

# The phenotypic spectrum of *SCN8A* encephalopathy

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## ABSTRACT

**Objective:** *SCN8A* encodes the sodium channel voltage-gated  $\alpha 8$ -subunit ( $Na_v1.6$ ). *SCN8A* mutations have recently been associated with epilepsy and neurodevelopmental disorders. We aimed to delineate the phenotype associated with *SCN8A* mutations.

**Methods:** We used high-throughput sequence analysis of the *SCN8A* gene in 683 patients with a range of epileptic encephalopathies. In addition, we ascertained cases with *SCN8A* mutations from other centers. A detailed clinical history was obtained together with a review of EEG and imaging data.

**Results:** Seventeen patients with de novo heterozygous mutations of *SCN8A* were studied. Seizure onset occurred at a mean age of 5 months (range: 1 day to 18 months); in general, seizures were not triggered by fever. Fifteen of 17 patients had multiple seizure types including focal, tonic, clonic, myoclonic and absence seizures, and epileptic spasms; seizures were refractory to antiepileptic therapy. Development was normal in 12 patients and slowed after seizure onset, often with regression; 5 patients had delayed development from birth. All patients developed intellectual disability, ranging from mild to severe. Motor manifestations were prominent including hypotonia, dystonia, hyperreflexia, and ataxia. EEG findings comprised moderate to severe background slowing with focal or multifocal epileptiform discharges.

**Conclusion:** *SCN8A* encephalopathy presents in infancy with multiple seizure types including focal seizures and spasms in some cases. Outcome is often poor and includes hypotonia and movement disorders. The majority of mutations arise de novo, although we observed a single case of somatic mosaicism in an unaffected parent. *Neurology*® 2015;84:480-489

## GLOSSARY

**EE** = epileptic encephalopathy; **SCN8A** = sodium channel, voltage-gated, type VIII,  $\alpha$  subunit; **SUDEP** = sudden unexplained death in epilepsy.

The epileptic encephalopathies (EEs) are a group of severe epilepsies that predominantly begin in infancy and childhood. They are characterized by refractory seizures with the child typically experiencing multiple seizure types in the setting of developmental delay or regression; frequent epileptiform activity is seen on EEG studies.<sup>1</sup> The genetic etiology of these disorders has become increasingly recognized with de novo mutations in many patients. The prototypic example is Dravet syndrome in which >80% of patients have mutations of the sodium channel  $\alpha 1$ -subunit gene, *SCN1A*. Gene discovery in Dravet syndrome has fueled clinical and basic research informing diagnosis and therapeutic approaches.

Recently, the application of next-generation sequencing approaches has led to the identification of multiple new genes for EEs although each is responsible for a small proportion of patients. Once a gene is identified, studies of patients who have a mutation of the same gene facilitate clinical recognition of the phenotypic spectrum of a specific genetic encephalopathy.

Sodium channel genes have emerged as very important in causation of EEs with *SCN1A* being the most well studied. Mutations of the  $\alpha$ -subunit genes *SCN1A* and *SCN2A* are

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associated with a wide spectrum of epilepsy syndromes ranging from EEs to mild disorders such as febrile seizures.<sup>2–6</sup> Recently, de novo mutations in *SCN8A*, encoding one of the main voltage-gated sodium channel subunits (Na<sub>v</sub>1.6) in the brain,<sup>7</sup> have been described in patients with severe epilepsy,<sup>8–13</sup> although a clear clinical presentation has yet to be described or investigated. Herein, we report the phenotype of 17 patients with EE and disease-causing mutations in *SCN8A*.

**METHODS** **Patients.** *SCN8A* patients were identified from a large cohort of 683 patients with EEs from Denmark, Australia, North and South America, and Europe. A detailed epilepsy, developmental, and general medical history was obtained for each patient together with examination findings. EEG and imaging data were reviewed. Seizures were diagnosed according to the International League Against Epilepsy Organization, and epilepsy syndromes were established where possible.<sup>1</sup>

**Standard protocol approvals, registrations, and patient consents.** The study was approved by the ethics committee of Western Zealand and Austin Health and the institutional review board of the University of Washington. The parent or legal guardian of each patient gave informed consent.

