

ARTICLE

Loss of Function of *KCNC1* is associated with intellectual disability without seizures

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p.(Arg320His) mutation in the *KCNC1* gene in human 11p15.1 has recently been identified in patients with progressive myoclonus epilepsies, a group of rare inherited disorders manifesting with action myoclonus, myoclonic epilepsy, and ataxia. This *KCNC1* variant causes a dominant-negative effect. Here we describe three patients from the same family with intellectual disability and dysmorphic features. The three affected individuals carry a c.1015C>T (p.(Arg339*)) nonsense variant in *KCNC1* gene. As previously observed in the mutant mouse carrying a disrupted *KCNC1* gene, these findings reveal that individuals with a *KCNC1* loss-of-function variant can present intellectual disability without seizure and epilepsy.

European Journal of Human Genetics (2017) 25, 560–564; doi:10.1038/ejhg.2017.3; published online 1 February 2017

INTRODUCTION

Potassium channels constitute a very diverse group of ion channels and are composed of variable combinations of subunits encoded in large multigene families.¹ This diversity contributes to the ability of specific neurons to respond uniquely to different inputs. One of these groups of genes, the Kv family, encodes subunits of tetrameric voltage-gated K⁺ channels and is divided into several subfamilies based on sequence similarities and hence probable evolutionary relationships.^{1,2} Subunits of the same subfamily, but not from different subfamilies, can form heteromeric channels, suggesting that each group of genes encodes a distinct set of channels.¹ One of the Kv subfamilies known as Kv3 has generated particular interest because their members show large unit conductances, high thresholds of activation, and very fast activation and deactivation kinetics.³ Kv3-type channels are involved in the rapid repolarization of the action potential (AP), and their presence in neurons correlates with narrow APs, fast after hyperpolarization (AHP), and high-frequency firing beyond 200 Hz.

The Kv3 subfamily consists of four genes (Kv3.1 (also known as *KCNC1*), Kv3.2, Kv3.3, and Kv3.4). Each Kv3 gene encodes multiple products by alternative splicing of 3' ends resulting in the expression of K⁺ channel proteins that differ only in short C-terminal sequences.⁴ The variant C termini contains or regulates targeting signals and raises the possibility that alternative splicing of 3' ends of other K⁺ channel genes has similar roles.^{5,6} Although Kv3.1 and Kv3.3 subunits are expressed throughout the brain, their expressions are restricted to distinct neuronal subpopulations.⁷ In neocortex, thalamus, hippocampus, and striatum, Kv3-type channels are found in inhibitory GABAergic interneurons that also express the calcium-binding protein parvalbumin, a marker for fast-spiking neurons. However, Kv3.1 channels were identified not only in neurons, but also in T-lymphocytes, oligodendrocyte precursor cells, astrocytes, and NPCs.^{8–10}

Ten years ago, *de novo* or autosomal dominantly *KCNC3* (Kv3.3) missense variants were identified in spinocerebellar ataxia.¹¹ In 2013,

Rajakulendran *et al*¹² identified a 670 Kb deletion of chromosome 12q21 including *KCNC2* and *ATXN7L3B* in patients with neurodevelopmental delay and ataxia. More recently, recurrent *de novo* mutation c.959G>A (p.(Arg320His)) in *KCNC1* was identified as a novel major cause for progressive myoclonus epilepsies, a group of rare, inherited disorders manifesting with action myoclonus, tonic-clonic seizures, and ataxia.¹³

During the genetic characterization of a large cohort of patients with syndromic and non-syndromic forms of intellectual disability (ID), we identified a nonsense variant in the *KCNC1* gene for three affected individuals of the same family. Affected father, his daughter, and his son suffered from intellectual disability and dysmorphic features without seizures.

SUBJECTS AND METHODS

Patients

We report a family with three affected people: a father, his daughter and his son. All presented with delayed motor milestones, speech delay, hypotonia, lability of attention, intellectual disability, normal head circumference (Figure 1a), minor dysmorphic features included epicanthal folds, ptosis, short philtrum, prognathism, fetal pads, and protruding ears. The grandparents were unaffected. The couple was a pair of cousins but the grandparents were not related.

