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SCN1A-related epilepsy with recessive inheritance: two further families

AUTHORS

Raffaella Moretti¹, Lionel Arnaud², Delphine Bouteiller³, Oriane Trouillard^{2,3}, Patricia Moreau², Julien Buratti², Agnès Rastetter³, Boris Keren², Vincent Des Portes^{4,5}, Joseph Toulouse⁴, Isabelle Gourfinkel-An⁶, Eric Leguern^{2,3}, Christel Depienne^{3,7}, Cyril Mignot^{*2,3,8}, Caroline Nava^{*2,3,#}

1. APHP.Sorbonne Université, Département de Physiologie, Hôpital Trousseau, Paris, France.

2. APHP.Sorbonne Université, Département de Génétique, Groupe Hospitalier Pitié Salpêtrière, Paris, France.

3. Sorbonne Université, Institut du Cerveau, ICM, Inserm U1127, CNRS UMR 7225, Paris, France.

4. Reference Center for rare epilepsies CRÉER, Lyon University Hospital, F-69677, Bron, France

5. University Lyon 1, F-69008, Lyon, France.

6. APHP.Sorbonne Université, Département de Neurologie, Centre de Référence des Épilepsies Rares, Groupe Hospitalier Pitié Salpêtrière, Paris, France.

7. Institute of Human Genetics, University Hospital Essen, University of Duisburg-Essen, Essen, Germany.

8. Centre de Référence Déficiences Intellectuelles de Causes Rares, GH Pitié-Salpêtrière, Paris, France.

* Common last authors: C Nava and C Mignot contributed equally to the manuscript.

Corresponding author: Caroline Nava, Département de Génétique, Hôpital Pitié-Salpêtrière, 75013 Paris, France. Email : caroline.nava@aphp.fr

KEY WORDS

SCN1A, Dravet syndrome, GEFS+, febrile seizures, recessive inheritance

Abbreviations

DEE: developmental and epileptic encephalopathies; DS: Dravet syndrome; GS: Grantham score

INTRODUCTION

Pathogenic variants in *SCN1A*, encoding the alpha 1 subunit of the voltage-gated sodium channel, result in a broad spectrum of disorders ranging from simple febrile seizures and genetic epilepsy with febrile seizures plus (GEFS+) to more severe, developmental and epileptic encephalopathies (DEEs), whose prototypical disease is Dravet syndrome (DS). DS typically presents in the first year of life with prolonged, febrile, generalized clonic or hemiclonic epileptic seizures in children with normal initial development. Other seizure types, including myoclonic, focal and atypical absence seizures, appear between the age of 1 and 4 in the absence of hyperthermia. They are often pharmacoresistant¹. From the age of 2 years, cognitive, behavioral and motor impairments become obvious. Pathogenic variants in *SCN1A* gene account for 80% of the etiologies of DS¹. Such variants are also rare causes of other epileptic disorders like myoclonic-atonic epilepsy, epilepsy with migrating focal seizures or unclassified DEEs. Finally, *SCN1A* pathogenic variants have also been reported in patients with non epileptic phenotypes, like familial hemiplegic migraines, autism spectrum disorders and arthrogryposis²⁻⁵.

The spectrum of pathogenic *SCN1A* variants causing DS and other epilepsies includes missense and truncating (nonsense, frameshift and splice site) variants, as well as intragenic microrearrangements⁶. Almost all *SCN1A* variants reported so far are heterozygous, mainly *de novo* or associated with parental mosaicism when causing DS, or with a dominant mode of inheritance for those associated with less severe phenotypes. However, families with GEFS+ in which rare members develop DS also exist⁷. Most of these variants are expected to lead to a loss-of-function. Animal models suggest that this loss-of-function reduces the excitability of specific γ -aminobutyric acid (GABA)ergic interneurons⁸⁻¹⁰, thereby lowering the global seizure threshold and the susceptibility to epileptic seizure precipitated by fever. To date, a single article suggested the existence of recessive *SCN1A* variants in two consanguineous families including individuals with GEFS+ or DS carrying homozygous variants¹¹.

