



Short communication

Complex SCN8A DNA-abnormalities in an individual with therapy resistant absence epilepsy



Bianca Berghuis^{a,*}, Carolien G.F. de Kovel^b, Loretta van Iterson^{a,c}, Robert J. Lamberts^a, Josemir W. Sander^{a,d,e}, Dick Lindhout^{a,b}, Bobby P.C. Koeleman^b

^a Stichting Epilepsie Instellingen Nederland SEIN, Zwolle, The Netherlands

^b Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands

^c De Waterlelie School, Expertisecentrum voor Onderwijs en Epilepsie, Cruquius, The Netherlands

^d NIHR UCL Hospitals Biomedical Research Centre, UCL Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom

^e Epilepsy Society, Chalfont St Peter SL9 0RJ, United Kingdom

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ABSTRACT

Background: *De novo* SCN8A missense mutations have been identified as a rare dominant cause of epileptic encephalopathy. We described a person with epileptic encephalopathy associated with a mosaic deletion of the SCN8A gene.

Methods: Array comparative genome hybridization was used to identify chromosomal abnormalities. Next Generation Sequencing was used to screen for variants in known and candidate epilepsy genes. A single nucleotide polymorphism array was used to test whether the SCN8A variants were in *cis* or in *trans*. **Results:** We identified a *de novo* mosaic deletion of exons 2–14 of SCN8A, and a rare maternally inherited missense variant on the other allele in a woman presenting with absence seizures, challenging behavior, intellectual disability and QRS-fragmentation on the ECG. We also found a variant in SCN5A.

Conclusions: The combination of a rare missense variant with a *de novo* mosaic deletion of a large part of the SCN8A gene suggests that other possible mechanisms for SCN8A mutations may cause epilepsy; loss of function, genetic modifiers and cellular interference may play a role. This case expands the phenotype associated with SCN8A mutations, with absence epilepsy and regression in language and memory skills.

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1. Introduction

Voltage-gated sodium channels (VGSCs) play essential roles in the initiation and firing of action potentials. In the adult brain there are four subtypes: NaV1.1 (SCN1A), NaV1.2 (SCN2A), NaV1.3 (SCN3A) and NaV1.6 (SCN8A). Gain and loss of function mutations in SCN1A and SCN2A are associated with epilepsy; around 80% of all cases of Dravet syndrome (severe myoclonic epilepsy of infancy) are associated with (mainly nonsense) mutations in SCN1A. Missense mutations in SCN1A and SCN2A can cause a much milder phenotype in families with genetic epilepsy with febrile seizures (GEFS+) (Meisler et al., 2010).

SCN8A mutations in epilepsy have only recently been described. To date mainly missense mutations have been associated with

severe epileptic encephalopathy (EE) in humans (O'Brien and Meisler, 2013, Larsen et al., 2015).

We describe the pheno- and genotype of an individual with an SCN8A deletion and discuss possible pathological mechanisms linking SCN8A with epilepsy.

2. Methods

2.1. Molecular genetic analysis

Copy-number variants (CNVs) were initially identified by array comparative genomic hybridization (aCGH) during diagnostic work-up. Mutation detection of SCN8A was performed as part of an epilepsy candidate gene screen. Sequencing was performed using a customized target array-based enrichment followed by Next Generation Sequencing to screen for mutations in 346 candidate genes (0.87 Mb coding region). These candidate genes include 64 genes known to be associated to epilepsy, and genes related to these genes including all brain expressed ion channel genes (www.ncbi.nlm.nih.gov/omim, Klassen et al., 2011). We fragmented the

* Corresponding author at: PO Box 563, 8000 AN Zwolle, The Netherlands. Tel.: +31 643044750.

E-mail address: bberghuis@sein.nl (B. Berghuis).

Table 1
Possibly deleterious polymorphisms found in the proband. Prediction of pathology by www.clinvar.com, sorting intolerant from tolerant (SIFT) and polyphen-2.

Variant	ExAC allele freq	ClinVar	SIFT	Polyphen-2
SCN8A: rs201458257; NM_014191.3:c.4748T>C p.I1583T, Chr 12:52188378 T/C (hg19)	0.0002	Unknown significance	Tolerated	Benign for most transcripts, possibly damaging for full-length transcript ENST00000599343
SCN5A: rs41313691; NM_198056.2:c.1571C>A p.S524Y, Chr 3:38645522 G/T (hg19)	0.005115	Benign/unknown significance	Tolerated	Probably damaging for at least one of the transcripts

human genome, enriched for coding regions of genes and then sequenced on a SOLiD 5500 platform. Alignment, variant calling and annotation was done using a BW-aligner (Nijman et al., 2010). Putative damaging variants were selected from all variants using common filtering steps. To determine whether the variant and the deletion were in *cis* or in *trans*, the proband and her parents were analyzed with an oligonucleotide array Infinium CytoSNP-850k BeadChip SNP (Illumina®), and phased single-nucleotide polymorphisms (SNPs) in the deletion region allowed determination of the parental haplotype in which the deletion originated.

