Published in final edited form as:
*Neurosci Lett*. 2008 March 5; 433(1): 65–70.

# Mutation of sodium channel *SCN3A* in a patient with cryptogenic pediatric partial epilepsy

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## Abstract

Mutations in the sodium channel genes *SCN1A* and *SCN2A* have been identified in monogenic childhood epilepsies, but *SCN3A* has not previously been investigated as a candidate gene for epilepsy. We screened a consecutive cohort of 18 children with cryptogenic partial epilepsy that was classified as pharmacoresistant because of nonresponse to carbamazepine or oxcarbazepine, antiepileptic drugs that bind sodium channels. The novel coding variant *SCN3A*-K354Q was identified in one patient and was not present in 295 neurological normal controls. Twelve novel SNPs were also detected. K354Q alters an evolutionarily conserved amino acid in the pore domain of *SCN3A*. Functional analysis of this mutation in the backbone of the closely related gene *SCN5A* demonstrated an increase in persistent current that is similar in magnitude to epileptogenic mutations of *SCN1A* and *SCN2A*. This observation of a potentially pathogenic mutation of *SCN3A* (Nav1.3) indicates that this gene should be further evaluated for its contribution to childhood epilepsy.

### Keywords

Sodium Channels; *SCN3A*; Epilepsy

## Introduction

Voltage-gated sodium channels are essential for the generation and propagation of action potentials in neurons. The mammalian genome contains nine genes encoding the sodium channel pore-forming α subunits. Three of these genes, *SCN1A*, *SCN2A* and *SCN3A*, are clustered within 600 kb on human chromosome 2q24. These neuronal channels are blocked by

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nanomolar concentrations of tetrodotoxin and exhibit similar kinetics, except for the slower inactivation of the *SCN3A* channel [1]. In human brain, the channels encoded by *SCN1A* ($Na_v1.1$) and *SCN3A* ($Na_v1.3$) exhibit a predominantly somtato-dendritic localization while the *SCN2A* channel ($Na_v1.2$) is predominantly axonal [2]. Mutations in *SCN1A* and *SCN2A* have been associated with the inherited childhood epilepsy syndromes GEFS+, SMEI, and BFNIS [3–5]. No mutations have been identified to date in *SCN3A*. In contrast to the rodent gene, which is downregulated after birth, human *SCN3A* is widely expressed in adult brain [2]. As part of a pilot pharmacogenetic study of children with cryptogenic partial epilepsy, we screened patients who were resistant to the sodium channel blockers carbamazepine or oxcarbazepine to detect sodium channel mutations.

## Materials and Methods

### Participants

Patients were identified from a consecutive cohort of 218 children, 1–18 years of age, who were newly diagnosed with epilepsy in the New Onset Seizure Clinic at Cincinnati Children's Hospital between January 1, 2003 and April 2005. The initial evaluation included EEG and MRI. History, physical examination, MRI, and EEG were used for classification in accordance with the guidelines of the International League Against Epilepsy (1989). Classification was done independently by two pediatric epileptologists. Children were considered to have partial onset seizures if the clinical history was indicative of partial onset seizures and there was no EEG evidence of generalized epilepsy. These criteria were selected because, while the yield of interictal epileptiform abnormalities on routine EEG is <50% [6], studies on ictal determination of seizure type in children demonstrated that absence epilepsy could be easily diagnosed on outpatient EEGs [7]. Etiology was classified as cryptogenic (i) if seizures were not the result of static brain damage or other structural brain abnormality (e.g. cortical dysplasia); (ii) if seizures were not the result of another neurological disorder; and (iii) if clinical and EEG features were not characteristic of an idiopathic epilepsy syndrome.

