


De Novo SCN8A Mutation Identified by Whole-Exome Sequencing in a Boy With Neonatal Epileptic Encephalopathy, Multiple Congenital Anomalies, and Movement Disorders

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Abstract

Epileptic encephalopathies represent a clinically and genetically heterogeneous group of disorders, majority of which are of unknown etiology. We used whole-exome sequencing of a parent-offspring trio to identify the cause of early infantile epileptic encephalopathy in a boy with neonatal seizures, movement disorders, and multiple congenital anomalies who died at the age of 17 months because of respiratory illness and identified a *de novo* heterozygous missense mutation (c.3979A>G; p.Ile1327Val) in *SCN8A* (voltage-gated sodium-channel type VIII alpha subunit) gene. The variant was confirmed in the proband with Sanger sequencing. Because the clinical phenotype associated with *SCN8A* mutations has previously been identified only in a few patients with or without epileptic seizures, these data together with our results suggest that mutations in *SCN8A* can lead to early infantile epileptic encephalopathy with a broad phenotypic spectrum. Additional investigations will be worthwhile to determine the prevalence and contribution of *SCN8A* mutations to epileptic encephalopathies.

Keywords

SCN8A, epileptic encephalopathy, exome sequencing

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Epileptic encephalopathies refer to a severe condition where epileptic activity itself can contribute to progressive cognitive, behavioral, and motor dysfunction. However, the encephalopathic effect of seizures can occur in association with any form of epilepsy.¹ Children with severe early-onset epilepsies are thought to be at more risk and typically carry a poor prognosis.² Several genes have been associated with early infantile epileptic encephalopathy, but determining the underlying cause can be challenging because of genetic and phenotypic heterogeneity; therefore, the majority of cases remain unexplained.³

Mutations in neuronal sodium channel genes such as *SCN1A*, *SCN2A*, *SCN3A*, and *SCN9A* have been described in a growing number of epileptic disorders.⁴⁻⁷ Recently, novel missense mutations in *SCN8A* have been reported in 2 patients with early infantile epileptic encephalopathy, the first case with epileptic encephalopathy presenting at 6 months of age and with seizures, autism, intellectual disability, ataxia, and sudden unexplained death in epilepsy at 15 years of age.⁸ The second

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case was reported in a patient presenting with epileptic encephalopathy beginning at 18 months of age with no further clinical data available.⁴

SCN8A gene encodes the neuronal sodium channel α -subunit Nav1.6, with high expression levels in the central and peripheral nervous system where it regulates neuronal firing.⁹ Nav1.6 forms the ion-conduction pore with voltage sensors in 4 internally repeated domains, each containing 6 transmembrane segments.¹⁰ Nav1.6 channels are localized at nodes of Ranvier, dendrites, and synapses.⁹

In this study, we report a novel *SCN8A* mutation contributing to early infantile epileptic encephalopathy with a broad spectrum of clinical symptoms. Our results propose *SCN8A* as a compelling candidate gene to be screened in sporadic cases of epileptic encephalopathies with unknown etiology and demonstrate the value of whole-exome sequencing in clinical practice.

Case Summary

The proband was born at term by emergency cesarean section due to fetal hypoxia with a birth weight of 4575 g and Apgar scores 5/5/6. He was the first child of a nonconsanguineous healthy couple and had a 14-year-old half-sister from the mother's first marriage, who has benign epilepsy with centrotemporal spikes and normal cognitive functioning. Seizures were present immediately after birth, leading to development of epileptic status. He was intubated and therapeutical hypothermia in general anesthesia was applied for 72 hours, but seizures continued despite the treatment. After weaning of general anesthesia, movement disorders like coarse tremor, myoclonias, and voice expression appeared in addition to seizures. All these symptoms were exaggerated by tactile stimuli. Seizures were generalized tonic with apnea and bradycardia or focal tonic with eye squeezing and mouthing in series up to 60 times per day. His seizures were refractory to treatment and intensive care was needed several times. The patient also had central hypotonia alternating with stiffness. He did not have active movements and head control, except slight head turning, and his eye contact was occasional. The course of seizures and movement disorders were waxing and waning. The boy had dysmorphic facial appearance (high frontal hairline, micrognathia, dysmorphic ears, full cheeks), congenital multiple arthrogryposis, hip dysplasia, inguinal hernia, and hydrocele. Kidney stones were diagnosed at the age of 7 months. He died at the age of 1 year and 5 months because of progressive respiratory failure during respiratory illness. Parents refused autopsy.

