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## Partial loss-of-function of sodium channel *SCN8A* in familial isolated myoclonus

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**Abstract (121/150 words)**

Variants in the neuronal sodium channel gene *SCN8A* have been implicated in several neurological disorders. Early infantile epileptic encephalopathy type 13 results from *de novo* gain-of-function mutations that alter the biophysical properties of the channel. Complete loss-of-function variants of *SCN8A* have been identified in cases of isolated intellectual disability. We now report a novel heterozygous *SCN8A* variant, p.Pro1719Arg in a small pedigree with five family members affected with autosomal dominant upper limb isolated myoclonus without seizures or cognitive impairment. Functional analysis of the p.Pro1719Arg variant in transfected neuron-derived cells demonstrated greatly reduced  $Na_v1.6$  channel activity without altered gating properties. Hypomorphic alleles of *Scn8a* in the mouse are known to result in similar movement disorders. This study expands the phenotypic and functional spectrum of *SCN8A* variants to include inherited non-epileptic isolated myoclonus. *SCN8A* can be considered as a candidate gene for isolated movement disorders without seizures.

**Key Words:** sodium channel, movement disorder, myoclonus, Nav1.6

The *SCN8A* gene (MIM# 600702) encodes the neuronal voltage-gated sodium channel  $Na_v1.6$ , which is localized at the axon initial segment and functions in initiation and propagation of action potentials.  $Na_v1.6$  is also concentrated at nodes of Ranvier in myelinated neurons, including sciatic nerve, and is expressed at a high level in cerebellar Purkinje cells. During the past 5 years, *SCN8A* has been found to have a major role in sporadic epilepsy. *De novo* missense mutations of *SCN8A* have been identified in more than 200 individuals with early-onset epileptic encephalopathy (EIEE13; MIM# 614558).

Approximately 25% of affected individuals carry recurrent mutations, many of which occur at CpG dinucleotides in arginine codons (Wagnon & Meisler, 2015). Functional analysis has demonstrated that the most common mechanism is gain-of-function alterations in biophysical properties of Na<sub>v</sub>1.6 that result in neuronal hyperexcitability (Meisler et al., 2016; Veeramah et al., 2012).. A small number of inherited variants have been associated with less severe conditions, including benign familial infantile seizures accompanied by paroxysmal dyskinesia (BFIS5, OMIM #617080) (Anand et al., 2016; Butler et al., 2017; Gardella et al., 2016; Han, Jang, Lee, Shin, & Park, 2017). Heterozygous loss-of-function mutations of *SCN8A* have been associated with isolated cognitive impairment in two individuals with *de novo* mutations (Wagnon et al., 2017) and one family with the protein truncation variant p.Pro1719Argfs\*6 (OMIM #614306). In the mouse, homozygosity for partial loss-of-function alleles of *Scn8a* results in movement disorders including ataxia, tremor, and dystonia, while homozygosity for null alleles results in paralysis and juvenile lethality (Kearney et al., 2002; O'Brien & Meisler, 2013).

We now describe a missense variant of *SCN8A* in a family with non-epileptic essential myoclonus. The proband is a 35-year-old female with onset of essential myoclonus at age 5 (**Figure 1A**, II-2). She was diagnosed with upper limb action-induced non-epileptic myoclonus, part of the myoclonus-dystonia spectrum (MIM# 159900). The myoclonus is alcohol-responsive in this individual. The proband has normal cognition and no other neurological abnormalities including dystonic posturing. Her affected nephew (**Figure 1A**, III-2) is a 7-year-old male with onset of action-induced myoclonus in the upper limbs at age 4, and evidence of subcortical origin from electrophysiological studies. EMG recordings in the upper limbs showed irregular, multifocal, myoclonic bursts, with duration ranging from 50 to 200 msec. Myoclonic jerk frequency was increased with maintenance of a posture or with action. EEG back-averaging did not reveal a cortical correlate. His motor milestones were delayed, e.g.

independent walking at age three, but he does not have intellectual disability and does not exhibit dystonia or other neurological abnormalities. The clinical features of these two family members are summarized in **Supplemental Table 1**. Three additional family members were reported as affected by a similar movement disorder (**Figure 1A**, I-2, II-3, and III-1), but were not examined directly. The pedigree is consistent with autosomal dominant inheritance.

