Original article

*Epileptic Disord 2014; 16 (1): 13-8*

# Confirming an expanded spectrum of *SCN2A* mutations: a case series

Dena Matalon, Ethan Goldberg, Livija Medne, Eric D. Marsh

Division of Child Neurology, Departments of Pediatrics and Neurology, Children's Hospital of Philadelphia and the Perelman School of Medicine at the University of Pennsylvania, Philadelphia, USA

Received August 1, 2013; Accepted January 20, 2014

**ABSTRACT** – Mutations in sodium channel genes are highly associated with epilepsy. Mutation of *SCN1A*, the gene encoding the voltage gated sodium channel (VGSC) alpha subunit type 1 (Nav1.1), causes Dravet syndrome spectrum disorders. Mutations in *SCN2A* have been identified in patients with benign familial neonatal-infantile epilepsy (BFNIE), generalised epilepsy with febrile seizures plus (GEFS+), and a small number of reported cases of other infantile-onset severe intractable epilepsy. Here, we report three patients with infantile-onset severe intractable epilepsy found to have *de novo* mutations in *SCN2A*. While a causal role for these mutations cannot be directly established, these findings contribute to growing evidence that mutation of *SCN2A* is associated with a range of epilepsy phenotypes including severe infantile-onset epilepsy.

**Key words:** *SCN2A*, early epileptic encephalopathy, channelopathy

Voltage-gated sodium channels (VGSCs) exist as macromolecular complexes composed of one alpha (pore-forming) subunit and one or more beta subunits (Brackenbury and Isom, 2011). There are 10 alpha subunit genes, 4 of which (*SCN1A*, *2A*, *3A,* and *8A*) are known to be highly expressed in the central nervous system (Catterall, 2000). Mutations of these sodium channel genes have been identified in multiple epilepsy syndromes (Meisler *et al*., 2010). Mutations in *SCN1A* cause generalised epilepsy with febrile seizures plus (GEFS+) (Escayg *et al*., 2000) and Dravet syndrome (Claes *et al*., 2001; Harkin *et al*., 2007). Mutations in *SCN2A*, which encodes Nav1.2, were first identified in patients with benign familial neonatal-infantile epilepsy (BFNIE) (Heron *et al*., 2002), an epilepsy syndrome with onset of focal seizures in the neonatal or infantile period and characterised by a benign course with remission typically in the first or second year of life (Berkovic *et al*., 2004). However, mutations in *SCN2A* have also been found in patients with GEFS+ and Dravet-like syndrome, and in a small number of patients with severe intractable infantile-onset epilepsy and infantile epileptic encephalopathies (Kamiya *et al*., 2004; Ogiwara, 2009; Shi *et al*., 2012). Homozygous disruption of *SCN2A* in mice is perinatal lethal but heterozygosity for *SCN2A* did not lead to a clear phenotype

**Correspondence:**
Eric D. Marsh
Division of Child Neurology,
Children's Hospital of Philadelphia,
Abramson Research Building- Room 502E,
3615 Civic Center Boulevard,
Philadelphia PA 19014, USA
<marshe@email.chop.edu>

doi:10.1684/epd.2014.0641

(Planells-Cases *et al.*, 2000). In contrast, transgenic mice harbouring a gain-of-function mutation in *SCN2A* exhibited spontaneous seizures originating in the temporal lobe (Kearney *et al.*, 2001). Together, the mouse and human data suggest that mutations in *SCN2A* may result in a similar spectrum to that of the *SCN1A* related epilepsies, though to date less well characterised.

Here, we report three patients with severe intractable infantile-onset epilepsy found to have novel *de novo* *SCN2A* mutations.