Magnetic resonance imaging (MRI) at 1.5 Tesla including diffusion-weighted sequences and spectroscopy performed at the ages of 9 days and 1.5 months did not show any abnormalities. However, at the age of 5 months, mild frontotemporal atrophy and thinning of the corpus callosum and at the age of 11 months moderate bilateral frontotemporal atrophy, thinning of the corpus callosum, and mild delay in white matter myelination were described (Figure 1).

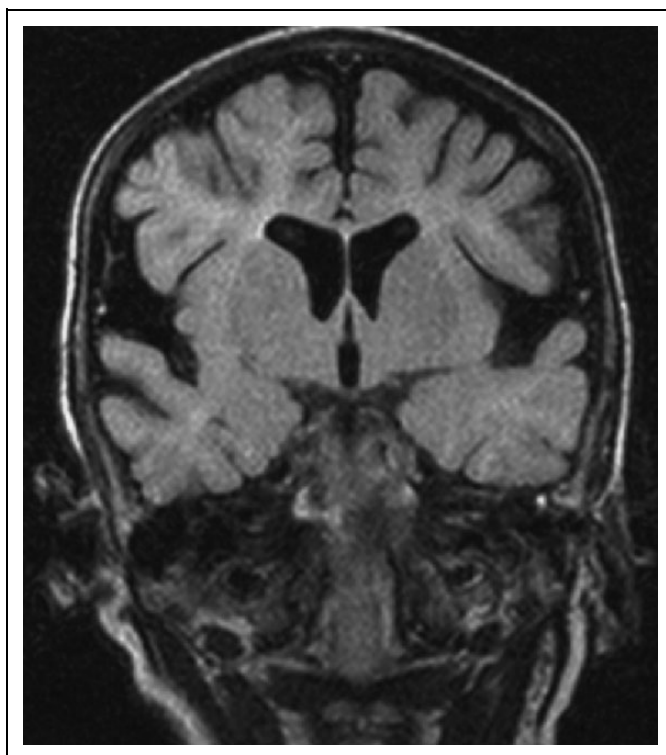


Figure 1. Coronal fluid-attenuated inversion recovery image of magnetic resonance imaging (MRI) at the age of 11 months showing frontotemporal atrophy and thinning of the corpus callosum.

Electroencephalography (EEG) showed mild changes with slight low-voltage background activity and occasional interictal epileptiform discharges in bilateral temporal areas during the first months. Burst-suppression-like pattern (low-voltage 12-16 μ V periods alternating with higher-voltage 150 μ V bilateral irregular spike-wave bursts) in sleep recording was noticed at 11 months of age. Electroneuromyography and visual evoked potentials measured at the age of 5 months did not show any abnormalities. Results of metabolic investigations including levels of organic acids, amino acids, sugars, purines/pyrimidines, acylcarnitines, oligosaccharides, glycosaminoglycans, creatine guanidinoacetate, very long chain fatty acids, homocysteine, uric acid, copper, and ceruloplasmin were in the normal range. In addition, GM1- and GM2-gangliosidosis, sialidosis, galactosialidosis, neuronal ceroid lipofuscinosis-1, and neuronal ceroid lipofuscinosis-2 were excluded by enzymatic analysis. Neurotransmitter, amino acid and pipecolic acid analysis of cerebrospinal fluid were normal. At the age of 1 month 3 weeks, transitory hypoglycorrachia (glucose in cerebrospinal fluid 2.5 mmol/L and glucose serum/cerebrospinal fluid ratio 0.5) was seen, but it normalized by the age of 4 months. Electron microscopy and immunohistochemistry analysis of muscle biopsy showed features of muscle pathology: damaged hyperchromophilic fibers with increase of lipid deposits and lysosomal enzymes, abnormal mitochondria with loss of inner cristae, and inflammation. Investigation of mitochondrial function revealed no damage of adenosine triphosphate synthesis, but impairment of glutamate connected respiratory function was found. However, respiratory

