The evolving genetic and pathophysiological spectrum of migraine

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The novel p.L1649Q mutation in the *SCN1A* epilepsy gene is associated with familial hemiplegic migraine: genetic and functional studies

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Abstract

Familial hemiplegic migraine (FHM) is a severe subtype of migraine with hemiparesis during attacks. We scanned 10 families with FHM without mutations in the *CACNA1A* (FHM1) and *ATP1A2* (FHM2) genes. We identified the novel p.L1649Q mutation (c.4946T>A) in $Na_v1.1$ sodium channel gene *SCN1A* (FHM3) in a North American kindred with FHM without associated ataxia or epilepsy. Functional analysis of the mutation, introduced in the highly homologous human SCN5A, revealed markedly slowed inactivation and a two-fold faster recovery from fast inactivation predicting enhanced neuronal excitation. Our findings establish the role of neuronal $Na_v1.1$ sodium channels in FHM and reinforce the involvement of ion channel dysfunction in the pathogenesis of this episodic brain disorder.

Introduction

Familial hemiplegic migraine (FHM) is a rare monogenic form of migraine with hemiparesis during aura. Mutations in three genes for FHM have been identified, in the *CACNA1A* calcium channel gene (MIM# 601011) for FHM1 (MIM# 141500)[1], the *ATP1A2* Na,K-ATPase gene (MIM# 182340) for FHM2 (MIM# 602481)[2] and, recently, the p.Q1489K mutation (c.4465C>A; p.Gln1489Lys) in the *SCN1A* sodium channel gene (MIM# 182389) for FHM3 (MIM# 609634)[3]. All three gene products are intimately involved in the modulation of ion fluxes across neuronal and glial cell membranes, suggesting that FHM, and possibly also common types of migraine, are cerebral ionopathies.[4] The p.Q1489K *SCN1A* mutation is remarkable as it represents the first among more than 150 mutations in this gene that is not associated with either severe myoclonic epilepsy of infancy (SMEI, MIM# 607208) or generalized epilepsy with febrile seizures (GEFS+, MIM# 604233).[5,6] The mutation spectrum in SMEI differs from that in GEFS+ as the majority of SM EI mutations occurred *de novo*. Approximately half of the SMEI mutations are nonsense or frameshift mutations resulting in protein truncation and consequent loss-of-function. Almost 40% of SMEI mutations are missense mutations, with functional consequences that range from complete loss-of-function, gain-of-function to minimal functional effects.[7] The milder GEFS+ phenotype is associated with missense mutations only, showing either loss- or gain of-function effects.[8,9] Functional studies of the p.Q1498K mutation expressed and analyzed in the highly homologous human SCN5A revealed a more rapid recovery from fast inactivation (Dichgans et al., 2005). A limitation in that study was that the mutation was found in three families of common ancestry leaving the possibility of an isolated finding rather than a prominent FHM gene. In order to firmly establish the *SCN1A* gene as a gene for FHM3, independent confirmation in other families is necessary.

Here we performed mutation scanning in the *SCN1A* gene in 10 FHM families that were negative for mutations in the *CACNA1A* and *ATP1A2* genes. We identified the novel *SCN1A* p.L1649Q mutation (c.4946T>A) in a large kindred with pure FHM without epilepsy and show that this mutation severely interferes with voltage-gated sodium channel functioning.

Subjects and methods

Patients

We investigated 10 families with pure FHM (without associated epilepsy or ataxia) and without mutations in the *CACNA1A* (FHM1) and *ATP1A2* (FHM2) genes. Two to seven affected members were available per family. Diagnoses were made according to the IHS criteria.[10] All subjects gave written informed consent. Detailed information on the clinical characteristics of the *SCN1A* mutation carriers is shown in Table 1. Clinical diagnosis was made blinded for the genetic data.

Genetic analysis

Genomic DNA was isolated from peripheral blood using a standard salting out extraction method.[11] All 26 exons of *SCN1A* were amplified by polymerase chain reaction (PCR), and primer details are available from the authors upon request. For several exons, primers were improved compared to our original paper[3], for instance the alternatively spliced exon 5N, reported by Tate et al.[12] is now included in the scan. All PCR products were analyzed for mutations by direct sequencing. DNA numbering for *SCN1A* is based on cDNA reference sequence AB093548.1. Nucleotide numbering uses the A of the ATG translation initiation codon as nucleotide +1. Mutation nomenclature follows guidelines of the Human Genome Variation Society (http://www.hgvs.org/mutnomen/).