Patients were followed for ≥ 1 year after initiation of therapy. Seizure control, adherence, random serum levels, and adverse events were recorded at each visit. Medication doses were adjusted based on clinical response, toleration, and serum levels. Adherence was assessed by direct questioning and measurement of serum carbamazepine or oxcarbazepine concentration at routine clinic visits and at other times if clinically indicated by seizure exacerbation or symptoms consistent with medication toxicity. Drug responsiveness was defined as freedom from seizures for at least 12 months on a stable dose of carbamazepine or oxcarbazepine in monotherapy, or breakthrough seizures only with missed doses of medication, in patients with evidence of good AED adherence based on measurement of random levels. Dose-response curves were determined from the cumulative distribution of doses of carbamazepine or oxcarbazepine used to control seizures in the entire cohort of partial onset drug responsive seizure patients. Pharmacoresistant epilepsy was defined as continued seizures while on carbamazepine or oxcarbazepine at doses above those at which 95% of the drug responsive patients stopped having seizures (17 mg/kg/day for carbamazepine and 33 mg/kg/day for oxcarbazepine). All experiments on human subjects were conducted in accordance with the Declaration of Helsinki and received prior approval by the Cincinnati Children's Hospital Institutional Review Board. Informed consent was obtained from the parent or guardian of each patient.

### Mutation detection

DNA was extracted from peripheral blood using the PUREGENE kit (Gentra systems). Primers for exon amplification of *SCN1A*, *SCN2A*, and *SCN3A* were previously described [8;9]. Primers for *SCN1B*, *SCN2B*, and *SCN3B* were designed to amplify fragments of ≤ 800 bp at an annealing

temperature of 60°C with at least 25 bp of exon/intron splice junction. Larger exons were covered by multiple amplicons overlapping by at least 50 bp. An 18 bp M13 and M13R tail was added to the 5′ end of each primer to facilitate sequencing. Primers and nucleotides were removed from the PCR reaction by enzymatic degradation. PCR products were sequenced bidirectionally on an Applied Biosystems model 3730xl 96 capillary sequencer. Mutation Surveyor v2.63 software (SoftGenetics, State College, PA) was used to compare patient sequences with the reference DNA sequence. All variants in the coding region, splice sites, and within 25 bp of exons are reported.

### Control samples

NINDS Neurologically Normal Caucasian Control Panels (Coriell NDPT006 and NDPT009; n=184) and a panel of 111 subjects who were >60 years of age without personal or family history of neurological disease [10] were screened for K354Q and G1862C by heteroduplex analysis using conformation-sensitive gel electrophoresis as previously described [8].

### Functional properties of the SCN3A missense mutation

Site-directed mutagenesis to introduce the K343Q mutation into the $Na_v1.5$ cDNA was carried out in the vector pcDNA3.1 (Invitrogen) using the Quikchange Site-Directed Mutagenesis Kit (Stratagene) according to manufacturer's protocols. The mutation was confirmed by DNA sequencing.

Wildtype $Na_v1.5$ and $Na_v1.5$_K343Q cDNA constructs were transiently transfected into HEK293 cells, together with the humanβ1 subunit, using Lipofectamine (Invitrogen) and currents were measured with whole cell patch clamp procedures as previously described [11]. In brief, whole cell $Na^+$ current was recorded at room temperature (22°C) using the following solutions (mmol/L). The internal solution contained aspartic acid 50, CsCl 60, $Na_2$-ATP 5, EGTA 11, HEPES 10, $CaCl_2$ 4.27, and $MgCl_2$ 1, with pH adjusted with CsOH. The external solution contained NaCl 130, $CaCl_2$ 2, CsCl 5, $MgCl_2$ 1.2, HEPES 10, and glucose 5, with pH 7.4 adjusted with CsOH. For the internal solution, the final Ca2+ concentration after EGTA buffering is 100 nM.

