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# A novel Kir2.6 mutation associated with hypokalemic periodic paralysis

Jinfan Zheng [a], Zonglai Liang [a], Ying Hou [a], Fuchen Liu [b], Yuanyuan Hu [c], Pengfei Lin [c,*], Chuanzhu Yan [c,*]

[a] Laboratory of Neuromuscular Disorders and Department of Neurology, Qilu Hospital, Shandong University, Jinan, China
[b] Department of Neurobiology, Yale School of Medicine, New Haven, CT 06510, USA
[c] Key Laboratory for Experimental Teratology of the Ministry of Education, Brain Science Research Institute, Department of Neurology, Qilu Hospital, Shandong University, Jinan, China

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HIGHLIGHTS

- A novel mutation of *KCNJ18*, G169R, was found to be associated with hypokalemic periodic paralysis with normal thyroid function.
- Functional characterization of this mutant Kir2.6 using patch clamp showed abnormally reduced inward and outward current densities.
- Findings extend previous knowledge on dysfunctions of Q126X, K360T, and E388K mutant Kir2.6 channels, which are associated with thyrotoxic periodic paralysis.

ABSTRACT

*Background and objective:* Mutations in *KCNJ18*, which encodes the inwardly rectifying potassium channel Kir2.6, have rarely been reported in hypokalemic periodic paralysis. We describe the clinical phenotype of a novel *KCNJ18* gene mutation and perform functional characterization of this mutant Kir2.6.
*Methods:* A long-term exercise test (ET) was conducted based on the McManis method. Whole-cell currents were recorded using patch clamp, and the HEK293 cells were transfected with wild-type or/and mutant Kir2.6 cDNA.
*Results:* A de novo conserved heterozygous mutation in Kir2.6, G169R, was found in a hypokalemic periodic paralysis patient. ET led to a decrease in the amplitude of compound muscle action potential (CMAP) by 64%. Patch clamp results showed that the potassium inward and outward current densities of the G169R mutant were, respectively, reduced by 65.6% and 84.7%; for co-expression with wild type, which more closely resembles the physiological conditions in vitro, the inward and outward current densities decreased, respectively, by 48.2% and 47.4%.
*Conclusions:* A novel *KCNJ18* mutation, G169R, was first reported to be associated with hypokalemic periodic paralysis without hyperthyroidism. Electrophysiological results demonstrated a significant functional defect of this mutant, which may predispose patients with this mutation to paralysis.
*Significance:* This new G169R mutation of the potassium channel Kir2.6 provides insight into the pathogenic mechanisms of hypokalemic periodic paralysis.


## 1. Introduction

Hypokalemic periodic paralysis (hypoKPP) is a rare autosomal dominant skeletal muscle channelopathy clinically characterized by recurrent paralytic episodes with concomitant hypokalemia (<2.5 mmol/L), and patients may occasionally develop late-onset proximal myopathy (Vicart et al., 1993–2006). The paralytic attacks usually lead to paraparesis or tetraparesis (Stedwell et al., 1992). Typically, patients awaken paralyzed and regain strength in several hours to days. The major triggering factors are

*Abbreviations:* hypoKPP, hypokalemic periodic paralysis; TPP, thyrotoxic periodic paralysis; $FT_3$, free triiodothyronine; TSH, thyroid stimulating hormone; CK, creatine kinase; PCR, polymerase chain reaction; HEK 293, human embryonic kidney 293; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; ET, exercise test; CMAP, compound muscle action potential; ADM, abductor digiti minimi.

* Corresponding authors at: Key Laboratory for Experimental Teratology of the Ministry of Education, Brain Science Research Institute, Shandong University, No. 107, West Wenhua Road, Jinan, Shandong Province 250012, China. Tel./fax: +86 531 82169217.

*E-mail addresses:* lpfsdu@foxmail.com (P. Lin), chuanzhuyan@163.com (C. Yan).

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carbohydrate-rich meals and rest after strenuous exercise. To date, hypoKPP has been primarily associated with mutations in three genes: (i) *CACNA1S*, encoding the alpha subunits of the skeletal muscle L-type calcium channel (Matthews et al., 2009); (ii) *SCN4A*, encoding the skeletal muscle sodium channel (Sternberg et al., 2001; Kuzmenkin et al., 2002); and (iii) *KCNJ18*, encoding the skeletal muscle inwardly rectifying potassium channel Kir2.6 (Ryan et al., 2010). Recently, the physiological functions of Kir2.6 have been described in detail (Ryan et al., 2010; Cheng et al., 2011; Dassau et al., 2011; Li et al., 2015). Given the limited number of reports on pathogenic *KCNJ18* mutations, more patients with mutant Kir2.6 channels and their characterizations are needed.

This study presents the clinical, molecular, and electrophysiological findings in a Chinese hypoKPP patient with normal thyroid functions. For providing more insights into the underlying mechanism, we further studied the functional properties of relative mutant Kir2.6 channels in thyrotoxic periodic paralysis patients previously reported in the literature of the Chinese mainland using patch clamp.

## 2. Methods

### 2.1. Subjects

This study was approved by the ethics committee of Qilu Hospital, Shandong University, China. Informed consent was obtained from all subjects according to the Declaration of Helsinki. This study included 100 healthy subjects without neuromuscular disorders and hyperthyroidism as normal controls for genome analysis.