3. Results

3.1. Clinical description

The 23-year-old female proband was diagnosed with absence epilepsy at age 6. She is one of four siblings born of two phenotypically unaffected parents. The older sister is healthy; the older of the two younger brothers was diagnosed with ADHD and the younger with dyslexia.

Retrospectively, seizure onset was probably within the first year of life. There were no fever-related seizures. Absences were brief (2–3 s) but frequent, occurring 60–70 times a day. During absences she stared with upward rotation and blinking of the eyes, and contact was disturbed. Her seizures were not controlled by valproic acid combined with ethosuximide (ETX) or lamotrigine. At age 9, she had a tonic clonic seizure, with postictal aphasia lasting 60 min. For the last 2 years she has also been experiencing myoclonic jerks. EEGs at ages 9, 12 and 21 years showed diffuse slowing, bifrontal runs of theta and delta activity, and 4–12 atypical absences per hour with bilateral synchronous generalized high voltage (poly)spike slow wave discharges with a frequency of 2–3/s. A photoparoxysmal response was seen at high and low frequencies with, during the last EEG, myoclonic and tonic clinical symptoms. Computed tomography of the brain at age 6 was normal. Electrocardiogram showed fragmented QRS complexes (fQRS) with notching in the nadir of the S wave (QRS duration < 120 ms) in two contiguous leads (V2, V3).

Early development was just within normal limits. At age 3, developmental delay became evident. Lack of speech intelligibility was consistently reported throughout the years. Intelligence testing at age 6 showed a full scale (FS)-IQ of 63 on the Wechsler Preschool and Primary scale of Intelligence. At age 18, her FS-IQ score on the Wechsler Adult Intelligence Scale (WAIS-III) was 46 (Supplementary Fig. S1). She scored zero in three verbal subtests and had an unusually low score for auditory short-term memory. Her behavior was characterized by severe attention problems and periods of aggressiveness. Her movements were somewhat rigid, but there were no signs of ataxia.

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3.2. Molecular findings

Diagnostic aCGH identified a deletion at 12q13.13 (chr12:52,055,075–52,154,800) (hg19), encompassing exons

2–14 of the *SCN8A* gene (NM.001177984). This was not detected in either parent, and was considered *de novo*.

The proband was screened using a research epilepsy gene panel. A single putative damaging variant was detected in the known epilepsy genes – a rare variant in *SCN8A* (rs201458257, Table 1) previously reported in 2 of 12,622 chromosomes in the exome sequencing project (<http://evs.gs.washington.edu>) and in 24 of 120,296 alleles in the Exome Aggregation Consortium (ExAC) browser (<http://exac.broadinstitute.org/about>), but never in a homozygous state. Sanger sequencing of the proband's and parents' DNA confirmed the presence of this heterozygous variant in the proband and her mother. A variant in *SCN5A* (rs41313691, Table 1) was also detected. This variant was reported in 142 of 12,462 chromosomes in the exome sequencing project and in 432 of 84,450 alleles in ExAC.

To identify the origin of the *SCN8A* deletion, genome-wide dense SNP genotyping was performed in the proband and her parents. This confirmed the presence of a deletion of exons 2–14 as shown by a decreased fluorescence signal. The reduction of total fluorescence (log(R)-ratio) was, however, approximately half that expected for a heterozygous deletion (observed average log(R)-ratio of –0.23 versus expected –0.5). Some of the probes for the wildtype and alternative alleles in the deleted region showed a B-allele frequency of, on average, 0.31 or 0.74 rather than 0 or 1 as would be expected for a complete deletion. Log R-ratio and B-allele frequency in the parents were normal (Fig. 1). We deduced that the paternal allele was present at lower frequencies given the lower paternal allele fluorescence in the proband. We interpreted these data as a deletion on the paternal chromosome, present as mosaic in the proband. We estimated that around 50% of cells carry the deletion, given the observed B-allele frequencies and log R-ratio. The deletion on the paternal chromosome and the *SCN8A* variant rs201458257 inherited from the mother affected different alleles of the *SCN8A* gene in the daughter (Fig. 2).

