# Variants in the $SK_2$ channel gene ($KCNN_2$) lead to dominant neurodevelopmental movement disorders

# Variants in the SK2 channel gene (*KCNN2*) lead to dominant neurodevelopmental movement disorders

Fanny Mochel,[1,2] Agnès Rastetter,[1] Berten Ceulemans,[3] Konrad Platzer,[4] Sandra Yang,[5] Deepali N. Shinde,[6] Katherine L. Helbig,[7,8] Diego Lopergolo,[9,10] Francesca Mari,[9,10] Alessandra Renieri[9,10] Elisa Benetti,[11] Roberto Canitano,[12] Quinten Waisfisz,[13] Astrid S. Plomp,[13] Sylvia A. Huisman,[14] Golder N. Wilson,[15] Sara S. Cathey,[16] Raymond J. Louie,[16] Daniela Del Gaudio,[17] Darrel Waggoner,[17] Shawn Kacker,[18] Kimberly M. Nugent,[19,20] Elizabeth R. Roeder,[19,20] Ange-Line Bruel,[21,22] Julien Thevenon,[23,24] Nadja Ehmke,[25] Denise Horn,[25] Manuel Holtgrewe,[26] Frank J. Kaiser,[27] Susanne B. Kamphausen,[28] Rami Abou Jamra,[4] Sarah Weckhuysen,[29,30,31] Carine Dalle,[1] and Christel Depienne[1,27,*]

[1] Institut du Cerveau et de la Moelle épinière (ICM), Sorbonne Université, UMR S 1127, Inserm U1127, CNRS UMR 7225, F-75013 Paris, France

[2] AP-HP, Hôpital Pitié-Salpêtrière, Département de Génétique and Centre de Référence Neurométabolique Adulte, F-75013, Paris, France

[3] Division of Paediatric Neurology, Antwerp University Hospital, University of Antwerp, Edegem, Belgium

[4] Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany.

[5] GeneDx, Gaithersburg, MD 20877, USA

[6] Department of Clinical Diagnostics, Ambry Genetics, Aliso Viejo, CA, USA

[7] Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA, USA

[8] The Epilepsy Neurogenetics Initiative, The Children's Hospital of Philadelphia, Philadelphia, PA, 19104

[9] Medical Genetics, University of Siena, Siena, Italy

[10] Genetica Medica, Azienda Ospedaliera Universitaria Senese, Siena, Italy

[11] Department of Medical Biotechnologies, University of Siena, Siena, Italy

[12] Division of Child and Adolescent Neuropsychiatry, University Hospital of Siena, Siena, Italy

[13] Department of Clinical Genetics, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

[14] Department of Pediatrics, Amsterdam UMC, Amsterdam, the Netherlands; and Prinsenstichting, Purmerend, the Netherlands

[15] Department of Pediatrics, Texas Tech University Health Science Center, Lubbock, Texas, USA

[16] Greenwood Genetic Center, Greenwood, South Carolina, 29646, USA

[17] Department of Human Genetics, University of Chicago, Chicago, IL, 60637, USA

[18] Department of Pediatrics, Section of Child Neurology, University of Chicago, Chicago, IL, 60637, USA

[19] Department of Pediatrics, Baylor College of Medicine, San Antonio, TX, 78207, USA

[20] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, 77030, USA

[21] UMR1231 GAD, Inserm - Université Bourgogne-Franche Comté, Dijon, France

[22] Centre de référence maladies rares « déficiences intellectuelles de causes rares », Centre de Génétique, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France

[23] Service de Génétique et Procréation, CHU Grenoble-Alpes, Grenoble, France

Service de Génétique, Génomique, et Procréation, Centre Hospitalier Universitaire Grenoble Alpes, 38700 La Tronche, France

[24] INSERM 1209, CNRS UMR 5309, Institute for Advanced Biosciences, Université Grenoble Alpes, 38706 Grenoble, France

[25] Institute for Human Genetics and Medical Genetics, Charité - Universitaetsmedizin Berlin, Berlin, Germany

[26] Core Unit Bioinformatics - CUBI, Berlin Institute of Health (BIH), Berlin, Germany

[27] Institute of Human Genetics, University Hospital Essen, University of Duisburg-Essen, Hufelandstraße 55, 45147 Essen, Germany

[28] Institute of Human Genetics, University Hospital Magdeburg, Magdeburg, Germany

[29] Applied&Translational Neurogenomics Group, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium

[30] Translational Neurosciences, Faculty of Medicine and Health Science, University of Antwerp, Antwerp, Belgium

[31] Department of Neurology, University Hospital Antwerp, Antwerp, Belgium

*Correspondence: Prof. Christel Depienne, Institut für Humangenetik, Universitätsklinikum Essen, Virchowstraße 171, 45147 Essen, Germany. Email: christel.depienne@uni-due.de

**Abstract**

*KCNN2* encodes the small conductance calcium-activated potassium channel 2 (SK2). Rodent models with spontaneous *Kcnn2* mutations show abnormal gait and locomotor activity, tremor and memory deficits, but human disorders related to *KCNN2* variants are largely unknown. Using exome sequencing, we identified a *de novo KCNN2* frameshift deletion in a patient with learning disabilities, cerebellar ataxia and white matter abnormalities on brain MRI. This discovery prompted us to collect data from nine additional patients with *de novo KCNN2* variants (one nonsense, one splice site, six missense variants and one in-frame deletion) and one family with a missense variant inherited from the affected mother. We investigated the functional impact of six selected variants on SK2 channel function using the patch-clamp technique. All variants tested but one, which was reclassified to uncertain significance, led to a loss-of-function of SK2 channels. Patients with *KCNN2* variants had motor and language developmental delay, intellectual disability often associated with early-onset movement disorders comprising cerebellar ataxia and/or extrapyramidal symptoms. Altogether, our findings provide evidence that heterozygous variants, likely causing a haploinsufficiency of the *KCNN2* gene, lead to novel autosomal dominant neurodevelopmental movement disorders mirroring phenotypes previously described in rodents.



**Keywords:** *KCNN2*, SK2 channel, calcium-activated potassium channels, developmental delay, movement disorder, intellectual disability, tremor, autism spectrum disorder, epilepsy, ataxia.

## Introduction

Small conductance calcium-activated potassium channels (SK) are a subfamily of potassium channels comprising three members (SK1-3) encoded by different genes (*KCNN1-3*) in humans, all widely expressed in the brain (Bond et al., 2005; Faber, 2009). A fourth paralogue (*KCNN4,* IK/SK4 channel) is primarily expressed in non-neuronal tissues. SK channels share the same topology as voltage-gated potassium channels, with each subunit containing six hydrophobic transmembrane domains (S1-S6) and assembling to form homo- or hetero-tetrameric channels (Lee et al., 2018). However, contrary to voltage-gated potassium channels, SK channel activation is voltage independent and depends on intracellular calcium levels. SK channels are activated via calmodulin, which is constitutively attached to a C-terminal region of each subunit (calmodulin binding domain, CaMBD), and triggers channel opening upon Ca2+ binding (Faber, 2009; Lee et al., 2018). SK channels can be selectively blocked by the bee venom toxin apamin, which directly binds to the pore of the channel (Weatherall et al., 2010).

