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Variable neurologic phenotype in a GEFS+ family with a novel mutation in SCN1A

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ABSTRACT

Purpose: To describe the spectrum of clinical disease in a mutliplex family with an autosomal dominant form of generalized epilepsy with febrile seizures plus (GEFS+) and determine its genetic etiology.
Methods: Medical and family history was obtained on 11 clinically affected individuals and their relatives across three generations through medical chart review and home visits. A candidate gene approach including haplotype analysis and direct sequencing was used.
Results: An epilepsy-associated haplotype was identified on 2q24. Direct sequencing of the entire *SCN1A* gene identified seven sequence variants. However, only one of these, c.1162 T > C, was not found in population controls. This transition in exon 8 of *SCN1A* predicts a substitution (Y388H) of a highly conserved tyrosine residue in the loop between transmembrane segments S5 and S6 of the sodium channel protein ($Na_v1.1$). Clinical features in mutation carriers of this novel missense mutation were highly variable, ranging from febrile seizures to severe refractory epilepsy.
Conclusion: A novel missense mutation in the pore-forming region of the sodium channel gene *SCN1A* causes GEFS+ with a variable phenotype that includes mood and anxiety disorders, as well as ataxia, expanding the GEFS+ spectrum to include neuropsychiatric disease.

1. Introduction

Generalized epilepsy with febrile seizures plus (GEFS+) is a diagnostic label coined by Scheffer and Berkovic in 1997 to describe a dominantly inherited epilepsy disorder associated with febrile and afebrile seizures. The phenotype is variable and can range from febrile seizures to severe intractable epilepsy with developmental delay in affected individuals within the same family.[1,2] GEFS+ is also genetically heterogeneous with five causative genes identified to date. The most widely reported is *SCN1A*, which encodes the α1-subunit of a voltage-gated sodium channel and accounts for mutations in approximately 11% of previously described GEFS+ families.[3,4] Mutations in genes encoding the β1 and αII-subunits of voltage-gated sodium channels, *SCN1B* and *SCN2A*, can also cause GEFS+.[5,6] One splicing and six missense mutations associated with GEFS+ have been identified in *SCN1B*,[5,7,8] and one missense mutation in the *SCN2A* gene has been associated with GEFS+ in a single patient.[6] In addition to mutations in subunits of the sodium channel, GEFS+ may also be associated with abnormalities in gamma-aminobutyric acid (GABA) neurotransmission, resulting from mutations in the *GABRG2* and *GABRD* genes, which encode subunits of the $GABA_A$, receptor.[9,10] Three missense mutations segregating in GEFS+ families have been reported in the $GABA_A$ receptor, gamma 2 (*GABRG2*) gene,[9,11] while two have been reported in the $GABA_A$ receptor, delta (*GABRD*) gene.[10]

The most clinically relevant GEFS+ gene, *SCN1A*, has 26 exons and is highly conserved across species.[3] Mutations in *SCN1A* have been associated with a spectrum of epilepsy phenotypes, including severe myoclonic epilepsy of infancy (SMEI, also known as Dravet's Syndrome), GEFS+, and infantile spasms (IS).[12,13] Of the 155 *SCN1A* mutations currently reported in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk), the vast majority are associated with SMEI, while 19 missense mutations in the *SCN1A* gene have been associated with GEFS+.[14–16]

Recently, the phenotypic spectrum resulting from *SCN1A* mutations has been extended to include cryptogenic generalized and focal epilepsy, familial hemiplegic migraine, and familial

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sudden unexpected death in epilepsy (SUDEP).[13,17,18] Studies involving comprehensive clinical data on mutation carriers from multiplex families are necessary to determine the natural history and full clinical spectrum associated with the GEFS+ phenotype. We report a four generation family with autosomal dominant GEFS+ with a novel mutation in *SCN1A*, which extends the phenotypic spectrum associated with GEFS+ to include ataxia and social anxiety.

2. Methods

2.1. Clinical features

The proband (IV:1, Fig. 1) was ascertained from a registry of all epilepsy patients treated at the Pediatric Neurology Clinic in the Janeway Child Health Centre (St. John's, NL, Canada). The pedigree was extended through family visits and interviews and further clinical data was obtained from EEG recordings, standardized questionnaires (see Supplementary material), and medical records. Epilepsy diagnoses were made by pediatric neurologists (DB, MA) using the International League Against Epilepsy (ILAE) classification system.[19] Informed consent was obtained from all participants or parents/legal guardians. The study was approved by the Human Investigations Committee of Memorial University (Study No. 4.15).