In this report, we aim at reporting two additional families in which seizures are likely related to biallelic *SCN1A* variants.

MATERIAL AND METHODS

Patients. Patients were referred to the genetic laboratory for the targeted study of *SCN1A*. Referring clinicians made clinical data available. Biological samples were obtained following written informed consent from the legal representatives. The study was approved by the local ethic committee.

Molecular analyses. Molecular analyses were performed on genomic DNA extracted from patients venous blood and comprised i) sequencing of all 26 exons and introns/exons boundaries of the *SCN1A* gene (NM_001165963.2) by Sanger sequencing¹² or microfluidic PCR amplification (Fluidigm Access Array) followed by MiSeq sequencing (Illumina, USA) according to the manufacturers, ii) MLPA (Multiplex Ligation dependent Probe Amplification), as previously described¹². We then performed the study of DNA samples from patients with a gene panel comprising 117 genes involved in epilepsy and DEEs (list of genes available on request) and whole exome sequencing (WES) to rule out other genetic variants possibly accounting for their epilepsy, as previously described^{13,14}

Bioinformatics analyses of SCN1A variants. Variant interpretation was assessed using Alamut Visual version 2.15 (SOPHiA GENETICS, Lausanne, Switzerland). Grantham chemical distance between amino acids, Align GVGD, SIFT version 6.2.1, Mutation Taster and PolyPhen-2 v2.2.2r398 scores were provided by Alamut software¹⁵⁻¹⁷. We also scored variant pathogenicity using CADD (Combined Annotation Dependent Depletion) and REVEL (Rare Exome Variant Ensemble Learner scores) which integrate scores from several *in silico* prediction tools^{18,19}.

CASE STUDIES

Family 1 originated from Lebanon and included three children born from consanguineous, distant cousins, parents (sup figure 1). The proband, a 30-year-old male, was born after an uneventful pregnancy and had normal early psychomotor development. Febrile seizures started when he was 19 months. He then had recurrent febrile and afebrile hemiclonic seizures, with some of them being prolonged. Seizures were pharmacoresistant for several years. At 21, seizures became controlled with a combination of levetiracetam and zonisamide. EEG

recordings were either normal or showed minor focal fronto-temporal abnormalities. Brain MRI was normal.

The patient had a heterogeneous cognitive profile associating learning difficulties and slow reasoning, but no intellectual disability. He did not develop dyskinesias or gait disturbances, but he was described as “clumsy”.

He had an asymptomatic sister aged 27 and a younger sister, aged 21, who had similar phenotypes. The proband’s medical record mentioned that her epilepsy started at 10 months, but no further information was available.

Sequencing of *SCN1A* in the proband revealed the homozygous c.2768T>G, p.(Ile923Ser) variant. MLPA study did not show any deletion, thus confirming the homozygous state of this variant. Similar results were found on the second study with the gene panel further showing that the patient had no other variants in 117 genes of epilepsy. **WES data did not reveal any other likely pathogenic or pathogenic variant in genes related to epilepsy or developmental disorders.** The p.(Ile923Ser) variant is not reported in gnomAD, is predicted deleterious by 5/5 of the in-silico tools (Align GVGD, SIFT, Mutation Taster, Polyphen 2, Mutation Assessor), has a CADD prediction score of 26.9 and a REVEL score of 0.934 (table 1). DNA from his parents and his two sisters were unavailable.

Family 2 was from Turkey and included a single child **born from first cousin parents (sup figure 1)**. The proband was a 19-year-old girl born after an uneventful pregnancy. First febrile generalized seizure was observed at 3 months of age. Between the age of 3 months and 6 years old, she had 7 febrile seizures. At the age of 6 years, she had partial febrile or afebrile seizures with tonic head rotation, tonic-clonic seizures and absences, that becomes very frequent (several per month), but without status epilepticus.

Under lamotrigine and carbamazepine the frequency of seizures increased. Levetiracetam, topiramate and clobazam were inefficient. Only sodium valproate was effective, with failure to withdraw three times. Most EEG recordings were normal but one of them showed diffuse spike-waves predominating in temporal and frontal regions. Brain MRI at age 7 was normal. At the time of the study, she was treated with sodium valproate only, and has been seizure-free for 2.5 years.