In neurons, SK channels play important roles in the regulation of membrane excitability by calcium and serve distinct physiological roles by coupling with different calcium sources. For instance, they contribute to regulate action potential firing by controlling the medium duration of the afterhyperpolarization phase when activated by the transient elevation of intracellular calcium during action potentials (Bond et al., 2005; Faber, 2009). At the postsynaptic level, SK channels can also be activated by calcium influx through N-methyl-D-aspartate (NMDA) receptors, or by a release of calcium from intracellular stores. At this juncture, SK channels play an important role in hampering excitatory synaptic transmission and synaptic plasticity, regulating dendritic excitability and modulating long-term potentiation (Faber, 2009). *In vivo* pharmacological blockade with apamin or overexpression of SK channels

in animals have an impact on learning and memory, although there is a debate on whether their inhibition enhances or impairs learning processes (Baker et al., 2011; Hammond et al., 2006; van der Staay et al., 1999).

*KCNN1* has not yet been associated with a human disorder whereas *de novo* gain-of-function variants in *KCNN3* cause Zimmermann-Laband syndrome, a neurodevelopmental disorder characterized by intellectual disability and gingival enlargement (Bauer et al., 2019). One sporadic patient and one family with *KCNN2* variants have independently been reported in the literature (Balint et al., 2020; Raghuram et al., 2017) but evidence is still lacking to definitely link *KCNN2* to human genetic disorders. Yet, several mouse or rat models with spontaneous *Kcnn2* mutations have been described. 'Frissonnant' mice exhibit abnormal gait and locomotor activity, early-onset tremors, and memory deficits (Callizot et al., 2001). This mouse line carries a homozygous deletion encompassing exons 1-2 of *Kcnn2* (Szatanik et al., 2008). The recessive *Jitter* mutation (*Kcnn2* Leu168Pro) is associated with stiff gait, hunched posture and tremors starting from three weeks of age (Mutagenetix database). More recently, a rat model (Tremor dominant Kyoto, Trdk) exhibiting tremor from weaning was shown to carry the dominant *Kcnn2* Ile289Asn variant (Kuramoto et al., 2017). In this study, we assembled molecular and clinical data of eleven patients with *KCNN2* variants and demonstrate that *KCNN2* variants underlie a dominant channelopathy characterized by developmental delay and movement disorders.

## Material and methods

### *Genetic studies*

Patient 1 was recruited from a series of individuals with leukoencephalopathies of unknown origin for whom exome sequencing was undertaken to identify a genetic cause (Fig. 1). *Trio*

exome sequencing was performed using the SeqCap EZ MedExome Enrichment Kit (Roche). Libraries were sequenced as paired-end 150 bp reads on a NextSeq500 (Illumina). Bioinformatic analyses were conducted using BWA-0.7.12, picard-tools-1.121, GenomeAnalysisTK-2014.3-17-g0583013, SNPEff-4.2, and variant filtering was performed using the Polyweb interface (Paris-Descartes University, France). We collected clinical and molecular data from additional patients with *KCNN2* variants through GeneMatcher (Sobreira et al., 2015) and the EuroEPINOMICS-RES consortium. Exome or genome sequencing was performed in *trio* or singleton for Patients 2 to 10 in diagnostic or research settings from blood samples (details in Table 1 and Supplementary Table 1). For singleton analyses, *KCNN2* variants were validated and searched for in parents using Sanger sequencing. Variants were classified using the Intervar interface with manual adjustment (Li et al., 2017). *KCNN2* variants are described on Refseq isoform NM_021614.3 (ENST00000264773.7), which encodes the well-characterized 579 amino-acid SK2 channel subunit (Q9H2S1-1 in Uniprot). Of note, several other *KCNN2* isoforms (four in UCSC and six in Ensembl) have been described. *KCNN2* variants described in this study have been submitted to ClinVar (accessions SCV001371847 - SCV001371856). Referring physicians filled out a table with detailed developmental, neurological and behavioral history, including brain MRI data when available. Informed written consent was obtained for each individual or his/her parents before blood sampling. Experiments were performed in accordance with European guidelines and legislation. This study received the ethics approval of Assistance Publique des Hôpitaux de Paris (RCB 2010-A01395-34). Ethics approval were locally obtained for genetic analyses and/or data sharing for patients 2-10. Patient information was anonymized before data sharing and no documents allowing to establish patient identity was transferred.

***Electrophysiology***

The first six non-truncating variants identified were introduced into a plasmid containing the human *KCNN2* NM_021614.3 cDNA (Origene, Rockville, MA, USA) using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, San Diego, CA, USA). Plasmids were verified by Sanger sequencing. Chinese hamster ovary (CHO)-K1 cells were transiently cotransfected with 4 µg of plasmid expressing the wild-type (WT) or mutant human KCNN2 (hKCNN2) and a plasmid expressing the green fluorescent protein (pEGFP) at a ratio of 75: 1, using the NeonTM transfection system (Invitrogen). Currents were recorded from green-fluorescent cells using the whole-cell configuration of the patch-clamp technique 16 to 24 hours after transfection at room temperature. Voltage-clamp recordings were performed using an Axopatch 200B amplifier and a Digidata 1440 Analog/Digital interface (Axon Instruments, Molecular Devices, USA). Data were low-pass filtered at 2 kHz, digitized at 10 kHz and analysed offline using Clampfit software. Current were recorded during a 400 ms voltage ramp from -120 mV to +60 mV applied from a holding potential of -80 mV every 5 s after establishment of the whole-cell configuration. The resistance of a typical patch pipette was 3-4 MΩ when filled with the intracellular solution. Serie resistance was not compensated, and no leak subtraction was performed. Data were not corrected offline for voltage error and liquid junction potential. The pipette solution contained (in mM): K-gluconate 144, $MgCl_2$ 1.15, $CaCl_2$ 0.85, MgATP 2, EGTA 1, HEPES, 10, pH adjusted to 7.2 with KOH. This composition gives 1.97 µM calcium-free at 25°C based on the Maxchelator program. The extracellular solution contained (in mM): NaCl 140, KCl 2.5, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 10, Glucose 10, pH adjusted to 7.3 with NaOH.

**Results**

*Trio* exome sequencing in a 30-year-old male with learning disabilities, cerebellar ataxia and white matter abnormalities (Fig. 1) led to the identification of a *de novo* 4-nucleotide deletion introducing a premature termination codon in *KCNN2* (c.800_803del, p.Tyr267*). Through collaborative efforts, we identified nine additional patients with *de novo KCNN2* variants and an affected mother-daughter pair with an inherited variant (p.Leu432Pro) in the CaMBD domain (Fig. 2, Table 1). All eleven variants were absent from gnomAD. *De novo* variants include one nonsense (p.Tyr160*), one splice site (c.1254+2T>C), six missense variants (p.Glu30Gln, p.Ile288Ser, p.Ile359Met, p.Tyr361Cys, p.Gly362Ser, p.Leu388Val), and one deletion of a single amino acid (p.Leu321del). Except p.Glu30Gln located in the N-terminal domain, *de novo* amino-acid substitutions and deletions cluster in or near functional SK2 domains, *i.e.* between S4 and S5 (p.Ile288Ser), in S5 (p.Leu321del) or S6 (p.Leu388Val) transmembrane domains, or in the pore region (p.Ile359Met, p.Tyr361Cys, p.Gly362Ser). Missense variants and p.Leu321del alter amino acids highly conserved in mammals, and except p.Glu30Gln, they also affect amino acids conserved in SK1 (*KCNN1*) and SK3 (*KCNN3*) channels (Fig. S1).

