



Short communication

Evaluation of *SCN8A* as a candidate gene for autosomal dominant essential tremorLisa M. Sharkey^{a,*}, Julie M. Jones^a, Peter Hedera^b, Miriam H. Meisler^a^a Department of Human Genetics, University of Michigan, 4812 Med Sci. II, 1241 Catherine Street, Ann Arbor, MI 48109-5618, USA^b Department of Neurology, Vanderbilt University, Nashville, TN 37232-8552, USA

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ABSTRACT

Objectives: Essential tremor (ET) is a common inherited movement disorder whose causes remain unknown. The presence of spontaneous tremor in murine mutants may provide clues into the pathogenesis of ET. *SCN8A* encodes the neuronal voltage gated sodium channel Na_v1.6 that is widely expressed in the central nervous system. Several mutations of *Scn8a* in the mouse result in congenital postural tremor of the extremities and head.

Methods: We screened *SCN8A* as a candidate gene in a cohort of 95 Caucasian patients with ET and a positive family history, including 48 patients with early onset in the first two decades of life. Early and adult onset ET subgroups did not differ in disease severity, but early onset patients had longer disease duration. Observed sequence variants were also screened in an ethnically matched control group.

Results: We did not detect *SCN8A* mutations affecting amino acid sequence or splice sites in our cohort of ET patients.

Conclusions: Although mutations of *Scn8a* cause congenital tremor in mice, mutations in the sequence of the exons and splice sites of human *SCN8A* do not appear to be a common cause of autosomal dominant essential tremor in Caucasian patients.

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1. Introduction

Essential tremor (ET) is one of the most common neurological disorders of adults, affecting 0.4–3.9% of the population [1]. ET is characterized by bilateral, predominantly symmetrical, postural and/or kinetic tremor involving mostly the upper extremities, including head and voice tremor [1]. ET is clinically heterogeneous, and additional movement abnormalities such as dystonia may be associated with postural or kinetic tremor [1]. Patients with advanced ET also develop predominantly midline ataxia. Most reported pathologic changes are localized in the cerebellum, supporting the emerging view that that ET is primarily a cerebellar disorder [2].

ET appears to be inherited in a significant proportion of cases. A positive family history is present in 30–70% of ET cases, suggesting a strong genetic component [3]. Childhood onset is observed in ~5% of ET patients, and among these early onset patients, approximately 80% belong to families with autosomal dominant inheritance [4]. There is considerable variation in age of onset within families, suggesting that childhood onset ET is unlikely to be a separate condition [5]. Genetic loci responsible for

ET in individual families have been mapped to chromosome 3q13, 2p24.1, 6p23, and 5q31.1–q33.1, but disease causing genes have not been conclusively identified. The Ser9Gly variant in the dopamine receptor gene *DRD3* may increase susceptibility to the disease [2], but this finding was not replicated in a large cohort of patients [6]. A SNP in the *HS1-BP3* gene, which is located within the ET locus on chromosome 2p is also likely to be a benign polymorphism [2].

Since the genetics of ET is fraught with difficulties, animal models with similar clinical phenotypes may be useful in identifying genes with a role in pathogenesis. Several mouse mutants of the voltage gated sodium channel gene *Scn8a* exhibit postural and kinetic tremor of the extremities, in combination with ataxia and dystonia [7]. For example, mice with the *med^{jolting}* mutant that alters the voltage dependence of the channel display a wide unsteady gait and a rhythmic tremor in the head and neck.

Voltage gated sodium channel α subunits are large transmembrane proteins composed of four homologous domains with voltage sensor and pore regions. Voltage gated sodium channels are responsible for the initiation and propagation of neuronal action potentials. The nine orthologous mammalian sodium channels differ in biophysical properties, subcellular localization and tissue specificity. Mutations in different neuronal sodium channel genes are responsible for inherited forms of epilepsy, migraine, and pain disorders [8].

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SCN8A encodes the voltage gated sodium channel $Na_v1.6$, one of the most abundant channels in the central and peripheral nervous system. $Na_v1.6$ is localized at axonal initial segments, dendrites, and nodes of Ranvier in myelinated axons. Expression of $Na_v1.6$ is required for repetitive firing of neuronal populations implicated in tremorogenesis, including cerebellar Purkinje neurons, cortical pyramidal neurons, and subthalamic nucleus neurons [7]. Heterozygotes for a null mutation of human *SCN8A* exhibit ataxia and cognitive defects [9]. Because tremor is a common feature of mice with mutations in *Scn8a*, we explored the role of the human gene as a candidate gene for autosomal dominant (AD) form of ET.