2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood.[20] Sixteen family members were genotyped for four microstatellite and two intragenic SNPs spanning *SCN1A* on chromosome 2q24 (Fig. 1). Sequencing primers were custom designed using Primer3 (http://frodo.wi.mit.edu/) to amplify all 26 exons, intron/exon boundaries and 5′ and 3′ UTRs of *SCN1A* (GenBank accession no. AB093548). Samples were sequenced using BigDye Terminator v.3.1 Cycle Sequencing Kit and run on an ABI 3130xl (Applied Biosystems). Sequence traces were inspected manually for quality control and analyzed using Mutation Surveyor v.3.20. The frequency of variants were tested against ethnically matched population controls and the effects of variants were predicted using several bioinformatics tools available online. Information on specific analysis is available upon request.

3. Results

3.1. Clinical features

The founders of this Newfoundland family originate from County Cork, Ireland. The complete pedigree consists of 97 relatives across five generations segregating an autosomal dominant form of epilepsy. The core pedigree depicts 4 generations (affected branches only) of this family, which includes 11 individuals diagnosed with epilepsy in three successive generations (Fig. 1). Marked clinical heterogeneity between affected relatives with a variety of seizure types and neurological deficits was obtained by reviewing medical records and EEGs, and also through seizure questionnaires and home visits. All clinically affected relatives ($n = 11$) had seizure disorders starting in childhood with a mean age of onset of 2.1 years (range 0.75–5.5 years) (Table 1). Of these, nine (IV:1, IV:2, IV:3, III:3, III:2, II:7, II:9, III:12 and III:16) had generalized tonic-clonic seizures (GTCS) which occurred with and without fever, and two (II:2 and IV:5) had febrile seizures only. Regarding seizure presentation, five (IV:1, IV:2, IV:3, II:7 and III:12) had multiple episodes of status epilepticus, four (IV:1, IV:2, IV:3 and III:12) had absence seizures, one (IV:1) had myoclonic seizures, and one (IV:5) had atonic seizures. Other neuropsychiatric disorders were also prominent, with five (IV:1, IV:2, III:2, III:12 and III:16) individuals presenting intellectual disabilities, three (II:7, II:9 and III:16) with debilitating psychiatric disease, and two, including the proband (IV:1, III:12), with ataxia. None of the eight spouses had any personal or family history of seizures.

The proband (IV:1, Fig. 1) had his first seizure (afebrile GTCS) at 11 months of age. He began having myoclonic seizures at 2 years and by 3 years of age was experiencing approximately 10 myoclonic seizures per day. These seizures were controlled with divalproic acid and ethosuximide to a rate of one per 2–3 days. In