Mutagenesis, Cell Culture, and Electrophysiology

As in the first study, we used the closely related *SCN5A* cDNA because of known difficulties in stability of recombinant bacteria with *SCN1A* cDNA.[3] p.L1636Q, which corresponds to p.L1649Q in *SCN1A* was introduced by site-directed mutagenesis into full-length human *SCN5A* cDNA subcloned in pCDNA3.1 (QuikChange XL Kit, Stratagene, La Jolla, CA, USA). *SCN5A-L1636Q* and *SCN5A-WT* cDNA constructs were transfected into human tsA201 cells using the calcium phosphate method and were each coexpressed with accessory human sodium channel subunit β1 (ratio of cDNA 2:1) and CD8 cDNA. Before recording, DMEM medium was exchanged with bath solution and anti-CD8 coated microbeads (Dynabeads M-450 CD8, Oxoid, Basingstoke, UK) were added to the cell suspension. The bath solution contained (in mM): 110 Naglutamate, 35 NaCl, 4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 mM HEPES (pH 7.3). Macroscopic sodium currents were recorded using the whole-cell configuration of the patch clamp technique, filtered at 10 kHz by a low-pass Bessel filter, and acquired by a patch clamp L/M-EPC7 amplifier (List Medical Electronics, Darmstadt, Germany) interfaced with a National Instruments interface (PCI-6052E) and a custom acquisition program

(GePulse). Temperature was controlled (20 ± 0.5 °C) with a Peltier device. Patch pipettes were pulled from aluminium silicate glass (Hilgenberg Gmbh, Malsfed, Germany) and fire polished with a microforge. Electrode resistance was 1.5-2.0 MΩ with a pipette solution containing (in mM): 110 CsGlu, 30 NaCl, 2 $MgCl_2$, 5 Cs-EGTA, 10 mM HEPES (pH 7.3). Access resistance was between 2 and 5 MΩ, and the cell capacitance was between 5 and 15 pF, as measured by the compensating circuit of the amplifier. Data from cells with a current amplitude of 0.5-1.5 nA were used for the analysis of the voltage-dependent parameters. Thus, the series resistance error was less than 4 mV. The holding-potential was –120 mV and steady state activation, steady-state inactivation, time constants of inactivation (e.g. time constants τ_{fast} and τ_{slow}), and recovery from inactivation were measured using protocols, as described before.[3] Data analysis was performed using the program Ana (available at http://www.ge.cnr.it/ICB/conti_moran_pusch/programs-pusch/software-mik.htm), and Sigma Plot (SPSS Inc., Chicago, IL, USA).

Results

Clinical genetic analysis of FHM families

Mutation scanning of the *SCN1A* gene in the probands of the 10 families revealed one mutation in a North American family of Caucasian descent (Fig. 1A). Clinical details of mutation carriers from this family are shown in Table 1. The proband (III-4), aged 51 years, has hemiplegic migraine attacks since the age of 10 with a frequency that varies from twice a month to once a year. The attacks always start with blurred vision with dark spots, followed within minutes by spreading hemiparasthesia and hemiparesis with dysarthria and dysphasia. After 20-30 minutes, this is followed by a hemicranial throbbing headache, which is always located on the side opposite to the hemiparesis and is accompanied by nausea, vomiting, photo- and phonopobia. Six additional family members suffer from typicial hemiplegic migraine attacks as well, with an age of onset varying from 11 to 24 years. We classified individual IV-3 also as affected, despite the fact that he only has had one attack of FHM so far. We feel that because of his young age (22) subsequent attacks are still likely to occur. Besides hemiplegic attacks, individual III-2 suffers from migraine with and without aura and individual IV-3 suffers from migraine without aura. No cerebellar signs or epilepsy symptoms were reported in this family.

Mutation analysis in the proband revealed a heterozygous point mutation in exon 26 (c.4946T>A; p.L1649Q), resulting in an amino acid substitution of glutamine for leucine. The mutation co-segregated completely with the hemiplegic migraine phenotype in this family and was not found in a panel of 400 control chromosomes. Sequence alignments indicated high conservation of Leucine[1649] among several vertebrate sodium channel α1 subunits (Fig. 1B). Mutation p.L1649Q is located in the S4/D4 domain that is implicated in voltage sensing of fast inactivation (Fig. 1C-D).