Late $Na^+$ channel current was detected as the tetrodotoxin (TTX, 30 μM)-sensitive current measured at 150 ms during depolarization to −10 mV from a holding potential of −100 mV. Percent late current was normalized to peak TTX-sensitive $Na^+$ channel current and plotted as % peak current in relevant figures. The voltage-dependence of inactivation was determined after application of conditioning pulses (500 ms) applied once every 2 s to a series of voltages followed by a test pulse (20ms) to voltages from −130 mV to −20 mV. In experiments designed to measure the voltage dependence of activation, external $Na^+$ was reduced to 30 mM using n-methyl-glucamine as a $Na^+$ substitute. Current was measured using test pulses (40 ms) from a holding potential of −100 mV to voltages ranging from −80mV to +75mV. Membrane currents were measured using the Axopatch 200B amplifiers (Axon Instruments, Foster City, CA). Capacity current and series resistance compensation were carried out using analog techniques according to the amplifier manufacturer. PClamp8 (Axon Instruments, Foster City, CA) was used for data acquisition. Data are mean ± SEM.

## Results

### Characterization of children with new-onset epilepsy

In a series of 218 consecutive children seen in the New Onset Seizure clinic, 118 were diagnosed with cryptogenic partial onset epilepsy and started on monotherapy with carbamazepine (n=87) or oxcarbazepine (n=27). Approximately 15% of the carbamazepine-treated patients (n=14) and oxcarbazepine-treated patients (n=4) were nonresponsive, a rate

similar to that reported by others in new-onset seizure patients [12;13]. Sixteen pharmacoresistant patients were Caucasian and 2 were African American. Development and neurological examination were normal with the exception of one patient with cranial nerve IV palsy and one with delayed development. One patient had a history of febrile seizures. These 18 patients were screened for mutations.

## Identification of a potential pathogenic mutation in *SCN3A*

The nonsynonymous coding variant K354Q in *SCN3A* was detected in a Caucasian male and was not present in 295 neurologically normal controls (Figure 1A,B). The proband presented at two years of age with 4 complex partial seizures over a 2 day period, with staring and unresponsiveness for up to 2 minutes followed by postictal confusion. The seizures occurred in the setting of a 3 week history of a viral upper respiratory tract infection and otitis media treated with antibiotics but no documented fever. During the following 3 years, he had 13 similar seizures, without documented fever. Carbamazepine monotherapy (25 mg/kg/day; serum level 7.2 μg/mL) was discontinued due to an intolerable level of CNS sedation. At the present age of 5 years, 8 months, seizures continue to occur at 1–2 month intervals, in the presence of oxcarbazepine monotherapy (33 mg/kg/d; MHD serum level 21.5 μg/mL). Evaluation included a normal MRI and two normal routine (awake and asleep) electroencephalograms. Because of the low seizure frequency, ictal activity has not been recorded. The low frequency, duration of >30 seconds, presence of a postictal state, and normal EEG are more consistent with partial seizures than with generalized epilepsy. The patient is developmentally normal with normal physical and neurological examinations.

Analysis of DNA from both parents demonstrated that the *SCN3A* variant was inherited from the patients' father, who is also heterozgyous. Although the father has no history of seizures, the paternal grandfather was diagnosed with childhood absence epilepsy. DNA from the grandfather has thus far been unavailable for analysis.

The variant residue, lysine 354, is located in the pore region of domain I of $Na_v1.3$ (Figure 1C). Lysine 354 is conserved in all human sodium channel genes except *SCN11A*, which contains another basic residue, histidine (Figure 1D). K354 is invariant in the orthologs of *SCN3A* in other vertebrates (Figure 1E). This evolutionary conservation indicates strong selection against amino acid substitutions at this position.

## Altered function associated with mutation of the mutated lysine residue

To investigate the functional consequences of K354Q, the mutation was introduced into the corresponding residue, K343, of the closely related channel $Na_v1.5$, which shares sequence homology and electrophysiological properties with Nav1.3 [14;15]. This approach circumvented the instability of Nav1.3 cDNA during cloning and mutagenesis. HEK293 cells were transfected with mutant or wildtype Nav1.5 cDNA and wildtype β1 subunit cDNA. After 1–2 days of culture, sodium currents were measured in whole cell patch clamp recording mode. The mutant channel exhibited slowing of the onset of fast inactivation and significantly increased persistent current during prolonged depolarization (Fig. 2A, B). These alterations would be expected to prolong the duration of action potentials in neurons expressing Nav1.3. Both abnormalities have been observed in epileptogenic mutations of *SCN1A* and *SCN2A* [16–19]. Wildtype and mutant channels did not differ with respect to voltage-dependence of activation and steady state inactivation (500 ms conditioning pulses) (Fig. 3), or the kinetics of recovery from inactivation ($t_{1/2}$(WT)=6.86 ± 0.36 ms, n=4; $t_{1/2}$(K343Q)=8.19 ± 0.76 ms, n=4; n.s.).