**Mutation analysis.** Genomic DNA was extracted using standard methods. Mutations in 7 cases were identified using targeted capture of all exons and at least 5 base pairs of flanking intronic sequence of *SCN8A* with molecular inversion probes (RefSeq, hg19 build, transcript ID ENST00000354534).<sup>14</sup> Data analysis, variant calling and filtering, as well as depth of coverage statistics were generated as previously described.<sup>10</sup> Variants were assumed to be pathogenic if they were nonsynonymous, splice-site altering, or frameshift changes, not present in 6,500 control samples (Exome Variant Server—see URLs/resources), and had arisen de novo in the patient (or was inherited from a parent with somatic mosaicism). The remaining 10 *SCN8A* mutations were identified by clinical or research testing at 6 centers. Traditional Sanger sequencing was used to confirm all mutations and to perform segregation analysis in parental DNA. Where possible, parental status was confirmed by microsatellite analysis.

**RESULTS** **Mutation analysis.** We identified 16 patients with de novo pathogenic heterozygous mutations of *SCN8A* and one pathogenic *SCN8A* mutation inherited from an unaffected somatic mosaic parent as described previously.<sup>10</sup> Seven cases were identified using high-throughput capture and resequencing in a cohort of 683 probands with a range of EEs, accounting for approximately 1% (7/683) of cases. We obtained additional phenotypic information for one case who was previously published<sup>10</sup> and for 10 additional patients who were referred with a de novo *SCN8A* mutation (table 1). None of these mutations have been identified previously in 6,500 control individuals.

The 17 pathogenic mutations were distributed throughout the entire *SCN8A* gene (figure 1). Sixteen

of 17 were missense and altered evolutionarily conserved amino acids. Notably, 4 patients (D, G, K, Q) had mutations altering the same amino acid. We identified a single individual with a 3 base pair deletion that abolished the donor splice site (predicted by MutationTaster<sup>15</sup>—see URLs/resources). Although we were unable to test the effects of this deletion on splicing, in silico analysis predicts that the disruption of this donor splice site will result in skipping of exon 24 during pre-mRNA splicing. Skipping of this 138 base pair exon would lead to an in-frame deletion of 46 amino acids from the third transmembrane domain and the intracellular loop to transmembrane domain 4.

**Clinical features of *SCN8A* encephalopathy.** The 17 patients ranged in age from 8 months to 44 years (mean 8 years) at diagnosis; 12 were female. Seizure onset occurred at a mean of 5 months (median 4 months, range 1 day to 18 months) (table 2). Seizure semiology at onset was variable and included focal clonic seizures evolving to a bilateral convulsive seizure (7), afebrile tonic-clonic seizures (3), tonic seizures (3), epileptic spasms (2), febrile seizures (1), and myoclonic seizures (1). Fifteen of 17 patients developed additional seizure types, including generalized tonic-clonic seizures (11), epileptic spasms (5), atypical absence seizures (5), myoclonic seizures (4), and atonic seizures (1). Eight of 17 patients had episodes of convulsive (7) or nonconvulsive (1) status epilepticus.

All patients had refractory epilepsy, although 4 patients had extended seizure-free periods. Patient E was seizure-free for 6 months on valproic acid and oxcarbazepine; patient J for 17 years on carbamazepine; patient K for 8 months on valproic acid + phenytoin; patient P is currently responding to oxcarbazepine (6 weeks seizure-free); and patient O remains seizure-free after 3 years on no treatment.

The developmental pattern varied from normal development with slowing or regression after seizure onset in 12 patients, to one of abnormal development from birth in 5 with regression in one. All patients older than 18 months (n = 15) had intellectual disability that ranged from mild (1) to moderate (4) to severe (10). Of the 15 patients older than 18 months, 7 could sit and walk unassisted.

Neurologic features were prominent in the majority of cases and included hypotonia (8), dystonia (4), hyperreflexia (2), choreoathetosis (2), and ataxia (1). Psychiatric features were observed in 4 of 17 patients: 3 had autistic features and one had attention deficit hyperactivity disorder. Seven of 17 patients gradually lost eye contact during the course of the disease; only one of these had autistic features.