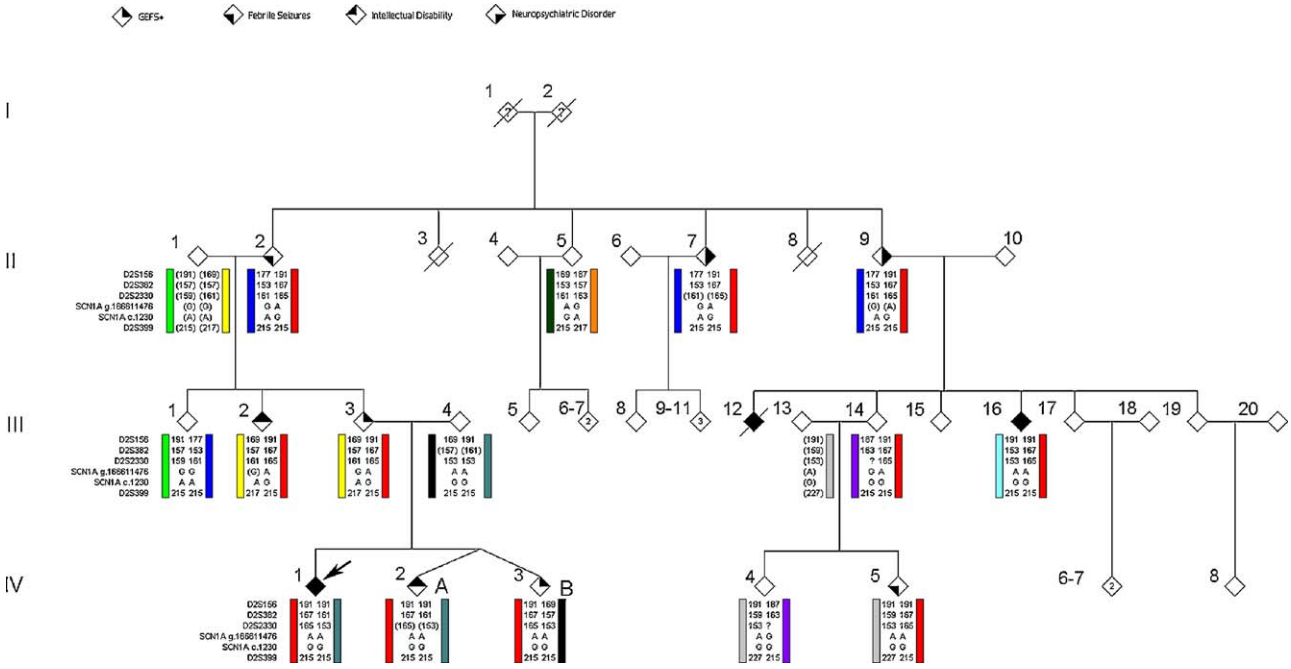

Fig. 1. Core pedigree of Newfoundland family showing both typical and novel features of GEFS+ associated with the epilepsy-associated haplotype (red) on chromosome 2q24 in the vicinity of *SCN1A*. Markers are listed on the left and alleles given in size (base pairs) or specific nucleotide. The proband is indicated by an arrow. Gender has been masked using diamond symbols to protect confidentiality.

Table 1
Clinical features of affected family members.

Pedigree reference [sex; age]	Age of onset/ remission	Type of seizures/ number	EEG	Neuroimaging (MRI/CT)	AEDs	Intellectual disability	Psychiatric/other neurological disorders	Clinical classification of epilepsy
IV:1 [m; 8 y]	11 mo./cont.	Ab, FS, GTCS, SE, myoclonus/>100	GSW	MRI-normal, CT-normal	Cont.	Moderate DD	Ataxia	GEFS+
IV:2 [f; 5 y]	1 y/cont.	FS, Ab, GTCS, SE/>50	Bilateral epileptiform discharges in frontal region	CT-normal	Cont.	Mild DD		GEFS+
IV:3 [m; 5 y]	1 y/cont.	FS, Ab, GTCS, SE/>50	Normal	CT-normal	Cont.			GEFS+
III:3 [m; 32 y]	1 y/29 y	FS, GTCS/many		CT-normal	Cont.			GEFS+
III:2 [m; 34 y]	1 y/15 y	FS, GTCS/5–10	Normal	CT-normal	Stopped	Learning difficulty, probable ADHD		GEFS+
II:2 [f; 57 y]	5–6 y/teens	FS/2			None			FS
II:7 [f; 63 y]	Infancy/20–22 y	GTCS, SE/>50			Stopped		Anxiety/Depression[a]	GTCS
II:9 [f; 61 y]	4–5 y/23 y	FS, GTCS/4			Stopped		Social anxiety/ depression	GEFS+
III:12 [f; deceased 27 y] DNA not available	9 mo./27 y	Ab, FS, GTCS, SE/100s	GSW, Rhythmic and paroxysmal slowing	CT-normal	Cont.	Severe MR	Ataxia	GTCS Cause of death: multiple seizures
III:16 [m; 41y]	5 y/20 y	FS, partial to GTCS/many	Normal	CT-normal	Cont.	Mild DD	Social anxiety[b]	GEFS+
IV:5 [f; 4 y]	16 mo./22 mo.	FS, atonic/2	Normal		None			Two atonic febrile seizures

Key: m, male; f, female; y, years; mo. months; Ab, absence; GTCS, generalized tonic-clonic seizures; FS, febrile seizures; SE, status epilepticus; GSW, generalized spike wave; AEDs, anti-epileptic drugs; DD, developmental delay; MR, mental retardation; ADHD, attention deficit hyperactivity disorder; cont., continuing.
[a] Patient was treated in a psychiatric unit.
[b] Patient lives with his parents and is unable to work due to social anxiety.

