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## SHORT REPORT

Rare *KCNJ18* variants do not explain hypokalaemic periodic paralysis in 263 unrelated patientsMarius Kuhn,<sup>1,2</sup> Karin Jurkat-Rott,<sup>1</sup> Frank Lehmann-Horn<sup>1</sup>

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**ABSTRACT**

**Objective** To examine rare *KCNJ18* variations recently reported to cause sporadic and thyrotoxic hypokalaemic periodic paralysis (TPP).

**Methods** We sequenced *KCNJ18* in 474 controls (400 Caucasians, 74 male Asians) and 263 unrelated patients with periodic paralysis (PP), including 30 patients with TPP without mutations in established PP genes.

**Results** In 10 patients without TPP, we identified 9 heterozygous, novel variations (c.-3G>A, L15S, R81C, E273X, T309I, I340T, N365S, G394R, R401W) and a questionable heterozygous causative R399X stop variant. Studies on 40 relatives of these 10 patients showed that none of the variants were de novo in the patients and that R399X occurred in 3 non-affected relatives. Most affected amino acids lacked conservation and several clinically affected relatives did not carry the patient's variant. T309I, however, could be pathogenic under the pre-requisite of strongly reduced penetrance in females. Of the controls, 17 revealed 12 novel rare variants including the heterozygous E273X stop variant in three individuals.

**Conclusions** Our study shows many different, rare *KCNJ18* alterations in patients as well as controls. Only perhaps one meets the requirements of a disease-causing mutation. Therefore, *KCNJ18* alterations are seldom pathogenic. Additional studies are required before patients with PP can be genetically diagnosed on the basis of a *KCNJ18* variant alone.

might result from a reduced outward conductance of this channel.

The most frequent form of hypokalaemic PP is thyrotoxic periodic paralysis (TPP). It resembles HypoPP1/2 with respect to provocative factors. However, the hypokalaemia is more pronounced in TPP (between 1.0 and 2.5 mM) and therefore, often alters the ECG. Patients with TPP only experience paralytic attacks in the hyperthyroid state whereby clinical signs of hyperthyroidism may not be obvious. Although hyperthyroidism is much more frequent in females, the male-to-female ratio for TPP in Asians is about 6:1 and the onset is usually after the age of 20 years.<sup>5</sup> Paralytic attacks cease when the euthyroid state is restored.<sup>6</sup>

Mutations in *KCNJ18* gene encoding an inwardly rectifying potassium channel (Kir2.6) cause TPP and sporadic, that is, non-familial cases of HypoPP.<sup>7,8</sup> Mutant Kir2.6 proteins form a heterotetrameric Kir channel complex with Kir2.1 and thereby reduce cell surface expression of the complex.<sup>9</sup>

The aim of our work was to identify potentially disease-causing *KCNJ18* variations (defined as <1% in the normal population) in 263 unrelated patients with PP, in whom mutations in the established PP genes have been excluded. Then the identified variations were analysed by (1) conservation of *KCNJ18* variants, (2) segregation studies, (3) prediction programmes and (4) comparison with non-synonymous variants of a control group of same origin without neuromuscular disease.

**INTRODUCTION**

Hypokalaemic periodic paralysis (PP) are a group of diseases characterised by episodes of flaccid muscle weakness associated with hypokalaemia. These episodes usually begin in the first or second decade of life, occur spontaneously and can be triggered by serum potassium reduction due to insulin (following carbohydrate-rich meals), glucocorticoids (stress, infection) and muscle reuptake at rest after strenuous work. The genes responsible for hypokalaemic PP are *CACNA1S* encoding the calcium channel Cav1.1 (HypoPP1) and *SCN4A* coding for the sodium channel Nav1.4 (HypoPP2).<sup>1,2</sup> Both are voltage-dependent channels of the skeletal muscle fibre membrane. A mutation-induced aberrant current leads to a paradoxical membrane depolarisation that renders muscle fibres unexcitable.<sup>3</sup> A third gene responsible for PP with concomitant arrhythmia and dysmorphia is *KCNJ2*, encoding the inwardly rectifying potassium channel Kir2.1 of skeletal and cardiac muscle.<sup>4</sup> The ictally observed hypokalaemia

**METHODS****Patients and volunteers**

Samples of DNA were collected from a total of 263 unrelated patients with a history of at least two episodes of quadriparesis associated with hypokalaemia. The phenotype was defined as mild if the episodes were majorly paretic (60% of cases) and severe if the episodes were majorly plegic (40% of cases). Thirty of these patients (17 Caucasians, 13 Asians, all males) were diagnosed as TPP according to accepted criteria.<sup>10</sup> Forty relatives of the 10 patients with novel variants were also studied. Additionally 474 DNA samples from individuals without muscle disease were examined (400 Caucasians, 74 Asians). Genomic DNA was isolated from EDTA blood using the QIAamp DNA Blood Kit (Qiagen, <http://www.qiagen.com>) according to the instructions of the producer. Informed consent was obtained from patients, relatives and volunteers with no evidence of muscle disease.