Table 1. *Clinical Characteristics of SCN1A Mutation Carriers*

		Aura symptoms during hemiplegic attacks				Hemiplegic attacks		Headache characteristics during hemiplegic attacks			
ID	Age at onset (yrs)	H	S	V	A	Duration Hemiplegia	Frequency	Duration	Side	Character	Nausea/ vomiting/ photophobia/ phonophobia
II-1	12	+	+	+	+	1-6 h	2/week	24-48 h	Both sides	Throbbing	+/+/+/+
II-2	11	+	+	+	+	10-60 min	4-5/year	4-72 h	Unilateral	Nagging	+/+/+/-
III-2*	21	+	+	+	+	1-14 h	3-4/year	2 days	Both sides	Pulsating	+/+/+/+
IV-2	15	+	+	+	+	10-60 min	2/year	4-72 h	Both sides	Throbbing	+/+/+/+
III-4	10	+	+	+	+	>60 min	2/month-1/year	24 h	Unilateral	Throbbing	+/+/+/+
III-5	24	+	+	+	+	15 min	5/life	3 h	Both sides	Pounding	-/-/-/-
IV-3**	19	+	+	+	+	45 min	1 till present	1.5 h	Unilateral	Throbbing	+/+/+/+

*H: hemiparesis or hemiplegia; S: sensory disturbance; V: visual disturbance; A: aphasia; +: symptom consistently present in all or most attacks; -: symptom never present; *Patient III-2 also suffers from migraine with and without aura attacks. **Patient IV-3 also suffers from migraine without aura attacks.*