This family was ascertained in 2011 when individual-2 was referred for genetic counseling, because her husband and daughter have intellectual disability (Figure 1a). The affected girl (individual-3) was born at term after an uneventful pregnancy and delivery. At birth, measurements were normal (weight (2740 g), height (51 cm), and head circumference (34 cm)). She had mild developmental delay. She began crawling at 11 months and walking at 19 months. She had dysmorphic features including prognathism, cup-shaped ears, protruding tongue, salivary incontinence, and clinodactyly of the fifth finger. At the age of 6, she had intellectual disability with severe attention and learning difficulties. Neurological examinations appeared to be normal with no ataxia and pyramidal disorder. She did not exhibit any signs of breathing or

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Received 3 June 2016; revised 9 December 2016; accepted 24 December 2016; published online 1 February 2017

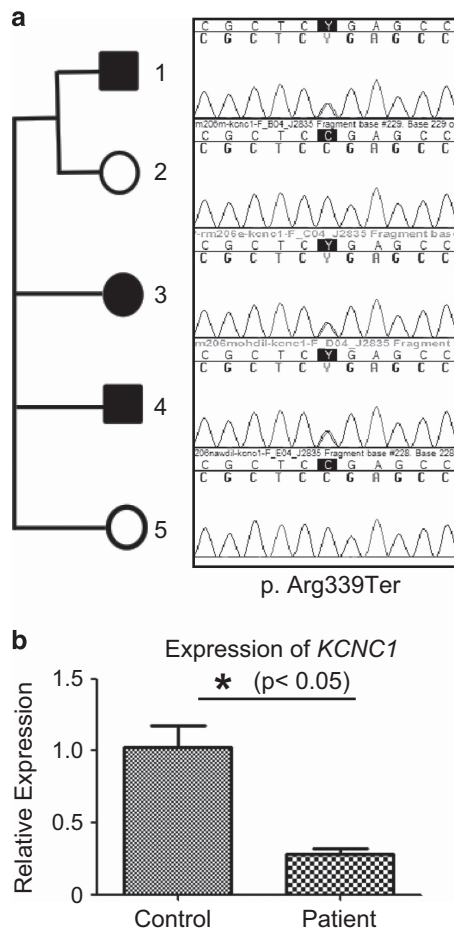


Figure 1 (a) Pedigree of the ID family, Electropherogram of the *KCNK1* nonsense mutation (p.(Arg339Ter)) (indicated by the symbol Y) identified in one, three, and four family members. (b) Consequence of the nonsense mutation at the mRNA level. Analysis of *KCNK1* mRNA extracted from fibroblasts by real-time quantitative reverse-transcriptase PCR. Ctrl: controls ($n = 13$). Patient: 3.

sleeping anomalies. At the age of 10, the measurements were within normal ranges (weight (29 kg), height (135 cm; +0.9 DS) and head circumference (52 cm)). Brain MRI and EEG were normal. The father also presented prognathism, dysplastic ears, and coarse face with moderate intellectual disability. Unlike his brothers and sisters, he was unable to read and write, and developed simple language. He was admitted and worked in a special institution. He is under guardianship to manage his money and administrative procedures. He is now taken care for a brain tumor discovered at the age of 40 after an epileptic seizure. The second boy sibling (individual-4) exhibited intellectual disability with hypotonia and the same face. Any regression and no progressive myoclonus epilepsy were reported for all affected patients.

