The clinical and genetic features in a cohort of mainland Chinese patients with thyrotoxic periodic paralysis

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Abstract

**Background:** Thyrotoxic periodic paralysis (TPP) is a life-threatening channelopathy manifesting as recurrent episodes of hypokalemia and muscle weakness in the presence of hyperthyroidism. Recent findings indicate hypofunction of inward rectifying K+ (Kir) channels are associated with a lot of TPP patients in Caucasian population. However, potential genetic risk factors for mainland Chinese patient who are the largest group of TPP cases in the world have been largely unexplored. **Methods:** Samples of DNA from 127 individuals with TPP and 102 hyperthyroidism male controls self-reported as mainland Chinese were collected from 5 clinical centers from Jan 2011 to Jan 2014. The \( \text{KCNJ2} \) gene, \( \text{KCNJ18} \) gene, as well as loci polymorphism (rs623011 and rs312691) at 17q24.3 were directly sequenced in TPP patients and controls. Clinical data were obtained on TPP participants for genotype/phenotype correlations. **Results:** About 3.15% of TPP cases harbored \( \text{KCNJ18} \) gene mutations in mainland Chinese patients. The risk alleles at 17q24.3 (rs623011 and rs312691) were more common in patients with TPP than in the controls and were therefore a significant risk factor for TPP (odds ratio, 11.94 and 10.57; 95% CI, 5.93-24.05 and 5.48-20.40; \( P=1.81 \times 10^{-14} \) and \( 1.07 \times 10^{-14} \) respectively).

**Conclusions:** This study demonstrates that the \( \text{KCNJ18} \) variants are responsible for a little part of TPP patients in mainland China. In addition, the rs623011 and rs312691 loci are significantly associated with TPP patients in mainland China and highlight the Kir2.1 channel as a causative target in TPP.

**Keyword:** thyrotoxic periodic paralysis; \( \text{KCNJ18} \) gene; hyperthyroidism; polymorphism
1. Background

Thyrotoxic periodic paralysis (TPP) is a disorder manifesting as recurrent episodes of hypokalemia and muscle weakness in the presence of hyperthyroidism. The condition may be life-threatening if weakness of the breathing leads to respiratory failure, or if the low potassium levels cause cardiac arrhythmias [1,2]. TPP is more prevalent in Asian populations, especially in males of Chinese, Japanese, Vietnamese, Filipino, and Korean descent [3,4]. In Chinese populations, TPP occurs in up to 13% of male thyrotoxic patients, while women are predominantly affected hyperthyroidism in the general population [3,5]. The high incidence of TPP among Asian people suggests that the basic defects may be genetically determined [6].

Although the mechanism of TPP remains largely uncertain, a few risk factors have been identified, such as carbohydrate loads and rest following exercise [7]. When the symptoms of thyrotoxicosis are separated from the clinical picture, many features of this disease are similar to those described for familial hypokalemic periodic paralysis [8]. The condition has been linked with genetic mutations in genes that encode for certain ion channels that transport electrolytes across cell membranes [9]. So TPP is considered as an endocrine channelopathy with genetic background [10]. Recent findings indicate that loss of function mutations of the skeletal muscle-specific inward rectifying K+ (Kir) channel, Kir2.6, encoding by the KCNJ18 gene, associate with a part of TPP patients mainly from the United States, Brazil, France and Singapore [11]. Other important developments are that gene polymorphisms (rs623011 and rs312691) at 17q24.3 may affect the expression of KCNJ2 gene (encoding Kir2.1) in Hong Kong and Thai populations [12,13]. In mainland
China, there exists the largest group of patients with TPP in the world [14]; however, the clinical and genetic features are seldom explored in these patients. In our study, we aimed to determine whether mutations in \textit{KCNJ2} and \textit{KCNJ18} exist in mainland Chinese patients, and assess whether loci polymorphisms (rs623011 and rs312691) at 17q24.3 are associated with mainland Chinese patients.