To investigate the functional consequences of *KCNN2* variants, whole-cell currents were recorded from CHO-K1 cells transfected with plasmids expressing the WT or six selected mutants using the patch-clamp technique. Large currents recorded upon voltage ramp from cell transfected with hKCNN2 WT were generated within few seconds of whole-cell formation and increased to a steady state by 2 min (Fig. 3A-B). This current was sensitive to the specific blocker of SK2 channel apamin (Fig. S2). Currents recorded from cell transfected with hKCNN2 Glu30Gln also increased with calcium dialysis after the establishment of whole-cell configuration, whereas only endogenous currents were recorded from cells transfected with all

other mutants (Leu321del, Ile359Met, Gly362Ser, Leu388Val and Leu432Pro; Fig.3A-B). Current densities from cells transfected with hKCNN2 Glu30Gln were not statistically different from those recorded from cell expressing hKCNN2 WT (Fig.3C, $p$ = 0.121, Mann-Whitney Rank Sum test). Altogether, these findings indicate that all variants studied except Glu30Gln lead to a loss-of-function (LoF) of SK2 homomeric channels.

Excluding Patient 11, whose variant p.Glu30Gln was reclassified to unknown significance (Supplementary Table 1), this patient series comprises ten subjects, including six males and four females, with pathogenic or likely pathogenic *KCNN2* variants, aged from 2 to 60 years (mean: 17.0 years, median: 12.0 years; Table 1). Nine patients presented with developmental delay, including delays in acquiring motor milestones and a speech delay or a regression of language abilities. All patients had intellectual disability (ID) although the degree of cognitive impairment was variable. Four patients exhibited mild ID, three patients had moderate ID and two had severe ID. Autism spectrum disorder (ASD) or autistic features were present in two and four patients, respectively. Movement disorders were observed in six patients including tremor in five. Four patients presented with cerebellar ataxia, with an early onset from childhood to adolescence, whereas four patients exhibited extrapyramidal symptoms with dyskinesia (n=2), parkinsonism (n=2), myoclonus-dystonia (n=1) and choreic movements (n=1). Other neurological features included motor tics (head jerking, eye blinking) (n=3), nystagmus (n=2), peripheral neuropathy (n=2), pyramidal signs (n=1) and microcephaly (n=1). The four patients (Patients 2, 4, 7 and 9) without movement disorders were under the age of 15 years at the time of the study. Still, movement disorders might appear later on and progress over time, as observed in Patient 5, who developed rigidity after initially presenting with tremor. Two patients had epilepsy: Patient 7 (p.Gly362Ser) had one febrile seizure at 20 months of age and afebrile generalized seizures from the age of 25 months, and Patient 9 (c.1254+2T>C) had

a generalized clonic-tonic seizure (GTCS) at 14 years followed by nocturnal focal seizures. Brain MRI data was available for six patients, with three displaying white matter abnormalities. Only sparse information was available for the mother of Patient 9 who also carried the p.Leu432Pro variant. She was reported to have mild ID, a dyskinetic movement disorder with dystonia, and an unspecified psychiatric disorder. She had a brother with similar symptoms and ASD, who was unavailable for genetic testing. Noticeably, Patients 1 and 8, who were aged 30 and 60 years, also had psychotic episodes, suggesting that psychiatric features can be part of the clinical picture of this new disorder in older subjects.



**Discussion**

Genetic channelopathies are a group of disorders resulting from pathogenic variants in any of the 400 human genes encoding ion channels. More than 30 neurological disorders related to ion channels primarily expressed in the brain have been described so far. These 'cerebral channelopathies' commonly include intellectual disability, epilepsy and cerebellar ataxia as primary features (Imbrici et al., 2016; Spillane et al., 2016). In this study, we describe a new channelopathy resulting from pathogenic variants in *KCNN2*. This dominant disorder is characterized by developmental delay and ID, often associated with an early-onset movement disorder manifesting mainly as cerebellar ataxia and/or extrapyramidal symptoms.

A *de novo* frameshift variant (c.581dupA, p.Leu195Val*fs**10) was previously reported in a female patient with developmental delay, epilepsy, spasticity, cerebellar ataxia and dystonia (Raghuram et al., 2017). However, this patient also had a homozygous in-frame deletion in *ZNF135* and the cause of her phenotype remains uncertain. This phenotype being remarkably similar to those described in our series, the *KCNN2* frameshift likely accounts for at least part of her disorder. More recently, a missense variant (c.1112G>A, p.Gly371Glu) located between

the S5 and S6 segment in the pore region was shown to segregate with myoclonus-dystonia and anxiety in a three-generation family (Balint et al., 2020). No functional study was performed and the impact of this variant on SK2 channels therefore remains unknown. Nevertheless, myoclonus-dystonia was also diagnosed in Patient 3 and it clinically overlaps with movement disorders observed in other patients, in particular tremor, dyskinesia, parkinsonism and motor tics, further supporting both the pathogenicity of the p.Gly371Glu variant, and myoclonus-dystonia as part of the clinical spectrum of *KCNN2* disorders.

Our results suggest that heterozygous *KCNN2* variants predominantly lead to a LoF of the SK2 channel (*i.e.* haploinsufficiency). Interestingly, p.Ile288Ser (Patient 3) alters the same amino acid than the mutation present in Trdk rats (*Kcnn2* I289N, corresponding to *KCNN2* p.Ile288Asn), which also significantly reduced SK2 currents (Kuramoto et al., 2017). The observation of three truncating variants that either trigger nonsense-mediated decay or truncate the channel before functional domains further supports haploinsufficiency as the main mechanism. The electrophysiological study performed by Raghuram *et al*. confirmed that, if expressed in patient's cells, truncated channels would not be functional (Raghuram et al., 2017). This variant spectrum, comprising both truncating and missense variants altering key functional domains of the channel, is observed in other channelopathies associated with haploinsufficiency, such as Dravet syndrome (Depienne et al., 2009). The intolerance of *KCNN2* to haploinsufficiency is strongly supported by a significant depletion of truncating variants in this gene in gnomAD (pLI = 0.99). Only ten individuals with consistent LoF variants were observed out of the 141,456 individuals included in this database, and half, located at the C-terminal end, spare functional SK domains, contrasting with the early location of truncating variants found in patients. We cannot exclude, however, that some missense variants reported herein may also have gain-of-function or dominant-negative effects on WT SK2 or other SK

subunits, as observed for many missense variants in ion channels, including SK3/*KCNN3* (Bauer et al., 2019).

Contrary to variants altering key functional domains, we could not confirm the pathogenicity of p.Glu30Gln, located in the less conserved N-terminal region of the channel. This patient has a more severe epileptic encephalopathy, possible related to a yet unidentified cause. However, this patient also shares common features with patients of this series, including tremor and dystonia/dyskinesia and seizures. Further investigations are needed to determine the possible contribution of *KCNN2* Glu30Gln to this epileptic encephalopathy phenotype.