Early death occurred in childhood in 2 patients: patient A died at 3 years during a seizure and

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Supplemental data  
at Neurology.org

**Table 1 Pathogenic SCN8A mutations in 17 patients**

Proband	Inheritance	DNA change	Amino acid change	Function	PolyPhen score
Patient A	De novo	g.52200120G>A	Arg1617Gln	Missense	1.00
Patient B	De novo	g.52159789T>A	Val960Asp	Missense	1.00
Patient C	De novo	g.52200671C>G	Gln1801Glu	Missense	0.99
Patient D	De novo	g.52200885G>A	Arg1872Gln <sup>a</sup>	Missense	1.00
Patient E	De novo	g.52159578G>A	Ala890Thr	Missense	1.00
Patient F	De novo	g.52184197A>G	Ile1479Val	Missense	0.97
Patient G	De novo	g.52200884C>T	Arg1872Trp <sup>a</sup>	Missense	1.00
Patient H	Inherited, somatic 13% mosaic	g.52180374C>G	Leu1331Val <sup>b</sup>	Missense	0.99
Patient I	De novo	g.52093426T>C	Phe260Ser	Missense	0.92
Patient J	De novo	g.52200083A>G	Ile1605Arg	Missense	0.83
Patient K	De novo	g.52200885G>A	Arg1872Gln <sup>a</sup>	Missense	1.00
Patient L	De novo	g.52099294G>C	Val410Leu	Missense	0.03
Patient M	De novo	g.52183202+1_+4del <sup>c</sup>	Pro1428_Lys1473del (predicted)	Splice-site	NA
Patient N	De novo	g.52200218G>A	Ala1650Thr	Missense	1.00
Patient O	De novo	g.52082570A>G	Asn215Arg	Missense	0.98
Patient P	De novo	g.52188404C>G	Val1592Leu	Missense	0.77
Patient Q	De novo	g.52200884C>T	Arg1872Trp <sup>a</sup>	Missense	1.00

Abbreviation: NA = not available.

Variants are annotated according to the transcript ENST00000354534.

<sup>a</sup>Mutations affecting the same amino acid.

<sup>b</sup>Reported in reference 9.

<sup>c</sup>Splice-site mutation.

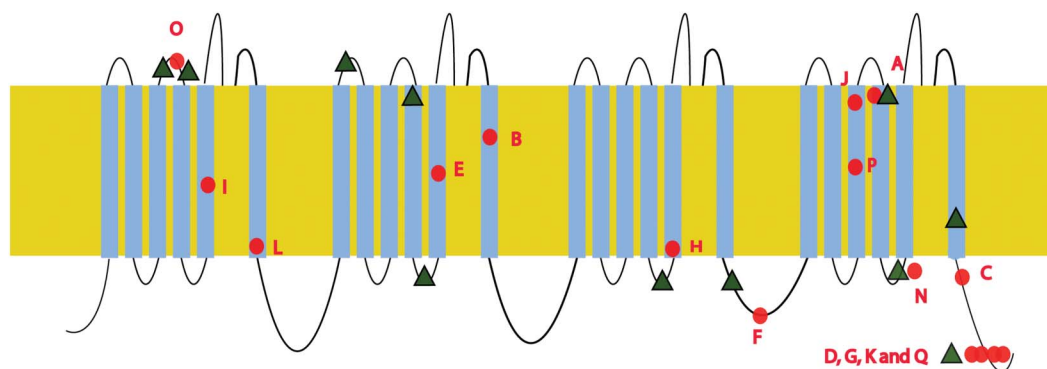
patient G had sudden unexplained death in epilepsy (SUDEP) at 5 years.

**MRI studies.** Brain MRI at onset was normal (9), abnormal (4), or not available (4). The abnormal findings included cerebral atrophy (3) and hypoplasia of the corpus callosum (1). Patients B and C had

normal MRI at seizure onset with follow-up studies at ages 5 years and 15 months, respectively, showing diffuse atrophy.

**EEG findings.** EEG at onset was available for 14 of 17 patients and showed focal or multifocal epileptiform activity in 6 patients and was normal in 8 (table 3).