4. Discussion

The first case of epilepsy with a *de novo* heterozygous missense mutation in *SCN8A* was described in 2012; the mutation caused an epileptic encephalopathy with onset 6 months after birth and sudden unexpected death in epilepsy (SUDEP) at age 15. Functional analysis showed a gain of function of *SCN8A* (Veeramah et al., 2012). Targeted sequencing in large cohorts of people with epileptic encephalopathy and three case reports confirmed the inclusion of *SCN8A* mutations as causative in infantile epilepsy (O'Brien and Meisler, 2013; Larsen et al., 2015; Estacion et al., 2014). Nearly all cases reported to date carried missense mutations. Our case with high frequency brief generalized seizures and a regression of speech and language skills is remarkably similar to the first individual described, although she carries a mosaic deletion of *SCN8A*. As a large part of the gene is deleted, including exon 2, the starting site for translation, we expected expression of *SCN8A* in these cells to be reduced by half and no functional protein to be formed from this allele. We attempted to explain EE phenotype as a result of loss of function of *SCN8A*.

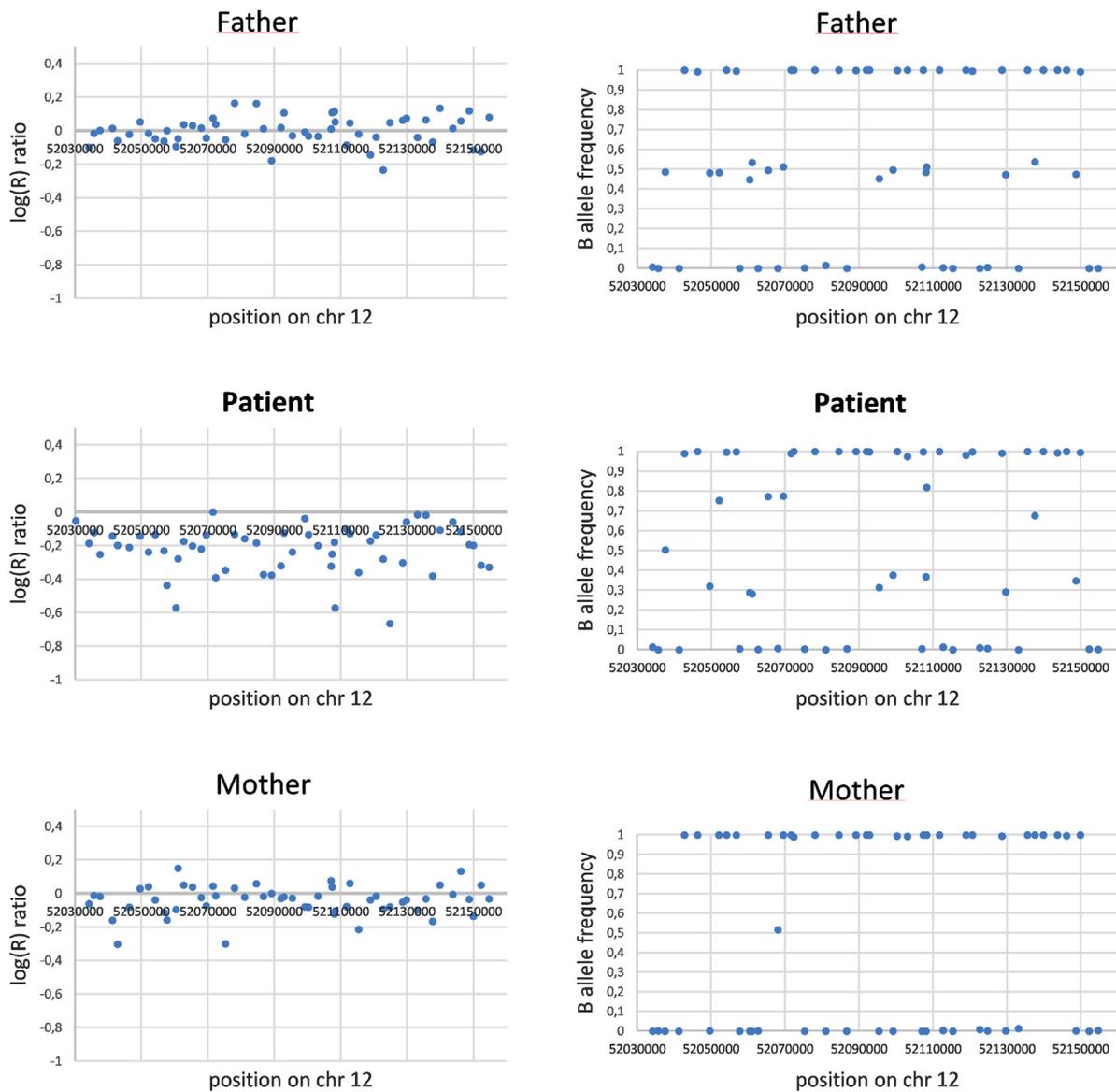


Fig. 1. Log R-ratio (left) and B-allele frequency (right) in proband and parents. The ratio of probe fluorescence for the wildtype and alternative alleles (B-allele frequency) in the mother were either 0 or 1 (homozygous SNPs), in the father frequencies of 0.5 were also found (heterozygous SNPs). For SNPs in the deleted region frequencies of either 0 or 1 were expected. In the proband B-allele frequencies were averaging 0.31/0.74, indicating a mosaicism of the deletion.

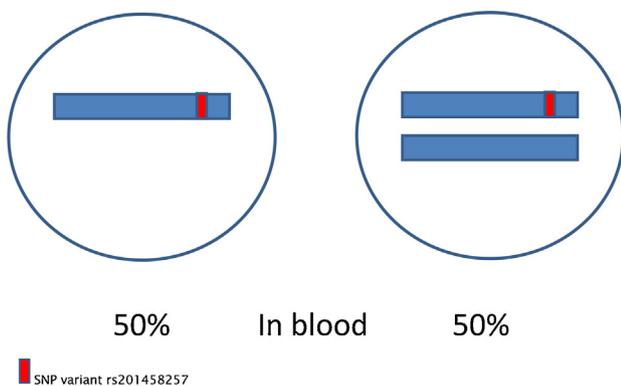


Fig. 2. Distribution of *SCN8A* transcripts in blood cells. The proband carries the deletion and the alternative SNP allele in 50% of her nuclear blood cells, and in the remaining 50% the healthy and the alternative SNP allele are present.

Intractable epilepsy can be caused by loss of function of *SCN2A* despite the predominant expression in excitatory neurons, as with *SCN8A*. One of the three functional analyses of *SCN8A* mutations in people with EE showed an unstable protein with reduced channel activity. The individual carrying this loss of function allele exhibited a severe phenotype with seizure onset at 6 months old, tonic clonic seizures, diffuse brain atrophy and profound developmental impairment (de Kovel et al., 2014).