## Case studies

### Patient A

Patient A was born at 38 weeks gestation to unrelated, healthy parents of Puerto Rican and European descent. Pregnancy, labour, and delivery were uncomplicated. The patient exhibited poor feeding, sleepiness, and failure to thrive in the first few days of life. Seizure activity was first noted on the fifth day of life, characterised by head deviation to the left, progressing to whole-body stiffening, then clonic movements of all extremities, with apnoea and oxygen desaturation. An EEG at 2 weeks of age (*figure 1A*) showed a suppression-burst pattern and numerous multifocal seizures. MRI of the brain performed at that time was unremarkable. The patient was diagnosed with Ohtahara syndrome and treatment was initiated with phenobarbital, levetiracetam, and topiramate.

The patient continued to have intractable epilepsy. Repeat EEG performed at three months showed hypsarrhythmia, which continued until age 14 months (*figure 1B*), at which time topiramate was continually raised with resolution of both the hypsarrhythmia and tonic seizures and spasms.

Despite improved seizure control, the patient's development remains globally delayed. The patient had tapered fingers on physical examination. On neurological examination, the patient was unable to fix or track objects. She could roll over but was unable to sit up. She exhibited axial hypotonia with peripheral spasticity and prominent head lag.

Workup, including genome-wide microarray and specific genetic testing for *FOXG1*, *CDKL5*, *MECP2*, *ARX*, *SCN1A*, *STXBP1*, *SLC25A22*, *PCDH19*, pyridoxine dependent seizure panel, *SPTAN1*, *ARHGEF9*, and *KCNQ2,* was negative. Cerebrospinal fluid neurotransmitters, pyridoxal-5-phosphate (P5P), amino acids, and pyruvate/lactate were all normal, except slight elevation in 3-O-methyldopa (considered insignificant as P5P was normal). Sequencing of *SCN2A* revealed a c.1289A>G missense mutation in exon 10 leading to a glutamine to glycine (p.Glu430Gly) amino acid substitution. The mutation was absent in the parents.

### Patient B

This patient was a dizygotic twin, born at 36 weeks to unrelated, healthy parents of mixed European ancestry. There were no prenatal issues. Delivery was complicated by a breech presentation. The patient was noted to be hypotonic at birth and remained in the neonatal intensive care unit (NICU) for several days due to poor feeding. The patient continued to have failure to thrive after hospital discharge. At age 3 months, the patient developed seizures described as behavioural arrest with eye deviation, tonic arm movements, and associated oxygen desaturation. On examination, the patient displayed no rooting reflex, a weak suck, and did not actively track objects presented in her visual field. An EEG at this time showed modified hypsarrhythmia (*figure 1C*). Initial MRI of the brain was unremarkable. Seizures evolved to include partial, myoclonic, and atonic seizures. Treatment with topiramate was initiated and after medication adjustments, good seizure control was achieved.

At the last follow-up visit, at age 39 months, her epilepsy was relatively well controlled with levetiracetam monotherapy but EEG remained abnormal (*figure 1D*). Repeat MRI of the brain demonstrated interval supratentorial volume loss, diminutive corpus callosum with deficient splenium, and non-specific white matter signal abnormality. The patient's development remained globally delayed. Physical examination was significant for mild dysmorphisms, including plagiocephaly, tapered fingers, and bushy eyebrows. She exhibited cortical visual impairment and inconsistent behavioural response to sound, as well as diffuse hypotonia. The patient displayed involuntary choreiform movements of all extremities.

The patient underwent an extensive workup in order to investigate a cause for her condition. Metabolic screening was unremarkable. Mitochondrial testing, including a muscle biopsy, electron transport chain (ETC) testing on muscle, and mtDNA analysis by qPCR on muscle, was negative. Genome-wide microarray was normal. Specific gene testing for mutations in Rett-like (*FOXG1, CDKL5, MECP2, MEF2C, SLC25A22*) and infantile spasms (*ARX, STXBP1, PCDH19*) were negative. The GeneDx 38-gene infantile epilepsy gene panel (a next-generation sequencing technology method for sequencing multiple genes in parallel) revealed a novel c.4025T>C missense mutation in exon 22 of *SCN2A*, leading to a leucine to proline (p.Leu1342Pro) substitution. The mutation was absent in the parents.