First, we screened the whole coding regions of 20 previously ID-genes (*MECP2*, *CDKL5*, *ARX*, *STXBP1*, *MEF2C*, *ARID1B*, *OPHN1*, *TBR1*, *PHF8*, *KDM5C*, *SYNGAP1*, *DYRK1A*, *GRIN2B*, *IQSEC2*, *FOXG1*, *UBE3A*, *SYNGAP1*, *SLC9A6*, *TCF4*, *ZEB2*) using Ampliseq approach developed by Life Technologies. All blood samples were obtained after receiving informed consent. This study was prospectively reviewed and approved by our local research ethics committee. Genomic DNA of the affected girl was amplified by Ampliseq approach, and subjected to sequencing on PGM Life Technologies sequencers (Platform at Jean Dausset, Institut Cochin, Paris, France). No pathogenic anomalies were identified by sequencing and CGH microarray. Second, taking into account these data, we performed whole-exome sequencing (WES).

Whole-exome sequencing

Library generation, exome enrichment, and WES were performed at the French National Centre for Genotyping (CNG, Evry, France). Briefly, libraries were prepared from 3 μ g genomic DNA extracted from whole blood using an optimized SureSelect Human Exome kit (Agilent, Santa Clara, CA, USA) following the manufacturer's instructions. Captured, purified, and clonally amplified libraries targeting the exome were then sequenced on a HiSeq 2000 (Illumina, San Diego, CA, USA) according to the manufacturer's recommendations. Obtained sequence reads were aligned to the human genome (hg19) using BWA software. Downstream processing was carried out with Genome analysis toolkit (GATK), SAMtools, and Picard Tools (<http://picard.sourceforge.net/>).^{14,15} Single-nucleotide variants and indels were subsequently called by SAMtools suite (mpileup, bcftools, vcfutil). All calls with a read coverage $\leq 5 \times$ and a Phred-scaled SNP quality of ≤ 20 were filtered out. Substitution and variation calls were performed with SAMtools pipeline (mpileup). The variants were annotated with an in-house Paris Descartes bioinformatics platform pipeline based on the Ensembl database (release 67).¹⁶ An autosomal dominant approach was applied for candidate gene identification. After exome analysis, each selected variants were confirmed by polymerase chain reaction (PCR) using genomic DNA from the patients and their parents and direct sequencing using BigDyedideoxy terminator chemistry and an ABI3130xl genetic DNA analyzer (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). Primer sequences and positions, PCR conditions, and product sizes are available on request.

RNA isolation, cDNA synthesis, and quantitative real-time PCR

After informed consent was obtained from the parents, a skin biopsy was carried out for the female individual with intellectual disability. Total RNA from primary cultures of fibroblasts was converted to cDNA with Maxima First Strand cDNA synthesis Kit (ThermoScientific, Rochester, NY, USA) for quantitative real-time PCR with SYBR Green as detection agent with Light-Cycler480 detection system (Roche Applied Bioscience, Indianapolis, IN, USA). Primer sequences are described in Supplementary Table S1. After PCR amplification, a dissociation protocol was performed to determine the melting curve of PCR product. Reactions with melting curves indicating a single amplification product were considered positive for further analysis. The identity and expected size of the single PCR product were also confirmed by sequencing. For each cycle, a relative quantification of each individual gene amount was calculated by the comparative $\Delta\Delta$ CT method as described by the manufacturer, providing a relative measure of expression for genes of interest, which were all normalized. Normalization factor was based on the mean of control gene *UBC* (ubiquitin conjugating enzyme) selected because of its expression stability in human fibroblasts.¹⁷ Statistical analysis was carried out using nonparametric Mann-Whitney test (Prism, GraphPad software Inc., La Jolla, CA, USA). A P -value < 0.05 was considered as significant.

Description of sequence variants

Sequence variants in *KCNK1* gene are numbered starting from the first base of the ATG codon, numbering based on reference sequence NM_004976.4. Description of the sequence (Human Genome Variation Society, <http://www.hgvs.org/mutnomen/recs.html>) was done with the assistance of Alamut Visual software version 2.4.2 (Interactive Biosoftware, Rouen, France). *KCNK1* variant has been submitted to the specific gene variant database LOVD (<http://databases.lovd.nl/shared/variants/0000147175>). The patient ID is 00089106 and the variant ID is 0000147175. The other potential variants are 0000147174, 0000147176-0000147181.