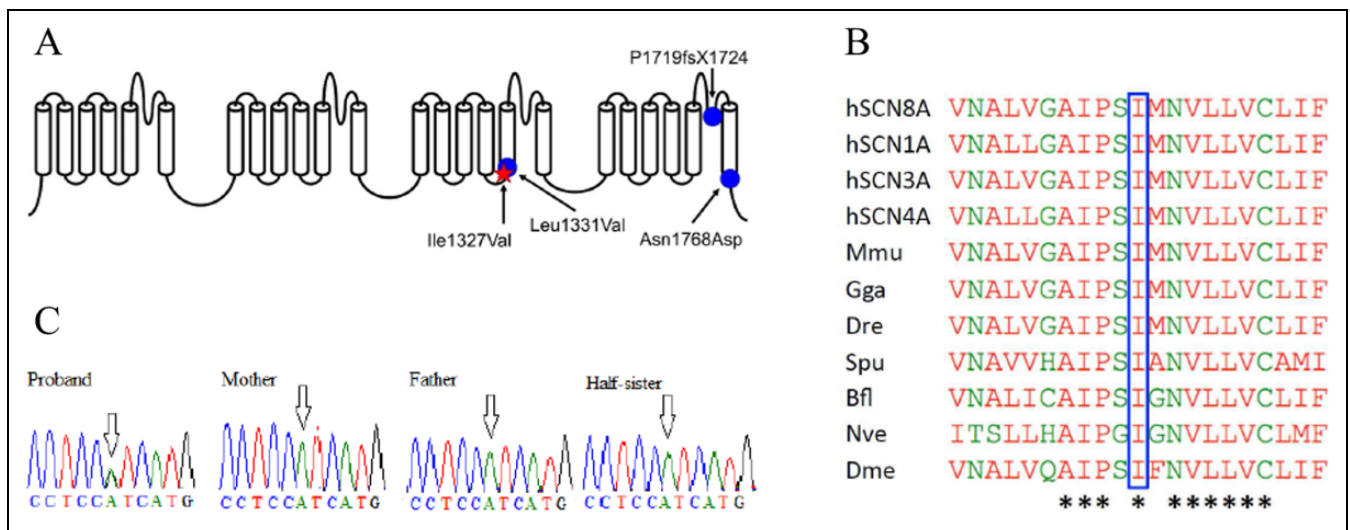


Figure 2. Sequence analysis of a novel missense *SCN8A* mutation c.3979A>G, together with previously reported mutations in *SCN8A*. (A) Structure and distribution of previously reported amino acid-altering mutations in *SCN8A*. Red star denotes a variant identified in this study, and blue circles indicate previously reported mutations in epileptic encephalopathy: p.Leu1331Val by Carvill et al,⁴ p.Asn1768Asp by Veeramah et al⁸; and cerebellar atrophy, ataxia, and mental retardation caused by a truncating mutation p.P1719fsX1724 reported by Trudeau et al. Note that Carvill et al originally annotated the mutation as Leu1290Val based on a different alternatively spliced transcript, NM_001177984.2, which lacks 41 amino acids preceding the mutated position. Diagram is based on the crystal structure information of related voltage-gated sodium channels by Payandeh et al.¹⁴ (B) Multiple sequence alignment of *SCN8A* sequences across species. Asterisks mark the positions of fully conserved amino acids. hSCN8A, human *SCN8A*; hSCN1A, human *SCN1A*; hSCN3A, human *SCN3A*; hSCN4A, human *SCN4A*; Mmu, mouse; Gga, chicken; Dre, zebrafish; Spu, sea urchin; Bfl, amphioxus; Nve, sea anemone; Dme, fruit fly. Alignment was generated by ClustalW. (C) Partial DNA sequences of *SCN8A* exon 22 showing the presence of c.3979A>G variant (indicated by an arrow) in a heterozygous state only in the proband.

chain enzymes in cultured skin fibroblasts and frozen muscle showed normal activity. Further studies, including chromosome analysis by HumanCytoSNP-12 array (Illumina Inc, San Diego, CA), mutation analysis of *SLC2A1*, *POLG*, *AGC1*, and mitochondrial DNA sequencing, revealed no abnormalities.