### Patient C

Patient C was born at 39 weeks to unrelated, healthy parents of mixed European ancestry. Delivery was *via* Caesarian section for macrosomia. Pregnancy was

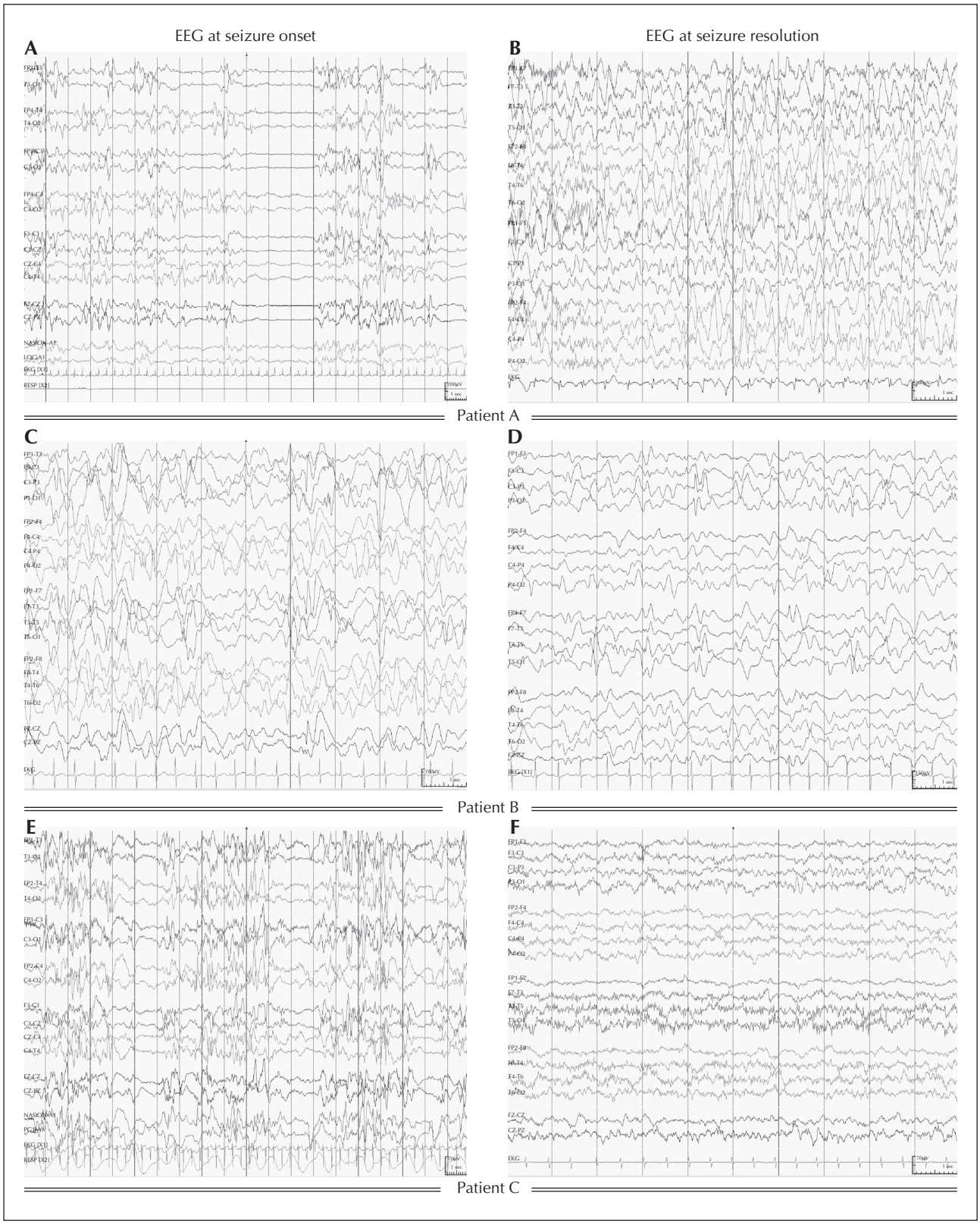


**Figure 1.** EEG of all three patients at seizure onset as well as during resolution and improvement of seizures. All EEGs at onset are on the left while repeat EEGs, performed later, are on the right. (A and B): Patient A; (C and D): Patient B; and (E and F): Patient C.

complicated by a deep vein thrombosis and a urinary tract infection in the mother.

