# The phenotypic spectrum of *SCN8A* encephalopathy

Jan Larsen, MSc*‡
Gemma L. Carvill, PhD*
Elena Gardella, MD, PhD
Gerhard Kluger, MD
Gudrun Schmiedel, MD
Nina Barisic, MD, PhD‡
Christel Depienne, PhD‡
Eva Brilstra, MD‡
Yuan Mang, MSc
Jens Erik Klint Nielsen, MD
Martin Kirkpatrick, MD
David Goudie, MD
Rebecca Goldman, MD
Johanna A. Jähn, MD‡
Birgit Jepsen, MD
Deepak Gill, FRACP
Miriam Döcker, MSc
Saskia Biskup, MD, PhD
Jacinta M. McMahon, BSc
Bobby Koeleman, PhD‡
Mandy Harris, MD
Kees Braun, MD, PhD
Carolien G.F. de Kovel, PhD‡
Carla Marini, MD, PhD‡
Nicola Specchio, MD, PhD
Tania Djémié, MSc‡
Sarah Weckhuysen, MD, PhD‡
Niels Tommerup, MD, PhD‡
Monica Troncoso, MD, PhD
Ledia Troncoso, MD, PhD
Andrea Bevot, MD
Markus Wolff, MD
Helle Hjalgrim, MD, PhD‡

*Author list continued on next page*

## ABSTRACT

**Objective:** *SCN8A* encodes the sodium channel voltage-gated α8-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, α 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.[1] 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 α1-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 α-subunit genes *SCN1A* and *SCN2A* are

*These authors contributed equally to this work.
Authors' affiliations are listed at the end of the article.
EuroEPINOMICS RES Consortium coinvestigators are listed on the *Neurology*® Web site at Neurology.org.
Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Renzo Guerrini, MD‡
Ingrid E. Scheffer, MBBS, PhD*
Heather C. Mefford, MD, PhD*
Rikke S. Møller, PhD*‡
‡On behalf of the EuroEPINOMICS RES Consortium CRP

Correspondence to
Prof. Scheffer:
i.scheffer@unimelb.edu.au
Dr. Mefford:
hmefford@uw.edu
or Dr. Møller:
rimo@filadelfia.dk

associated with a wide spectrum of epilepsy syndromes ranging from EEs to mild disorders such as febrile seizures.[2–6] Recently, de novo mutations in *SCN8A*, encoding one of the main voltage-gated sodium channel subunits ($Na_v1.6$) in the brain,[7] have been described in patients with severe epilepsy,[8–13] 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.[1]

**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).[14] Data analysis, variant calling and filtering, as well as depth of coverage statistics were generated as previously described.[10] 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.[10] 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[10] 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[15]—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

Editorial, page 446

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[a] | 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[a] | Missense | 1.00 |
| Patient H | Inherited, somatic 13% mosaic | g.52180374C>G | Leu1331Val[b] | 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[a] | Missense | 1.00 |
| Patient L | De novo | g.52099294G>C | Val410Leu | Missense | 0.03 |
| Patient M | De novo | g.52183202+1_+4del[c] | 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[a] | Missense | 1.00 |

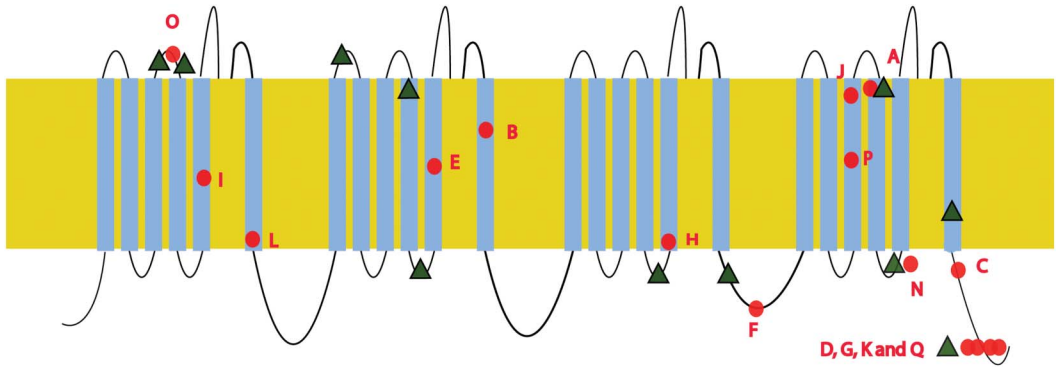
Abbreviation: NA = not available.
Variants are annotated according to the transcript ENST00000354534.
[a] Mutations affecting the same amino acid.
[b] Reported in reference 9.
[c] Splice-site mutation.