2. Research methods and procedures

2.1. Subjects

Every patient was diagnosed by a movement disorder neurologist (PH) and signed an informed consent, approved by the Institutional Review Board at Vanderbilt University. The cohort of unrelated Caucasian patients of a northern European ancestry was diagnosed with definite ET based on the following criteria: the presence of bilateral postural and kinetic arm tremor without prominent asymmetry lasting at least for more than five years, the absence of additional neurologic abnormalities, no history of exposure to tremorogenic drugs before the onset of symptoms, and no history or examination suggestive of psychogenic tremor or sudden onset with stepwise deterioration [1]. Tremor was quantified using the NIH ET consortium grading and the rating scale from the Washington Heights-Inswood Genetic Study of Essential Tremor (WHIGET). We did not include patients with a significant asymmetry of right and left arm tremor severity to minimize a risk of misdiagnosis, such as an atypical dystonic tremor or Parkinson's disease. Patients with slight tremor (scores 0 and 1) were not included. Furthermore, the degree of disability was also judged by self-reporting of questions adapted from the Tremor disability questionnaire.

We included only subjects with a positive family history of tremor, defined by the presence of at least one affected living first degree relative who met diagnostic criteria for definite ET and was examined by the same neurologist (PH), and vertical transmission of the disease (parent to offspring) consistent with autosomal dominant (AD) inheritance. Because the tremor in *Scn8a* mutant mice is congenital with very early onset, we enriched the patient cohort with early onset ET defined as onset before the age of 18 years. Not all affected family members of these probands exhibited juvenile onset of disease. The age of tremor onset was self-reported by adults and obtained from parents, when available, for juvenile onset cases. Controls for determining SNP frequencies consisted of 178 neurologically normal, ethnically matched individuals from the Coriell Institute (panels NDPT006 and NDPT009), as well as 431 previously described, ethnically matched patients with the following neurological disorders: ataxia ($n = 150$); autism ($n = 91$); epilepsy ($n = 95$), and Charcot Marie Tooth disease ($n = 95$). The four unique SNPs reported here were not observed in any of these ethnically matched individuals, demonstrating that they are not common polymorphisms in the Caucasian population.

2.2. Variant detection and genotyping

Heteroduplex analysis was carried out by conformation sensitive gel electrophoresis (CSGE) as described previously [9]. Twenty-seven coding exons of *SCN8A*, including the alternatively spliced exons 5A and 5N, were amplified by PCR from genomic DNA using previously described primers. PCR products with altered mobility were sequenced by the University of Michigan Sequencing Core. Sequence analysis was carried out with Sequencher software (Gene Codes Corporation, Ann Arbor, Michigan). The effect of novel variants on splice enhancer sites was evaluated using ESEfinder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/ese_finder.cgi).

3. Results

We included 95 ET patients (42 men, 53 women, average current age 53 ± 22 years) with mean WHIGET score 16.3 ± 3.7 (range = 6–25). The subgroup of early onset ET patients had average age of disease onset at 11 ± 7 years (23 men, 25 women, average current age 44 ± 13 years). The subgroup of adult onset ET had average age of onset of 49 ± 12 years (19 men, 28 women, average current age 62 ± 9 years). Disease severity did not differ between the early onset patients (mean WHIGET score 13.8 ± 4.7 , range = 6–22) and the adult onset patients (mean WHIGET score 18.2 ± 6.8 , range = 7–25). The two groups differed, as expected, in the duration of the disease (29 ± 9 years for early onset and 9.3 ± 4.4 years for late onset, $p < 0.05$). Within the subgroup of 45

probands with juvenile onset, 40 kindreds included first or second degree relatives with typical adult onset. Exclusively, juvenile onset was observed in five small pedigrees containing only two or three affected individuals.

Five SNPs in *SCN8A* were observed in our cohort of 95 ET patients (Fig. 1). None of these variants changed the amino acid sequence or consensus splice sites (Table 1). The three SNPs located in exons did not change any predicted exonic splice enhancer sites (ESEfinder, http://rulai.cshl.edu/cgi-bin/tools/ESE3/ese_finder.cgi). The intron 5 SNP is located 19 bp upstream of the start of exon 6 and is not predicted to influence splicing. Each of these four SNPs were observed in a single ET patient and were not present in >600 controls. The fifth SNP is located in intron 17 at a position 34 bp downstream of exon 17 and does not change any recognizable functional element. The intron 17 SNP was present in 6% of patients (6/95) and 2% of controls (6/178) ($p = 0.12$). Among the patients, the intron 17 SNP was present in 5/48 early onset cases and 1/47 late onset cases; this trend was not statistically significant ($\chi^2 = 1.5$; $p < 0.22$).