\textbf{2. Methods:}

\textbf{2.1 Subjects}

127 Chinese patients with TPP were collected in 5 clinical centers (the first affiliated hospital of Nanchang University, Navy general hospital of China, Affiliated hospital Guiyang medical College, People hospital of Shanxi province, and Affiliated Hospital of Jiujiang College) from January 2011 to January 2014. Diagnostic criteria for TPP include: (1) acute limb paralysis with lower motor neuron; (2) blood potassium concentration <3.5mmol/L; (3) hyperthyroidism supported by laboratory tests; (4) excluding familial hypokalemia periodic paralysis and hypokalemic periodic paralysis caused by other causes. All subjects were examined and interviewed by at least two clinicians (Li X. Yao S. Xiang Y. Zhang X. Wu X. Wan H. and Hong D.). 102 control subjects were recruited from hyperthyroid male patients without episodic weakness during their hyperthyroid states. All cases and controls self-reported as mainland Chinese were male and their etiology of hyperthyroidism was Graves’ disease. This study was approved by the ethics committee of the first affiliated hospital of Nanchang University. All participants gave their written informed consent in compliance with the Chinese bioethics laws as well as the Declaration of Helsinki.
2.2 Genetic screening

Genomic DNA was extracted from peripheral blood of TPP cases and controls. Coding exons of the \textit{KCNJ2} and \textit{KCNJ18} genes were amplified using polymerase chain reaction (PCR) with intronic primers. The primers for amplification of the \textit{KCNJ2} gene (NM000891.2) include as fellows F1: 5’GCTCCCAGAGACCATCTC3’, R1: 5’CATGACTGCGCCAATGATG3’; and F2: 5’GACCCAGACCAACCATAGGCTC3’, R2: 5’CCCATCTTGACCAGTACCCT3’. The primer for the \textit{KCNJ18} gene (NG033093) is F: 5’ATGCTGTCCTCTCTGTTCC3’ and R: 5’GGGCCTCTCCCCCAGGCA3’. The primers for rs623011 and rs312691 were F: 5’TCAGTCAACCACAAACCAC3’, R: 5’CCAGCCAAGGAGACAAAT3’ and F: 5’TCACTGGAAGGTTGGG3’, R: 5’AGCAGTGAAGGAGGTTGGG3’ respectively. After purification, PCR products were directly sequenced with an ABI 3730 DNA analyzer (Applied Biosystems, Inc., CA, USA). To exclude PCR errors, all nucleotide variations were sequenced in reverse. To exclude the possibility that \textit{KCNJ18} mutation represents polymorphisms, identical genomic fragments from 200 healthy Chinese controls were examined for the novel mutations.

2.3 Statistical analysis

For association studies, the allele and genotype distributions in cases and controls were compared and evaluated in allelic, dominant and recessive inheritance models by two-tail Fisher’s exact test. Odds ratios (OR) and confidence intervals (CI) were calculated using the non-risk genotype (12+22) as reference.

3. Results

3.1 \textit{KCNJ18} gene mutation
In the \textit{KCNJ18} gene screening, 3 novel and 1 previously reported heterozygous point mutations were identified in 4 out of 127 (3.15\%) patients with TPP. One nonsense variant was discovered (c.376C>T) introducing a stop codon (p.Q126X). Two other novel missense mutations caused c.1079A>C substituting a threonine for a lysine at residue 360 (p.K360T); and c.1162G>A resulting the change of glutamic to lysine at residue 388 (p.E388K). We also identified a variant leading to the change of alanine to proline at residue 200 (p.A200P) that was described in a Taiwanese with sporadic periodic paralysis [15]. These novel mutations were not identified in 200 healthy controls of Chinese descent.