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)[8,9,12,13,16,23]




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 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Patient A (F)** | 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 |
| **Patient B (F)** | 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 |
| **Patient C (M)** | 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 |
| **Patient D (F)** | 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 |
| **Patient E (F)** | 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 |
| **Patient F (F)** | 8 mo | 1 d | Nocturnal M | Nocturnal T, SE, perioral cyanosis | T | NA | Delayed | Moderate ID | 2.5 mo: circumscript hypoplasia of corpus callosum | Unclassified EE | Hypotonic, movement disorder |
| **Patient G (F)** | 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 |
| **Patient H (M)** | 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 |
| **Patient I (F)** | 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 |
| **Patient J (M)** | 44 y | 4 mo | T | GTC | Sz-free for 17 y | Normal | Delayed | Mild ID | Bilateral reduction in cerebellar volume | "Vaccine encephalopathy" | None |
| **Patient K (F)** | 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 |
| **Patient L (M)** | 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 |
| **Patient M (F)** | 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 |
| **Patient N (F)** | NA | 3 mo | FC evolving to BC | SE, asymmetric T, AA | Asymmetric F, asymmetric C | Delayed | Delayed | Severe ID | NA | EOEE | Quadriparesis 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 starring | 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.[8–13,16]

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.[8,11] 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.[13] 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.[13] 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.[17] 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

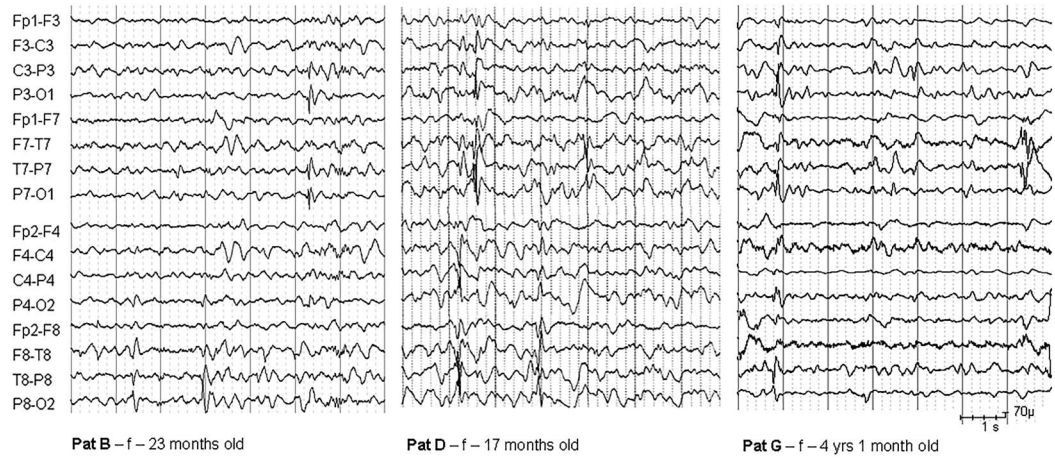
| Patient identification (sex) | EEG at onset | EEG at follow-up | Last EEG |
|---|---|---|---|
| **Patient A (F)** | Normal | 2 y: bilateral temporo-occipito-parietal delta activity with superimposed beta activity; single left temporal S/SSW | 2 y: bilateral temporo-occipito-parietal delta activity 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.[18,19] 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.[8] 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.[20] 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 μV/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.[13] 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).[9,13] 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 Thr176Ile mutations identified in patients with EE.[8,16] 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.[8] Similar studies for the Thr767Ile mutation demonstrated analogous biophysical properties of the mutant channel.[16] 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.[20] 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,[20] and homozygous null mice present with ataxia and impaired learning.[21,22] 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.[13] 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/.