The functional duality is also seen in mice, where heterozygous *Scn8a* null mice rescue compound heterozygous *Scn1a* knockout mice from seizures and lethality (Martin et al., 2007), but also exhibit the hallmark of absence epilepsy: ETX-sensitive spike-wave discharges (SWD). Importantly, strain background had a strong effect on the incidence of SWD in *Scn8a* mice: an *Scn8a* loss of function mutation causes absence seizures in a C57BL/6J strain, but the incidence and severity of SWD was significantly less in the C3HeB/FeJ strain (Oliva et al., 2014).

A similar factor contributing to the epilepsy phenotype in this case may be the presence of the *SCN8A* variant (rs201458257). This variant could work as a genetic modifier by influencing the net channelome expressed in the cell, comparable to the modification of the severity of *SCN1A*-related Dravet syndrome by co-segregation of mutations in the *SCN9A* gene (Singh et al., 2009).

Our case might be less severely affected because of mosaicism. Mosaicism itself is also described as an epileptogenic mechanism through functionally different cell populations causing pathology by cellular interference in epilepsy and mental retardation limited to females (EFMR) (Lindhout, 2008; Dibbens et al., 2008).

SCN8A and *SCN5A* mutations have both been associated with SUDEP (Larsen et al., 2015; Veeramah et al., 2012; Tu et al., 2011) and *SCN5A* mutations have been implicated in long QT and Brugada syndrome (Shimizu, 2014). Our case showed fQRS in two contiguous ECG leads. fQRS represents a distortion of signal conduction and the depolarization process within cardiac ventricles, and may be associated with a higher risk of sudden cardiac death in people with cardiac disease (Rosengarten et al., 2015). The *SCN5A* variant rs41313691 has not been described in relation to cardiac disease, but we believe that thorough cardiac monitoring is indicated in this individual carrying mutations in *SCN8A* and *SCN5A*, and at least an ECG is recommended in similar people with EE.

In conclusion, complex alterations in the *SCN8A* gene may underlie epilepsy and cognitive impairment. To understand fully the genotype–phenotype association, a comprehensive screen of *SCN8A* in people with EE needs to be performed taking mosaicism and background genetics into account.

5. Conflict of interest

There are no conflicts of interest.

6. Financial disclosures

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