### Additional sodium channel variants

The missense variant G1862C in the C-terminal region of *SCN3A* was present in 1/18 patients and 2/295 neurologically normal controls, and is unlikely to be pathogenic (Table 1). Other previously described nonsynonymous variants in *SCN1A* and *SCN2A* did not differ in frequency between patients and controls (Table 1). No nonsynonymous variants were found in the β subunit genes *SCN1B*, *SCN2B*, and *SCN3B*. Twelve novel synonymous SNPs were also identified (Table 2).

## Discussion

The coding variant K354Q in *SCN3A* in a patient with pharmacoresistant partial-onset epilepsy represents the first reported mutation of *SCN3A*. While we cannot rule out the possibility that this variant is not disease causing, pathogenicity is indicated by the evolutionary conservation of the altered residue, the location in the pore domain of the channel, the increase in persistent current, the family history, and the absence of this variant in 590 alleles from neurologically normal controls.

Functional characterization of the K354Q mutation was carried out in Nav1.5 rather than Nav1.3, in order to circumvent the instability of the Nav1.3 cDNA, which accumulates point mutations, insertions and deletions during bacterial cloning, like other neuronal sodium channel cDNAs. The *SCN5A* cDNA has been used in this way to evaluate migraine associated mutations in *SCN1A* [14;15]. The pore region of domain 1 from *SCN3A* and *SCN5A* are identical at 28/36 residues (78%) and the channels display similar inactivation kinetics, voltage dependence of activation and inactivation, and non-inactivating currents during prolonged depolarization [20].

The transmembrane segments of the sodium channel α subunit associate within the membrane to form a sodium permeable pore lined by the S5–S6 pore-loops. Transmembrane segments S5 and S6 and the associated S5–S6 linker can generate a functional channel in the absence of the other segments [21]. The pore region also contains the binding site for AEDs, including phenytoin, carbamazepine and lamotrigine [22]. K354Q could therefore account for seizure activity and/or drug resistance in the proband. The mutation was inherited from the proband's unaffected father, indicating that there is reduced penetrance of the associated phenotype. Since the paternal grandfather was affected with childhood absence epilepsy, it will be important in the future to determine whether he is heterozygous for SCN3A-K354Q.

Dominant missense mutations in the pore regions of *SCN1A* contribute to SMEI and GEFS+ [4;23;24]. The mutations of the nicotinic acetylcholine receptor in patients with ADNFLE are located in the pore region [25]. Mutations in potassium pore regions are associated with benign familial neonatal convulsions [26], and mutations in the pore region of *SCN8A* result in movement disorders in the mouse [27]. While most pore mutations result in reduced channel activity, at least one P-loop mutation and two S6 mutations have been reported to slow inactivation kinetics and promote late currents, similar to the results in our study [28–30]. These observations support the proposed pathogenicity of *SCN3A*-K354Q.

Several epileptogenic mutations of *SCN1A* and *SCN2A* result in impaired inactivation and increased persistent current [8;17;18;31;32]. The level of persistent current in *SCN3A*-K354Q, 0.5% of peak current, is comparable to the known epileptogenic mutations (0.9% to 4%) [17]. Defective inactivation and increased persistent current are also characteristic of *SCN5A* mutations resulting in cardiac arrhythmias [33] and *SCN4A* mutations resulting in myotonia or periodic paralysis [34].