Seizures were refractory to treatment with different anti-epileptic drugs (carbamazepine, phenobarbital, phenytoin, levetiracetam, clonazepam, vigabatrin), vitamins (pyridoxine, pyridoxal phosphate, biotin, folic acid, vitamin B₁₂, carnitine), and ketogenic diet. Levo-dopa/carbi-dopa did not control the movement disorders.

Exome Sequencing

Exome sequencing was performed on genomic DNA of the affected boy and his healthy parents. Exome capture was performed using TruSeq Exome Enrichment Kit according to the manufacturer's protocol (Illumina). The captured libraries were sequenced on Illumina HiSeq2500 Sequencer. Sequence reads were aligned to the human reference genome (hg19) using Burrows-Wheeler Aligner 0.6.1.¹¹ Single-nucleotide variants and indels were called with SAMtools 0.1.18, Picard tools 1.60, and the Genome Analysis Toolkit 1.6-7 and annotated with custom scripts.^{12,13} Genotypes were filtered to retain single-nucleotide polymorphisms and indels with Phred-like quality scores of at least 20. We focused on variants altering protein-coding regions and canonical splice-sites and excluded variants with the frequency of >1% present in public databases

(including dbSNP135 and the 1000 Genomes Project) and our in-house exome and full-genome database. Mutation analysis was performed by Sanger sequencing in the proband, his parents, and half-sister.

Results

The average read depth for sequenced exome was 43×. After variant prioritization, we identified a novel heterozygous missense mutation c.3979A>G in exon 22 of *SCN8A* (NM_014191.3), predicting a p.Ile1327Val substitution. *In silico* analysis using PolyPhen-2, SIFT, PhyloP, and Condel suggested that the mutation has a deleterious effect on protein function. Also, the affected amino acid is located at the extremely conserved position at the cytosolic interface of S4-S5 linker and transmembrane segment 5 in domain 3 (Figure 2A, B). Additional evaluation of dbSNP, NHLBI-ESP Exome Variant server, and other available databases did not reveal the c.3979A>G mutation in *SCN8A*. This variant was also absent from our local data set of 69 exomes and 86 full genomes previously sequenced in our unit. The variant c.3979A>G was confirmed as a *de novo* in the proband with Sanger sequencing (Figure 2C).

Discussion

In this study, we describe a single nucleotide substitution in *SCN8A* that changes the isoleucine at position 1327 to a valine in a patient who had early infantile epileptic encephalopathy

with variable clinical symptoms. The identified heterozygous missense mutation c.3979A>G is located at the extremely conserved position at the cytosolic interface of S4-S5 linker and transmembrane segment 5 in domain 3 of the Nav1.6 sodium channel (Figure 2A, 2B).^{14,15}

To date, this is the fourth report of a human *SCN8A* mutation (Figure 2A). In 2 of the previous cases, novel *SCN8A* mutations have been associated with the pathogenesis of early infantile epileptic encephalopathy, and in 1 case with mental retardation, ataxia and pancerebellar atrophy.^{4,8,16} The significant differences in clinical symptoms suggest that the clinical manifestation of *SCN8A* mutations might depend on the localization of amino acid substitution in the Nav1.6 sodium channel and the nature of the mutation. The first reported *de novo* pathogenic missense mutation (c.5302A>G) in epileptic encephalopathy was described in a girl with the onset of seizures at 6 months of age, who also manifested autism, intellectual disability, ataxia, and sudden unexplained death in epilepsy at the age of 15 years. The altered amino acid residue was located at the cytoplasmic end of transmembrane segment 6 in domain 4. Veeramah et al⁸ performed detailed functional analysis of the p.Asn1768Asp mutant of *SCN8A*, which showed incomplete channel inactivation and a large increase in persistent sodium current. Based on functional studies performed by Veeramah et al and the phenotype of our patient, we suggest that the p.Ile1327Val mutation could also represent a dominant gain-of-function variant.⁸ The second identified *SCN8A* missense mutation in a patient with early infantile epileptic encephalopathy (encoding p.Leu1331Val) was located at the cytosolic end of transmembrane segment 5 in a domain 3. The patient had epileptic encephalopathy beginning at 18 months with no further clinical data available.⁴ In a patient with mental retardation, ataxia, and pancerebellar atrophy, but without seizures, a truncating mutation p.P1719fsX1724 in *SCN8A* was found.¹⁶ Comparing the clinical manifestations, it is important to stress that symptoms in our patient started with fetal hypoxia and very-early-onset refractory seizures. Also, despite frequent and refractory seizures, EEG changes were mild until 11 months of age, when burst-suppression-like pattern developed. Brain atrophy on MRI was first seen at the age of 5 months with progression on later images. Global developmental delay and movement disorders were present in our case since birth, whereas in previous cases it followed epilepsy. Besides the involvement of the nervous system, multiple congenital anomalies were present and other organ systems were affected in our patient. Neither multiple-organ involvement nor neonatal beginning of the disease has been described previously.