4. Discussion

Animal models with spontaneous tremor provide an opportunity for identification of causative ET genes. The postural and action extremities tremor in *Scn8a* mutant mice are associated with neurologic abnormalities such as dystonia and ataxia that are commonly present in ET patients. In spite of the phenotypic

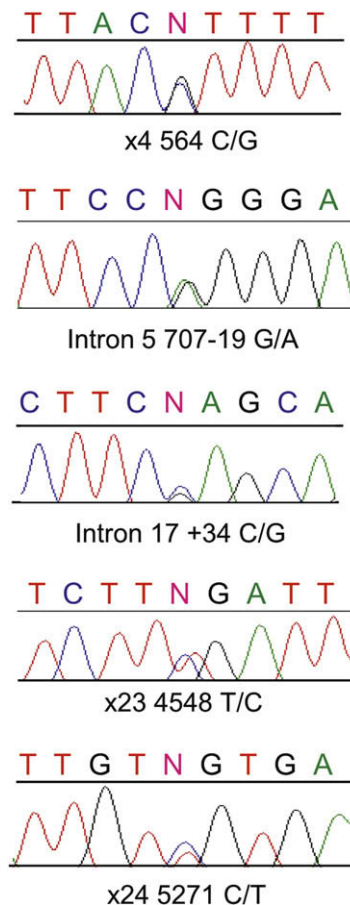


Fig. 1. Novel variants of *SCN8A*. Sequence chromatograms demonstrate heterozygosity for each variant.

Table 1
Novel SNPs in human *SCN8A*

Location	Nucleotide	Protein	Alleles	ET patients		Controls	
				Heterozygote frequency	Minor allele frequency	Heterozygote frequency	Minor allele frequency
exon 4	c564	T188T	C/G	1/95	0.005	0/431	0
intron 5	c707 – 19	Noncoding	G/A	1/95	0.005	0/431	0
intron 17	c3819 + 34	Noncoding	C/G	6/95	0.032	6/178	0.017
exon 23	c4548	F1516F	T/C	1/94	0.005	0/431	0
exon 24	c5271	V1757V	C/T	1/95	0.005	0/431	0

Nucleotides are numbered from the translation start site.

similarities, our results indicate that mutations in the *SCN8A* gene are not a common cause of autosomal dominant familial ET. We studied Caucasian individuals only and cannot exclude the possibility that this gene plays a more important role in patients with other ethnic backgrounds.

The relationship between the tremor phenotype in mutant mice and human ET is controversial [10]. ET is clinically and genetically heterogeneous, and it is possible that tremor in mouse mutants could have a different pathology and pathophysiology. Although alcohol responsive tremor has been reported in mice lacking the GABA_A receptor α 1 subunit [10], genetic analysis of a large cohort of ET patients did not identify pathogenic variants in this gene [11]. In spite of the negative outcome for *SCN8A* and the GABA_A receptor, ligand and voltage gated ion channels remain important candidate genes for this disorder.

Inherited ET is characterized by vertical transmission. Autosomal dominant inheritance with reduced penetrance is the most widely accepted genetic model. However, many multigenerational families with a large number of affected individuals do not demonstrate linkage to a single genetic locus. Thus, autosomal dominant ET may have a complex mode of inheritance involving genetic interaction between multiple susceptibility genes [5]. Age of onset also exhibits significant intrafamilial variability, consistent with the effects of genetic modifiers. Indeed, 93% of our kindreds with individuals suffering from a juvenile onset of the disease had affected relatives with a later age of ET onset, arguing against the possibility that juvenile AD ET represents a genetically distinct subtype of ET, and further supporting the role of modifying genes [4]. Interactions between mutations in different ion channel genes are known to modify epilepsy severity in mouse models [12], and may provide a model for polygenic ET.

Our data suggest that mutations of *SCN8A* are unlikely to be a major cause of autosomal dominant ET in Caucasian patients, but rare mutations may influence clinical expression in some patients.

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