Remarkably, patients with *KCNN2* variants have phenotypes highly similar to those described in *Kcnn2* mutant mouse and rat models (Callizot et al., 2001; Kuramoto et al., 2017; Szatanik et al., 2008). All showed rapid constant tremor and abnormal (ataxic and/or stiff) gait, mirroring movement disorders observed in patients, as well as memory deficits and behavioral disturbances (*e.g.* lack-luster coat in *Jitter* mice), reminiscent of ID, autistic and psychiatric features. A major difference, however, is that *Kcnn2* pathogenic variants are recessive in mice whereas they are dominant in rats and humans. The reason why *KCNN2* is more sensitive to gene dosage in these species remains unexplained. Possible explanations include distinct baseline *Kcnn2* expression levels, gene regulation, or compensatory mechanisms in response to *Kcnn2/KCNN2* haploinsufficiency. 'Frissonnant' mice and Trdk rats have been proposed as models for Parkinson's disease (Callizot et al., 2001) and essential tremor (Kuramoto et al., 2017), and interestingly parkinsonism and tremor are part of the clinical features seen in patients with *KCNN2* variants. Another interesting observation is that tremors observed in 'frissonnant' mice respond to dopamine (Callizot et al., 2001), as also reported for one patient (Raghuram et al., 2017), suggesting that dopamine or dopamine agonists may be used to treat movement

disorders in patients with *KCNN2* variants. Yet, levodopa was not beneficial to treat myoclonus-dystonia in family members with p.Gly371Glu (Balint et al., 2020), and was not tested in any of our patients.

The exact pathophysiological mechanisms involved in this novel *KCNN2* disorder have to be studied in more details, but given the neurological manifestations observed in patients, brain regions affected by *KCNN2* haploinsufficiency are likely multiple and include cerebellum, where *KCNN2* is highly expressed (Ballesteros-Merino et al., 2012; GTEx Consortium, 2015), basal ganglia and cerebral cortex. The mechanisms linking *KCNN2* alteration and leukoencephalopathy remains unclear. According to the Protein Atlas Database, *KCNN2* is mainly expressed in neurons of the cortex and cerebellum, but the SK2 protein is also detected in endothelial cells, which alterations may also cause leukodystrophies (Belleri et al., 2016).

The ID observed in *KCNN2* patients indicates that germline alteration of SK2 channel does impair memory and learning in humans. The results contrast with previous findings that blockade of SK channels with apamin enhances learning while overexpression of SK2 channel impairs learning in mice (Hammond et al., 2006). Increased *Kcnn2* expression was observed in the cortex of a mouse model for fetal alcohol spectrum disorder and pharmacologic blockade of SK2 channels was suggested as a possible treatment strategy (Mohammad et al., 2020). However, our results suggest that this strategy could be potentially hazardous in humans.

Recently, an association between a common intronic variant (rs13188074) of *KCNN2* and ASD was reported (Alonso-Gonzalez et al., 2019). Autistic features were present in half of the patients in our series, confirming the association of *KCNN2* variants with ASD with

incomplete penetrance. *KCNN2* variants may also predispose to additional psychiatric features that become more obvious with age since all older patients experienced psychotic episodes.

In conclusion, our study provides compelling evidence that heterozygous *KCNN2* variants lead to a novel syndrome associating impairment of cognitive development and movement disorders in humans. This new channelopathy is clinically variable and its characteristics lie at the interface of genetics, pediatrics, neurology and psychiatry.

**Web resources**

Ensembl, https://www.ensembl.org/

GeneMatcher, https://www.genematcher.org/

gnomAD, http://gnomad.broadinstitute.org/

InterVar, http://wintervar.wglab.org/

Maxchelator, https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/

Mutagenetix, https://mutagenetix.utsouthwestern.edu/phenotypic/phenotypic_rec.cfm?pk=88

Protein Atlas, https://www.proteinatlas.org/

UCSC, https://genome.ucsc.edu/

Uniprot, https://www.uniprot.org/

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**Declaration of interests**

S. Yang is an employee of GeneDx, Inc., a wholly owned subsidiary of OPKO Health, Inc. D.N. Shinde is a full-time employee of Ambry Genetics. Exome sequencing is one of Ambry’s commercially available tests. Dr. R. J. Louie is a clinical laboratory director at the Greenwood Genetic Center, which receives fee income from clinical laboratory testing. Other authors declare no competing interests.



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**Table 1. Molecular and clinical characteristics of patients with pathogenic or likely pathogenic *KCNN2* variants**.
GTCS: generalized tonic-clonic seizures; m: months; y: years; ADHD: attention-deficit hyperactivity disorder; NA: not available.

| Patient ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Sex M/F | M | M | F | F | M | M | M | M | F | F |
| Age at study | 30 y | 9 y 2 m | 17 y | 2 y | 15 y | 5 y 9 m | 7 y 11 m | 60 y | 16 y | 7 y |
| Genomic change (Ch5(GRCh37)) | g.113740352_113740355del | g.113698952C>A | g.113740414_113740415delinsTC | g.113740514_113740516del | g.113798821T>G | g.113798826A>G | g.113798828G>A | g.113808769C>G | g.113808863T>C | g.113822787T>C |
| cDNA change (NM_021614.3) | c.800_803del | c.480C>A | c.862_863delinsTC | c.962_964delTAT | c.1077 T>G | c.1082A>G | c.1084G>A | c.1162C>G | c.1254+2T>C | c.1295T>C |
| protein change | p.(Tyr267*) | p.(Tyr160*) | p.(Ile288Ser) | p.(Leu321del) | p.(Ile359Met) | p.(Tyr361Cys) | p.(Gly362Ser) | p.(Leu388Val) | p.? | p.(Leu432Pro) |
| Inheritance | *de novo* | *de novo* | *de novo* | *de novo* | *de novo* | *de novo* | *de novo* | *de novo* | *de novo* | inherited from affected mother |
| Identification method | WES (trio) | WES (trio) | WES (trio) | WES (trio) | WES (trio) | WES (trio) | WES (trio) | WGS (trio) | WES (singleton) | WES (singleton) |
| CADD score | 33 | 39 | 24 | 20.7 | 24.3 | 28.9 | 32 | 23.8 | 33 | 28.9 |
| ACMG classification | pathogenic | pathogenic | likely pathogenic | pathogenic | pathogenic | likely pathogenic | pathogenic | pathogenic | likely pathogenic | pathogenic |
| Microcephaly | no | no | no | no | no | no | yes | no | no | no |
| Motor delay | no | yes | yes | yes | yes | yes | yes | yes | yes | yes |
| Language delay | no | yes (regression) | yes | yes | yes | yes (regression) | yes | yes | yes | no |
| Intellectual disability | yes (mild) | yes (severe) | yes (mild) | developmental delay | yes (moderate) | yes (severe) | yes (mild) | yes (moderate) | yes (moderate) | yes (mild) |
| Autism spectrum disorder | no | yes | yes | no | no | autistic features | autistic features | autistic features | autistic features | no |
| Other behavioral problems | psychotic episodes in adulthood | manual stereotypies (hand clapping), difficulty falling asleep | anxiety, panic attacks, extreme shyness | none | ADHD, anxiety, severe shyness, fearful of routine activities | afraid of water, auto-aggressive behavior (hand biting), stereotypic hand clapping, | aggressive behavioral change after seizures | ADHD, manual stereotypies | ADHD, anxiety | none |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | intermittent hyperventilation | | | | |
| **Seizures (age at onset; type)** | no | no | no | no | no but epileptiform activity on EEG | no | yes (2 y 1 m; febrile, generalized tonic clonic) | no | yes (14 years, GTCS, nocturnal focal seizures) | no |
| **Movement disorder** | yes | no | yes | no | yes | yes | no | yes | no | yes |
| **Tremor (age at onset; type)** | yes (17 y; intention tremor) | no | yes (10 y; intention and resting tremor) | no | yes (10 y; intention tremor and resting tremor) | no | no | yes (mild tremor) | no | yes (3 m; intention tremor) |
| **Cerebellar ataxia (age at onset)** | yes (childhood) | no | no | no | no | yes (since starting to walk) | no | yes | no | yes (12 m) |
| **Extrapyramidal symptoms (age at onset)** | no | no | myoclonus-dystonia | no | bradykinesia, cogwheel rigidity (15 y) | no | no | tardive dyskinesia and parkinsonism; choreic movements | no | dyskinesia (6 y) |
| **Other neurological sign(s)** | pyramidal signs, demyelinating neuropathy | motor tics, bilateral tendon retraction | motor tics, muscular hypotonia | nystagmus | motor tics including head jerking and eye blinking (10 y) | nystagmus (3 m), discrete demyelinating neuropathy | no | no | no | no |
| **Treatments** | none | bilateral Achilles tendon lengthening | none | NA | propanol (no improvement of tremor), methylphenidate (some improvement on ADHD) | physiotherapy, occupational therapy | levetiracetam (seizures control) | haloperidol (link with movement disorders?) | lamotrigin and zonisamide (seizures control | physiotherapy, occupational therapy |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Brain MRI** | diffuse periventricular white matter abnormalities | NA | normal | delayed myelination at 15 m | normal | dilatation of 3rd ventricle; mild cerebellar heterogeneity (peduncles, white matter) | white matter abnormalities | NA | two isolated arachnoidal cysts | NA |
| **Other significant variants** | none | none | arr [hg19] 4q13.3(75024534_75117789)x1 pat; 5p14(23214244_23968379)x1 mat | *MED12L* (NM_053002.5: c.2666 G>A, p.R889H (mat) c.6002 C>T, p.S2001L (pat)) | *RYR2* (NM_001035.3:c.6800G>A/p.Arg2267His (maternal, incidental finding) | *TNK2* (NM_001010938.1:c.278T>G, p.Leu93Arg) *de novo* | none | none | none | none |

