Supplementary Information

A dominant-negative mutation in the TRESK potassium channel is linked to familial migraine with aura

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SUPPLEMENTARY TABLE 1
List of variants in the human *KCNK18* gene

			Occurrence†															
			110 migraine				80 CEPH				AUS migraine				AUS non-migraine			
nt change*	aa change*	dbSNP	11	12	22	0	11	12	22	0	11	12	22	0	11	12	22	0
c.28 A>G	Arg10Gly	rs67346047	98	11	1	0	67	13	0	0	405	50	4	52	402	73	0	30
c.101 C>T	Ala34Val	none	102	0	0	8	77	0	0	3	450	1	0	60	475	0	0	30
c.309 C>T	Phe103Phe	none	109	0	0	1	79	0	0	1	479	0	0	32	492	2	0	11
c.328 T>C	Cys110Arg	none	109	1	0	0	77	3	0	0	471	8	0	32	482	12	0	11
c.414-415 del CT	Phe139TrpfsX24	none	109	1	0	0	79	0	0	1	465	0	0	46	496	0	0	9
c.427 C>T	Leu143Leu	none	105	0	0	5	78	0	0	2	464	1	0	46	496	0	0	9
c.489 T>C	Tyr163Tyr	none	105	0	0	5	78	0	0	2	458	5	0	48	495	1	0	9
c.691 T>C	Ser231Pro	rs363315	105	1	0	4	72	6	0	2	449	20	0	42	484	11	1	9
c.698 C>T	Ala233Val	rs363360	105	0	0	5	78	0	0	2	465	0	0	46	494	2	0	9
c.718 C>T	Leu240Leu	none	105	1	0	4	78	0	0	2	464	1	0	46	494	2	0	9
c.756 G>T	Ser252Ser	none	106	0	0	4	75	0	0	5	401	0	0	80	436	1	0	68
c.846 C>T	Pro282Pro	none	106	0	0	4	75	0	0	5	398	1	0	82	435	0	0	70
c.867 T>A	Ile289Ile	none	106	0	0	4	75	0	0	5	402	0	0	79	434	1	0	70
c.966 C>G	Thr322Thr	none	106	0	0	4	75	0	0	5	402	0	0	79	434	1	0	70

*nt change: nucleotide change in relation to the cDNA, with the 1st base of the start codon numbered 1; aa change: amino acid change

†Occurrence: number of individuals homozygous for common allele (11), heterozygous (12), homozygous for rare allele (22), or no data (0)

SUPPLEMENTARY TABLE 2
PCR primers used to amplify regions of the human *KCNK18* gene

Exon	Forward primer	Reverse primer	Size (bp)
1	5'CCTGGCGGGGCTTAGATGCTC3'	5'GGGGGACTGCTGCCACCAC3'	345
2	5'GGGGCGGGGCTTGTCTTTACC3'	5'CACCCAGAAACCCACCCTTCACAG3'	252
3A*	5'AAGGGAAGGGGCCAGATGC3'	5'ACACCAGTTCGGGACACGAGTTAC3'	467
3B*	5'AGCATGGAGCTGTTTGAGAG3'	5'ATGACCCTGAAAGACAACACA3'	555

*Exon 3 was amplified in two separate minimally-overlapping PCR fragments.

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SUPPLEMENTARY METHODS

Amplicon design and PCR. To screen the human *KCNK18* gene, we designed amplicons to minimize the amount of intronic sequence for each amplicon, and to avoid repetitive elements such as Alu repeats. We designed PCR primers using PrimerSelect (DNASTAR, Madison WI) and purchased them from BioCorp (Montreal). The primer sequences, amplicon sizes and amplification conditions are given in **Supplementary Table 2**. We amplified all samples using the following touch-down PCR protocol: initial denaturation for 4 min. at 94 ºC, followed by 17 cycles of denaturation at 94 ºC for 30 s, annealing at a temperature starting at 70 ºC and ending at 54 ºC (–1 ºC per cycle), and 45 s elongation at 72 ºC, followed by 25 cycles of denaturation at 94 ºC for 30 s, annealing at 54 ºC for 30 s and elongation at 72 ºC for 45 s, followed by a single cycle at 72 ºC for 5 min. We amplified samples using Taq DNA polymerase (Qiagen) on a PE 9700 PCR thermocycler (Perkin Elmer). We tested all amplified fragments using agarose gel electrophoresis.