In order to identify the genetic basis for the disorder, we performed whole-exome sequencing (WES) in individuals II-2 and III-2. The study was approved by the University College London ethics committee (UCLH project 06/N076), and written informed consent was obtained from all participating subjects. DNA was extracted from peripheral lymphocytes following a standard protocol. Point mutations and copy-number variants in *SGCE*, the most common genetic cause of autosomal dominant myoclonus-dystonia (OMIM #159900), were previously excluded (Carecchio et al., 2013).

WES was carried out at the Institute of Neurology, University College London, using Illumina's Nextera Rapid Capture according to the manufacturer's recommendations. Indexed and pooled libraries were sequenced on Illumina's HiSeq3000. Bioinformatic analysis was performed as previously described (Mencacci et al., 2015).

Synonymous and non-coding variants were excluded from further examination. Variants were filtered to include only those that were shared by the two affected individuals and absent from public genomic databases (dbSNP 137, 1000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69 and Exome Aggregation Consortium [ExAC]). Finally, variants were required to have a CADD score greater than 20, indicative of greater potential for pathogenicity (<http://cadd.gs.washington.edu/info>) (Kircher et al., 2014).

After filtering, 11 variants remained (**Supplemental Table 2**). Among these, *SCN8A* is the only gene with previous association with movement disorders and a high intolerance score for missense and loss-of-function variants ( $z=7.7$  and  $pLI=1.0$ )

([exac.broadinstitute.org](http://exac.broadinstitute.org)). Sanger sequencing analysis showed that the *SCN8A* mutation was also present in individual III-1 (**Figure 1A**).

Affected individuals are heterozygous for the single nucleotide variant c.5156C>G in the *SCN8A* gene (RefSeq NM\_014191.3), resulting in the amino acid substitution p.Pro1719Arg (P1719R). This variant is absent in ~130,000 individuals listed in the gnomAD database (<http://gnomad.broadinstitute.org>). Proline residue 1719 is located in the pore loop of domain IV of the protein that confers sodium selectivity to the channel (**Figure 1B**). The pore loops are highly conserved through evolution and *SCN8A* residue 1719 is invariant in vertebrate orthologs and the human paralogs *SCN1A* and *SCN5A*, which diverged from *SCN8A* more than 400 million years ago (**Figure 1C**). Substitution of a charged arginine for the rigid proline residue is a chemically nonconservative change likely to alter secondary structure of the channel protein. This variant has been submitted as entry # 00163785 to the locus specific public database <http://www.lovd.nl/SCN8A>.

To determine experimentally whether P1719R is a deleterious variant, we carried out functional comparison of mutant and wildtype Na<sub>v</sub>1.6. The substitution P1719R was introduced into the tetrodotoxin (TTX)-resistant derivative of the full-length Na<sub>v</sub>1.6 cDNA clone, as previously described (Wagnon et al., 2016). Site-directed mutagenesis was carried out with the QuikChange II XL kit (Agilent Technologies). The 6-kb open reading frame was re-sequenced to confirm the absence of other mutations.

Channel activity was characterized in DRG-neuron derived ND7/23 cells (Sigma Aldrich). Cells were cultured and transfected as previously described (Wagnon et al., 2016). Forty-eight hours after transfection with 5 μg of Na<sub>v</sub>1.6 alpha subunit cDNA and 0.5 μg of the fluorescent m-Venus bioreporter, electrophysiological recordings of fluorescent cells were made. Recording was carried out in the presence of 500 nM tetrodotoxin (TTX) to block endogenous sodium currents. Currents were recorded using the whole-cell

configuration of the patch clamp recording technique as previously described (Wagnon et al., 2016).

Cells expressing the Na<sub>v</sub>1.6-P1719R variant demonstrated inward sodium current that was significantly reduced compared to cells expressing wildtype channels:  $-27.3 \pm 5.3$  pA/pF (n=9) for mutant channel compared with  $-108 \pm 21$  pA/pF (n=9) for wild-type channel (p<0.01) (Figure 2 A, B). The voltage dependence of mutant channel activation and inactivation did not differ from wildtype (Figure 2, C-F). The finding of partially reduced channel activity for P1719R is novel, since previously characterized deleterious variants resulted either in shifted voltage dependence of activation (Estacion et al., 2014), delayed channel inactivation (Wagnon et al., 2016), or complete loss of channel activity (Wagnon et al., 2017).