**Figure legends**

**Figure 1. Brain MRI characteristics**. MRI in Patient 1 at the age of 20 years showed diffuse periventricular white matter abnormalities with hyperintensenties on FLAIR sequences (A-B-C-D-E) and hypointensities (arrows) on T1-weigthed sequences.

**Figure 2. Overview of *KCNN2* pathogenic variants**. (A) Schematic representation of the *KCNN2* variants identified in patients in this study (above) and mutations described in rodents (below) on the gene (isoform NM_021414.3) and on the protein. (B) Schematic representation of the SK2 channel showing the localization of the pathogenic variants identified in patients (red: truncating variants; black: pathogenic or likely pathogenic missense variants; grey: *de novo* variant of unknown significance) and rodents (in green).

**Figure 3. Impact of six selected *KCNN2* variants on SK2 channel functional expression**. **A**) Representative traces of whole-cell current recorded from CHO-K1 cells transiently transfected with human wild-type (WT) or mutant hKCNN2 plasmid during a voltage ramp protocol using the patch-clamp technique**.** Each current trace is the steady-state current obtained after calcium dialysis following the establishment of the whole-cell configuration. hKCNN2 Leu321del (n = 5), Ile359Met (n = 5), Gly362Ser (n = 5), Leu388Val (n = 8) and Leu432Pro (n = 9) mutants do not conduct functional SK2 currents whereas hKCNN2 Glu30Gln (n = 8) conducts a SK2 current similar to that expressed by hKCNN2 WT (n = 22). **B**) Time course of current amplitudes at 55 mV recorded from the cells shown in (A), voltage ramp protocols being applied every 5 s. **C**) Box plots of the current density for WT and Glu30Gln (E30Q) showing the median (horizontal line), the 25-75% percentile range (box) and range within 1.5 IQR (whiskers). Numbers of experiment from different cells are shown in parentheses. Statistical

analysis between groups was performed with the Kruskal-Wallis ANOVA on ranks (Mann-Whitney Rank Sum test, $p = 0.121$).

**Supplementary Figure 1.** Orthologous (A) and paralogous (B) SK protein alignments correspond to transmembrane domains. P corresponds to the pore region. CaMBD: Calmodulin-binding region. ERS: endoplasmic reticulum retention signal. Alignment of SK channel (B) only displays highly conserved regions and do not include less conserved N- and C-terminal regions of SK channels.

**Supplementary Figure 2**. Inhibitory effect of apamin on whole-cell current recorded from GFP-labelled cells transfected with WT hKCNN2 plasmid using the patch-clamp technique. Representative superimposed current traces (top) obtained with voltage ramp protocol from -120 to 60 mV (bottom) in the absence or presence of 100 nM apamin (black and red traces, respectively). Blue trace represents apamin-sensitive current obtained by digitally subtracting the currents recorded in the absence of apamin from this one recorded in the presence of the blocker. The inhibitory effect of apamin was observed in n=4 cells.

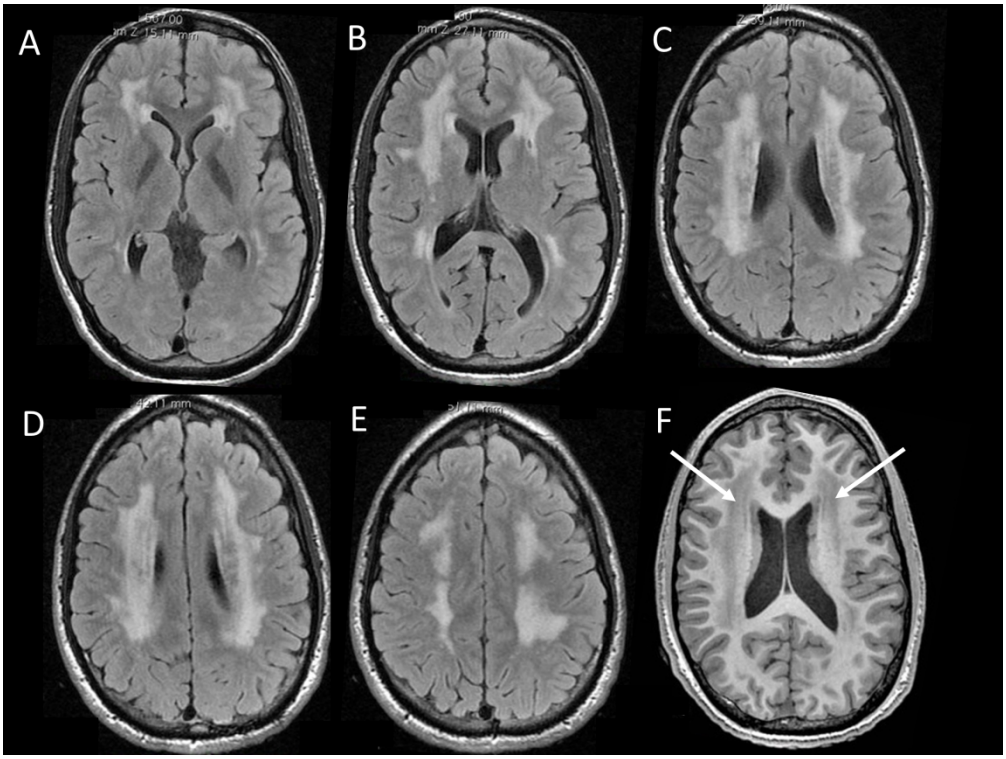


Figure 1. Brain MRI characteristics. MRI in Patient 1 at the age of 20 years showed diffuse periventricular white matter abnormalities with hyperintensenties on FLAIR sequences (A-B-C-D-E) and hypointensities (arrows) on T1-weigthed sequences.