**Figure 1 De novo SCN8A mutations in patients with epileptic encephalopathy reported in this study (red dots) and in the literature (green triangles)<sup>8,9,12,13,16,23</sup>**



The letters associated with each dot correspond to the patient identification in the tables. Mutations are observed in the cytoplasmic loops, extracellular loops, and transmembrane helices. There are 3 amino acid residues that are found to be recurrently mutated: 5 occurrences at Arg1872, 3 of Arg1617Gln, and 2 of Ala1650Thr have been reported.

**Table 2** Clinical features of patients with *SCN8A* mutations

Patient identification (sex)	Current age	Seizure onset	Seizure type at onset	Other seizure types	Seizure outcome	Development before seizure onset	Development after seizure onset	Intellect	MRI	Diagnosis at clinical assessment	Additional features
<b>Patient A (F)</b>	Deceased (3 y)	5.5 mo	GTC during afebrile gastroenteritis	C, T, AA, M	Continuing sz until death	Normal	Delayed with regression	Severe ID	At onset: normal	Unclassified EE	No speech, loss of eye contact from 30 mo, hypotonia, dystonia, wheelchair-bound
<b>Patient B (F)</b>	6 y	2.5 mo	FC	C, GTC, A, E, AA	T, FC evolving to BC	Normal	Delayed with regression	Severe ID	At onset: normal; 5 y: diffuse atrophy	Unclassified EE	No speech, loss of eye contact from 24 mo, hypotonia, dystonic cerebral palsy, stereotypies, wheelchair-bound
<b>Patient C (M)</b>	4 y	3 mo	F, GTC, E with eye deviation	F, T, E, GTC preceded by apnea and deep cyanosis, FC evolving to BC, G, SE	F, GTC, SE, FO	Normal	Delayed with regression	Severe ID	At onset: normal; 15 mo: cerebral atrophy	Dravet-like	No speech, loss of eye contact, generalized hypotonia, fatigable muscle weakness and ptosis, dyskinesia, stereotypic hand movements, not sitting, autistic features
<b>Patient D (F)</b>	16 y	7 mo	FC evolving to BC	C, GTC, A, AA, rare myoclonic jerks, F, SE	FC evolving to BC	Normal	Delayed	Moderate ID	NA	Dravet-like	Repetitive language, macrocephaly, generalized hyperreflexia, clumsiness, autistic features
<b>Patient E (F)</b>	4 y	9 mo	Nocturnal BC with cyanosis	BC with cyanosis	Sz-free for 6 mo, except for a single short sz	Delayed	Delayed	Moderate ID	NA	Unclassified EE	Speaks single words, moderate hypotonia, ADHD
<b>Patient F (F)</b>	8 mo	1 d	Nocturnal M	Nocturnal T, SE, perioral cyanosis	T	NA	Delayed	Moderate ID	2.5 mo: circumscribed hypoplasia of corpus callosum	Unclassified EE	Hypotonic, movement disorder
<b>Patient G (F)</b>	Deceased (5 y)	4 mo	T with SE and cyanosis	GTC with SE, T with cyanosis, AB with myoclonic jerks, E	Until death continuing sz	Normal	Delayed	Severe ID	At onset and follow-up: normal	EIEE	No speech, loss of eye contact, hypertonia, generalized hyperreflexia, wheelchair-bound
<b>Patient H (M)</b>	12 y	18 mo	GTC with SE	FD, M, SE, T	GTC, FD	Delayed	Delayed with regression	Moderate ID	Nonspecific foci of high signal in white matter of frontal lobes	Unclassified EE	Ataxia, autistic features
<b>Patient I (F)</b>	10 y	4 mo	Clusters of GTC	T, GTC, FD, focal M	GTC, A, FD	Normal	Delayed with regression	Moderate ID	At onset and follow-up: normal	Dravet-like	Speaks short sentences, ataxic gait
<b>Patient J (M)</b>	44 y	4 mo	T	GTC	Sz-free for 17 y	Normal	Delayed	Mild ID	Bilateral reduction in cerebellar volume	"Vaccine encephalopathy"	None
<b>Patient K (F)</b>	7 y	4 mo	FC evolving to BC	GTC, SE, T	Ongoing	Normal	Delayed with regression	Moderate to severe ID	Normal	Unclassified EE	Reflex component to seizures with fall or pain, autistic features
<b>Patient L (M)</b>	19 mo	4 mo	E	None	E	Delayed	Delayed	Severe ID	5 mo: myelination delayed, residual superficial hemosiderin with frontal predominance	Unclassified EE	No speech, loss of eye contact, severe hypotonia, secondary microcephaly
<b>Patient M (F)</b>	4 y	10 mo	Clonic alternating	BC with tonic posture, prolonged BC with vomiting	Ongoing	Delayed	Delayed	Severe ID	17 mo: normal	EIEE	No speech, loss of eye contact, severe extrapyramidal movement disorder, severe dystrophy, gastroparesis, microcephaly
<b>Patient N (F)</b>	NA	3 mo	FC evolving to BC	SE, asymmetric T, AA	Asymmetric F, asymmetric C	Delayed	Delayed	Severe ID	NA	EOEE	Quadriplegia with dystonic posturing, dystonic-dyskinetic movement disorder, hypotonia, wheelchair-bound