## AUTHOR AFFILIATIONS

From the Danish Epilepsy Centre (J.L., E.G., B.J., H.H., R.S.M.); ICMM (J.L., Y.M., N.T.), Panum Institute, University of Copenhagen, Denmark; Department of Pediatrics (G.L.C., H.C.M.), Division of Genetic Medicine, University of Washington, Seattle; Clinic for Neuropediatrics and Neurorehabilitation (G.K.), Epilepsy Center for Children and Adolescents, Schön Klinik Vogtareuth, Germany; Departments of Medical Genetics and Child Neurology (E.B., B.K., K.B.), University Medical Center Utrecht, the Netherlands; Clinic for Children and Adolescents (G.S.), Esslingen, Germany; Université Pierre et Marie Curie (C.D.), Faculté de Médecine, Paris; INSERM UMRS 975 (C.D.), CNRS UMR 725, Paris, France; Institüt für Humangenetik (C.D.), Universität Würzburg, Germany; Department of Clinical Medicine (J.E.K.N.), Section of Gynaecology, Obstetrics and Paediatrics, Roskilde Hospital; UMC Utrecht (C.G.F.d.K.), Medical Genetics Research; Child Neurology Service Hospital San Borja Arriarán (M.T., L.T.), University of Chile, Santiago; Florey Institute Melbourne (I.E.S.); Epilepsy Research Centre (J.M.M., I.E.S.), Department of Medicine, University of Melbourne, Austin Health; Department of Paediatrics (I.E.S.), Royal Children's Hospital, University of Melbourne, Australia; Department of Pediatric Neurology (A.B., M.W.), University Children's Hospital, Tübingen, Germany; Institute of Regional Health Services Research (J.L., E.G., H.H., R.S.M.), Faculty of Health Sciences, University of Southern Denmark; Department of Pediatrics (N.B.), University Hospital Centre Zagreb; Neurogenetics Group (T.D., S.W.), Department of Molecular Genetics, VIB, Antwerp; Laboratory of Neurogenetics (T.D., S.W.), Institute Born-Bunge, University of Antwerp, Belgium; Child Neurology Unit (N.S., R. Guerrini), Children's Hospital A. Meyer and University of Florence; Child Neurology Unit (C.M.), Pediatric Hospital Bambino Gesú, Rome, Italy; Tayside Children's Hospital (M.K., R. Goldman), Dundee; Ninewells Hospital and Medical School (D. Goudie), Dundee, UK, for the DDD Study; CeGaT GmbH–Center for Genomics and Transcriptomics (M.D., S.B.), Tübingen; University Medical Center Schleswig-Holstein (J.A.J.), Christian-Albrechts University, Kiel, Germany; T.Y. Nelson Department of Neurology (D. Gill), The Children's Hospital at Westmead, Sydney, Australia; and Department of Neurology (M.H.), Division of Child Neurology, Riley Hospital for Children, Indiana University Health.