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### Analysis of *KCNJ18* and evaluation of rare variants

Amplification, nested PCR, sequencing and our reference sequence are described elsewhere (see online supplementary file S1). Sequence analyses were evaluated using software SeqPilot of JSI (<http://www.jsi-medisys.de/>). Predictions regarding missense changes were made with PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>) and Mutations Taster (<http://www.mutationtaster.org/>). Splicing behaviour was predicted with MaxEnt ([http://genes.mit.edu/burgelab/maxent/Xmax\\_entscan\\_scoreseq\\_acc.html](http://genes.mit.edu/burgelab/maxent/Xmax_entscan_scoreseq_acc.html)). Homologous areas were compared with 15 non-human species using NCBI BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Alignments of several sequences of these species were carried out using the program ClustalW (<http://www.genome.jp/tools/clustalw/>) to check conservation.

### RESULTS

In our 263 unrelated patients with PP we found the known amino acid substitutions R6Q, Q39R, H40R, V100I, H192Q, V249I, F281L and Y338F which are not considered to be pathogenic.<sup>7</sup> We also identified eight synonymous, presumably non-pathogenic changes. Of greater interest are the following nine novel alterations (table 1): one prestart base change (c.-3G>A), seven non-synonymous amino acid substitutions (L15S, R81C, T309I, I340T, N365S, G394R, R401W), a novel E273X and a known questionably causative R399X stop mutation.<sup>7</sup> These 10 variants were heterozygous in 10 index patients with normal thyroid function and were studied more deeply

according to conservation, concordance of predictions on disease causality and segregation:

- ▶ Base exchange c.-3G>A is considered 'improbable' by MaxEnt to generate a splice site and is carried by an unaffected brother of the male patient.
- ▶ L15S is not conserved and occurs in two controls and for *KCNJ12* in 5%, but does not occur in an affected family member of the patient.
- ▶ R81C is conserved and is predicted as pathogenic; however, R81P is found in one control.
- ▶ T309 is conserved and T309I is pathogenic according to the prediction programmes and two non-affected female relatives are carriers (mother and daughter).
- ▶ I340 is different in four species and I340T is predicted as a benign polymorphism.
- ▶ N365 is conserved, but predictions on N365S are discordant and the index patient's daughter is affected although she is hypothyroid.
- ▶ G394R is not conserved and is predicted as benign by both programmes and two non-affected relatives are carriers.
- ▶ R401 is not conserved and the substitution R401W is excluded in three affected family members (see online supplementary figure 1S).
- ▶ E273X is found in three unrelated controls.
- ▶ R399X is identified in a 10-year-old boy whose mother and maternal grandfather are R399X carriers without PP history. As previously described, R399X also occurred in 1 of 100 controls.<sup>7</sup>

**Table 1** Novel variants and known questionably causative mutations of 263 patients with PP and 474 controls

Exchange nucleotide	amino acid	Index, n	Localisation	PolyPhen2	Mutation taster	Conservation	dbSNP ( <i>KCNJ12</i> )	Severity/segregation
Patients with PP								
-3G>A	-	1	Intron 2-3, no splice site predicted				No entry	-/-
44T>C	L15S	1	N	-	+	-	50 (1089)	-/-
241C>T	R81C	1	N	+	+	+	No entry	-/0
759insT	E273X	1	C				NA	+/0
926C>T	T309I	1	C	+	+	+	No entry	-/-
1019T>C	I340T	1	C	-	-	-	No info	+/0
1094A>G	N365S	1	C	-	+	+	No entry	-/0
1180G>A	G394R	1	C	-	-	-	No info	+/-
1195C>T	R399X*	1	C				NA	-/-
1201C>T	R401W	1	C	(+)	-	-	No entry	+/-
Controls								
-7C>T	-	1	Intron 2-3, no splice site predicted				No entry	NA
44T>C	L15S	2	N	-	+	-	50 (1089)	NA
100G>A	G34S	1	N	(+)	+	+	No entry	NA
242G>C	R81P	1	N	+	+	+	No entry	NA
578C>T	T193M	1	C	+	+	+	No info	NA
754G>A	D252N	3	C	-	+	-	No info	NA
782G>A	R261H	1	C	+	+	+	No info	NA
759insT	E273X	3	C	-	+	-	No entry	NA
1037A>G	H346R	1	C	+	+	+	No entry	NA
1137C>A	N379K	1	C	+	+	+	No entry	NA
1153A>C	S385R	1	C	-	+	-	No entry	NA
1219C>T	Q407X*	1	C				NA	NA
1228C>T	H410Y	1	C	(+)	+	-	No entry	NA