Continued

**Table 2** Continued

Patient identification (sex)	Current age	Seizure onset	Seizure type at onset	Other seizure types	Seizure outcome	Development before seizure onset	Development after seizure onset	Intellect	MRI	Diagnosis at clinical assessment	Additional features
Patient O (F)	7 y	6 mo	E	Episodes of prolonged staring	Sz-free for 3 y	Normal	Delayed	Severe ID	Infancy: normal	Rett-like syndrome	No speech, loss of eye contact, hand stereotypies similar to Rett syndrome but superimposed by general chorea involving arms, legs, and body, wheelchair-bound
Patient P (F)	8 mo	5 mo	FO with eye deviation, lip smacking, apnea, perioral cyanosis	None	Sz-free on oxcarbazepine for 6 wk	Normal	Delayed	NA	At onset: mild diffuse white matter volume loss with prominent frontal arachnoid spaces	Unclassified EE	Hypotonia
Patient Q (F)	6 y	1 mo	Prolonged T	FO evolving to BC, M, TC, SE, nonconvulsive SE	Ongoing	Normal	Delayed with regression	Severe ID	Normal	EOEE	Dystonia, intention tremor with some spasticity

Abbreviations: A = atonic seizures; AA = atypical absence seizures; AB = absence seizures; ADHD = attention deficit hyperactivity disorder; BC = bilateral convulsive seizures; C = clonic seizures; E = epileptic spasms; EE = epileptic encephalopathy; EIEE = early infantile epileptic encephalopathy; EOEE = early onset epileptic encephalopathy; F = febrile seizures; FC = focal clonic seizures; FD = focal dyscognitive seizures; FO = focal seizures; G = gelastic seizures; GTC = generalized tonic-clonic seizures; ID = intellectual disability; M = myoclonic seizures; NA = not available; SE = status epilepticus; sz = seizure; T = tonic seizures; TC = tonic-clonic seizures.

Fifteen patients developed an abnormal EEG showing moderate to severe background slowing (12) and focal or multifocal sharp waves or spikes (12), most often observed in the temporal region (8). Patients A, B, C, D, and G showed almost continuous delta slowing in the temporo-parietal-occipital regions, with superimposed beta frequencies in some and bilateral asynchronous spikes or sharp waves (figure 2).

**DISCUSSION** Our results confirm the importance of *SCN8A* as a cause of EEs with 7 of 683 (1%) previously unexplained cases having a causative mutation. Mutations frequently arise de novo, but we show that inherited mutations from a mosaic parent can also occur. We describe the phenotype of 17 cases with *SCN8A* encephalopathy bringing the total number of patients with EE due to *SCN8A* mutations to 30.<sup>8-13,16</sup>

Combining our series with previously published cases, seizures began in infancy at a mean age of 5 months, typically with focal seizures. Tonic-clonic seizures were seen in the majority of cases (18/30). Epileptic spasms were reported in one-third of cases either at presentation or as the disease evolved.<sup>8,11</sup> Myoclonic and absence seizures occurred in approximately 30% of patients. Seizures were usually refractory. Notably sodium channel blockers appeared effective and allowed 4 of our patients a period of seizure freedom.