## AUTHOR CONTRIBUTIONS

Jan Larsen: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data, statistical analysis, study supervision. Gemma Louise Carvill: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval. Elena Gardella: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval. Gerhard Kluger: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval, acquisition of data. Gudrun Schmiedel: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval, acquisition of data. Nina Barisic: analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data, study supervision. Christel Depienne: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval, contribution of vital reagents/tools/patients. Eva Brilstra: analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data. Yuan Mang: analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval. Jens Erik Klint Nielsen: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval. Martin Kirkpatrick: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval. David Goudie: analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data. Rebecca Goldman: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval, acquisition of data. Johanna A. Jähn: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval, study supervision. Birgit Jepsen: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval. Deepak Gill: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval. Miriam Döcker: analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval. Saskia Biskup: analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data. Jacinta M. McMahon: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data. Bobby Koeleman: drafting/revising the manuscript, study concept or design, accepts responsibility for conduct of research and will give final approval, acquisition of data. Mandy O. Harris: analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval. Kees Braun: analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data. Carolien de Kovel: analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data. Carla Marini: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval, acquisition of data. Nicola Specchio: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data. Tania Djémié: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data. Sarah Weckhuysen: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval, contribution of vital reagents/tools/patients, acquisition of data. Niels Tommerup: study concept or design, accepts responsibility for conduct of research and will give final approval, contribution of vital reagents/tools/patients. Monica Troncoso: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval, contribution of vital reagents/tools/patients. Ledia Troncoso: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval, contribution of vital reagents/tools/patients. Andrea Bevot: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data. Markus Wolff: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of

data. Helle Hjalgrim: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval, acquisition of data, study supervision. Renzo Guerrini: drafting/revising the manuscript, accepts responsibility for conduct of research and will give final approval, acquisition of data. Ingrid E. Scheffer: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data, study supervision, obtaining funding. Heather C. Mefford: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, contribution of vital reagents/tools/patients, acquisition of data, study supervision, obtaining funding. Rikke S. Møller: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, study supervision.


## ACKNOWLEDGMENT

The authors thank the families for their participation in this study.

## STUDY FUNDING

The research is supported by Wilhelm Johannsen Centre for Functional Genome Research, ICMM, University of Copenhagen, NIH (National Institute of Neurological Disorders and Stroke 1R01NS069605) to H.C.M., the American Epilepsy Society and the Lennox and Lombroso Fund to G.L.C., National Health and Medical Research Foundation to S.F.B. and I.E.S., the Lundbeck Foundation (2012–6206) to N.T., Institüt für Humangenetik, Universität Würzburg, Patient P was part of the DDD Study, which presents independent research commissioned by the Health Innovation Challenge Fund (grant HICF-1009-003), a parallel funding partnership between the Wellcome Trust and the Department of Health, and the Wellcome Trust Sanger Institute (grant WT098051). The research team acknowledges the support of the National Institute for Health, funds from the Italian Minister of Health, RF 2009-1525669; special thanks to Eurocores program EuroEPINOMICS of the European Science Foundation (P.D.J.).


## DISCLOSURE

J. Larsen, G. Carvill, E. Gardella, G. Kluger, G. Schmiedel, N. Barisic, C. Depienne, E. Brilstra, Y. Mang, J. Nielsen, M. Kirkpatrick, D. Goudie, R. Goldman, J. Jähn, B. Jepsen, D. Gill, M. Döcker, S. Biskup, J. McMahon, B. Koeleman, M. Harris, K. Braun, C. de Kovel, C. Marini, N. Specchio, T. Djémié, S. Weckhuysen, N. Tommerup, M. Troncoso, L. Troncoso, A. Bevot, M. Wolff, H. Hjalgrim, and R. Guerrini report no disclosures relevant to the manuscript. I. Scheffer has served on scientific advisory boards for UCB and Janssen-Cilag EMEA; serves on the editorial boards of the *Annals of Neurology*, *Neurology*®, and *Epileptic Disorders*; may accrue future revenue on pending patent WO61/010176 (filed 2008): Therapeutic Compound; has received speaker honoraria from GlaxoSmithKline, Athena Diagnostics, UCB, Biocodex, and Janssen-Cilag EMEA; has received funding for travel from Athena Diagnostics, UCB, Biocodex, GlaxoSmithKline, and Janssen-Cilag EMEA; and receives/has received research support from the National Health and Medical Research Council of Australia, NIH, Australian Research Council, Health Research Council of New Zealand, CURE, American Epilepsy Society, US Department of Defense Autism Spectrum Disorder Research Program, the Jack Brockhoff Foundation, the Shepherd Foundation, Perpetual Charitable Trustees, and The University of Melbourne. H. Mefford has received a grant from the NIH/National Institute of Neurological Disorders and Stroke, Burroughs Wellcome Fund, and is a consultant for the Simons Foundation (SFARI Gene Advisory Board). R. Møller reports no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.