### 2.2. Exercise test

The exercise test (ET) proposed by McManis was modified and performed in the selected patient after resting for 5 min (McManis et al., 1986). Surface electrodes were taped on the abductor digiti minimi (ADM) muscle, and compound muscle action potentials (CMAPs) were recorded. Supramaximal stimulation was performed on the ulnar nerve. The ET procedure was as follows: (i) CMAPs were first recorded every 10 s for 1–2 min for obtaining a stable baseline amplitude. (ii) The rest period was set as 3–4 s at every 30–45 s, and then the ADM muscle was exercised as strongly as possible for 5 min. (iii) Immediately and then 1 h after this 5 min of exercise, CMAPs were recorded for 2 s during each 3–4-s rest.

### 2.3. Genomic analysis

Genomic DNA was extracted from the peripheral blood of this patient and 100 healthy control subjects using the Wizard Genomic DNA Purification Kit (Promega). The exons of *KCNJ18* gene were amplified via polymerase chain reaction (PCR) using primers as previously reported (Ryan et al., 2010). In addition, screening of *CACNA1S* and *SCN4A* gene was also performed. Direct sequencing of the amplified fragments was performed on an ABI prism 3130 genetic analyzer.

### 2.4. Construction of Kir2.6 mutant channels

The wild-type (*WT*) cDNA of Kir2.6, pEGFP-Kir2.6, was provided by Professor Louis Ptacek (Ryan et al., 2010). Point mutations were induced through PCR including 1-μM forward- and reverse-specific primers, that is, for Q126X: GTGTGATGTAGGTGCA/TGCACCTACAT CACAC; G169R: CCATCGTGCGCTGCATC/GATGCAGCGCACGATGG; K360T: CAGTGCGACGGATCTGG/CCAGATCCGTCGCACTG; and E388K: GCCGTGACGAAGAGGAT/ATCCTCTTCGTCACGGC. All final plasmids were confirmed by sequencing.

### 2.5. Expression of WT and mutant Kir2.6 channels

COS-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and transfected with *WT* and mutant cDNA of Kir2.6 using Turbofect (Life Technologies). Following 1-day transfection, the cells were fixed at −20 °C with cold 100% methanol for 15 min and blocked with 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 1 h. Afterward, the cells were incubated with rabbit anti-GFP (green fluorescent protein; 1:300) primary antibodies at 4 °C overnight, then rinsed thrice with PBS, and incubated with goat anti-rabbit Alexa Fluor 488 (1:600) secondary antibodies for 1 h. Finally, the slides were covered using ProLong Gold Antifade Reagent overnight at room temperature.

### 2.6. Electrophysiological recording

Human embryonic kidney (HEK) 293 cells were cultured in DMEM (Hyclone), containing 10% FBS and transfected with *WT* and mutant cDNA of Kir2.6 using Turbofect. After 1-day transfection, whole-cell currents were recorded using an Axopatch 200B Amplifier (Axon Instruments) and pClamp6 for data export at room temperature. Data were analyzed through Igor Pro. The pipette had a resistance of 1.5–3.5 MΩ. The series resistance and capacitance were compensated (95%) for all whole-cell recordings.

Whole-cell currents were first recorded at the membrane potential for 50 ms and then at every potential for 100 ms ranging from −60 to +60 mV with a 10-mV increment. Each procedure was repeated thrice. Data were sampled at 5 kHz with a 2-kHz low-pass filter. The pipette solution contained 5 mM glucose, 20 mM KCl, 110 mM K-aspartate, 1 mM $MgCl_2$, 5 mM $Na_2$-ATP (adenosine 5′-triphosphate), 10 mM EGTA (ethylene glycol tetraacetic acid), and 10 mM HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; pH 7.4). The extracellular solution comprised 1 mM $MgCl_2$, 117 mM NaCl, 2 mM $CaCl_2$, 5 mM glucose, 30 mM KCl, 10 mM HEPES, and 2 mM $NaHCO_3$ (pH 7.3).

### 2.7. Statistical analysis

Data analysis and curve fitting of the electrophysiological experiments were performed with Microsoft Excel. Values were shown as mean ± standard error. The statistical significances were analyzed using the two-tailed unpaired Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

## 3. Results

### 3.1. Clinical data

A 34-year-old man presented with periodic episodes of paralysis for 17 years. He experienced his first attack of muscle weakness at the age of 17. During this episode, he awoke with quadriplegia, and the paralysis was worse in the lower than in the upper extremities. The blood potassium level was 1.8 mmol/L. The creatine kinase level was found to be 1964 IU/L. No arrhythmia was reported. His symptoms returned to normal after around 24 h without any treatment. Subsequently, similar symptoms occurred once or twice monthly from the age of 17 years, more frequently in September, October, and November. Each attack lasted for 24–72 h. The patient reported that attack could be induced by exhaustion and prolonged immobility, especially carbohydrate-rich meals. He denied any family history of thyroid or neurological problems. His muscle strength was well maintained between attacks, and no fasciculation or percussion myotonia was noted. The thyroid-stimulating levels were normal.