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Mutation spectrum of the *SCN1A* gene in a Hungarian population with epilepsy

Ágnes Till[a], Judith Zima[a], Anett Fekete[a,1], Judit Bene[a,b], Márta Czakó[a,b], András Szabó[a,b], Béla Melegh[a,b,*], Kinga Hadzsiev[a,b]

[a] Department of Medical Genetics, Medical School, Clinical Center, University of Pécs, Pécs, Hungary
[b] Szentágothai Research Center, University of Pécs, Pécs, Hungary

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ABSTRACT

Purpose: The vast majority of mutations responsible for epilepsy syndromes such as genetic epilepsy with febrile seizures plus (GEFS+) and Dravet syndrome (DS) occur in the gene encoding the type 1 alpha subunit of neuronal voltage-gated sodium channel (*SCN1A*).
Methods: 63 individuals presenting with either DS or GEFS + syndrome phenotype were screened for *SCN1A* gene mutation using Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA).
Results: Our research study identified 15 novel pathogen mutations in the *SCN1A* gene of which 12 appeared to be missense mutations with addition of two frameshift-deletions and one in-frame deletion. The distribution of clinical phenotypes in patients carrying *SCN1A* mutations was as follows: twelve patients had classical DS, three patients had GEFS + syndrome and two relatives of DS patients were suffering from febrile seizures.
Conclusions: Our study highlights the phenotypic and genotypic heterogeneities of DS and GEFS + with the important aim of gaining a deeper understanding of *SCN1A*-related disorders. This study also represents the first genetic analysis of the *SCN1A* gene in a Hungarian cohort with the DS and GEFS + syndrome phenotype.

1. Introduction

Epilepsy, conventionally classified as idiopathic by etiology in up to 70 % of the cases, has started to reveal its genetic roots with the advent of widely available genetic testing [1]. The majority of the genes identified as disease causing mutation encode ion channels or receptors including voltage-gated sodium, potassium, calcium and chloride channels; additionally, receptors for acetylcholine and γ-amino butyric acid (GABA) [2]. The most widely investigated association is between *SCN1A* mutations (OMIM 182389) with Dravet syndrome (DS) (OMIM 607208) and the genetic epilepsy with febrile seizure plus syndrome (GEFS+) (OMIM 604403) [3–5].

DS (previously known as severe myoclonic epilepsy in infancy, SMEI) is one of the most common epileptic encephalopathies of infancy. The incidence of DS is about 1 in 20,000–40,000 live births [6,7]. It is characterized by febrile and afebrile, generalized and unilateral, clonic or tonic-clonic seizures that are often prolonged and occur in the first year of life in an otherwise healthy infant. The condition may be later associated with myoclonus, atypical absences and partial seizures. Some children, however, do not develop myoclonic symptoms and will have a milder form of DS [8]. The seizures are frequently triggered by fever and refractory to antiepileptic treatment. The convulsions present commonly as febrile status epilepticus during the first year of life [9,10]. Brain magnetic resonance imaging (MRI) shows no pathological structural alterations in most cases and electroencephalogram (EEG) typically remains normal at the onset of the disease [11,12]. Valproate, stiripentol, topiramate and bromide are the mainstay of treatment while sodium channel blocking anticonvulsants have been shown to aggravate the seizures and therefore must be avoided [13]. Non-epileptic manifestations such as intellectual disability and ataxia may appear with age.

Alterations in the gene encoding the type 1 alpha subunit of neuronal voltage-gated sodium channel (*SCN1A*) are responsible for the symptoms in about 70–80% of the cases [14]. The majority of changes

Abbreviations: CNS, Central nervous system; DS, Dravet syndrome; EEG, Electroencephalography; GEFS+, Genetic epilepsy with febrile seizures plus; FS, Febrile seizure; MLPA, Multiplex ligation-dependent probe amplification; MRI, Magnetic resonance imaging; NGS, Next generation sequencing; *SCN1A*, Sodium channel alpha subunit type 1; SMEI, Severe myoclonic epilepsy in infancy; WES, Whole exome sequencing

* Corresponding author at: Department of Medical Genetics, Medical School, Clinical Center, University of Pécs, H-7624 Pécs, Szigeti street 12., Hungary.
E-mail address: melegh.bela@pte.hu (B. Melegh).
[1] Present address: I. Department of Pediatrics, Semmelweis University, Budapest, Hungary

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are *de novo* mutations; however, DS shows an autosomal dominant inheritance pattern in 10% of the cases. In the inherited cases, relatives carrying the same *SCN1A* mutation as the patient often develop milder forms of epilepsy consistent with the phenotypic spectrum of GEFS+, or might even stay unaffected suggesting the role of additional genes in disease severity [15]. Familial phenotypic variability might also be explained by mosaic *SCN1A* mutations in some cases [16–19]. Missense and truncating mutations are found at approximately equal frequencies in DS, while GEFS + is largely associated with missense mutations. *SCN1A* was first described as an epilepsy-causing gene in 2001 and more than 1200 mutations have been identified so far [20]. Despite considerable efforts no clear phenotype-genotype correlations have been described for the hundreds of *SCN1A* mutations identified [21].