Development was normal before seizure onset in 13 of 23 cases (57%) with subsequent developmental slowing often with regression. In the remaining patients, development was not normal and regression sometimes occurred with seizure onset. In an additional 8 cases, development before seizures was not fully documented.<sup>13</sup> Half of the patients had severe intellectual disability, and autistic features were noted in some cases (table 2). Hypotonia was often observed as well as movement disorders in some individuals manifesting as dystonia and choreoathetosis. Three patients exhibited atrophy on brain MRI, a finding that has been described in at least 2 previously reported patients.<sup>13</sup> The atrophy is more likely to be due to the underlying sodium channelopathy, but we cannot exclude that it is secondary to treatment of the seizure disorder.

A key differential diagnosis of *SCN8A* encephalopathy is Dravet syndrome, which is due to *SCN1A* mutations in >80% of cases.<sup>17</sup> Indeed, several of our patients were referred for genetic testing with a diagnosis of Dravet syndrome. While *SCN8A* encephalopathy shares some features with Dravet syndrome, there are notable differences. The mean age at onset of 5 months is similar to Dravet syndrome; however, the range of 0 days to 18 months in *SCN8A* encephalopathy is broader than that seen in Dravet syndrome. In contrast to the pronounced

**Table 3 EEG features of patients with SCN8A mutations**

Patient identification (sex)	EEG at onset	EEG at follow-up	Last EEG
Patient A (F)	Normal	2 y: bilateral temporo-occipito-parietal delta activity with superimposed beta activity; single left temporal S/SSW	2 y: bilateral temporo-occipito-parietal delta activity with superimposed beta activity; single left temporal S/SSW
Patient B (F)	Normal	21 mo: bilateral temporo-occipito-parietal delta activity with superimposed beta activity and bilateral asynchronous temporo-occipito-parietal S/SSW	2 y: bilateral temporo-occipito-parietal delta activity with superimposed beta activity and bilateral asynchronous temporo-occipito-parietal S/SSW
Patient C (M)	Normal	Frequent focal/multifocal activity, diffuse slow high-voltage activity	2 y: focal discharges predominantly in left hemisphere, slow diffuse irritative dysrhythmic activity
Patient D (F)	Normal	Multifocal epileptic activity with persistent focus in the left temporal region	13 y: multifocal epileptic activity
Patient E (F)	Discrete left temporal spikes	Normal	Normal
Patient F (F)	Normal	5 mo interictal EEG: temporo-occipital delta activity; 5 mo ictal EEG: generalized rhythmic epileptic activity with 4–5/s irregular SW followed by generalized slowing	8 mo: temporo-occipital slowing with rhythmic theta-delta activity, sporadic SW in the left temporal region
Patient G (F)	NA	17 mo: moderate background slowing	4 y: bilateral asynchronous sharp waves in temporo-centro-parietal regions
Patient H (M)	NA	9 y interictal EEG: generalized background slowing, multifocal activity; ictal EEG: paroxysmal fast activity during tonic seizures	10 y: frequent SW activity from left frontal region; bilateral frontal activity seen with tonic posturing
Patient I (F)	Normal	6 mo: right temporal SW complexes; 9 mo: 2 subclinical EEG seizures arising from central midline	7.5 y: normal
Patient J (M)	Minor slowing over the right midparietal and midtemporal regions	13 mo: normal	13 y: normal
Patient K (F)	NA	10 mo: slow background activity with generalized or multifocal epileptic activity, hypsarrhythmia, multifocal discharges	3 y: continuous slowing, bilateral SW activity
Patient L (M)	Hypsarrhythmia in the posterior regions and during sleep	Hypsarrhythmia in the posterior regions and during sleep	19 mo: hypsarrhythmia in the posterior regions and during sleep
Patient M (F)	Bifrontal delta activity with intermittent spikes	18 mo: bifrontal delta activity with intermittent spikes	3 y, 8 mo: frontotemporal or posterior delta activity
Patient N (F)	Multifocal epileptiform activity	Slow waves in the central regions, frontal asynchronous epileptiform activity	Slow background, multifocal spikes
Patient O (F)	Multifocal epileptiform activity with secondary generalization; epileptic spasm captured with EEG decrement after event	20 mo: bilateral temporo-occipito-parietal delta activity with superimposed beta activity	20 mo: bilateral temporo-occipito-parietal delta activity with superimposed beta activity
Patient P (F)	Frequent central spike and wave discharges from vertex and right central region; normal background	Repetitive spike discharges over vertex and left and right central head regions, slow posterior rhythm	Repetitive spike discharges over vertex and left and right central head regions, slow posterior rhythm
Patient Q (F)	Normal	3 mo: bilateral occipital discharges, tonic seizures captured with left occipital onset followed by bilateral spread	6 y: diffuse slowing