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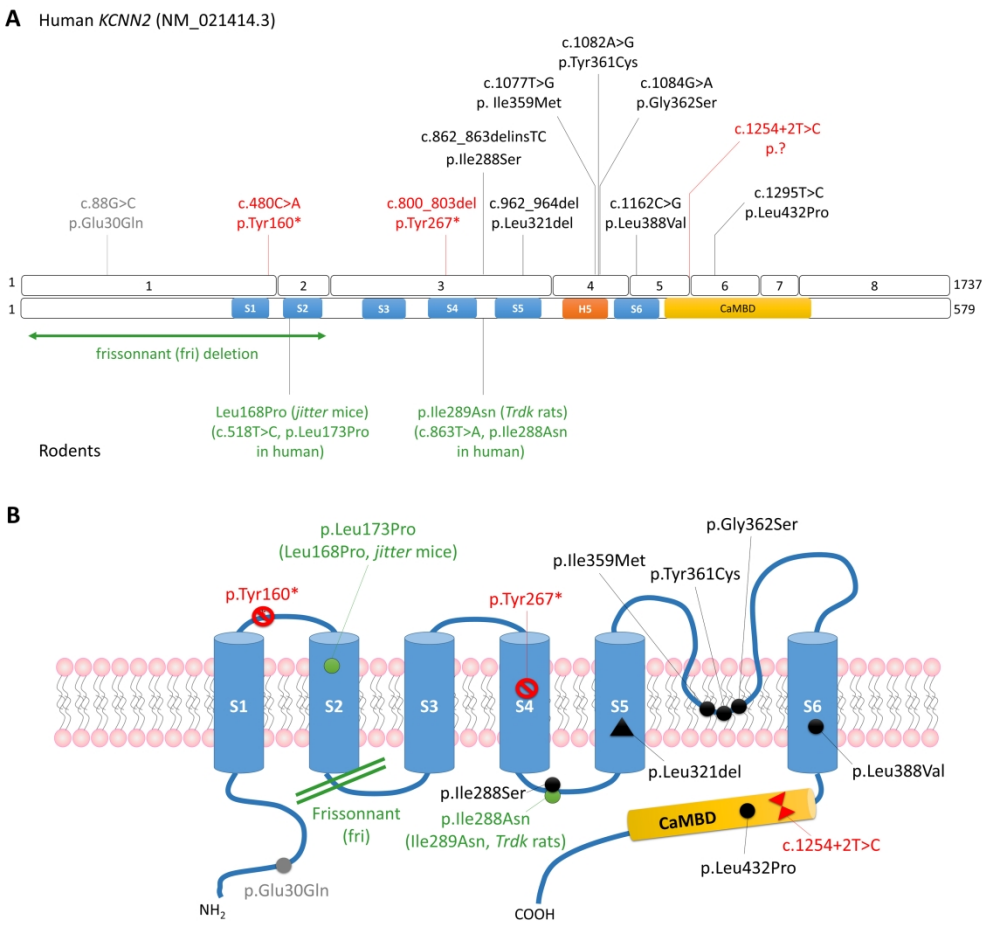


Figure 2. Overview of KCNN2 pathogenic variants. (A) Schematic representation of the KCNN2 variants identified in patients in this study (above) and mutations described in rodents (below) on the gene (isoform NM_021414.3) and on the protein. (B) Schematic representation of the SK2 channel showing the localization of the pathogenic variants identified in patients (red: truncating variants; black: pathogenic or likely pathogenic missense variants; grey: de novo variant of unknown significance) and rodents (in green).

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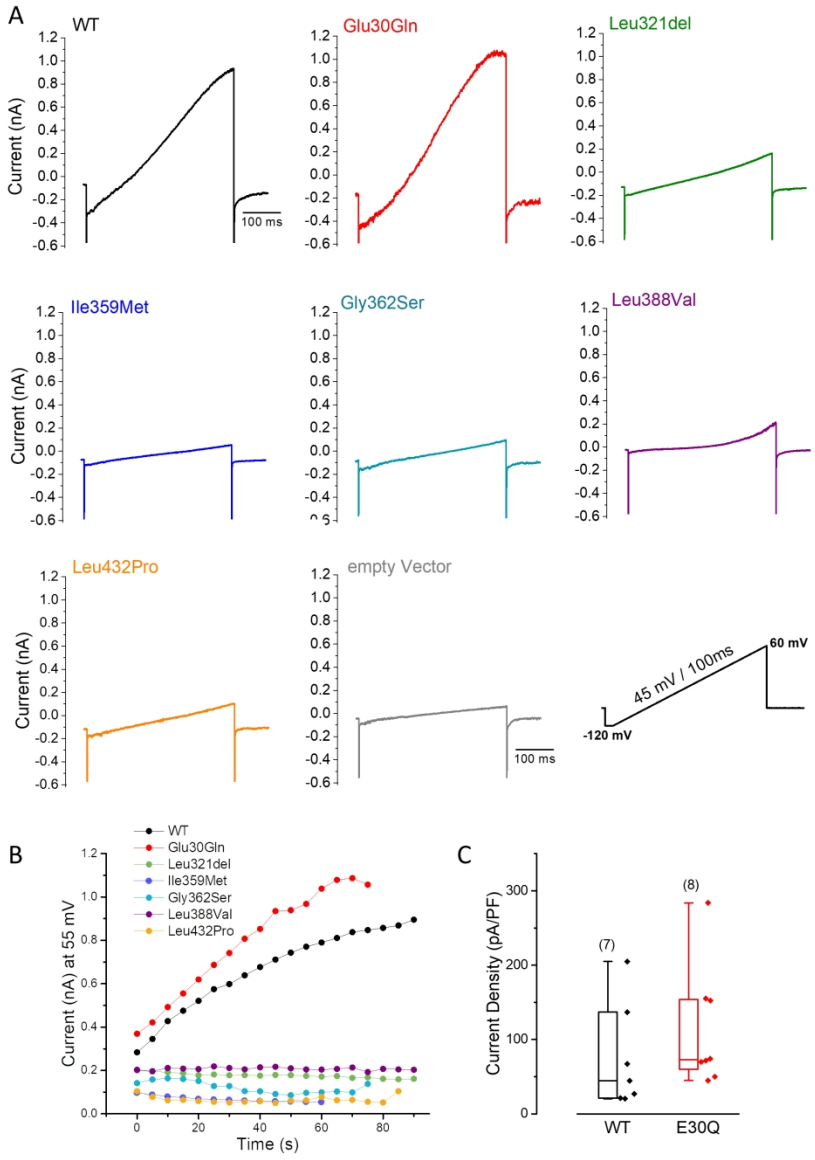


Figure 3. impact of six selected KCNN2 variants on SK2 channel functional expression. A) Representative traces of whole-cell current obtained from CHO-K1 cells transiently transfected with human wild-type (WT) or mutant hKCNN2 plasmid and recorded during a voltage ramp protocol using the patch-clamp technique. Each current trace is the steady-state current obtained after calcium dialysis following the establishment of the whole-cell configuration. hKCNN2 Leu321del (n = 5), Ile359Met (n = 5), Gly362Ser (n = 5), Leu388Val (n = 8) and Leu432Pro (n = 9) mutants do not conduct functional SK2 currents whereas hKCNN2 Glu30Gln (n = 8) conducts a SK2 current similar to that expressed by hKCNN2 WT (n = 22). B) Time course of current amplitudes at 55 mV recorded from the cells shown in (A), voltage ramp protocols being applied every 5 s. C) Box plots of the current density for WT and Glu30Gln (E30Q). Boxes indicate the 25% to 75% range, whiskers indicate the 10/90 percentiles, the median is shown as line and numbers of experiment from different cells are shown in parentheses. Statistical analysis between both groups was performed with the Kruskal-Wallis ANOVA on ranks (Mann-Whitney Rank Sum test, p = 0.121).