Individuals with epileptic encephalopathy due to gain-of-function mutations of *SCN8A* often exhibit movement disorders and other neurological signs such as hypotonia, ataxia, dystonia, hyperreflexia and choreoathetosis (Meisler et al., 2016). Severe hypotonia in these individuals is responsible for loss of intentional movement in 50% of cases (Meisler et al., 2016). *SCN8A* variants have not been previously reported in individuals with isolated movement disorders. This absence may reflect the lack of sequencing data in this population or the failure to recognize the relevance of missense variants. The identification of *SCN8A*-P1917R co-segregating with isolated non-epileptic myoclonus demonstrates that mutation of *SCN8A* can result in isolated movement disorders in patients.

Variants of *SCN8A* with gain-of-function and elevated channel activity, either due to premature channel opening or delayed channel closing, result in epileptic encephalopathy (Meisler et al., 2016). At the other extreme, heterozygosity for complete loss-of-function alleles of *SCN8A* can result in isolated intellectual disability (Trudeau, Dalton, Day, Ranum, & Meisler, 2006; Wagnon et al., 2017). In rare cases, seizures have been seen in individuals

with loss-of-function alleles of *SCN8A* lacking residual activity (Blanchard et al., 2015; Moller et al., 2016). Partial loss-of-function with residual activity, as demonstrated here for P1719R, has not previously been described for a pathogenic variant of human *SCN8A*. The dominant phenotype of this pore loop mutation could result from impaired trafficking of the co-expressed wildtype type Nav1.6, possible because of the recently described dimerization of the  $\alpha$  subunit (Clatot et al., 2017). As another possibility, this mutation could impair glycosylation or trafficking of the channel protein (e.g. Jones et al., 2016). The data presented here demonstrate that heterozygosity for partial loss-of-function of *SCN8A* can be associated with movement disorders without seizures.

In the mouse, homozygosity for the hypomorphic *Scn8a<sup>medJ</sup>* allele, a splice site variant with 90% reduction in Nav1.6 protein, results in severe progressive dystonia (Sprunger, Escayg, Tallaksen-Greene, Albin, & Meisler, 1999). A second hypomorphic allele, *Scn8a<sup>9J</sup>*, results in early onset tremor and adult onset dystonia (Jones et al., 2016). Complete loss of mouse *Scn8a* associated with homozygosity for a null allele results in hind limb paralysis and death (Burgess et al., 1995). Conditional inactivation of *Scn8a* restricted to cerebellar Purkinje and granule layer neurons also leads to pronounced ataxia (Levin et al., 2006), and dysfunction of cerebellar neurons could contribute to myoclonus in the family described here. Cerebellum dysfunction has been implicated in the pathogenesis of other forms of non-epileptic myoclonus such as *SGCE*-related myoclonus-dystonia (Carbon et al., 2013; Hubsch et al., 2011). Furthermore, we observed improvement of myoclonus with alcohol intake in the adult subject with *SCN8A*-P1719R, an effect likely to occur through modulation of the activity of cerebellar neurons.

Three affected family members were demonstrated to carry the *SCN8A* variant. A fourth affected family member, II-3, is an obligate carrier since his sister and son carry the variant. The fifth affected individual, III-1, who was not available for testing, is likely to

carry this variant since her clinical symptoms correspond to the other four affected family members. *SCN8A*-P1719R thus appears to be a fully penetrant pathogenic variant responsible for autosomal dominant isolated myoclonus in this family, with no evidence of maternal imprinting as observed in myoclonus dystonia due to mutations of *SGCE*. In distinction from *SGCE*-related myoclonus dystonia, the disease course in affected individuals from our kindred seems to be non-progressive and no relevant psychiatric disturbances were observed. However other features (i.e. childhood-onset, distribution of symptoms in the upper part of the body, response to alcohol, and a subcortical origin of myoclonus) partially overlap with the clinical phenotype associated with mutations of *SGCE*, which is generally dominated by myoclonus accompanied by mild dystonia. These findings extend the phenotypic spectrum associated with *SCN8A* to include a movement disorder without seizures or intellectual disability.