With the exception of *SCN3A* and *SCN11A*, all of the other neuronal and muscle sodium channels were previously associated with inherited disorders [4]. This first reported functionally altered mutation in *SCN3A* was identified in a relatively mildly affected individual in a new-onset seizure clinic, rather than a subspecialty clinic. Our observations suggest that sodium channelopathies may be more widespread in childhood onset epilepsy than previously recognized. The identification of a potentially pathogenic *SCN3A* mutation in our small screen of 18 patients suggests that routine screening may yield many more cases.

**Acknowledgements**

This work was supported by the Board of Trustees at Cincinnati Children's Hospital Medical Center (KDH), the MCJ foundation (KDH), the Epilepsy Foundation (TAG) and NIH research grants NS34509 (MHM), NS053792 (JAK) and HL56810 (RSK).

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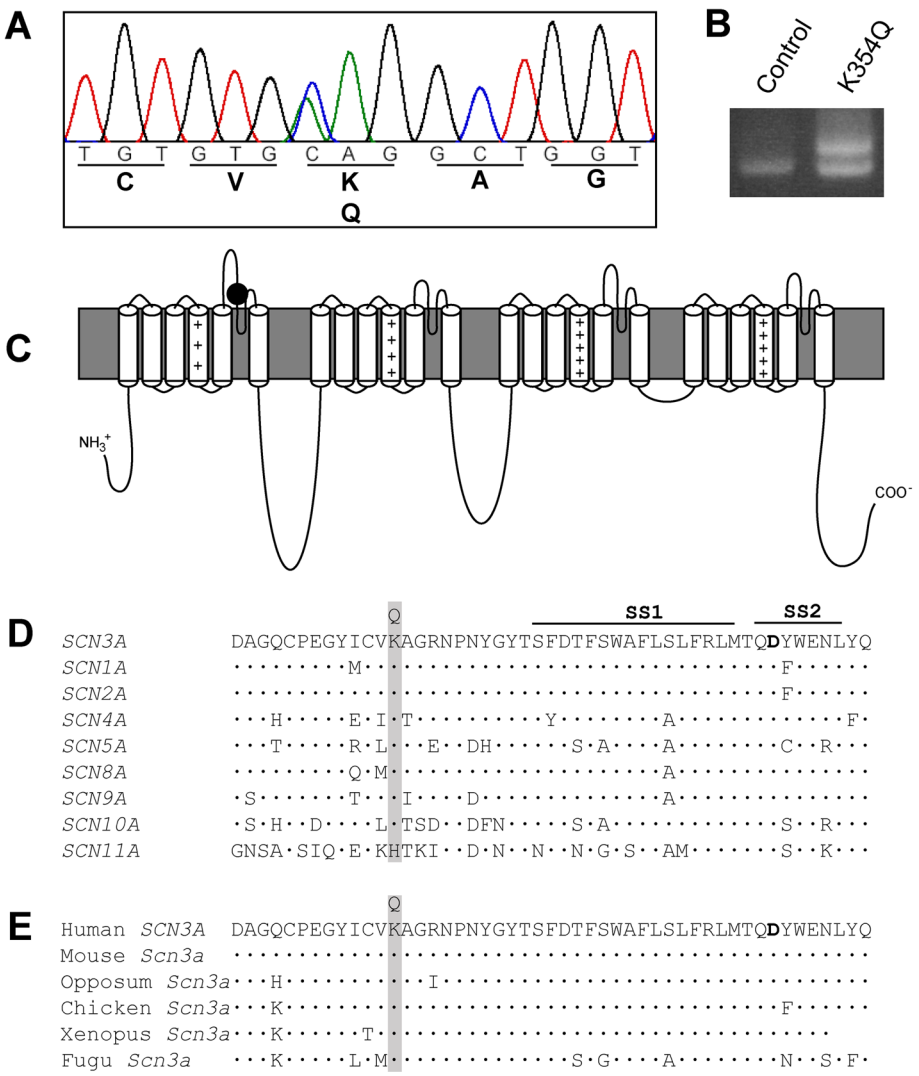