Finding the cause of severe and sporadic early infantile epileptic encephalopathy with unknown genetic etiology using traditional clinical practice guidelines represents a considerable challenge. However, we identified a novel pathogenic c.3979A>G missense mutation in *SCN8A* using whole-exome sequencing approach, demonstrating the value of next-generation sequencing in disease gene discovery as compared to single-gene testing.

In conclusion, this study implicates *SCN8A* in the pathogenesis of epileptic encephalopathy with neonatal beginning. In neonates with perinatal asphyxia and early-onset refractory seizures without signs of hypoxic-ischemic brain injury on neuroimaging, epileptic encephalopathy should be considered. Further investigations will be worthwhile to determine the prevalence and significance of *SCN8A* mutations in epileptic encephalopathies.

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Author Contributions

First authors UV and MN have contributed equally to this study. Patient referral and patient's clinical information was provided by UV, TR, KÕ, IT, and TT; patient's magnetic resonance image (MRI) was reviewed by PI; muscle tissue investigations were performed by AP and ES; sequencing was carried out and interpreted by MN, TN, MN, MK, TA, and AM; manuscript was written by UV and MN, cowritten and critically revised by TT; all authors contributed to and approved the final version of the paper.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

Research Ethics Committee of the University of Tartu approved this study (210/M-5 from December 19, 2011). Written patient consent was obtained from the patient's parents.

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 of Classification and Terminology, 2005-2009. *Epilepsia*. 2010;51:476-685.
2. Cross JH, Guerrini R. The epileptic encephalopathies. *Handb Clin Neurol*. 2013;111:619-626.
3. Kamien BA, Cardamone M, Lawson JA, Sachdev R. A genetic diagnostic approach to infantile epileptic encephalopathies. *J Clin Neurosci*. 2012;19:934-941.

4. 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.
5. Ogiwara I, Ito K, Sawaishi Y, et al. De novo mutations of voltage-gated sodium channel alphaII gene *SCN2A* in intractable epilepsias. *Neurology.* 2009;73:1046-1053.
6. Goeggel Simonetti B, Rieubland C, Courage C, et al. Duplication of the sodium channel gene cluster on 2q24 in children with early onset epilepsy. *Epilepsia.* 2012;53:2128-2134.
7. Singh NA, Pappas C, Dahle EJ, et al. A role of *SCN9A* in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. *PLoS Genet.* 2009;5:e1000649.
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. Caldwell H, Schaller KL, Lasher RS, et al. Sodium channel Na(v)1.6 is localized at nodes of Ranvier, dendrites, and synapses. *Proc Natl Acad Sci U S A.* 2000;97:5616-5620.
10. Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron.* 2000;26:13-25.
11. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 2009;25:1754-1760.
12. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics.* 2009;25:2078-2079.
13. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 2010;20:1297-1303.
14. Payandeh J, Scheuer T, Zheng N, Catterall WA. The crystal structure of a voltage-gated sodium channel. *Nature.* 2011;475:353-358.
15. Multiple sequence alignment with ClustalW: <http://www.ebi.ac.uk/clustalw/>. Accessed June, 2013.
16. Trudeau MM, Dalton JC, Day JW, Ranum LPW, Meisler MH. Heterozygosity for a protein truncation mutation of sodium channel *SCN8A* in a patient with cerebellar atrophy, ataxia, and mental retardation. *J Med Genet.* 2006;43:527-530.