SHORT REPORT

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

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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, 12 revealed 11 novel rare variations 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 (following carbohydrate-rich meals). Glucocorticoids (stress, infection) and muscle reuptake at rest (following carbohydrate-rich meals)

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 mostly 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. 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.


To cite: Kuhn M, Jurkat-Rott K, Lehmann-Horn F. J Neurol Neurosurg Psychiatry 2016;87:49–52.

To view: please visit the journal online. Click Here
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 mis-sense changes were made with PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and Mutations Taster (http://www.mutationtaster.org). Homologous areas were compared with MaxEnt (http://genes.mit.edu/burgelab/maxent/Xmaxentscan-mutationtaster.org). Splicing behaviour was predicted with Harvard (http://pph2.mpi-inf.mpg.de) and Mutations Taster (http://www.mutationtaster.org). Sense changes were made with PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and Mutations Taster (http://www.mutationtaster.org). Results regarding mis-

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.

The following amino acid substitutions (R6Q, Q39R, H40R, V100I, H192Q, V249I, F281L and Y338F) are considered to be pathogenic.

We also identified eight synonymous amino acid substitutions (L15S, T309I, I340T, N365S, G394R, R401W), a novel E273X and a known questionably causative R399X stop mutation.

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 unaffected brothers of the male patient.

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

<table>
<thead>
<tr>
<th>Exchange nucleotide amino acid</th>
<th>Index, n</th>
<th>Localisation</th>
<th>PolyPhen2</th>
<th>Mutation taster</th>
<th>Conservation</th>
<th>dbSNP (KCNJ12)</th>
<th>Severity/segregation</th>
</tr>
</thead>
</table>
| Patients with PP
| -3G>A                           | –        | 1 Intron 2–3, no splice site predicted | –         | –               | No entry       | NA               | –/–                 |
| 44T>C                           | L15S     | 1 N          | +         | +               | No entry       | 50 (1089)       | –/–                 |
| 241C>T                          | R81C     | 1 N          | +         | +               | No entry       | –/–             | –/–                 |
| 759insT                         | E273X    | 1 C          | –         | –               | No entry       | NA              | +/0                 |
| 926C>T                          | T309I    | 1 C          | +         | +               | No entry       | –/–             | –/–                 |
| 1019T>C                         | I340T    | 1 C          | –         | –               | No info        | +/0             | –/–                 |
| 1094A>G                         | N365S    | 1 C          | –         | +               | No entry       | –/–             | –/–                 |
| 1180G>A                         | G394R    | 1 C          | –         | –               | No info        | 4/–             | –/–                 |
| 1195C>T                         | R399X   | 1 C          | –         | –               | No entry       | NA              | –/–                 |
| 1201C>T                         | R401W    | 1 C          | (+)       | –               | No entry       | NA              | –/–                 |
| Controls
| -7C>T                           | L15S     | 2 N          | –         | +               | No entry       | 50 (1089)       | –/–                 |
| 100G>A                          | G34S     | 1 N (+)      | +         | +               | No entry       | NA              | NA                  |
| 242G>C                          | R81P     | 1 N          | +         | +               | No entry       | NA              | NA                  |
| 578C>T                          | T193M    | 1 C          | +         | +               | No info        | NA              | NA                  |
| 754G>A                          | D252N    | 3 C          | –         | +               | No info        | NA              | NA                  |
| 782G>A                          | R261H    | 1 C          | +         | +               | No info        | NA              | NA                  |
| 759insT                         | E273X    | 3 C          | –         | +               | No entry       | NA              | NA                  |
| 1037A>G                         | H346R    | 1 C          | +         | +               | No info        | NA              | NA                  |
| 1137C>A                         | N379K    | 1 C          | +         | +               | No entry       | NA              | NA                  |
| 1153A>G                         | S385R    | 1 C          | –         | +               | No entry       | NA              | NA                  |
| 1219C>T                         | Q407X    | 1 C          | –         | –               | No entry       | 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.7

NA, not applicable; PP, periodic paralysis.
Periodic paralysis (SPP). A sporadic disease on a monogenic basis like SPP is due to an autosomal-dominant gene defect, 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 their CACNAIS gene a heterozygous stop mutation (c.C709del) 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. However, the Kir2.6 channel itself is already shown to have a dominant negative effect on Kir2.1 function.

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-1.3,4 This interpretation cannot be excluded since 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 as well as others reviewed previously.

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, L155S, Q399R, H404R, V100I, H118R, L156P, H192Q, V249I).

In the 474 controls, we found the known amino acid substitutions R6Q, Q399R, H404R, 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 questionably causative mutation Q407X, a prestart base change (c.−7C>T), the E273X stop mutation and 10 novel non-synonymous heterozygous changes (L155S, G34S, R81P, T193M, D252N, R261H, H346R, N379K, S385R, H410Y).

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.
- L155S, 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. 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. No such effect has been demonstrated for any of other KCNJ18 variants.

KCNJ18 mutations have also been reported to cause sporadic periodic paralysis (SPP). 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. 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. 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.

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. All tetrameric channel complexes are therefore, normal—no matter whether the incomplete RNA is unstable and immediately destructed 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 their CACNAIS gene a heterozygous stop mutation (c.C709del) without showing a defect in muscle excitation-contraction coupling. With these individuals provocative stimuli do not elicit bouts of paralysis.

REFERENCES