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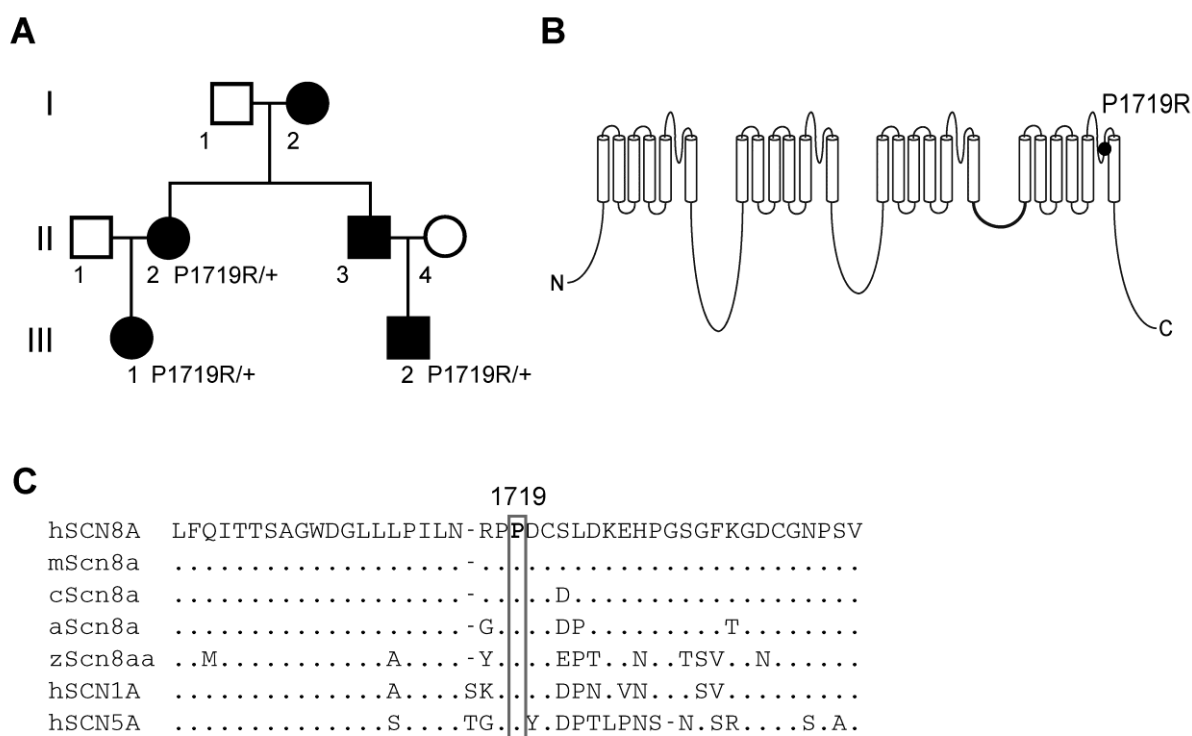
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## Figure Legends

**Figure 1. Inheritance of the *SCN8A* variant P1719R in a family with essential myoclonus.** (A) Pedigree demonstrating co-inheritance of myoclonus and the *SCN8A* variant (c. 5156C>G, P1719R). Filled symbols, essential myoclonus. (B) Four-domain structure of the voltage-gated sodium channel alpha subunit. P1719R is located in the pore loop of domain IV. (C) Evolutionary conservation of residue P1719R in multiple species. h, human; m, mouse; c, chicken; a, anole; z, zebrafish.

Figure 1



**Figure 2. The *SCN8A* variant P1719R results in partial loss of  $\text{Na}_v1.6$  function.**

(A) Representative traces of sodium currents recorded from ND7/23 cells that were transfected with  $\text{Na}_v1.6$  cDNA. black, wildtype (WT); red, P1719R. (B) Averaged current-voltage (I-V) relation for WT (black, n=9) and P1719R (red, n=9) channels, and non transfected cells (purple, n=12). (C) Voltage dependence of channel activation. Smooth lines correspond to the least squares fit when average data were fit to a single Boltzmann equation. (D) Scatter plot of voltage at half-maximal activation ( $V_{1/2}$ ) for cells expressing WT and P1719R channels. (E) Voltage dependence of steady-state inactivation. Smooth lines correspond to the least squares fit when average data were fit to a single Boltzmann equation. (F) Scatter plot of voltage at half-maximal inactivation ( $V_{1/2}$ ) for cells expressing WT and P1719R channels.

