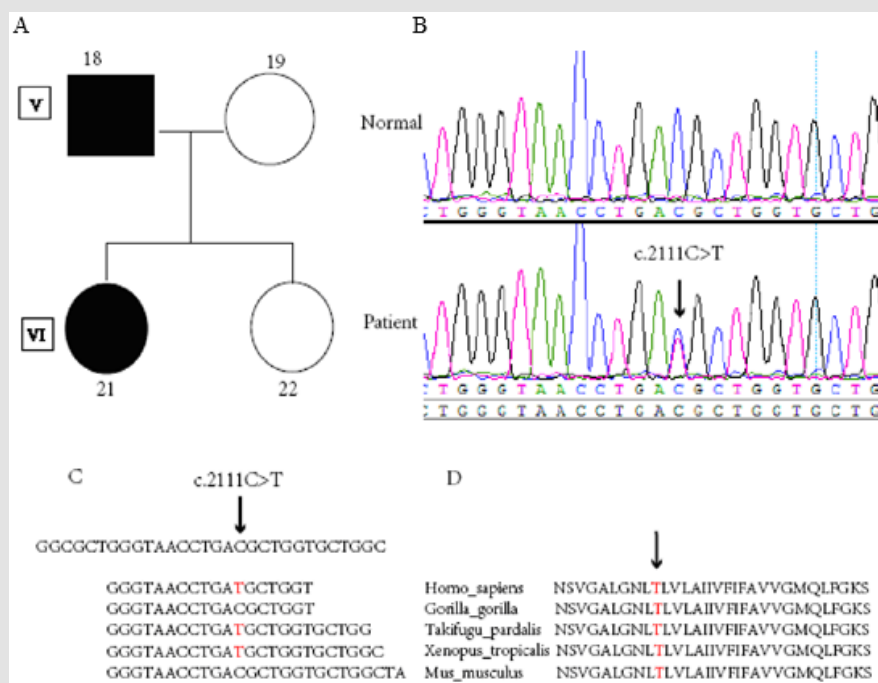


Figure 2: *SCN4A* mutation detection in a family with normokalemic periodic paralysis.

Note:

- Pedigree structure of a NormoKPP family carrying the c.2111C>T mutation in *SCN4A*.
- SCN4A* DNA sequencing results of the patient and a healthy control.
- SCN4A* Ion torrent DNA sequencing results for the patient.
- Sequence conservation of amino acid 704 in the *SCN4A* protein.

We performed whole-exome sequencing of a four-member Chinese family including one parent and one child affected with Normo KPP, resulting in > 100× coverage (Figure 2A). On average, 75 million high quality reads (AQ ≥ 20) were generated per run. The average depth of total coverage was 148x, and the minimal coverage was 28x. Candidate variants (n = 94) (supplementary data) were selected after mapping to the reference genome and filtering using relevant databases. Finally, one variant (SCN4A, p.T704M) was identified in a gene of interest (Figure 2C). The result of exome sequencing was verified by Sanger sequencing (Figure 2B) in affected patients (IV5, V1, V3, V5, V7, V9, V18, V20, VI2, VI8, VI10, VI12, VI14, VI16, VI21, VII1). The threonine residue at position 704 of SCN4A is highly conserved among humans, mice, gorilla, Fugu, and Xenopus (Figure 2D).

Discussion

SCN4A encodes the α -subunit, voltage-gated sodium channel, Nav1.4 whose structure consists of four domains (DI-DIV) of six transmembrane helical segments (S1-S6). Here, we report a pathological mutation in the SCN4A gene in a family presenting with hereditary Normo KPP. In the present study, whole-exome and Sanger sequencing identified C/T heterozygosity in the SCN4A gene at nucleotide position 2111 in the 13th exon. Both of the affected members in the family carried the p.T704M mutation, whereas the unaffected members did not. Pathophysiological studies show the mutation conduct gating pore current in both activated and slow-inactivated states, which would cause increased influx of sodium near the resting membrane potential, membrane depolarization, sodium overload, action potential failure [12]. Structural studies elucidate the molecular mechanism by which mutations in S4 cause pathogenic gating pore currents and suggest that ion permeation through the gating pore is controlled dynamically by the state of the voltage sensor and by rotamer conformations of R4 [13]. Patients of the family exhibited Normo KPP with atypical features, in that treatment with high dose physiological saline was ineffective, while calcium gluconate was effective. Different families with the p.T704M mutation of SCN4A showed various clinical picture. The mutation p. T704M identified in family accounts for the majority of patients with hyper KPP [14,15]. Some patients harbouring this mutation also exhibit periodica paramyotonia and paramyotonia congenita [16,17]. Some of families diagnosed as Normo PP were subsequently found to have the Hyper PP mutations T704M or M1592V, leading to the suggestion that Normo PP may be a phenotypic variant of HyperPP [18]. Patients with Normo PP may experience transient high serum potassium level before attacks. Why Genotype-phenotype correlations for SCN4A mutations are not straightforward? A potassium channel gene such as KCNE3, in which mutations are a rare cause of hypokalaemic or hyperkalaemic periodic paralysis, is an example of a possible modifying gene [19]. It now appears that Ca^{2+} -activated- K^{+} -(BK) channel encoded by one gene (slo1/KCNMA1) may have relevance in disorders associated

with abnormal K^{+} ion homeostasis including periodic paralysis and myotonia [20,21].

Saline solution is first choice for acute management of Normo KPP. The dose of saline solution usually needs more than three thousand milliliter a day and patients with NormoKPP regularly recovered within 2-3 days. Patients of the family with Normo KPP showed no response to saline solution, intravenous calcium gluconate was chosen to treat patients according to protocol of Neurology textbook [22]. We presumed the mechanism of which was calcium gluconate enhance opening of calcium-activated K channels [20,21]. The prophylactic therapy with diuretics is effective in treating Normo KPP. Acetazolamide was empiric treatment for NormoKPP, the mechanism of action is unclear. Another diuretics hydrochlorothiazide was confirmed being effective in the prophylactic treatment of normoKPP caused by the SCN4A mutation of p. Thr704Met [23]. The results of this study demonstrate potential new clinical features of PP associated with p.T704M mutation in SCN4A. In addition, we also demonstrate the feasibility of next generation sequencing for mutation detection in a complex monogenic disease.

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Conflict of Interest

We have no disclosure to make that qualifies as a conflict of interest.

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