The type 1 alpha subunit of the voltage-gated sodium channel is one of the four isoforms of the mammalian voltage-gated sodium channel alpha subunits that are expressed at high levels in the central nervous system (CNS) [22,23]. The *SCN1A* gene harbours 26 exons and encodes a 2009 amino acid-containing transmembrane protein which is a critical component of the voltage-gated sodium channels in the CNS [24]. If the alpha subunit, the core component of the transmembrane protein, does not function normally, the sodium channel can remain closed which, in turn, stops synaptic signal propagation to the next neuron [25]. Murine models suggest that the primary effect of both GEFS + and DS mutations is to decrease GABAergic inhibitory neuron activity, contributing to seizure generation in these patients. Impaired function of cerebellar GABAergic and inhibitory Purkinje cells may explain the observed ataxia and cognitive impairment in these patients [26].

The aim of this study was to investigate the mutational spectrum of the *SCN1A* gene in Hungarian patients with DS and GEFS + syndrome phenotype.

2. Materials and methods

Between January 2012 and December 2017 a total of 183 Hungarian individuals with fever-triggered and/or pharmacoresistant epilepsy were referred for genetic examination to the Department of Medical Genetics, University of Pécs. A total of 63 patients met the clinical and EEG characteristics diagnostic criteria of DS as per Guerrini and Oguni. DNA samples were examined for *SCN1A* gene mutations using Sanger sequencing analysis and multiplex ligation-dependent probe amplification (MLPA) [8]. In cases where an *SCN1A* mutation was found, segregation analysis was performed to determine the mutation's de novo or inherited origin. Written informed consent was obtained from all subjects. The collection and usage of DNA samples, and management of data followed the Helsinki Declaration of 1975 and also satisfying the Hungarian legal requirements of genetic examination, research and biobanking.

Genomic DNA was extracted from peripheral blood cells of the patients using the E.Z.N.A. Blood DNA Maxi Kit (Omega Bio-tek, USA) according to the protocol of the manufacturer. As the first stage of screening, mutation analysis was performed of the *SCN1A* gene (sequence reference: NM_001165963, NP_001159435) by direct sequencing. Exons 1–26 were amplified by PCR. Primers were designed in our laboratory (primer sequences and PCR conditions are available upon request in our institute). Sequencing was performed with the same primers as those applied for the PCR amplification using a BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions. Interpretation of the results was performed with the help of Mutation Taster (http://www.mutationtaster.org), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2) PROVEAN (http://provean.jcvi.org/index.php) and Mutation Assessor (http://mutationassessor.org/r3) prediction software. The identified mutations were validated on a second sample obtained from the patients. During the second stage of screening, 33 sequence-negative patients were tested with MLPA method. MLPA was performed with the SALSA MLPA Kit P-137 Probemix (MRC-Holland, Netherlands) in accordance with the manufacturer's instructions. All identified variants were classified by the standards and guidelines set by the American College of Medical Genetics and Genomics (ACMG) standards and guidelines [27].

3. Results

A total of 12 previously described *SCN1A* alterations (in 15 patients and three relatives) and 15 previously unknown pathogenic mutations (in 15 patients and two relatives) were identified by Sanger sequencing analysis. MLPA testing detected gross deletions of the *SCN1A* gene in three additional patients. Altogether, different types of *SCN1A* mutations were identified in 33 patients from our cohort. The inheritance patterns could not be determined in all cases as parental samples were not available in some families. The mutations proved to be inherited in six cases. The parents either have developed GEFS + syndrome (Patient 1b) or suffered from febrile seizures in childhood (Patient 3c, 21b, 24b). A couple of parents have remained unaffected (Patient 5b, 8b).

Among the previously described mutations, a recurrent, missense mutation in exon 17 was detected (p.Thr1174Ser) in three non-consanguineous patients and in the mother of one patient without any neurological symptoms (Patients 4, 5/a, b, 20) According to the study by Cestéle et al. (2013) the functional effects of this mutation are divergent. They reported a three-generation family segregating this mutation where three affected individuals had developed febrile seizures and/or focal occipital epilepsy while two family members suffered from typical familial hemiplegic migraine [28]. In exon 19, the recurrent truncating mutation p.Arg1245* was identified in two non-consanguineous patients presenting with classical DS phenotype (Patients 6, 7). A familial truncating mutation in exon 10 was detected in a father and in his two daughters. While the girls were affected by classical DS, the father merely had developed childhood febrile seizures (Patients 3/a, b, c). A mother with GEFS+, and his son with classical DS phenotype were harbouring a splice region variant (Patients 1/a, b). A frameshift causing deletion and a missense mutation associated with GEFS + phenotype (Patients 11, 13) and five other different, previously known missense alterations associated with classical DS were also found in our cohort (Patients 2, 8/a, 9, 10, 12). Missense and nonsense mutations, one splice region variant and one frameshift-causing deletion were detected so far (Table 1).