addition, he had frequent generalized tonic-clonic (GTC) and absence seizures despite treatment with valproic acid and ethosuximide. He has had two episodes of status epilepticus. CT head scan at 3 years of age was normal. EEG at 3 years of age showed generalized epileptiform discharges, slowing, and poly-spike and wave discharges. EEGs at 4, 6 and 7 years of age were normal. Of particular note are his neurologic signs. At 8 years old he displays moderate global developmental delay, ataxia and behavioral concerns. He repeated kindergarten and receives special education support at school, but has had no developmental regression. He continues to have GTCS approximately once every 3–4 months and remains on valproic acid. His most recent episode of status epilepticus was 4 years ago.

The sister of the proband (IV:2) was born at 34 + 3 weeks gestation (twin A; Fig. 1). Her first seizure, a febrile GTCS, occurred at the age of 1 year 2 weeks. She has since had many GTC and absence seizures, and has had seven hospital admissions for episodes of status epilepticus, lasting 30–80 min. EEGs (at 1, 2 and 3 years of age) and a CT head scan (at 2 years of age) were normal. She is now 5 years old, has mild developmental delay and infrequent seizures on treatment with topiramate and valproic acid. Kindergarten entry was delayed by 1 year.

The brother of the proband (IV:3, twin B) experienced his first seizure at the age of 1 year 2 weeks. This was an afebrile partial seizure progressing to secondary generalized tonic-clonic seizure. He has since had both febrile and afebrile onset generalized seizures. He has had two episodes of status epilepticus. EEGs (at 2 and 3 years of age) and a CT head scan (at 1 year of age) were normal. He is now 5 years old, shows normal cognitive development and his seizures persist at a rate of approximately one per 3–6 months, despite treatment with valproic acid. There have been no reported learning or behavioral issues.

Of the remaining eight affected individuals, all except two have developed afebrile seizures requiring antiepileptic medication. Three others have developmental delay, all of whom have a history of multiple seizures refractory to medication.

3.2. Genetic study

Haplotype analysis confirmed co-segregation of epilepsy with a haplotype on 2q24 (Fig. 1). Direct sequencing of *SCN1A* revealed seven sequence variants that co-segregated on the epilepsy-associated haplotype (Table 2). However, only one of these, a c.1162 T > C transition in exon 8 of *SCN1A* (Fig. 2), was found in all

Table 2
Sequencing variants identified in SCN1A (NM_006920) in clinically affected subjects.

Exon/Intron	Variant nomenclature	Classification	Amino acid change	Segregation of alleles/verification
Intron 1	c.265-83 A > T	Noncoding	No	Does not predict a splice site[a]
Intron 6	c.965-21 C > T	Noncoding	No	Does not predict a splice site[a]
Exon 8	c.1162 T > C, p.Y388	Missense	Yes (Y388H)	Heterozygous in all affecteds, Not present in 190 control alleles
Exon 9	c.1212 A > G, p.V404, rs 7580482	Synonymous	No (V404V)	Allele frequency of 42%[b]
Exon 13	c.2167 T > C	Synonymous	No (V723V)	Not heterozygous in affecteds
Exon 16	c.3310 G > A	Missense	Yes (A1104T)	Not heterozygous in affecteds
Exon 17	c.3472 G > A	Missense	Yes (E1158K)	Not heterozygous in affecteds

[a] BDGP: Splice Site Prediction by Neural Network.
[b] NCBI dbSNP.