The patient exhibited tonic seizures on the first day of life, with brief eye deviation and lip smacking. First evaluation with an EEG demonstrated an excessively discontinuous background with multifocal sharp waves (*figure 1E*). Brain MRI at the time was normal. Seizures were frequent in the first month of life but were eventually controlled on a combination of phenobarbital and topiramate.

With no seizures, an EEG performed at age 7 months was normal (*figure 1F*). Repeat MRI of the brain at 15 months showed interval age-appropriate myelination with mild thinning of the corpus callosum. Seizures recurred at age 20 months.

On examination at 3 years of age, growth parameters were normal. The patient reacted to light but did not fix on or track faces or objects. The patient exhibited axial hypotonia, with "slip through" on vertical suspension, and peripheral hypertonia. Developmentally, the patient exhibited profound global developmental delay, was non-verbal, not sitting, and had no fine motor control.

Genetic workup, including genome-wide microarray and specific genetic testing for *FOXG1*, *CDKL5*, *MECP2*, *ARX*, *TCF4*, *MEF2C*, *PCDH19*, Angelman syndrome methylation studies, *UBE3A*, and *KCNQ2,* was negative. CSF neurotransmitters, P5P, amino acids, and pyruvate/lactate were negative. Metabolic workup was negative. Sequencing of *SCN2A* revealed a c.396T>A missense mutation in exon 5, leading to an asparagine to lysine (p.Asn132Lys) amino acid substitution. This mutation was absent in the parents.

## Discussion

In summary, we have described three patients with early-onset epileptic encephalopathy phenotype with mutations in *SCN2A*. Voltage-gated sodium channel genes are strongly implicated in a range of epilepsy syndromes. The most prominent and well-studied of these is *SCN1A*, the mutation of which is the most common genetic cause of intractable epilepsy. Mutations in *SCN2A* are also increasingly recognised as a cause of epilepsy. However, to date, most reported mutations in *SCN2A* have been described in patients with BFNIE. The clinical phenotype of these patients was defined by seizure onset before 3 months of age, with the presence of at least tonic seizures. Early EEGs were abnormal, and two of the patients had a discontinuous EEG. Non-specific white matter changes were found on brain MRI in two of the patients. All patients were female, globally developmentally delayed, and hypotonic. No significant dysmorphic features were present on physical examination.

Extensive aetiological investigation in the three cases described in this report revealed *de novo* mutations in *SCN2A* as the most likely finding responsible for the pathogenesis of epilepsy in these patients. The assertion that these mutations are indeed pathogenic stems from a combination of factors. First, all three cases had severe, neonatal-onset epilepsy with a similar pattern of seizures. Second, all were found to have *de novo* mutation in *SCN2A* after extensive workup. Lastly, all three mutations were predicted to be pathogenic by *in silico* analysis. *In silico* analysis is a computer-based method to test the effect of an amino acid change on a protein. In all three patients, the mutations were localised to evolutionarily conserved regions of the channel protein at sites believed to be important for channel function. This, together with the other cases in the literature, supports our assertion that these mutations are indeed pathogenic and contributory to the epilepsy phenotype.