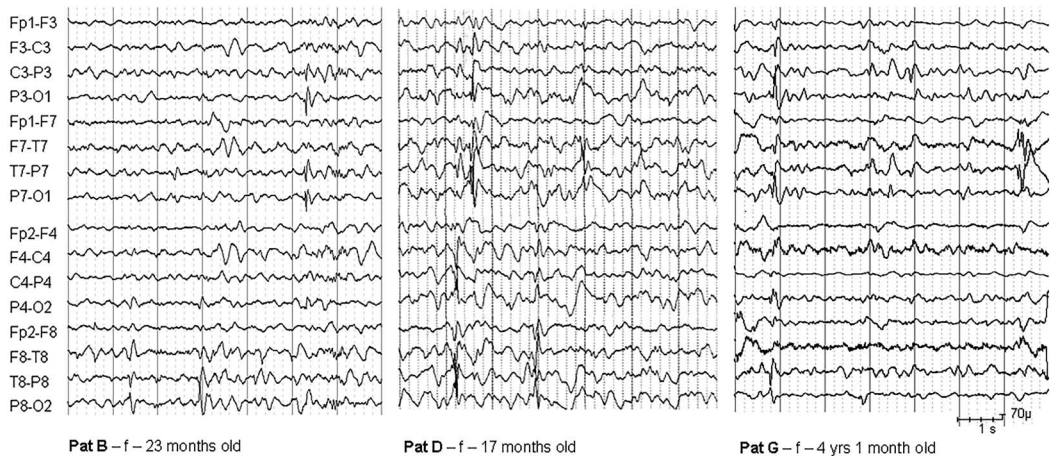
Abbreviations: NA = not available; S/SSW = spike/spike and slow wave; SW = spike wave.

susceptibility to seizures with fever in Dravet syndrome, only 2 of 17 patients with *SCN8A* encephalopathy had seizures with fever. Seizure types also differ. Seven of 17 patients with *SCN8A* encephalopathy had spasms, which are not a feature of Dravet syndrome. Myoclonic seizures, which are common in Dravet syndrome, only occurred in 5 patients. Hypotonia and movement disorders are not features of Dravet syndrome. The EEG findings also differ in that generalized spike wave was not seen in *SCN8A* encephalopathy and is a hallmark of Dravet syndrome after 1 to 2 years of age. We also observed that the sodium channel blockers carbamazepine, oxcarbazepine, and

phenytoin rendered a few of our *SCN8A* cases seizure-free but are reported to exacerbate seizures in Dravet syndrome.<sup>18,19</sup> Two of the patients in our study died aged 3 and 5 years, one of SUDEP, which has been reported in one other patient with an *SCN8A* mutation.<sup>8</sup> Larger series of patients with mutations in *SCN8A* will be required to determine the overall risk of SUDEP in this patient population.

The mutations reported in this study were distributed throughout the protein (figure 1). None were located in the known protein–protein interaction regions of *SCN8A*.<sup>20</sup> In 4 patients, we observed mutations altering the same amino acid (1872, transcript

**Figure 2** Interictal EEG features of patients B, D, and G



EEG discharges in the temporo-parieto-occipital regions consist of bilateral independent spikes and sharp waves, and intermittent biposterior quadrant delta activity. EEG parameters are as follows: speed: 20 mm/s; sensitivity: 300  $\mu$ V/mm; bandpass filter: 1,600–70 Hz; notch off. Pat = patient.