RESULTS

Exome analysis revealed a nonsense variant in the *KCNK1* gene

The pedigree structure of this family was consistent with autosomal dominant inheritance. Clinical examination did not suggest a specific syndromic form of intellectual disability. Previous candidate gene investigations and CGH array had not identified the causative gene. We performed exome sequencing on three members from the family (father, mother, and affected daughter). Summary of exome

Table 1 Summary of exome sequencing data for the proband (individual-3) and her parents (individuals 1 and 2)

| | Father | Mother | Proband |
|---|--------|--------|---------|
| <i>Total captured regions size (Mb)</i> | | | |
| Average depth for nucleotides in captured region | 180 | 178 | 129 |
| % Of captured regions with coverage >15 | 93 | 93 | 93 |
| % Of captured regions with coverage >30 | 85 | 88 | 78 |
| <i>Average coverage of captured region (%)</i> | | | |
| Total number of SNPs (essential splicing and coding regions) before filtering | 16 579 | 17 640 | 15 150 |
| Total number of INDELs (essential splicing and coding regions) before filtering | 814 | 875 | 772 |
| Total number of SNPs | 522 | 545 | 225 |
| Total number of INDELs | 15 | 19 | 11 |
| <i>N</i> rare homozygous | | | 5 |
| <i>N</i> compound heterozygous | | | 1 |
| <i>N</i> X linked | | | NA |
| <i>N de novo</i> events | | | 13 |
| Inherited from her father | | | 111 |

sequencing data for the proband and her parents is reported in Table 1. In total, 15 150 to 17 640 SNP (essential splicing and coding regions) and 772 to 875 small deletions/insertions (1–10 bp) (INDEL) were identified across the exomes. We tested an autosomal dominant inheritance and applied five consecutive filters to narrow down the variants: (i) variants heterozygous in the father and affected child and not present in the mother; (ii) variants with read depth >10; (iii) exclusion of X chromosome-linked variants; (iv) rare variants in the population (MAF<1% in the 1000 genomes, ExAC database, and dbSNP137); (v) variants with moderate high or functional impact (Polyphen-2 and SIFT scores). After filtering these data, it resulted in the identification of candidate variants in eight genes (*KCNC1*, *TPH1*, *MADD*, *EGFR*, *CES5A*, *GADLI*, *SLC35A5*, and *KIAA1024*; Table 2). Except one, all variants were missense variants. Segregation analysis by using affected (individual-4) and unaffected (individual-5) members of this family allowed us to exclude four variants (c.2842G>A (p.V948I) in *EGFR*; c.971T>C (p.L324P) in *SLC35A5*, c.211G>A (p.V71M) in *GADLI*, and c.473A>G (p.D158G) in *CES5A*). Four variants were not excluded (c.689G>A (p.R230H) in *TPH1*; c.1018G>A (p.V340M) in *MADD*, c.1015C>T (p.R339*) in *KCNC1*, c.1832A>T (p.E611V) in *KIAA1024*). Taking into account that *KCNC1* was previously involved in progressive myoclonus epilepsies, nonsense variant (c.1015C>T (p. (R339*))) in the *KCNC1* gene was identified as the most plausible candidate.¹³ *KCNC1* variant was confirmed by Sanger sequencing for both affected siblings (boy and daughter) and affected father (Figure 1a). Moreover, both unaffected individuals (mother and unaffected girl) do not carry this nonsense variant.

Relative quantification of *KCNC1* transcript showed that mutated transcript was target for the nonsense-mediated RNA decay

Despite an expected tendency for premature termination codons to yield shortened polypeptide products, formation of truncated proteins does not occur often *in vivo*. Many organisms, including humans, use a nonsense-mediated mRNA decay pathway, which degrades mRNAs containing nonsense variants before translation into nonfunctional polypeptides. To understand molecular consequences of this nonsense variant, we investigated mRNA processing by detection of its transcript

in fibroblasts obtained from a skin biopsy of one of the affected patients. Quantification of mRNA showed a very significant reduction (>50%) of *KCNC1* transcript in fibroblasts from the affected patient compared with 13 independent controls (Figure 1b). This result suggests that the mutant transcript is highly unstable leading to an absence of truncated protein synthesis and reduction of *KCNC1* expression.