Specifically, Patient A's mutation was at the highly conserved amino acid position 430, in the extracellular loop between the first and second transmembrane domains, resulting in a substitution from glutamic acid to glutamine. This is predicted to be damaging by multiple *in silico* algorithms and was previously identified in a large family with BFNIE and not detected in 88 controls (Herlenius *et al*., 2007). The National Heart, Lung, and Blood Institute exome sequencing project (a large sequencing effort looking for variation in the population) did not identify Glu430Gly in 6,500 individuals, indicating that it is not a common benign variant in the population. The mutation in Patient B localised to a highly conserved region in the S5 transmembrane segment of the third domain of Nav1.2. In this mutation, the amino acid leucine is replaced with the larger amino acid proline. *In silico* analysis using Polyphen-2 (Polymorphism Phenotyping v2) and SIFT (Sorting Tolerant From Intolerant) suggested that the mutation is highly pathogenic. Patient C had a missense mutation at position 132, leading to substitution from the highly basic amino acid, asparagine, to a less basic amino acid, lysine, in the first transmembrane domain of Nav1.2. This site is highly conserved across species, and multiple *in silico* analyses predicted this change to be pathogenic.

To date, mutations in *SCN2A* are less common and generally less severe than *SCN1A* mutations. Our patients were found to have *de novo* mutations in *SCN2A* and developed intractable generalised or tonic seizures within the first year of life, but with later remission/improvement of seizures without improvement in development. This is in contrast to most *SCN2A* mutations which have been identified in BFNIE, a mild phenotype. Our three patients now add to approximately 25 reported cases of *SCN2A* mutation causing intractable epilepsy. Until 2013, there were

only 9 patients reported with severe epilepsy associated with *SCN2A* mutations and only 2 had some level of seizure remission. All of these 9 patients had severe encephalopathy. In 2013, there were 19 patients reported (Touma *et al*., 2013; Epi4K Consortium, 2013; Sundaram *et al*., 2013; Nakamura *et al*., 2013); 14 in a large series spanning patients with Ohtahara syndrome, infantile spasms, and non-specific early-onset epileptic encephalopathy. Though these reports all have very similar clinical characteristics, some unique features have been reported in different studies. For example, Sundaram *et al*. (2013) found bitemporal glucose hypometabolism on PET in their patient with IS. Touma *et al*. (2013) found brainstem pathology of dentate olivary dysplasia and changes on MRI, described as basal ganglia and brainstem degeneration. In addition, there have even been reports of autistic phenotype without seizures, associated with mutations in *SCN2A* (Rauch *et al*., 2012; Sanders *et al*., 2012). As more mutations are identified and cases reported, *SCN2A* mutation associated epilepsy will likely represent a spectrum similar to that associated with *SCN1A* mutation, ranging from mild to severe phenotypes.

As more patients with *SCN2A* mutations are identified, this may lead to a more thorough characterisation of the *SCN2A* epilepsies and possible genotype-phenotype correlation. Targeted next-generation sequencing has already been used to identify a patient with *SCN2A* mutation and a mild phenotype, and will be a useful tool for identifying mutation in the future (Lemke *et al*., 2012; Touma *et al*., 2013; Nakamura *et al*., 2013; Epi4K Consortium, 2013). Until more patients with *SCN2A* mutation are identified to validate the relationship between *SCN2A* and an intractable phenotype, additional work is needed to further explore the influence of *SCN2A* mutation on epilepsy. As an example of functional *in vitro* expression studies, Lauxmann *et al*. (2013) investigated heterologous expression using a mutant Nav1.2 (c.4766A>G/p.Tyr1589Cys), identified from a family with BFNIE, and found a number of changes to channel current, with an overall conclusion of a gain in function. More data of this kind will provide additional evidence as to the pathogenicity of these mutations. Another line of support might be to generate a mouse knock-in model harbouring a human mutation, rather than a global null. A knock-in mouse line might better reproduce the pathology seen in patients with *SCN2A* mutation and epilepsy, and could be used to determine mechanisms of seizure generation in sodium channel disorders.

The finding of *SCN2A* mutation in our three patients further extends the phenotypic spectrum of *SCN2A*-related disorders. The emerging data that *SCN2A* can be associated with a variety of epilepsy syndromes supports the conclusion that mutations in *SCN2A* are, at least in part, strong genetic modifiers of epilepsy and suggests a prominent role for Nav1.2 in neuronal excitability. This study should encourage publication of other reports of patients with such mutation; or even lead to collaborative studies, in order to validate and further explore the influence of *SCN2A* mutation on epilepsy. Such work may lead to a better understanding of sodium channel-related epilepsies and, eventually, to better treatment options. □

**Acknowledgements and disclosures.**
The authors would like to thank the patients and their families for this work. We would also like to thank the primary doctors who referred the patients to our clinic.
The authors have no conflicts of interests to disclose.