PolyPhen2: benign -, possibly damaging (+), probably damaging +; mutation taster: disease-causing +, benign polymorphism -; conservation: 100% conserved (16/16) +, <100% conserved -; dbSNP (*KCNJ12*): alignment was performed with *KCNJ12* because *KCNJ18* data are not available and the identity of the two coding sequences is 98.7%; severity: mild -, severe +; segregation: no segregation -, no available relatives 0.

\*Published as potential causative mutations previously.<sup>7</sup>

NA, not applicable; PP, periodic paralysis.

In summary, only T309I fulfils the criteria of a disease-causing mutation—but only if the two female carriers without PP history are explained as reduced penetrance, as sometimes reported for HypoPP-1.<sup>11</sup> This interpretation cannot be excluded since the phenotype in the index case is clinically mild.

In the 474 controls, we found the known amino acid substitutions R6Q, Q39R, H40R, V100I, H118R, L156P, H192Q, V249I, F281L and Y338F which are not considered to be pathogenic.<sup>7</sup> Additionally, we identified seven synonymous, presumably non-pathogenic changes, the earlier reported questionably causative mutation Q407X,<sup>7</sup> a prestart base change (c.-7C>T), the E273X stop mutation and 10 novel non-synonymous heterozygous changes (L15S, G34S, R81P, T193M, D252N, R261H, H346R, N379K, S385R, H410Y). Again, we evaluated the novel changes according to the above criteria (table 1):

- ▶ The prestart exchange c.-7C>T is considered ‘improbable’ to generate a splice site by MaxEnt.
- ▶ L15S, D252N, S385R and H401Y are not conserved, and the predictions on disease causality are discordant.
- ▶ G34S, T193M, R261H, H346R and N379K are perfectly conserved and concordantly predicted to be damaging, but all occurred only in healthy controls.

## DISCUSSION

*KCNJ18* mutations have been reported to cause TPP.<sup>7</sup> A requirement for *KCNJ18* being a responsible TPP gene in the presence of hyperthyroidism would be a mutation-specific change in T3-induced expression or translocation of the *mutant* product. Previous functional studies showed that only L156P translocates to the cell surface.<sup>9</sup> No such effect has been demonstrated for any of other *KCNJ18* variants.

*KCNJ18* mutations have also been reported to cause sporadic periodic paralysis (SPP).<sup>8</sup> A sporadic disease on a monogenic basis like SPP is due to an autosomal-dominant gene defect, arising by a new mutation transmitted through a non-penetrant or very mildly affected parent, or by a clinically unaffected parent who carries a mosaic germ line mutation.<sup>12</sup> To clarify whether the 10 index patients have de novo variants, we have clinically studied and genotyped their parents and siblings. For each patient, a parent (or at least a sibling) carried the variant or was clinically affected. Therefore, we conclude that *KCNJ18* variants, if disease-causing at all, are neither frequent de novo mutations nor mosaic germ line mutations. Surprisingly, family members have not been studied in the article on SPP.<sup>8</sup> Assuming that *KCNJ18* is a PP gene, it also remains unclear why both gain-of-function and dominant-negative mutations should have the same clinical effects, that is, weakness episodes.<sup>7,8</sup>

The identified variants, here, except for T309I, do not meet the requirements of a disease-causing mutation. All heterozygous stop mutations, such as E273X, R399X and Q407X (as well as early frameshift mutations) should be excluded as relevant *KCNJ18* alterations. The missing carboxyterminus prevents the assembly with wild type proteins.<sup>13</sup> All tetrameric channel complexes are, therefore, normal—no matter whether the incomplete RNA is unstable and immediately destroyed or not. Often the other allele is overexpressed so that the number of normal channels is not reduced. For example, we are aware of healthy controls carrying in their *CACNA1S* gene a heterozygous stop mutation (c.C3709del) without showing a defect in muscle excitation-contraction coupling. With these individuals provocative stimuli do not elicit bouts of paralysis.