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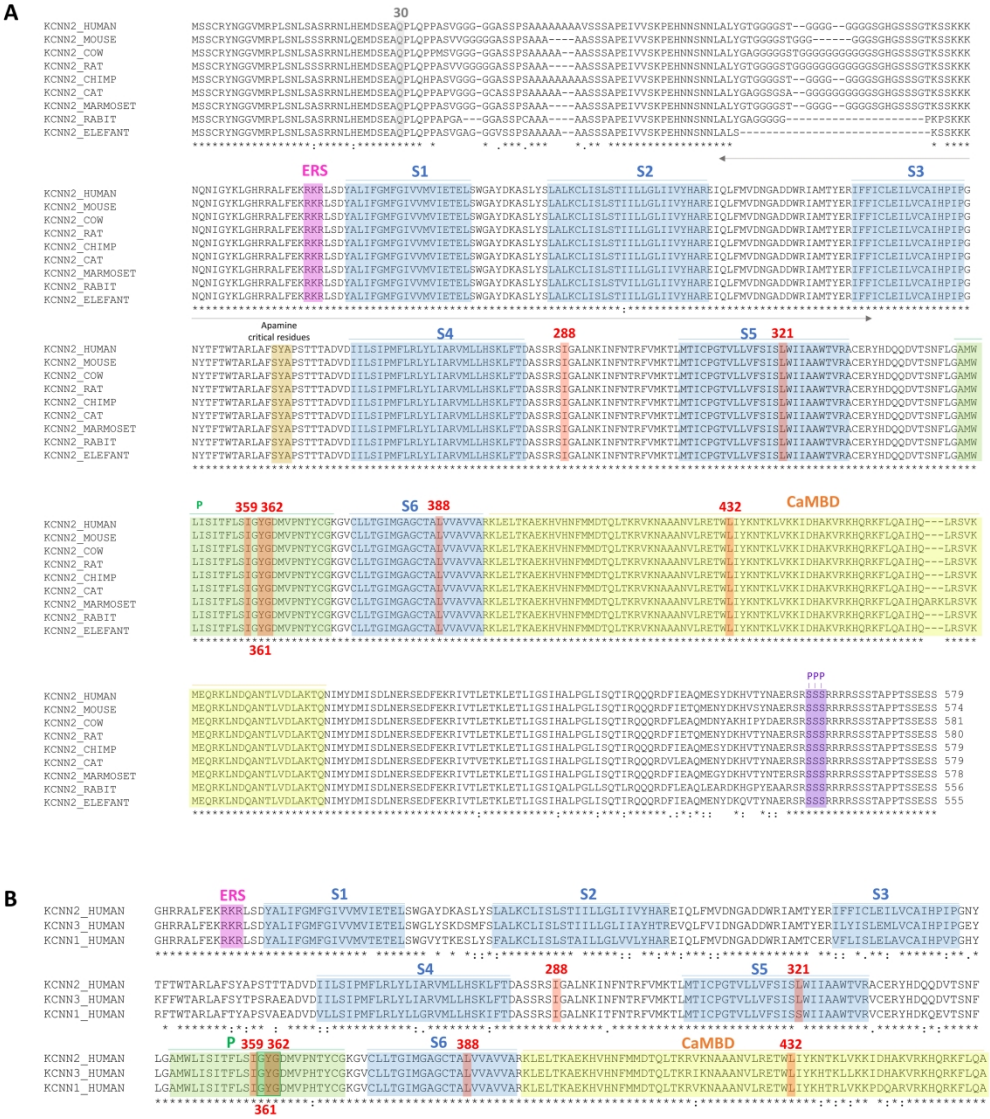


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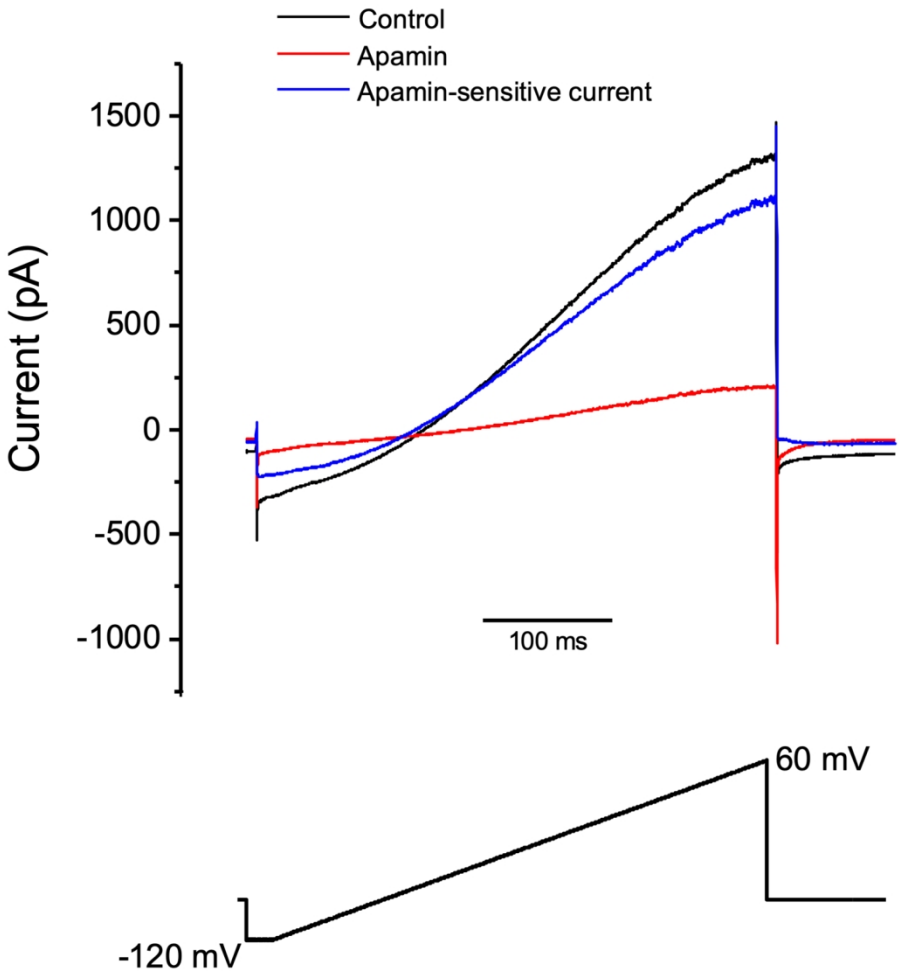