DISCUSSION

Using whole-exome sequencing, we identified eight candidate variants in this family with ID. Segregation analysis by comparing other unaffected and affected individuals from this family, allowed us to exclude four missense variants. Three of them (c.689G>A, p.(R230H) in *TPH1*; c.2842G>A p.(V948I) in *EGFR*; c.971T>C p.(L324P) in *SLC35A5*) were also previously described in the ExAC database (<http://exac.broadinstitute.org/>). We cannot exclude the *TPH1*, *MADD*, and *KIAA1024* variants by segregation analysis. However, Tryptophan hydroxylase-1 (*TPH1*) is only expressed by non-neuronal cells including enterochromaffin cells of gut, mast cells, and pineal gland, and it has not been found to have any differences between the *Tph1*-deficient and wild-type mice in general motor behavior as tested by rotarod, grip-strength test, open field, and beam walk.¹⁸ *KIAA1024* located in 15q25.1 presents more than 350 missense variants in ExAC database including several in the same region (p.(R608Q), p.(S614L), p.(R617C)). Finally, *MADD*, a splice variant of *IG20* gene, encodes a protein highly expressed in melanocytes, parotid acinar cells, and involved in physiological cell death.^{19–21} Taking into account of all these data, we focused on the *KCNC1* gene previously involved in myoclonus epilepsies.¹³ Up to now, only one loss-of-function variant was described in ExAC database (c.1504+1G>T, 1/117116). Patients described here are the first cases presenting a loss-of-function variant of *KCNC1* gene in intellectual disability context, suggesting that *KCNC1* variants can also cause ID.

KCNC1 encodes $K_{V3.1}$, which functions as a highly conserved potassium ion channel subunit of the K_{V3} subfamily of voltage-gated tetrameric potassium ion channels. K_{V3} channel subunits consist of six membrane-spanning segments (S1–S6), have overlapping expression patterns, and can form heterotetramers.²² S4 constitutes the main voltage sensor where specific positively charged arginine residues contribute to the gating charge.²³

Here, we showed that the mutated mRNA is degraded by nonsense-mediated mRNA decay pathway, suggesting that it is unlikely translated into a nonfunctional polypeptide that would alter all K_{V3} -mediated currents of the neurons in which it is expressed. Most likely reduction of *KCNC1* protein expression (haploinsufficiency) is at the origin of the pathology.

This result explains that reduction of *KCNC1* expression due to nonsense variant is associated with ID without PME, whereas the p.(R320H) substitution associated with mutant protein synthesis causes a dominant-negative effect on wild-type $K_{V3.1}$ channels and progressive myoclonus epilepsies.¹³

Interestingly, $K_{V3.1}$ homozygous mice are viable and fertile indicating that functional $K_{V3.1} K^+$ channels are neither essential for embryonic development nor for reproduction. In our family, the father bearing the nonsense mutation is also fertile. $K_{V3.1}$ homozygous mice show normal spontaneous locomotion, unaltered acquisition (learning), and retention (memory) of a conditioned response, and no spontaneous seizures. However, they are smaller in body size and have impaired coordinated motor skills.²⁴ As observed in $K_{V3.1}$ homozygous mice, our affected patients have relatively mild phenotypes without seizures.