Mutation analysis. We used denaturing high performance liquid chromatography (dHPLC) and direct sequencing to identify DNA variants. We optimized PCR fragment melting temperatures on a model 3500HT WAVE (Transgenomic Inc., Omaha, NB with Wavemaker software version 4.1.44) dHPLC apparatus. Fragments from patient samples were pooled 4×, denatured at 95 ºC in a heating block for 5 min and renatured slowly by cooling the block to 25 ºC over the course of 3 h. The pooled PCR fragments were run on the dHPLC apparatus at two different melting temperatures. The elution profiles of each sample pool were compared, and samples from 1 to 3 pools showing variant elution profiles were selected for sequence determination at the Genome Quebec Innovation Centre sequencing facility (Montreal). Sequencing reactions were performed on an ABI prism 3700 sequencing apparatus using manufacturer's recommended conditions. Sequence traces were aligned using SeqManII (DNASTAR, Madison, WI). Base-pair variants were identified and annotated. Publicly available predicted Single Nucleotide Polymorphisms (SNPs, from NCBI dbSNP) were identified, and those found in the sequence alignment were noted. Genotyping was done either by direct sequencing or by allele specific oligonucleotide (ASO) hybridization as described[1]. Briefly, each oligonucleotide was end-labeled with γ^{32}P-dATP using T4 kinase in a 25 µl total reaction. Then the probes were hybridized to nylon membranes to which was affixed the desired PCR amplified fragments, washed, and exposed to X-ray film. Films were scored and the genotypes for all successfully amplified samples were determined. Genotypes obtained from sequence reads were generated by Mutation Surveyor (SoftGenetics Inc.).

qRT-PCR. We performed quantitative RT-PCR (Applied Biosystems, TaqMan Gene Expression Assay Hs00699272_m1) on a panel of human mRNAs isolated from various tissues (FirstChoice Human Total RNA Survey Panel, Ambion, with DRG from Clontech). We used *GAPDH* expression as an endogenous control (Applied Biosystems, Cat#: 4326317E). PCR reagents (TaqMan Universal Mix 2× (ABgene)) were purchased from ABI and used according to the manufacturer's instructions. Optical 96-well plates and optical

seals were used for the experiments. The experiment was run on an ABI7000 thermocycler and the data analyzed using SDS v1.2.3 software. Three independent experiments were done. The difference between *GAPDH* and *KCNK18* Ct (i.e. cycle threshold) values is representative of *KCNK18* mRNA enrichment in a tissue (the bigger the difference, the smaller the enrichment). We calculated the average and standard deviation of cycle threshold (Ct) difference (also known as delta Ct) for all tissues and for the independent three experiments. Human ovary which has the lowest perceptible expression was chosen as the reference. We subtracted the delta Ct value of the human ovary to all other tissues (also known as the deltadelta Ct) and these values were then converted into fold-enrichment by performing 1/(2 EXP[deltadelta Ct]). A similar procedure was done for the standard deviation (STDEV); however the 1/(2EXP[deltadelta Ct]) was multiplied by the fold value.

Electrophysiological characterisation of TRESK channels. We transcribed messenger RNAs coding for the wild type and the mutant TRESK using the SP6 mMESSAGE mMACHINE kit (Ambion). Oocytes were continuously perfused with low [K^+] solution containing (mM): 95.4 NaCl, 2 KCl, 1.8 $CaCl_2$, 5 HEPES (pH 7.5 with NaOH) using a pump driven perfusion system. The high [K^+] solution contained (mM): 17.4 NaCl, 80 KCl, 1.8 $CaCl_2$, 5 HEPES (pH 7.5 with NaOH). Ionomycin was obtained from Calbiochem. We pulled recording electrodes from borosilicate glass capillaries using a two stage vertical puller (PP-830, Narishige), back-filled with 0.2 µm filtered 3M KCl and their resistances varied from 0.3 to 1 MΩ. Currents were filtered at 100 Hz and digitized at 1 kHz and transferred to a computer for analysis via an analog to digital converter (Digidata 1322A, Axon Instruments). Oocytes were held at –80 mV, voltage commands were applied, and currents were recorded using pClamp 9 software (Axon Instruments). We analyzed all data with pCLAMP9 (Axon Instruments), Origin 6.02 (Microcal Software). Molar ratios are as follow 1:1 = 0.45ng:0.45ng; 5:5=2.5ng:2.5ng; 1:5=0.45ng:2.5ng. Results were reproducible in at least three individual batches of oocytes from different donors. When error bars are not shown in the figures they are smaller than the size of the symbol.

References

1. Bourgeois, S. & Labuda, D. Dynamic allele-specific oligonucleotide hybridization on solid support. *Anal. Biochem.* **324**, 309-311 (2004).