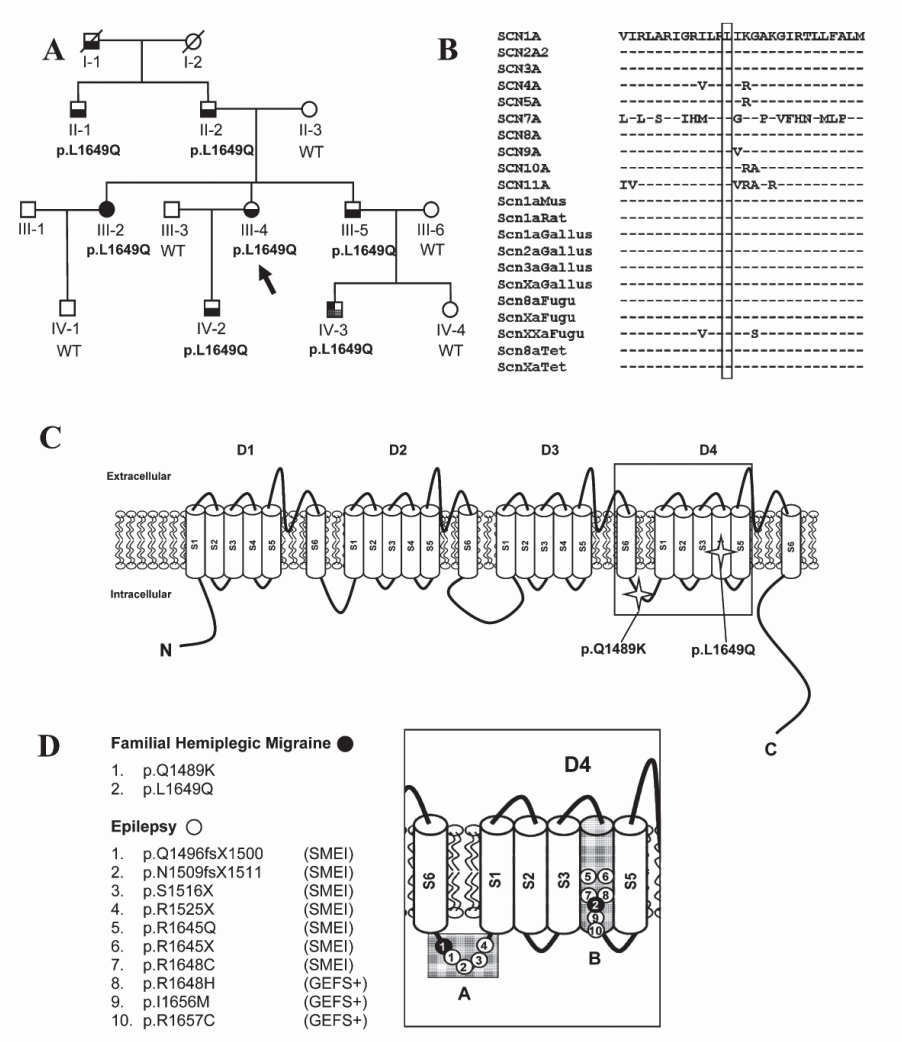

Figure 1. A: *Pedigree of the FHM3 p.L1649Q family. The arrow indicates the proband. Symbols present: FHM: black lower half; MA (migraine with aura): right upper square; MO (migraine without aura): left upper square; WT: wild-type; p.L1649Q heterozygous carrier of the pathogenic SCN1A mutation. Patient IV-3 (aged 22, gray lower half) had one typical (fulfilling the IHS criteria) hemiplegic migraine attack until present.* **B:** *Alignment of the amino acid sequence from several vertebrate sodium channel α subunits, showing complete conservation of the mutated amino acid Leucine*[1649]. **C:** *Topology of SCN1A, encoding the α1-subunit of a neuronal $Na_v1.1$ sodium channel. The subunit consists of 4 repeat domains (D1-D4), which contain 6 transmembrane domains (S1-S6). The location of the novel mutation p.L1649Q in the voltage sensor domain S4/D4 is depicted, as well as the previously identified p.Q1489K FHM3 mutation.* **D:** *Over 150 mutations have been identified for severe myoclonic epilepsy in infancy (SMEI) or generalized epilepsy with febrile seizures plus (GEFS+). For clarity, only SMEI and GEFS+ mutations are shown that are located in the (A) inactivation gate and (B) S4/D4 voltage sensor domain where FHM3 mutations were identified (all mutations are based on SCN1A cDNA reference sequence: AB093548.1). For review papers with all SCN1A epilepsy mutations see Meisler et al. (2005)*[5] *and Mulley et al. (2005)*[6].

Functional Consequences of the FHM3 Mutation

TsA201cells expressing construct *SCN5A-L1636Q*, which is equivalent to mutation p.L1649Q in *SCN1A* showed typical voltage-dependent sodium inward currents, similar to cells trans-

fected with construct *SCN5A-WT* (Fig. 2A), and with similar current density (Table 2). Mutant channels activated with the same voltage dependence as WT channels (Table 2). However, the time course of inactivation was slower for the mutant (Fig. 2A). Quantitative analysis of the inactivation time-course revealed that, at all tested voltages (-50 mV to 30 mV), both time constants of the double exponential fits (τ_{fast} and τ_{slow}) were two- to four-fold larger for the mutant compared to wild-type (Fig. 2B-C). The contribution of the fast component relative to the slow component (also expressed as the ratio C_{fast}/C_{slow}) of inactivation was reduced (Fig. 2D). Both effects lead to an overall slower fast inactivation of mutant channels (Fig. 2A). On the other hand, slow inactivation was unaffected (Table 2). The voltage dependence of steady state fast inactivation was shifted by ~ 10 mV towards more positive voltages and recovery from fast inactivation, measured after a 500 ms conditioning pulse to –10 mV, was 150% faster in the mutant than in the wild-type channels. Altogether, these functional analyses show that this mutation severely interferes with the fast inactivation process.