150x150mm (300 x 300 DPI)

| Patient ID | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Sex M/F | M | M | F | F | M |
| Age at study | 30 y | 9 y 2 m | 17 y | 2 y | 15 y |
| Genomic change (Ch5(GRCh37)) | g.113740352_113740355 del | g.113698952C>A | g.113740414_113740415 delinsTC | g.113740514_113740516 del | g.113798821T>G |
| cDNA change (NM_021614.3) | c.800_803del | c.480C>A | c.862_863delinsTC | c.962_964delTAT | c.1077 T>G |
| protein change | p.(Tyr267*) | p.(Tyr160*) | p.(Ile288Ser) | p.(Leu321del) | p.(Ile359Met) |
| Inheritance | *de novo* | *de novo* | *de novo* | *de novo* | *de novo* |
| Identification method | WES (trio) | WES (trio) | WES (trio) | WES (trio) | WES (trio) |
| CADD score | 33 | 39 | 24 | 20.7 | 24.3 |
| ACMG classification | pathogenic | pathogenic | likely pathogenic | pathogenic | pathogenic |
| Microcephaly | no | no | no | no | no |
| Motor delay | no | yes | yes | yes | yes |
| Language delay | no | yes (regression) | yes | yes | yes |
| Intellectual disability | yes (mild) | yes (severe) | yes (mild) | developmental delay | yes (moderate) |
| Autism spectrum disorder | no | yes | yes | no | no |
| Other behavioral problems | psychotic episodes in adulthood | manual stereotypies (hand clapping), difficulty falling asleep | anxiety, panic attacks, extreme shyness | none | ADHD, anxiety, severe shyness, fearful of routine activities |
| Seizures (age at onset; type) | no | no | no | no | no but epileptiform activity on EEG |
| Movement disorder | yes | no | yes | no | yes |
| Tremor (age at onset; type) | yes (17 y; intention tremor) | no | yes (10 y; intention tremor and resting tremor) | no | yes (10 y; intention tremor and resting tremor) |
| Cerebellar ataxia (age at onset) | yes (childhood) | no | no | no | no |
| Extrapyramidal symptoms (age at onset) | no | no | myoclonus-dystonia | no | bradykinesia, cogwheel rigidity (15 y) |
| Other neurological sign(s) | pyramidal signs, demyelinating neuropathy | motor tics, bilateral tendon retraction | motor tics, muscular hypotonia | nystagmus | motor tics including head jerking and eye blinking (10 y) |
| Treatments | none | bilateral Achilles tendon lengthening | none | NA | propanol (no improvement of tremor), methylphenidate (some improvement on ADHD) |
| Brain MRI | diffuse periventricular white matter abnormalities | NA | normal | delayed myelination at 15 m | normal |
| Other significant variants | none | none | arr [hg19] 4q13.3(75024534_751177 89)x1 pat; 5p14(23214244_2396837 9)x1 mat | *MED12L* (NM_053002.5*: c.2666 G>A, p.R889H (mat) c.6002 C>T, p.S2001L (pat))* | *RYR2* (NM_001035.3:c.6800G> A/p.Arg2267His (maternal, incidental finding) |

GTCS: generalized tonic-clonic seizures; m: months; y: years; ADHD: attention-deficit hyperactivity disorder; NA: not ava

| 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|
| M | M | M | F | F | F |
| 5 y 9 m | 7 y 11 m | 60 y | 16 y | 7 y | 6 y |
| g.113798826A>G | g.113798828G>A | g.113808769C>G | g.113808863T>C | g.113822787T>C | g.113698560G>C |
| c.1082A>G | c.1084G>A | c.1162C>G | c.1254+2T>C | c.1295T>C | c.88G>C |
| p.(Tyr361Cys) | p.(Gly362Ser) | p.(Leu388Val) | p.? | p.(Leu432Pro) | p.(Glu30Gln) |
| *de novo* | *de novo* | *de novo* | *de novo* | inherited from affected mother | *de novo* |
| WES (trio) | WES (trio) | WGS (trio) | WES (singleton) | WES (singleton) | WES (trio) |
| 28.9 | 32 | 23.8 | 33 | 28.9 | 28.5 |
| likely pathogenic | pathogenic | pathogenic | likely pathogenic | pathogenic | unknown significance |
| no | yes | no | no | no | yes |
| yes | yes | yes | yes | yes | yes |
| yes (regression) | yes | yes | yes | no | yes (nonverbal) |
| yes (severe) | yes (mild) | yes (moderate) | yes (moderate) | yes (mild) | yes (profound) |
| autistic features | autistic features | autistic features | autistic features | no | no |
| afraid of water, auto-aggressive behavior (hand biting), stereotypic hand clapping, intermittent hyperventilation | aggressive behavioral change after seizures | ADHD, manual stereotypies | ADHD, anxiety | none | hand biting when younger, bruxism |
| no | yes (2 y 1 m; febrile, generalized tonic clonic) | no | yes (14 years, GTCS, nocturnal focal seizures) | No | yes (3 m; daily; refractory; EEG with myoclonic jerks, generalized epileptiform discharges) |
| yes | no | yes | no | yes | yes |
| no | no | yes (mild tremor) | no | yes (3 m; intention tremor) | yes (6 years; generalized tremor but possibly medication related) |
| yes (since starting to walk) | no | yes | no | yes (12 m) | no |
| no | no | tardive dyskinesia and parkinsonism; choreic movements | no | dyskinesia (6 y) | dystonia and dyskinesia (limbs and trunk) |
| nystagmus (3 m), discrete demyelinating neuropathy | no | no | no | No | hypotonia; hyperreflexia; clonus |
| physiotherapy, occupational therapy | levetiracetam (seizures control) | haloperidol (link with movement disorders?) | lamotrigin and zonisamide (seizures control | physiotherapy, occupational therapy | Levetiracetam, clobazam, lamotrigin , diazepam, cannabidiol |
| dilatation of 3rd ventricle; mild cerebellar heterogeneity (peduncles, white matter) | white matter abnormalities | NA | two isolated arachnoidal cysts | NA | cortical / subcortical atrophy; hypoplastic bilateral hippocampal complexes; mega cisterna magna |
| *TNK2* (NM_001010938.1:c.278T >G, p.Leu93Arg) de novo | none | none | none | None | None |

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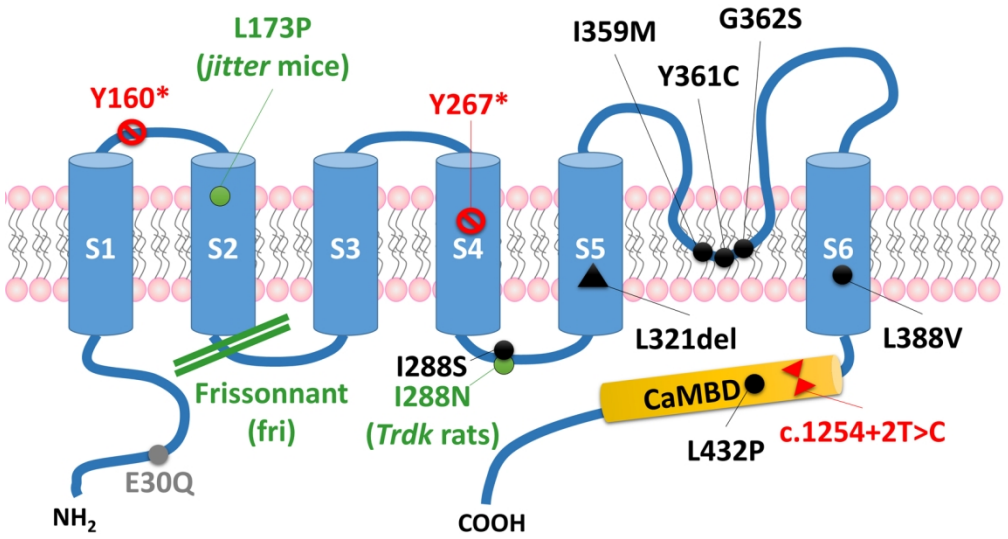


Schematic representation of the SK2 channel showing the localization of pathogenic KCNN2 variants identified in human individuals and rodents.

148x85mm (300 x 300 DPI)