**Figure 1.**

Location and evolutionary conservation of the SCN3A variant K354Q. (A) Identification of the sequence variant in *SCN3A*. The A>C substitution is observed as A/C heterozygosity in the sequence chromatogram. (B) The heterozygous variant appears as a heteroduplex by conformation sensitive gel electrophoresis; (C) The missense variant K354Q (shaded circle) is located in the pore region of domain 1 of *SCN3A*; (D) Alignment of K354Q pore region with other human voltage-gated sodium channels; (E) Alignment of sequences from vertebrate orthologs of *SCN3A*. SS1 is the N-terminal portion of the pore-lining segment and SS2 is the C-terminus of the pore-lining segment. The pore residue D383 shown in bold confers sodium selectivity to the channel.

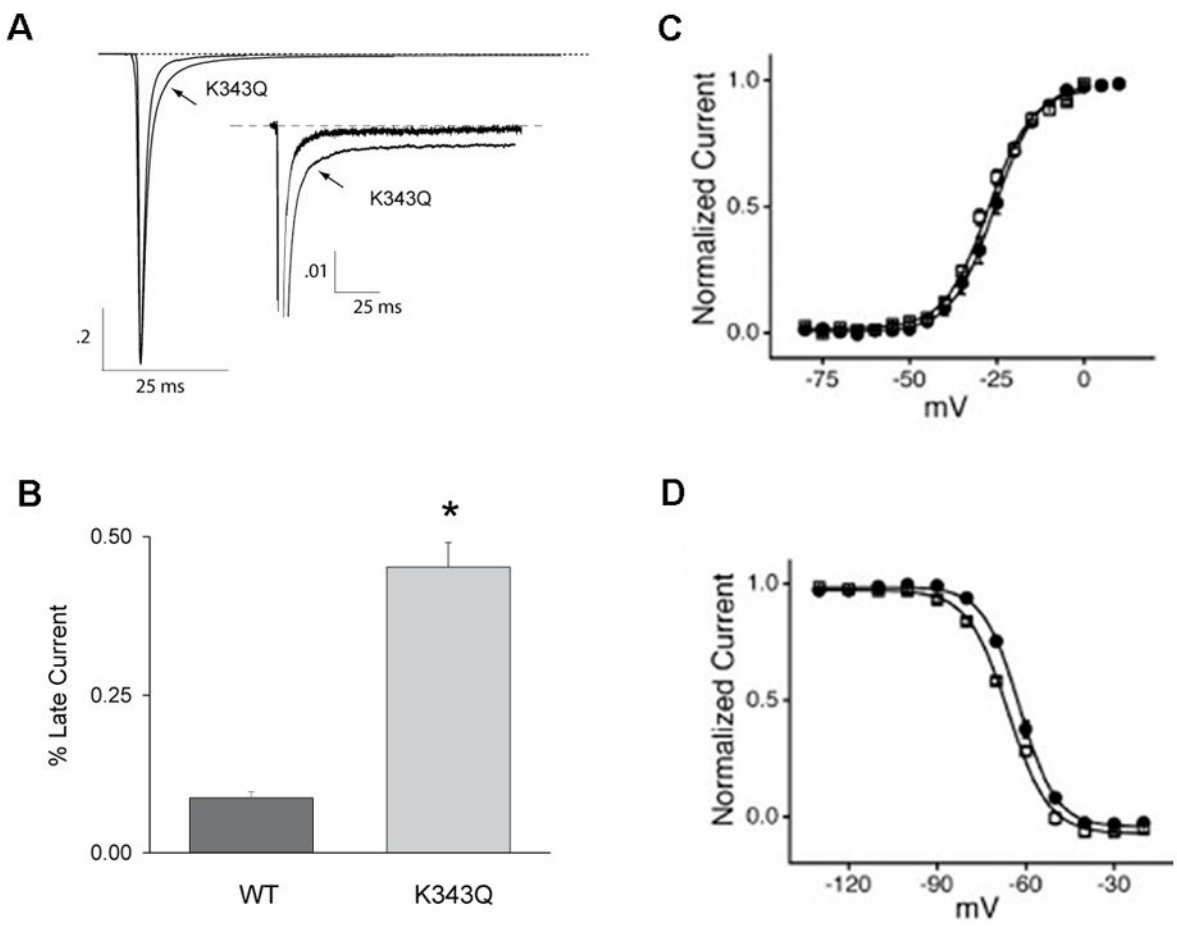


**Figure 2.**

Functional consequences of the K343Q mutation expressed in the Nav1.5 backbone. (A). Averaged normalized current traces in response to pulses to −10 mV at two gains and time bases. At low gain, with WT currents shown as dotted lines. There is an evident slowing of the onset of inactivation (the total time of the sweep is approximately 25 ms). At higher gain and slower sweeps (150 ms total time shown) non inactivating current is clear in the mutant traces and the WT channel traces exhibit more noise.. Averages of 6 cells for WT and 7 cells for K343Q channels. (B) TTX-sensitive late current at 150 ms relative to peak current for WT and K343Q channels (Mean +− SEM, WT: n=6; K343Q: n=7; * p<0.001). (C) Current measured at the indicated voltages was normalized to peak inward current and driving force. Mean activation parameters: WT: V½ = − 25.76 +/− 1.5 mV; K343G: V½ = −27.5 +/− 2.5 mV; n.s. (D) Currents measured at −10 mV following 500 ms conditioning pulses to the voltages indicated along the abscissa and normalized to peak inward current. Mean inactivation parameters: WT: V½ = −62.18 +/− 1.63 mV; K343G: V½ = −65.96 +/− 1.3 mV; n.s. In panels D and D black circles represent WT; open squares represent K343Q mutant channels; n=5 for both constructs.