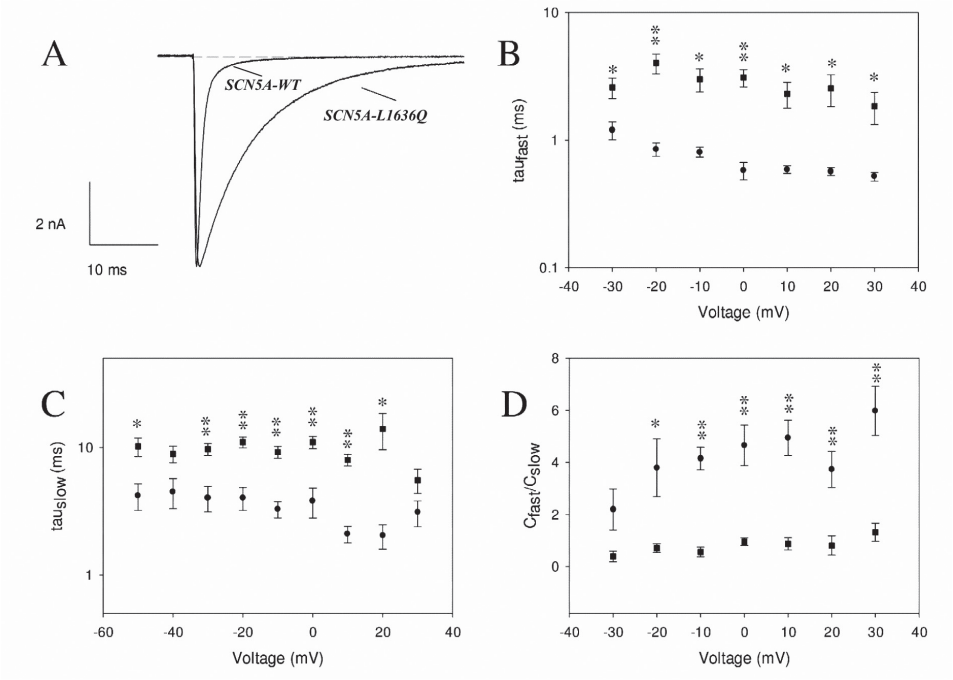

Figure 2. *Electrophysiological properties of wild-type and mutant sodium channels.* **A:** *Macroscopic sodium currents were recorded using the whole-cell configuration of the patch clamp technique. Depicted are current traces recorded at -10 mV showing slowed inactivation of mutant channels.* **B-D:** *Time constants of inactivation (tau_{fast} and tau_{slow}) and their respective contributions (C_{fast} and C_{slow}) were obtained from a bi-exponential fit to the decaying current phase as described earlier fast slow fast slow (Dichgans et al., 2005).* **B:** *fast time constant of inactivation (tau_{slow}).* **C:** *slow time constant of inactivation (tau_{slow}).* **D:** *ratio of the contribution of the fast and the slow component of inactivation (C_{fast}/C_{slow}). At -50 mV and -40 mV the fast component was negligible. Mean values ± SEM are given for SCN5A-WT (filled circles; n=6) and mutant SCN5A-L1636Q (filled squares; n=6). Values marked with asterisks are significantly different between wild-type and mutant (* p<0.05; ** p<0.01) with Student's unpaired t-test.*

Table 2. *Activation and Inactivation Parameters for Wild-type and Mutant Sodium Channels*

Parameters	*SCN5A-WT*	*SCN5A-L1636Q*
Current density (nA/pF)	0.24 ± 0.04 (30)	0.21 ± 0.03 (28)
Steady-state activation		
Voltage of half maximal activation (V*a*, mV)	-44.2 ± 5.1 (7)	-41.4 ± 1.5 (6)
Slope factor (k*a*)	6.1 ± 1.8	6.0 ± 1.5
Reversal potential (V*rev*, Mv)	45.6 ± 5.2	41.7 ± 7.0
Steady-state fast inactivation		
Voltage of half maximal inactivation (V*fi*, mV)	-77.4 ± 3.1 (8)	-67.8 ± 2.7 (5) **
Slope factor (k*fi*)	7.2 ± 1.4	7.5 ± 1.1
Steady-state slow inactivation		
Voltage of half maximal slow inactivation (V*si*, mV)	-99.1 ± 10.2 (3)	-92.8 ± 0.7 (3)
Slope factor (k*si*)	12.6 ± 3.3	13.0 ± 2.1
Recovery from inactivation (-120 mV)		
Fast time constant (τ_{fast}) (ms)	7.1 ± 1.6 (4)	4.6 ± 1.0 (5) *
Slow time constant (τ_{slow}) (ms)	322 ± 200	108 ± 48
Onset of slow inactivation		
Fast time constant (τ_{fast}) (s)	3.8 ± 1.4 (9)	5.1 ± 0.9 (4)
Slow time constant (τ_{slow}) (s)	302 ± 105	217 ± 98

Data are mean ± SD. Numbers in brackets indicate number of experiments. Values marked with asterisks are significantly different between wild-type and mutant (p<0.05; ** p<0.01) with Student's unpaired t-test.*