A functional change brought about by an ion channel variants is usually an important criterion for disease causality; but this

criterion is not sufficient to prove causality, especially when the functional defects do not explain the phenotype. In previous studies, Kir2.6 mutations have been shown to lead to loss of function defects and suppression of the main rectifier Kir2.1.<sup>7,8</sup> However, the Kir2.6 channel itself is already shown to have a dominant negative effect on Kir2.1 function.<sup>9</sup> Therefore, a loss of function of the dominant negative suppression should actually lead to a gain of function of Kir2.1 which does not fit with the known depolarisation-induced paralysis pathogenesis.<sup>3</sup> Testing for functional defects in a channel with unclear significance for muscle membrane potential would not be interpretable. Also, regardless of the outcome, it would not change the fact that the genetic criteria are not met for any of the variants but perhaps T309I. Finally, any functional effect could simply be the result of the variants being functional polymorphisms, such as sodium channel Nav1.4 variant S906T<sup>14</sup> as well as others reviewed previously.<sup>2</sup>

In summary, it remains unclear whether *KCNJ18* is a PP gene. Without doubt, Kir2.6 contains functional units that could make it a PP gene. However, the large homology with *KCNJ12* leaves us with some problems concerning the interpretation. *KCNJ18* might be a duplication of *KCNJ12*, because the SNPs that occur in *KCNJ18* are predominantly related to amino acid positions that discern *KCNJ18* and *KCNJ12* (eg, L15S, Q39R, H40R, V100I, H118R, L156P, H192Q, V249I). Similarly the high percentage of rare variants in patients and controls (10/263=3.8% vs 17/474=3.6%) additionally questions the pathogenicity of these variants taken all together. Up to the final clarification neurologists should not consider *KCNJ18* as an established periodic paralysis gene and should continue the genetic analysis of the three other known causative genes. If *KCNJ18* can be confirmed as PP gene by additional studies, the number of identified mutations<sup>7,8</sup> and our results demonstrate its rarity.

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**Contributors** MK established and performed gene analysis and assisted with manuscript revisions; KJ-R and FL-H designed the study, collected patient DNA and data, and authored the manuscript.

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**Competing interests** None.

**Patient consent** Obtained.

**Ethics approval** These studies were approved by the Institutional Review Board of Ulm University (IRB Study #30/12\_Pathogenesis of hypokalaemic periodic paralysis).

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### Supplementary File S1. Analysis of *KCNJ18* and our reference sequence

*KCNJ18* has one coding exon (exon 3). This exon was amplified using polymerase chain reaction (PCR) under the following conditions: 94 °C for 5 min, followed by 35 cycles with 94°C for 30 s, 65°C for 1 min and 72°C for 1.5 min as well as a final elongation at 72°C for 10 min. For this procedure the primers of Ryan et al. (2010) were slightly modified for higher specificity (CCAGACATGCTGTCCTCTCTGTTC/ GGCCTCTCCCCGGCCA) and amplified by means of AmpliTaq Gold with GeneAmp (Applied Biosystems by Life Technologies, Carlsbad, CA, USA). For each preparation of 50 µl we used 50 ng DNA.

For some products, a second amplification was done using a nested PCR with the primers (ATGCTGTCCTCTCTGTTC/ CGGCCAGGGGTGGATGCTGCATG) under the following conditions: 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 65 °C for 1 min und 72 °C for 1.5 min as well as a final elongation at 72 °C for 10 min. The resulting products were sequenced using BigDye terminator v1.1, v3.1 mix and the forward-primers (CCAGACATGCTGTCCTCTCTGTTC/CTGGCGCTACATGCTGCTCATC/ CGCCGTGGTGGCCCTGCGTGAC/GCCAATGAGATCCTGTGGGGTCCAC) on the capillary sequencer ABI 3130xl (Applied Biosystems by Life Technologies, Carlsbad, CA, USA).

