SHORT REPORT

Rare KCNJ18 variants do not explain hypokalaemic periodic paralysis in 263 unrelated patients

Marius Kuhn,1,2 Karin Jurkat-Rott,1 Frank Lehmann-Horn1

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, L155, 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.

INTRODUCTION

Hypokalaemic periodic paralyses (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 encoding the sodium channel Nav1.4 (HypoPP2). 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. A third gene responsible for PP with concomitant arrhythmia and dysmorphy is KCNJ2, encoding the inwardly rectifying potassium channel Kir2.1 of skeletal and cardiac muscle. The actually observed hypokalaemia 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. Paralytic attacks cease when the euthyroid state is restored. Mutations in KCNJ18 gene encoding an inwardly rectifying potassium channel (Kir2.6) cause TPP and sporadic, that is, non-familial cases of HypoPP.

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-syndromic variants of a control group of same origin without neuromuscular disease.

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 plégic (40% of cases). Thirty of these patients (17 Caucasians, 13 Asians, all males) were diagnosed as TPP according to accepted criteria. 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 QIamp 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.
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). Homologous areas were compared with 13 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.7 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.7 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 carriers are also 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.

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 (KCNJ12) | Severity/ segregation |
|--------------------------------|----------|--------------|-----------|----------------|--------------|-----------------|---------------------|
| Patients with PP               |          |              |           |                |              |                 |                     |
| –3G>A                          | –        | Intron 2–3, no splice site predicted |          |                 |              | No entry        | –                   |
| 44T>C                          | L15S     | N            | +         | +              | No entry     | 50 (1089)       | –                   |
| 241C>T                         | R81C     | N            | +         | +              | No entry     | –10             | –                   |
| 759insT                        | E273X    | C            |            |                |              |                 | –                   |
| 926C>T                         | T309I    | C            | +         | +              | No entry     | –10             | –                   |
| 1019T>C                        | I340T    | C            | –         | –              | No info      | +10             | –                   |
| 1094A>G                        | N365S    | C            | –         | +              | No entry     | –10             | –                   |
| 1180G>A                        | G394R    | C            | –         | –              | No info      | +/+              | –                   |
| 1195C>T                        | R399X    | C            | (+)       | –              | No entry     | –/–             | –                   |
| 1201C>T                        | R401W    | C            | (+)       | –              | No entry     | –/–             | –                   |
| Controls                       |          |              |           |                |              |                 |                     |
| –7C>T                          | L15S     | N            | –         | +              | No entry     | 50 (1089)       | NA                  |
| 100G>A                         | G34S     | N            | (+)       | +              | No entry     | NA              | –                   |
| 242G>C                         | R81P     | N            | +         | +              | No entry     | NA              | –                   |
| 578C>T                         | T193M    | C            | +         | +              | No info      | NA              | –                   |
| 754G>A                         | D252N    | C            | –         | +              | No info      | NA              | –                   |
| 782G>A                         | R261H    | C            | +         | +              | No info      | NA              | –                   |
| 759insT                        | E273X    | C            | –         | –              | No entry     | NA              | –                   |
| 1037A>G                        | H364R    | C            | +         | +              | No info      | NA              | –                   |
| 1137C>A                        | N379K    | C            | +         | +              | No entry     | NA              | –                   |
| 1153A>C                        | S385R    | C            | –         | –              | No entry     | NA              | –                   |
| 1219C>T                        | Q407X    | C            | +         | +              | No info      | NA              | –                   |
| 1228C>T                        | H410Y    | C            | (+)       | –              | No entry     | –/–             | –                   |

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.3

NA, not applicable; PP, periodic paralysis.

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In summary, only T309I fulfills 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.1,3 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. Additionally, we identified seven synonymous, presumably non-pathogenic changes, the earlier reported 

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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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