**Table 1**

Nonsynonymous variants in sodium channel genes observed in patients with childhood onset cryptogenic partial epilepsy.

| Gene | amino acid substitution | Patient heterozygote frequency | Control heterozygote frequency | Reference for control frequency |
|---|---|---|---|---|
| *SCN1A* | T1067A | 0.33 (n=18) | 0.33 (n=96) | Ref. 30 |
| *SCN2A* | R19K | 0.28 (n=18) | 0.14 (n=230) | Ref. 15, 36 |
| *SCN3A* | ΔN43 | 0.11 (n=18) | 0.09 (n=57) | Ref. 15 |
| | K354Q | 0.06 (n=18) | 0 (n=295) | this paper |
| | G1862C | 0.06 (n=18) | 0.01 (n=295) | this paper |

**Table 2**

Novel synonymous SNPs in sodium channel genes in 18 patients with cryptogenic partial epilepsy. (Genbank accession numbers NM_006920.3, NM_001040142.1, NM_006922.2, NM_001037.3, NM-199037.2, NM_004588.3, NM_018400.3)

| Gene | Location | cDNA nucleotide | codon | SNP alleles major/minor | Heterozygote frequency | Minor allele frequency |
|---|---|---|---|---|---|---|
| *SCN1A* | exon 19 | 3690 | Y1230Y | T/C | 1/18 | 0.03 |
| | exon 25 | 4617 | L1539L | C/G | 4/18 | 0.11 |
| *SCN2A* | intron 10 | 1671+49 | --- | G/A | 4/18 | 0.11 |
| *SCN3A* | intron 4 | 602+9 | --- | C/T | 2/18 | 0.06 |
| | exon 14 | 2547 | V849V | A/C | 5/18 | 0.17 |
| | exon 26 | 5160 | N1720N | C/A | 1/18 | 0.05 |
| | exon 26 | 5196 | H1732H | T/C | 3/18 | 0.17 |
| *SCN2B* | intron 1 | 501 | --- | A/G | 1/18 | 0.05 |
| | intron 4 | 448+296 | --- | A/G | 8/18 | 0.31 |
| | intron 4 | 448+301 | --- | A/G | 8/18 | 0.31 |
| *SCN3B* | intron 1 | 55+44 | --- | C/T | 3/18 | 0.17 |
| | exon 3 | 438 | T144T | C/T | 4/18 | 0.22 |