\textbf{3.2 \textit{KCNJ12} gene related mutations}

No genetic variants were identified in the \textit{KCNJ2} gene in this cohort of TPP patients. However, the genetic polymorphisms of rs312691 and rs623011 nearby the \textit{KCNJ2} gene showed significant associations with TPP (Table 1). In rs623011, A is the minor allele (0.488 in Han Chinese in Beijing, China (CHB)) [16]. The allele frequency increased to 0.772 (196/254) in TPP patients, while A allele frequency was 0.461 (94/204) in hyperthyroid controls. In rs312691, C is minor allele (0.463 in CHB) [16]. The allele frequency increased to 0.799 (203/254) in TPP patients, while C allele frequency was 0.471 (96/204) in hyperthyroid controls. The polymorphisms had great associations with TPP occurrence, but there are no significant differences between clinical phenotype and genotype. Interestingly, the 4 patients with \textit{KCNJ18} mutation carried with A/G or G/G at rs623011, and C/T or T/T at rs312691, while no homogenous minor alleles (A/A and C/C) were identified at the 2 alleles.
3.3 Clinical features of TPP

Four sporadic male patients harbored the mutation of \textit{KCNJ18} gene (Table 2). The age at onset ranged from 19 to 25 years old (mean $21.75\pm2.75$). All subjects suffered from severe limbs weakness when getting up in the morning. In addition, 3 out of the 4 patients complained muscle soreness, and one felt limb numbness. The disease course lasted for 2-8 (mean $4.75\pm2.75$) hours. Three patients had strenuous exercise before attack. Serum potassium was 1.6-2.4mmol/L (normal reference 3.5-5.1mmol/L) without abnormalities of serum magnesium and phosphorus. Serum creatine kinase (CK) of all patients moderately elevated, reaching 1205-1710U/L (normal reference 20-170U/L). ECG showed a typical u-wave without other abnormal electrophysiology. The diagnosis of hyperthyroidism was not made before weakness attack; however, medical history suggested that 3 patients had weight loss, 1 patient had mild goiter, and 2 patients had mild hand tremor. Diagnosis of hyperthyroidism was made in 4 patients basing on thyroid stimulating hormone (TSH) $<0.03$ mU/L (normal reference 0.35-4.95mU/L), elevated free triiodothyronine (17.2$\pm$5.4pg/L) (normal reference 3.1-6.8pg/L), increased free tetraiodothyronine (65.6$\pm$21.9pmol/L) (normal reference 12-22pmol/L). Weakness was relieved after supplementing potassium. The symptom recurred during the treatment in 3 patients, and one of them had 4 times in the follow-up 13-28 months period, however, weakness did not occur again after remission of hyperthyroidism.

The age at onset in 123 patients without \textit{KCNJ18} variants were from 16 to 62 years old (mean $25.23\pm16.67$). During the course of attacks, 21 patients felt muscle soreness or limb numbness. The duration of weakness ranged from 1 hour to 6 days (mean
27.55±11.61 hours), which was longer than those of patients with \textit{KCNJ18} variants. 66 patients had quadriplegia; 52 patients had weakness mainly in lower limbs; 4 patients had weakness mainly in upper limbs; and interestingly 1 patient had weakness both lower limbs plus right upper limb. Only 2 patients had mild dysphagia, while weakness involving in face, bulb and respiratory muscles was not observed in others. The first serum electrolyte tests in all patients were confirmed hypokalemia from 1.3 to 3.4mmol/L. The elevation of CK in 75 cases ranged from 720 to 7860U/L. All patients were confirmed hyperthyroidism through lab test, while different extent of hyperthyroidism symptoms was observed in 69 patients (tremor in 47 cases, weight loss in 30cases, and goiter in 12 cases). The attacks appeared again in 36 patients during the follow-up 2 months to 35 months, while weakness did not occur again after remission of hyperthyroidism.