She had a borderline intellectual functioning (WAIS-IV; Verbal Comprehension Index 66; Perceptual reasoning Index 80) and ADHD, and she attended a school for children with learning disabilities. She had no fine motor or gait problems.

The sequencing of *SCN1A* showed a homozygous c.824A>G, p.(Asn275Ser) variant which was inherited from both asymptomatic heterozygous parents. MLPA study did not reveal any deletion, confirming the homozygous state of the change. Sequencing of the 117 genes involved in DEE, **as well as WES**, confirmed the variant and the absence of deletion, and did not reveal any other pathogenic variant. The p.(Asn275Ser) variant is absent from gnomAD, is predicted deleterious by 4/5 of the in-silico tools, has a CADD prediction score of 23.1 and a REVEL score of 0.655 (table 1).

DISCUSSION

We report on two families with patients exhibiting a set of seizure types and epilepsy histories suggesting the involvement of *SCN1A* : i) febrile generalized tonic clonic or hemiclonic seizures, with some of them being prolonged, with onset between 3 and 19 months, ii) followed by partial or generalized afebrile seizures, iii) these seizures were either controlled or pharmaco-resistant. Interestingly, in one of the patients, epilepsy was aggravated by carbamazepine and lamotrigine, as in the majority of young DS patients. The hypothesis of *SCN1A*-related epilepsy led to the sequencing of the gene, which revealed a homozygous variant both cases. Like in the two previous families in which such results have been discussed, the « determination of the clinical significance of [biallelic] *SCN1A* missense mutations remains challenging »¹¹.

Most of the admitted pathogenic variants responsible for *SCN1A*-related epilepsy are heterozygous changes likely inducing a loss-of-function. The hypothesis of a recessively inherited GEFS+ phenotype assumes that hypomorphic variants may not necessarily cause epilepsy at the heterozygous state (in parents) but may decrease the seizure threshold when combined (in affected children). The only experimental data supporting such hypothesis is brought by the study of transgenic p.(Arg1648His)

mouse model showing that a *SCN1A* variant with a limited loss-of-function effect may have more detrimental clinical consequences when homozygous^{10,11}.

Protein location, physicochemical difference between amino acids (measured by Grantham distance, GS) and bioinformatic predictive tools can be helpful to establish the pathogenicity of variants. Previous studies about the pathogenicity of *SCN1A* variants showed that clinically significant missense variants mainly alter residues belonging to ion transport sequences, in the 4 homologous domains^{6,12}. Moreover, the median GS was 94 for *SCN1A* variants inducing DS, 81 for those responsible for GEFS+ syndrome and 58 for polymorphisms, without significant difference between DS and GEFS+⁶. A recent study based on 58 functionally characterized missense *SCN1A* variants, showed that 52 variants associated with an epileptic phenotype had a median CADD score of 28.3 (range [22.6–34]) and a median REVEL score at 0.96 (range [0.808–0.994])²⁰. 47/52 variants were predicted to have a functional impact (medium or high) by the five *in silico* software (Align GVGD, SIFT, Mutation Taster, Polyphen 2, Mutation Assessor). Notably, the Polyphen2 score was 0.916 or more, corresponding on a probably damaging effect, for all variants except one. Finally, this study showed that these *in silico* tools are useful in distinguishing disease-causing and benign variants but they cannot predict the severity of the disease, i.e. they do not discriminate DS vs GEFS+ phenotype²⁰.

The first missense homozygous variant p.(Met400Val) previously reported by Brunkaus et al. is located in a functional domain; however the GS and the CADD score are low, and it is predicted deleterious by only 4/5 *in silico* software (table 1). On the contrary, the second variant, p.(Arg618Cys), has high GS and CADD score, and it is predicted deleterious by all bioinformatics tools, although located in the DI-DII cytoplasmic linker, less important for the protein function.

