Epilepsy Research 115 (2015) 141–144

Contents lists available at www.sciencedirect.com

Epilepsy Research

journal homepage: www.elsevier.com/locate/epilepsyres

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*

ARTICLE INFO

Article history:
Received 12 March 2015
Received in revised form 22 May 2015
Accepted 12 June 2015
Available online 15 June 2015

Keywords:
SCN8A
Epileptic encephalopathy
SCN5A
fQRS
Mosaicism

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.

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).

http://dx.doi.org/10.1016/j.eplepsyres.2015.06.007
0920-1211/

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.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eplepsyres.2015.06.007

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*.