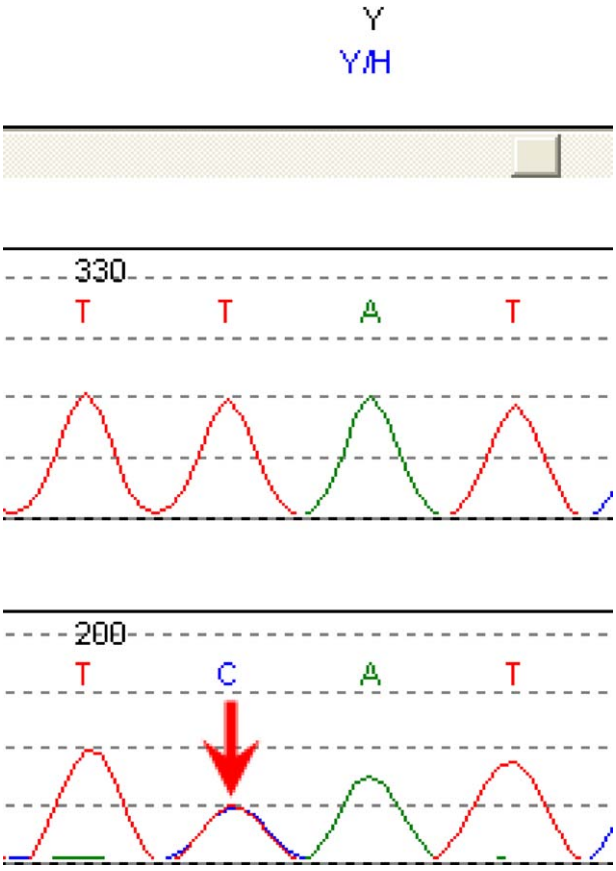

Fig. 2. Sequencing traces (forward direction) showing the heterozygous c.1162 T > C transition in exon 8 of *SCN1A* of the proband's (IV:1, Fig. 1) genomic DNA. The amino acid translations (top) show the Y388H amino acid substitution.

clinically affected relatives and was not seen in 190 ethnically matched population control chromsomes (Table 2). In addition, this novel mutation predicted a deleterious replacement of a highly conserved and uncharged tyrosine to a basically charged histidine residue within the pore domain of the SCNIA sodium channel (Fig. 3A and B).

4. Discussion

We report a comprehensive clinical study of a family with an autosomal dominant history of extended spectrum GEFS+ due to a novel missense mutation in *SCN1A*. Although the clinical presentation is variable, as is the case for GEFS+,[1,21–23] seven of the 11 affected individuals presented with neuropsychiatric deficits. Of the 10 affected individuals known to have the novel *SCN1A* mutation, four had developmental delay, three had debilitating psychiatric disease, and two had ataxia, in addition to epilepsy. The 11th affected individual (III:12; deceased) had severe mental retardation, ataxia and died as a result of multiple seizures, though her mutation status is unknown as her DNA was unavailable. This is the first report of familial ataxia in a family with GEFS+ as the primary presentation. These findings suggest that the GEFS+ phenotype may include a range of neuropsychiatric deficits, and testing mutation carriers with neuropsychological scales may be indicated.

Approximately 20% of cases of GEFS+ have an identified genetic cause and most of these have mutations in *SCN1A*.[24] The mutation identified in this family, a c.1162 T > C transition in exon 8 of *SCN1A*, is novel and located between S5 and S6 in the pore-forming region of domain 1 of *SCN1A*. Interestingly, the majority of mutations in the pore-forming loop regions (between S5 and S6) have been identified in patients with SMEI.[25] Similar to the GEFS+ phenotype, SMEI is associated with wide intrafamilial variability of phenotype which can include progressive myoclonic epilepsy and developmental delay.[26] In this family, none of the 11 clinically affected members fulfill the diagnostic criteria for SMEI. The proband (IV:1) had multiple seizure types including myoclonus, in