ID ENST00000354534); alteration of this residue was previously reported in a patient with EE.<sup>13</sup> There are 2 additional recurrent mutations: the Arg1617Gln reported here has been seen in 2 additional patients with severe intellectual disability and epilepsy (3 mutations total) and the Ala1650Thr in one additional patient (2 mutations total).<sup>9,13</sup> These findings reveal amino acid position 1872 as a mutation hotspot and positions 1617 and 1650 as likely emerging hotspots. It will be important for future studies to establish the effect of these pathogenic mutations on SCN8A function. It is likely that pathogenic SCN8A missense mutations act in a gain-of-function manner, similar to the de novo Asn1768Asp and Thr1761Ile mutations identified in patients with EE.<sup>8,16</sup> In vitro studies of the Asn1768Asp demonstrated that the mutant channel had incomplete channel activation, increase in persistent sodium current, and a depolarizing shift in voltage dependence of steady-state fast inactivation.<sup>8</sup> Similar studies for the Thr767Ile mutation demonstrated analogous biophysical properties of the mutant channel.<sup>16</sup> Collectively, these results suggest that the mutant channels lead to increased excitability of the neuron, and that this feature is likely to underlie the epilepsy in patients with de novo missense mutations.

We identified a single patient with a de novo splice-site mutation that is predicted to give rise to an in-frame deletion of 46 amino acids because of exon skipping. This deletion would remove a portion of the third transmembrane domain and the intracellular loop that serves as the inactivation gate.<sup>20</sup> It will be important to establish whether this mutation also acts in a gain-of-function manner. Loss-of-function mutations seem to be associated with intellectual

disability and ataxia in humans,<sup>20</sup> and homozygous null mice present with ataxia and impaired learning.<sup>21,22</sup> Future sequence-based studies in patients with these disorders, as well as functional validation of the effects of different types of SCN8A mutations, are needed to determine whether distinct mutations in SCN8A cause diverse neurologic manifestations.

It is also worth noting that despite the identification of 30 patients with de novo SCN8A mutations throughout the protein, there does not appear to be any genotype–phenotype correlation regarding the position of the mutation and the seizure onset, types, or severity of clinical presentation. This is exemplified by the recurrent missense mutation at protein position 1872, which has now been described in 5 patients, 4 here and one previously.<sup>13</sup> Even though these individuals have the same primary genetic lesion, clinical presentation was variable. Onset of seizures ranged from 1 to 7 months; 3 patients first presented with tonic or tonic-clonic seizures, and 2 with focal clonic seizures that evolved to bilateral convulsive seizures. Multiple seizure types were subsequently present in all cases, and intellectual disability ranged from moderate to severe. The only feature common to all patients was normal development before seizure onset. A greater number of patients with SCN8A mutations will need to be identified in the future to determine whether there are any phenotypic or genotypic subgroups that may be delineated.

SCN8A mutations are found in 1% of patients with previously unexplained infantile-onset EE. We present the largest series of patients with pathogenic SCN8A mutations to date. We observed a wide spectrum of phenotypes of the EEs in all patients; seizure onset, type, and neurodevelopment and progression

were all variable within our cohort. An emerging phenotype seems to consist of seizure onset by 18 months with multiple seizure types and developmental slowing. Whether this lack of a distinct clinical presentation is attributable to the relatively small number of patients who have been identified or reflects a true spectrum remains to be seen. Of note, despite the lack of clear phenotype, the observation that sodium channel blockers may be effective in these cases underscores the importance of a molecular diagnosis. It also argues for unbiased genetic testing as part of a gene panel or exome in children with EEs. Our study highlights the power of such an approach, whereby a genotype first paradigm can advance our understanding of the clinical presentation of patients, which may in turn guide therapeutic choices in the future, enable recognition of associated comorbidities, and inform prognostic counseling.

### URLS/RESOURCES

PolyPhen-2: <http://genetics.bwh.harvard.edu/pph2/>; SIFT: <http://sift.jcvi.org/>; Exome Variant Server: <http://evs.gs.washington.edu/EVS/>; MutationTaster: <http://www.mutationtaster.org/>; GATK: <http://www.broadinstitute.org/gatk/>.

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## DISCLOSURE

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