Our wild type sequence differed from the published *KCNJ18* sequence<sup>7</sup> in 11 bases and from the known *KCNJ12* sequence in 17 bases. Dr. Ryan's actual *KCNJ18* sequence (personal communication) differed from our most frequent *KCNJ18* sequence in one base (c.473 T>C) that did not show up in our study. Our reference sequence for *KCNJ18* is:

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gattgtgggtcaatcaggggtggaagcgtcctccagtcacgtctgggggcccctgggatgggtag
aggagcctggagccaccagcccagccagacatgctgtcctctctgtccaggagccgcccctgcc
tggagctagcctgggggtgagccaggggtccccaacccccggg
```

Exon 3

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ATG ACC GCG GCC AGC CGG GCC AAC CCC TAC AGC ATC GTG TCA TTG
GAG GAG GAC GGG CTG CAC CTG GTC ACC ATG TCG GGC GCC AAC GGC
TTC GGC AAC GGC AAG GTG CAC ACG CAG CAC AGG TGC CGC AAC CGC
TTC GTC AAG AAG AAT GGC CAG TGC AAC ATT GCG TTC GCC AAC ATG
```

GAC GAG AAG TCA CAG CGC TAC CTG GCT GAC ATG TTC ACC ACC TGT  
GTG GAC ATC CGC TGG CGC TAC ATG CTG CTC ATC TTC TCG CTG GCC  
TTC CTT GCC TCC TGG CTG CTG TTC GGC GTC ATC TTC TGG GTC ATC  
GCG GTG GCA CAC GGT GAC CTG GAG CCG GCT GAG GGC CAC GGC CGC  
ACA CCC TGT GTG ATG CAG GTG CAC GGC TTC ATG GCG GCC TTC CTC  
TTC TCC ATC GAG ACG CAG ACC ACC ATC GGC TAC GGG CTG CGC TGT  
GTG ACG GAG GAG TGC CTG GTG GCC GTC TTC ATG GTG GTG GCC CAG  
TCC ATC GTG GGC TGC ATC ATC GAC TCC TTC ATG ATT GGT GCC ATC  
ATG GCC AAG ATG GCA AGG CCC AAG AAG CGG GCA CAC ACG CTG CTG  
TTC AGC CAC AAC GCC GTG GTG GCC CTG CGT GAC GGC AAG CTC TGC  
CTC ATG TGG CGT GTG GGC AAC CTG CGC AAG AGC CAC ATT GTG GAG  
GCC CAT GTG CGC GCG CAG CTC ATC AAG CCG CGG GTC ACC GAG GAG  
GGC GAG TAC ATC CCG CTG GAC CAG GTC GAC ATC GAT GTG GGC TTC  
GAC AAG GGC CTG GAC CGC ATC TTT CTG GTG TCG CCC ATC ACC ATC  
TTG CAT GAA ATT GAC GAG GCC AGC CCG CTC TTC GGC ATC AGC CGG  
CAG GAC CTG GAG ACG GAC GAC TTT GAG ATC GTG GTC ATC CTG GAA  
GGC ATG GTG GAG GCC ACA GCC ATG ACC ACC CAG GCC CGC AGC TCC  
TAC CTG GCC AAT GAG ATC CTG TGG GGT CAC CGC TTT GAG CCC GTG  
CTC TTC GAG GAG AAG AAC CAG TAC AAG ATT GAC TAC TCG CAC TTC  
CAC AAG ACC TAT GAG GTG CCC TCT ACG CCC CGC TGC AGT GCG AAG  
GAT CTG GTA GAG AAC AAG TTC CTG CTG CCC AGT GCC AAC TCC TTC  
TGC TAT GAG AAC GAG CTG GCC TTC CTG AGC CGT GAC GAG GAG GAT  
GAG GCG GAC GGA GAC CAG GAC GGC CGA AGC CGG GAT GGC CTC AGC  
CCC CAG GCC AGG CAT GAC TTT GAC AGA CTC CAG GCT GGC GGC GGG  
GTC CTG GAG CAG CGG CCC TAC AGA CGG GGG TCA GAG ATC TGA  
gccaaccttgccgacatgcagcatccaccctggccggggagaggccccgcggtcgcctcaggggc  
cctggggttgagcagaacgggcccagtgccctgggttcagactcagtagcgtttagtcgttta  
tgtttcttgcaaaggcctcagaaggttgccggagaggggg

**Supplementary Figure 1S: Lack of segregation in a PP family with KCNJ18-p.R401W.**

Ten affected family members (and one clinically equivocal) are carriers as well as one non-affected relative. In contrast, three affected relatives do not carry R401W.