## References

Berkovic SF, Heron SE, Giordano L, *et al*. Benign familial neonatal-infantile seizures: characterization of a new sodium channelopathy. *Ann Neurol* 2004; 55(4): 550-7.

Brackenbury WJ, Isom LL. Na+ channel β subunits: overachievers of the ion channel family. *Front pharmacol* 2011; 2: 53.

Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* 2000; 26: 13-25.

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; 21: 615-21.

Epi4K Consortium; Epilepsy Phenome/Genome Project. De novo mutations in epileptic encephalopathies. *Nature* 2013; 501: 217-21.

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: 343-5.

Harkin LA, McMahon JM, Iona X, *et al*. The spectrum of *SCN1A*-related infantile epileptic encephalopathies. *Brain* 2007; 130: 843-52.

Herlenius E, Heron SE, Grinton BE, *et al*. *SCN2A* mutations and benign familial neonatal-infantile seizures: the phenotypic spectrum. *Epilepsia* 2007; 48: 1138-42.

Heron SE, Crossland KM, Andermann E, *et al*. Sodium-channel defects in benign familial neonatal-infantile seizures. *Lancet* 2002; 360: 851-2.

Kamiya K, Kaneda M, Sugawara T, *et al*. A nonsense mutation of the sodium channel gene *SCN2A* in a patient with intractable epilepsy and mental decline. *J Neurosci* 2004; 24: 2690-8.

Kearney JA, Plummer NW, Smith MR, *et al*. A gain-of-function mutation in the sodium channel gene *SCN2A* results in seizures and behavioral abnormalities. *Neuroscience* 2001; 102: 301-17.

Lauxmann S, Boutry-Kryza N, Rivier C, *et al*. An *SCN2A* mutation in a family with infantile seizures from Madagascar reveals an increased subthreshold Na(+) current. *Epilepsia* 2013; 54: e117-21.

Lemke JR, Riesch E, Scheurenbrand T, *et al*. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. *Epilepsia* 2012; 53: 1387-98.

Meisler MH, O'Brien JE, Sharkey LS. Sodium channel gene family: epilepsy mutations, gene interactions and modifier effects. *J Physiol* 2010; 588: 1841-8.

Nakamura K, Kato M, Osaka H, *et al*. Clinical spectrum of *SCN2A* mutations expanding to Ohtahara syndrome. *Neurology* 2013; 81: 992-8.

Ogiwara I, Ito K, Sawaishi Y, *et al*. *De novo* mutations of voltage-gated sodium channel αII gene *SCN2A* in intractable epilepsies. *Neurology* 2009; 73: 1046-53.

Planells-Cases R, Caprini M, Zhang J, *et al*. Neuronal death and perinatal lethality in voltage-gated sodium channel alpha(II)-deficient mice. *Biophys J* 2000; 78: 2878-91.

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: 1674-82.

Sanders SJ, Murtha MT, Gupta AR, *et al*. *De novo* mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature* 2012; 485: 237-41.

Shi X, Yasumoto S, Kurahashi H, *et al*. Clinical spectrum of *SCN2A* mutations. *Brain & Dev* 2012; 34: 541-5.

Sundaram SK, Chugani HT, Tiwari VN, Huq AH. *SCN2A* mutation is associated with infantile spasms and bitemporal glucose hypometabolism. *Pediatr Neurol* 2013; 49: 46-9.

Touma M, Joshi M, Connolly MC, *et al*. Whole genome sequencing identifies *SCN2A* mutation in monozygotic twins with Ohtahara syndrome and unique neuropathologic findings. *Epilepsia* 2013; 54: e81-5.