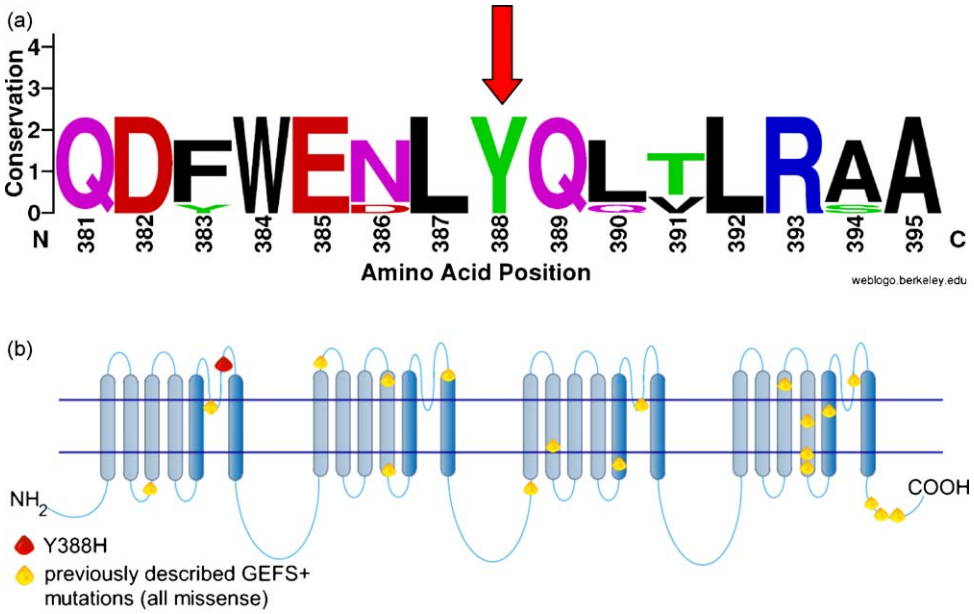

Fig. 3. Conservation of SCN1A in the vicinity of Y388 and location of Y388H within the SCN1A protein. (A) Weblogo was used to align orthologs from *Homo sapiens* (NP_008851), *Pan troglodytes* (XP_515872), *Mus musculus* (NP_061203), *Rattus norvegicus* (NP_110502), *Danio rerio* (NP_956426), *Drosophila melanogaster* (NP_001036280), and *Nasonia vitripennis* (NP_001128389). The tyrosine (Y) residue at position 388 of the SCN1A protein compared to other sodium channel proteins is marked by a red arrow. (B) Y388H (red triangle) results in the substitution of a neutral polar tyrosine residue to a polar basic histidine in the loop between the S5 and S6 segments of domain 1 of the SCN1A protein. Segments 5 and 6 (darker blue) form the ion channel pore and segment 4 is the voltage sensor.

addition to developmental delay, but has not regressed and now has only occasional seizures on a single antiepileptic medication.

Interestingly, mutations in sodium channel genes have also been associated with other neuropsychiatric disorders of similar pathology. For example, panic disorder and Asperger syndrome were present in two individuals in a GEFS+ family with a missense mutation in *SCN1A*, and variants in the *SCN1A* and *SCN2A* genes were observed in six patients with autism.[27,28] Heterozygotes for a null allele of another sodium channel gene, *SCN8A*, showed a variety of cognitive defects, including mental retardation and behavior disorders.[29] Recently, a novel missense mutation in *SCN1A* was identified in a family with familial hemiplegic migraine, widening the phenotype associated with mutation in this gene.[17]

To date, there have been three other reports of missense mutations in *SCN1A* causing GEFS+ located in the loop between S5 and S6. The first mutation was identified in 2001 in GEFS+ patients with febrile seizures associated with afebrile partial seizures (domain 3, S5-S6; V1428A, C4283T).[30] The second occurred in a South American family in whom an amino acid substitution was located in the pore-forming region in domain four of the *SCN1A* protein (domain 4, S5-S6; D1742E, C5226A or G). The phenotype was highly variable, with some individuals having only febrile seizures, and others having numerous seizures and mental retardation.[15] The third mutation was identified in a single patient for whom no family history was available. At 6 years of age the patient has well-controlled seizures and normal cognitive and neurological development (domain 1, S5-S6; R377Q, G1130A).[31] In addition, mutations in the pore-forming regions of *SCN1A* have been previously associated with ataxia amongst patients with an SMEI phenotype.[25]

The variable expressivity in this multiplex, multigenerational family is compatible with the broad phenotypic spectrum previously observed in other GEFS+ families.[1,21–23] Increasing reports of the high degree of variability in GEFS+ families implies the presence of modifying influences including other genes and environmental factors.[32] It also presents challenges in counseling families regarding prognosis and recurrent risk. Comprehensive clinical data in mutation carriers suggests that GEFS+ should be extended to include psychiatric and neurological deficits, based largely on observations and follow-ups from home visits. Although often infeasible in clinical studies, family visits and using open questions can facilitate the collection of phenotypic data that might otherwise be missed with structured interviews and standardized questionnaires. The use of neurological scales to further the extent of neuropsychiatric features resulting from this novel mutation in *SCN1A* is underway.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.seizure.2009.04.009.

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