Table 2 Details of the variants narrowed down using consecutive filters based on dominant model of inheritance

| Chromosome | 3 | 3 | 7 | 11 | 11 | 11 | 15 | 16 |
|---|----------------|----------------------|---|---|----------------------|-----------------|-----------------|-----------------|
| Position | 30898633 | 112299935 | 55266550 | 17793656 | 18048151 | 47298337 | 79750321 | 55903601 |
| Gene name | <i>GADL1</i> | <i>SLC35A5</i> | <i>EGFR</i> | <i>KCNC1</i> | <i>TPH1</i> | <i>MADD</i> | <i>KIAA1024</i> | <i>CES5A</i> |
| Refseq | NM_207359.2 | NM_017945.3 | NM_005228.3 | NM_001112741.1 | NM_004179.2 | NM_003682.3 | NM_015206.2 | NM_001190158.1 |
| variant reference | 3_30898633_C_T | exac_3_112299935_T_C | exac_7_55266550_G_A | 11_17793656_C_T | exac_11_18048151_C_T | 11_47298337_G_A | 15_79750321_A_T | 16_55903601_T_C |
| Reference sequence | C | T | G | C | C | G | A | T |
| Proband: number of reads with reference | 10 | 18 | 9 | 60 | 52 | 77 | 22 | 28 |
| Proband: mutant | T | C | A | T | T | A | T | C |
| Proband: number of reads with mutant | 16 | 12 | 20 | 48 | 36 | 71 | 21 | 32 |
| Father: number of reads with reference | 0 | 0 | 55 | 236 | 90 | 86 | 156 | 131 |
| Father: number of reads with mutant | 36 | 78 | 62 | 244 | 69 | 104 | 192 | 137 |
| Mutation type | Missense | Missense | Missense | Nonsense | Missense | Missense | Missense | missense |
| Mutation: cDNA (HGVS nomenclature) | c.211G>A | c.971T>C | c.2842G>A | c.1015C>T | c.689G>A | c.1018G>A | c.1832A>T | c.560A>G |
| Mutation: Protein (HGVS nomenclature) | p.V71M | p.L324P | p.V948I | p.R339* | p.R230H | p.V340M | p.E611V | p.D187G |
| Prediction SIFT | 0.999 | 1 | 0.997 | 0.998 | 0.998 | 0.999 | 0.999 | 1 |
| Prediction Polyphen-2 | — | — | 0.01 | — | — | — | — | — |
| Segregation into the family | No | No | | Yes | | | | |
| Omim | *615601 | NA | *131550 | *176258 | *191060 | *603584 | NA | NA |
| Pathology associated | | | Inflammatory skin and bowel disease, neonatal, 2 (616069-AR) | Epilepsy, progressive myoclonic 7 (616187-AD) | | | | |
| | | | Adenocarcinoma of lung, response to tyrosine kinase inhibitor in (211980-AR) | | | | | |
| | | | Nonsmall cell lung cancer, response to tyrosine kinase inhibitor in (211980-AR) | | | | | |

Early expression of K_v3 channels during an embryonic period of life in which the fast firing phenotype is not yet present suggests that K_v3 channels have other non-conventional functions. K_v3 channels have not only been involved in cell proliferation, migration, and neuronal growth cone, but may also have a role in regulating neuronal and glial properties during the earliest phases of brain development.^{25–27} Our data suggest that *KCNK1* haploinsufficiency may affect at least one of these functions and could have a role in the cognitive symptoms of our ID individuals. Further studies are needed to evaluate the impact of *KCNK1* loss-of-function variants on gating properties of $K_v3.1$ channels. Modulation of K_v3 channel function may provide a possibility for pharmacological intervention in patients with *KCNK1* variants. However, although anticonvulsant drugs targeting other K_v channels exist, there is no activator of K_v3 channels currently available. Nevertheless, taking into account the fact that $K_v3.1$ channels are reduced in untreated schizophrenia and its expression is normalized with antipsychotics drugs, a similar treatment should be a future pharmacological approach in these *KCNK1*-related disorders.²⁸

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the patients and their family for their participation in this study. We thank Aurélie Toussaint for her help to draft the manuscript. This work was supported by Fondation Maladies Rares. We thank the cell bank of Cochin Hospital (Patrick Nusbaum) for assistance with the samples used in this study.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (<http://www.nature.com/ejhg>)