4. Discussion

Although the detailed mechanism of TPP remains obscured, TPP has been considered as a kind of channelopathy disease, because its clinical pictures are very similar to those of familial or sporadic hypokalemic periodic paralysis [1-3]. A milestone progression was made by \textit{Ptacek LJ} and his colleagues in 2010 [11]. They found that the hypo-function mutation of Kir2.6 was responsible for partial TPP patients: one third Caucasian and Brazilian TPP patients harboring \textit{KCNJ18} mutations, 25.9% in Singapore, and 1.2% in Hong Kong. In addition, 1.7% TPP patients from Taiwan were identified with \textit{KCNJ18} variations, while mutations in \textit{KCNJ18} were also identified in sporadic periodic paralysis (SPP) suggesting both SPP and TPP had the same molecular basis [15]. Our systematic screening of 127 TPP cases found 3 novel and 1 previously reported heterozygous
mutations in the *KCNJ18* gene, indicating 3.15% mainland Chinese TPP patients harbored this gene mutations, which is a little higher than those in Hong Kong and Taiwan, but significant lower than Caucasian populations.

Since the first cases with *KCNJ18* mutations were reported in 2010, there were only 20 TPP cases with *KCNJ18* mutations described in the worldwide [11,15]. All of the patients plus our 4 cases were male individuals, while 2%-5% of TPP patients without *KCNJ18* mutation were female cases [5,6]. Comparing with wide span on onset age in non-*KCNJ18* mutation TPP patients, patients with *KCNJ18* mutation are generally around 20 years old. The episodic weakness of *KCNJ18* mutation patients was commonly relieved within several hours (4.75h), while the mean duration of our non-*KCNJ18* patients lasted for 27.55 hours. All of *KCNJ18* mutation patients presented with severe weakness, high level of serum CK, and muscle soreness in 3 cases, indicating clinical manifestations in *KCNJ18* mutation patients may be severer than those in non-*KCNJ18* mutation patients. The muscle weakness recurred in 3 out 4 patients with *KCNJ18* mutation, while the recurrence only appeared in 36 out of from 123 patients with non-*KCNJ18* mutation, suggesting the patients with *KCNJ18* mutation may have higher recurrence. At present, the total number of patients with *KCNJ18* mutation having been reported was relatively small. Whether the clinical phenotype had its own characteristics or not, more cases should be collected to analyze and summarize in the future.

The *KCNJ18* gene encodes Kir2.6 protein that is a specific muscle subtype of Kir2.x family [11]. Kir2.6 can physically associate with Kir2.1 and Kir2.2 to form heterotetramers on sarcolemma. Through heterotetramerization of subunits, Kir2.6 may control Kir2.1 and
Kir2.2 abundance on the muscle plasma membrane, thus providing a mechanism to fine tune electrical responses [17,18]. The KCNJ18 gene contains a thyroid-responsive cis element within its promoter that may regulate gene transcription [19]. Under the high thyroid hormone, mutational KCNJ18 transcripts express lots of abnormal Kir2.6, and thus a dominant negative trafficking phenomenon can lead to endoplasmic reticulum retention of Kir2.x subunits [18]. Thus, mutational Kir2.6 may alter a delicate protein abundance and balance of electrical activity in muscle. Similar to other reports, the 3 novel mutations in our study are also located in intracellular C-terminal of Kir2.6, which maybe affect downstream phosphorylation of ion channel signal conduction [11,18]. Interestingly, except for our TPP case, A200P was also found in a Taiwan SPP patient [15], but our case had not recurred periodic paralysis after remission of hyperthyroidism. In vitro study suggested A200P mutant could exert a significant dominant negative inhibition to wild type [15]. It still needs to investigate the delicate role of thyroid toxicity to function of Kir2.6 with A200P mutation.