In our study, both variants are located in a similar functional domain (DIS5-S6 and DIIS5-S6 extracellular regions) of the protein. The p.(Asn275Ser) variant found in family 2, exhibits low CADD and REVEL scores, and a small physicochemical difference between an asparagine and serine, which are both polar uncharged (GS 46). It is predicted deleterious only by Mutation Taster. The variant in the family 1, p.(Ile923Ser), seems more deleterious, with a high GS, as an isoleucine (hydrophobic) is exchanged for a serine (polar uncharged) and an effect on the protein predicted by 5/5 *in silico* software. However, the predictions of the

pathogenicity are moderated for two software (Polyphen 2 and Mut Assessor) and the CADD and REVEL scores are lower than the median observed with *SCN1A* pathogenic variants. These data are in line with tolerated variants at the heterozygous state, possibly pathogenic at the homozygous state.

On the contrary, over seven homozygous variants reported in the GnomADv2.1.1 control population in less than five individuals, six appear as possibly benign, even in the homozygous state: (i) 4 variants [p.(Ile1955Thr), p.(Met1619Val), p.(Thr1174Ser), p.(Ser1110Thr)] with low CADD and/or REVEL scores (respectively less than 20 and 0.6); (ii) one change resulting in a small physico-chemical difference between the two amino acids [p.(Arg604His), GS=29]; and (iii) one variant [p.(Arg1928Gly)] located in the C-terminal cytoplasmic domain, which is less important for the protein function. The pathogenicity of the last homozygous variant, p.(Val255Ala), cannot be excluded on the basis of the bioinformatics algorithms. This variant has been described once only in the homozygous state and never in the heterozygous state in gnomAD. Nevertheless, the clinical data of the individual are not known and a mild phenotype during infancy could not be excluded (sup table 1).

MLPA and NGS sequencing ruled out a deletion in *SCN1A* or a likely pathogenic or pathogenic variant in genes involved in epilepsy or developmental disorders in both cases. Although this is supportive, it is not a definite exclusion of other causes, a variant in a gene not yet associated to a disease, or in a regulatory intronic sequence cannot be excluded.

~~Interestingly, homozygous variants in *SCN1B*, encoding the $\beta 1$ subunit of the voltage-gated sodium channel, is a rare cause of DEE overlapping with DS. Only 10 patients in seven families have been reported so far. Heterozygous parents were asymptomatic or presented febrile seizures in childhood whereas homozygous children suffered from a severe DEE, which support the additive effects of biallelic variants²¹⁻²⁵.~~

In conclusion, we report two additional patients from consanguineous families with possibly pathogenic homozygous *SCN1A* variants. These variants as well as the two patients previously described exhibit amino acid property and/or protein location

and/or *in silico* predictions compatible with a milder effect on the protein and therefore could be tolerated at the heterozygous state.

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LEGENDS

Figure 1. Schematic representation of the homozygous variants identified in this study (red stars) or previously reported by Brunklaus et al. (black stars) on the Nav1.1 protein

Family	position (Hg19)	nucleotide change	aminoacid change	GnomAD	Grantham	GVGD	SIFT	Mut taster	Polyphen2	Mut Assessor	CADD	Revel	Protein location
1	166894464	c.2768T>G	p.(Ile923Ser)	0	142	C65	Deleterious (score: 0)	Disease causing	possibly damaging (0.763)	medium	26.9	0.934	DII extracellular (between S5 and S6)
2	166908369	c.824A>G	p.(Asn275Ser)	0	46	C0	Tolerated (score: 0.07)	Disease causing	benign (0.080)	low	23.1	0.655	DI extracellular (between S5 and S6)
Brunklaus et al. 1	166903459	c.1198A>G	p.(Met400Val)	0	21	C15	Deleterious (score: 0)	Disease causing	probably damaging (0.95)	low	23.7	0.917	DIS6
Brunklaus et al. 2	166900370	c.1852C>T	p.(Arg618Cys)	1/251358	180	C25	Deleterious (score: 0.02)	Disease causing	probably damaging (1)	medium	29.6	0.922	DI-DII cytoplasmic linker

Table 1. Homozygous SCN1A variants identified in this study and previously reported¹¹

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