Discussion

We here firmly establish that certain mutations in the *SCN1A* epilepsy gene may cause FHM. First, the p.L1649Q *SCN1A* mutation completely co-segregated with a pure FHM phenotype (without epilepsy) in our family (Fig. 1A) and was not found in the control panel. Second, Leucine[1649] is highly conserved among several vertebrate sodium channel α1 subunits (Fig. 1B). Third, the mutation is located in the S4 segment of domain 4 that acts as a voltage sensor and is known to play an important role in channel gating (Fig. 1C-D).[13,14] Finally, our functional studies of the mutation introduced in the highly homologous human SCN5A revealed clear functional effects: i) an overall slower inactivation of sodium channels; ii) a depolarizing shift by ~ 10 mV in the voltage dependence of the steady state inactivation; and iii) an accelerated recovery from fast inactivation (Table 2). Most likely, the p.L1636Q *SCN5A* mutation directly interferes with the inactivation process, as do other mutations in the S4/D4 domain[14], even though we cannot fully exclude a contribution of an altered interaction with the β1 subunit.[15] Although this study convincingly showed causality for p.L1649Q in FHM3, in future studies, these findings should be confirmed in SCN1A, or even better in a knockin mouse model.

The previously identified FHM3 mutation p.Q1489K[3] was also introduced in the highly homologous *SCN5A* cDNA, which allows comparison of the functional consequences of both FHM3 mutations. This mutation is located in the cytoplasmic linker between domains III and IV (Fig. 1C-D) and revealed a two-fold to four-fold accelerated recovery from fast inactivation.[3] Thus, both the p.Q1489K and the p.L1649Q mutation lead to impaired fast inactivation and predict enhanced neuronal excitation. This fits very well with our current understanding of the pathogenesis of FHM.[16,4] The *CACNA1A* gene encodes neuronal $Ca_v2.1$ calcium channels that modulate the release of neurotransmitters. FHM1 *CACNA1A* mutations were shown to cause gain-of-function effects in cellular models[17,18] and in a knockin mouse model.[19] In the transgenic model, FHM1 mutations increase the release of glutamate and other neurotransmitters (A. Tottene, A. van den Maagdenberg, and D. Pietrobon, unpublished observations) and reduce the threshold for cortical spreading depression (CSD).[19] CSD has been convincingly shown to be the underlying mechanism for the migraine aura[20] and, based on animal experiments, may also be responsible for triggering the headache phase of migraine attacks by activating the trigeminovascular system.[21] The *ATP1A2* gene encodes a Na,K-ATPase in glial cells. FHM2 *ATP1A2* mutations were shown to have altered kinetics or loss-of-function effects in cellular studies, predicting reduced re-uptake of both K^+ and glutamate from the synaptic cleft into glial cells.[2,22] Voltage-gated sodium channels are involved in the generation and propagation of action potentials in excitable tissues. FHM3 *SCN1A* mutations changed $Na_v1.1$ channel inactivation kinetics, predicting enhanced neuronal excitation leading to increased release of neurotransmitters, including glutamate. The common overall effect of FHM mutations in all three FHM genes seems to be an increase of the concentration of K^+ and glutamate in the synaptic cleft. This should translate into an enhanced propensity for CSD and may thus be responsible for triggering FHM, and possibly "normal" migraine attacks.

Migraine and epilepsy are comorbid disorders and seem to have some overlapping mechanisms related to dysfunction of ion transportation.[23] It is remarkable that the vast majority of *SCN1A* mutations are associated with severe forms of epilepsy, whilst the p.Q1489K and p.L1649Q mutations cause pure FHM. The p.L1649Q FHM3 mutation is even adjacent to two "epilepsy" mutations that affect Arginine[1648], the p.R1648C mutation causing SMEI[24] and p.R1648H causing GEFS+.[25] Both mutations have been examined in several expression systems with different outcomes all affecting channel inactivation.[5] From these and our studies it is evident that the voltage sensor in domain 4 (S4/D4) is pivotal to the fast inactivation of sodium channels but that there is no simple correlation between clinical phenotype and biophysical changes induced by *SCN1A* mutations. Dedicated functional studies comparing epilepsy and migraine mutations in the same gene may further the insight into both episodic brain disorders.

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