*Received May 15, 2014. Accepted in final form September 12, 2014.*


## REFERENCES


1. Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. Epilepsia 2010;51:676–685.
2. Heron SE, Crossland KM, Andermann E, et al. Sodium-channel defects in benign familial neonatal-infantile seizures. Lancet 2002;360:851–852.
3. Escayg A, MacDonald BT, Meisler MH, et al. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. Nat Genet 2000;24:343–345.
4. Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. Am J Hum Genet 2001;68:1327–1332.
5. Herlenius E, Heron SE, Grinton BE, et al. SCN2A mutations and benign familial neonatal-infantile seizures: the phenotypic spectrum. Epilepsia 2007;48:1138–1142.
6. Oliva M, Berkovic SF, Petrou S. Sodium channels and the neurobiology of epilepsy. Epilepsia 2012;53:1849–1859.
7. Lorincz A, Nusser Z. Molecular identity of dendritic voltage-gated sodium channels. Science 2010;328:906–909.
8. Veeramah KR, O'Brien JE, Meisler MH, et al. De novo pathogenic SCN8A mutation identified by whole-genome sequencing of a family quartet affected by infantile epileptic encephalopathy and SUDEP. Am J Hum Genet 2012;90:502–510.
9. Rauch A, Wieczorek D, Graf E, et al. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. Lancet 2012;380:1674–1682.
10. Carvill GL, Heavin SB, Yendle SC, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. Nat Genet 2013;45:825–830.
11. Epi4K Consortium, Epilepsy Phenome/Genome Project; Allen AS, Berkovic S, Cossette P, et al. De novo mutations in epileptic encephalopathies. Nature 2013;501:217–221.
12. Vaher U, Noukas M, Nikopensius T, et al. De novo SCN8A mutation identified by whole-exome sequencing in a boy with neonatal epileptic encephalopathy, multiple congenital anomalies, and movement disorders. J Child Neurol 2013;29:NP202–NP206.
13. Ohba C, Kato M, Takahashi S, et al. Early onset epileptic encephalopathy caused by de novo SCN8A mutations. Epilepsia 2014;55:994–1000.
14. O'Roak BJ, Vives L, Fu W, et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science 2012;338:1619–1622.
15. Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 2010;7:575–576.
16. Estacion M, O'Brien JE, Conravey A, et al. A novel de novo mutation of SCN8A (Na1.6) with enhanced channel activation in a child with epileptic encephalopathy. Neurobiol Dis 2014;69:117–123.
17. Helbig I, Lowenstein DH. Genetics of the epilepsies: where are we and where are we going? Curr Opin Neurol 2013;26:179–185.
18. Chiron C. Current therapeutic procedures in Dravet syndrome. Dev Med Child Neurol 2011;53(suppl 2):16–18.
19. Arzimanoglou A. Dravet syndrome: from electroclinical characteristics to molecular biology. Epilepsia 2009;50(suppl 8):3–9.
20. O'Brien JE, Meisler MH. Sodium channel (Na1.6): properties and mutations in epileptic encephalopathy and intellectual disability. Front Genet 2013;4:213.

21. Woodruff-Pak DS, Green JT, Levin SI, Meisler MH. Inactivation of sodium channel Scn8A (Na-sub(v)1.6) in Purkinje neurons impairs learning in Morris water maze and delay but not trace eyeblink classical conditioning. Behav Neurosci 2006;120:229–240.
22. Burgess DL, Kohrman DC, Galt J, et al. Mutation of a new sodium channel gene, Scn8a, in the mouse mutant "motor endplate disease". Nat Genet 1995;10:461–465.
23. Allen AS, Berkovic SF, Cossette P, et al. De novo mutations in epileptic encephalopathies. Nature 2013;501:217–221.