Kir2.1 (encoded by KCNJ2) is highly expressed in skeletal and cardiac muscle. It is one of important components in the Kir2.x subfamily that are involved in the determination of muscle electrical physiology, such as resting membrane potential and cell excitability [17,20]. It is also known that Kir2.1 can physically integrate with any one of the other Kir2.x proteins, such as Kir2.2, Kir2.3, Kir2.4, and Kir2.6, to form heterotetramers on cell membrane [17]. Although we could not find any deleterious KCNJ2 mutations in our TPP cases through direct sequencing, earlier studies showed that Kir2.1 mutations lead to Andersen–Tawil syndrome (ATS) manifesting as periodic paralysis, ventricular ectopy, and
dysmorphic features [21]. Both ATS and TPP are characterized by periodic paralysis. Periodic paralysis occurs in ATS independent of blood potassium concentration, but periodic paralysis in TPP can occur only in the presence of hypokalemia and a thyrotoxic state. Both rs623011 and rs312691 polymorphisms are located in the 17q24.3 locus, nearby ~75 kb and ~150 kb downstream of the KCNJ2 gene. Transcriptional regulatory elements of genes can be located at regions far away from the target transcriptional units [22]. In expression quantitative trait locus (eQTL) analysis, the loci variants might affect the expression of Kir2.1 and result in episodic weakness similar to one of the clinical characteristics in ATS patients [23].

5. Conclusion

The mutations in KCNJ18 are responsible to a little part of Chinese patients with TPP. The patients with KCNJ18 mutation might have a shorter disease course, severer manifestation, and higher prevalence of recurrence comparing with those TPP patients with non-KCNJ18 mutations. We also demonstrate that the genetic variants of rs623011 and rs312691 at 17q24.3 locus are important susceptible genetic polymorphisms for TPP patients in mainland China.
List of abbreviations

TPP: thyrotoxic periodic paralysis; Kir: inward rectifying K⁺; PCR: polymerase chain reaction; OR: odds ratios; CI: confidence intervals; CK: creatine kinase; TSH: thyroid stimulating hormone; SPP: sporadic periodic paralysis; AST: Andersen–Tawil syndrome; eQTL: expression quantitative trait locus.

Competing interests: None.

Author Contributions:

Dr. Daojun Hong completed study concept, drafting of the manuscript and takes responsibility for the integrity of the data and the accuracy of the data analysis. Dr. Xiaobing Li and Dr. Sheng Yao collected the most of TPP patients, and performed the genetic screening. All other authors contributed to collecting TPP patients and drafting the manuscript.

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References


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**Table 1.** Genotype associations of rs623011 and rs312691 with TPP in mainland Chinese male patients

<table>
<thead>
<tr>
<th></th>
<th>case</th>
<th>control</th>
<th>P-value</th>
<th>odds</th>
<th>95% confidence interval</th>
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<tbody>
<tr>
<td>allele(1/2)</td>
<td>frequency of risk allele(1)</td>
<td>frequency of risk allele(1)</td>
<td>1 vs 2</td>
<td>11 vs 12 + 22</td>
<td>11 + 12 vs 22</td>
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<tr>
<td>rs623011</td>
<td>A/G</td>
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<tr>
<td>rs312691</td>
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<td>39</td>
<td>6</td>
<td>0.799</td>
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</tbody>
</table>

Odds ratios and confidence intervals were calculated using the non-risk genotype (12+22) as reference.
<table>
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<tr>
<th>case</th>
<th>gender/age</th>
<th>attack duration</th>
<th>symptoms of hyperthyroidism</th>
<th>potassium mmol/L</th>
<th>CK IU/L</th>
<th>TSH uIU/ml</th>
<th>FT3 pg/ml</th>
<th>FT4 ng/dl</th>
<th>KCNJ18 mutation</th>
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<td>1</td>
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<td>2h</td>
<td>quadriplegia</td>
<td>1.9</td>
<td>2093</td>
<td>&lt;0.03</td>
<td>8.2</td>
<td>3.5</td>
<td>p.Q126X</td>
</tr>
<tr>
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<td>6h</td>
<td>quadriplegia</td>
<td>2.4</td>
<td>1476</td>
<td>&lt;0.03</td>
<td>12.3</td>
<td>5.8</td>
<td>p.A200P</td>
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<tr>
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<td>quadriplegia</td>
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<td>5.1</td>
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<td>9.3</td>
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</tr>
</tbody>
</table>

Abbreviation: CK, creatine kinase; TSH, Thyroid Stimulating Hormone; FT3, free triiodothyronine; FT4, free thyroxine.