Three patients carried two different mutations simultaneously: two known mutations in one case (Patient 5/a), one known and one previously undescribed variants in the other patient (Patient 20), and another patient with two novel *SCN1A* mutations were identified (Patient 15).

Among the novel, previously undescribed *SCN1A* mutations 12 missense variants, two frameshift causing and one in-frame deletions were identified (Table 2). A missense variant in the last exon of *SCN1A* was detected in monozygotic twins with classic DS phenotype (Patients 26/a, b). One patient with a novel frameshift causing deletion and another with a missense mutation had a mild type of DS as they both appeared to have near normal cognitive ability with satisfactory seizure-control at school-age (Patients 19, 20). One previously undescribed frameshift causing and one in-frame deletion in patients with DS phenotype (Patients 16, 18) and seven other, novel missense mutations associated with DS phenotype were also found in our cohort (Patients 15, 17, 21/a, 22, 25, 27). Three novel missense mutations associated with GEFS + phenotype were also identified (Patients 14, 23, 24/a). Two family members were detected as carriers of a pathogen *SCN1A* mutation having had childhood febrile seizures only (Patients 21/b, 24/b). Using the ACMG guidelines for the interpretation of sequence variants, 2 of 15 novel variants were classified as "pathogenic", 12 were classified as "likely pathogenic" and one remained a variant of uncertain significance [27]. Significant data concerning the previously unknown mutations and details of the important clinical features are

Table 1
Previously described mutations in the *SCN1A* gene.

Patient	Location	Sequence change	Amino acid change	Mutation type	Phenotype
1/a	intron 5-6	c.473 + 5 het G-A	–	splice site	DS
1/b	intron 5-6	c.473 + 5 het G-A	–	splice site	GEFS +
2	exon 9	c.1277A > G	p.Tyr426Cys	missense	DS
3/a	exon 10	c.1624 C > T	p.Arg542*	nonsense	DS
3/b	exon 10	c.1624 C > T	p.Arg542*	nonsense	DS
3/c	exon 10	c.1624 C > T	p.Arg542*	nonsense	FS
4	exon 17	c.3521C > G	p.Thr1174Ser	missense	GEFS +
5/a	exon 17	c.3521C > G	p.Thr1174Ser	missense	DS
5/a	exon 21	c.4219C > T	p.Arg1407*	nonsense	DS
5/b	exon 17	c.3521C > G	p.Thr1174Ser	missense	sine morbo
6	exon 19	c.3733C > T	p.Arg1245*	nonsense	DS
7	exon 19	c.3733C > T	p.Arg1245*	nonsense	DS
8/a	exon 20	c.3924A > T	p.Glu1308Asp	missense	DS
8/b	exon 20	c.3924A > T	p.Glu1308Asp	missense	sine morbo
9	exon 25	c.4793A > T	p.Tyr1598Phe	missense	DS
10	exon 26	c.4934 G > A	p.Arg1645Gln	missense	DS
11	exon 26	c.5189 T > C	p.Leu1730Pro	missense	GEFS +
12	exon 26	c.5264A > G	p.Asp1755Gly	missense	DS
13	exon 26	c.5536-5539delAAAC	p.Lys1846SerfsX11	frameshift	GEFS +

Sequence reference: NM_001165963 NP_001159435.
Patient 1/b is the mother of Patient 1/a; Patient 3/c is the father of Patient 3/a and 3/b; Patient 5/b is the mother of Patient 5/a; Patient 8/b is the mother of Patient 8/a.

listed in Tables 2 and 3.

The diagnosis of DS was confirmed with MLPA method in three additional patients. Phenotypes of two of them were not significantly different from those with point mutations (Patients 28, 30). On the other hand, the third patient (Patient 29) with a large heterozygous deletion of exon 1–17 ha d an unexpectedly mild DS phenotype. Table 4 contains relevant information about our MLPA-positive patients.

4. Discussion

SMEI or DS, as it was renamed in 1989, was first described in 1978 by Charlotte Dravet [29]. Her initial suggestion of a genetic cause was confirmed by Claes et al. in 2001 [14]. Noting the disease's association with fever, Claes et al. screened for mutations in *SCN1A* which had been known to cause GEFS + syndrome. Seven *de novo* mutations were eventually found by the group, and *SCN1A* has become one of the most relevant epilepsy genes since. Today DS is described as "a prototype of an epileptic encephalopathy" [30,31].

Among the 63 patients with DS or GEFS + phenotype investigated in this study pathogen alterations of the *SCN1A* gene have been confirmed in 33 patients (52,4%) and in five symptomless relatives. Twelve previously described mutations were detected in 18 participants and 15 novel mutations were found in 17 individuals. Most of the identified mutations proved to be missense mutations that probably alter but do not abolish the ion channel's function [23]. Among the discovered mutations there were only two previously known, recurrent mutations that were identified in more than one patient in our cohort (p.Thr1174Ser and p.Arg1245*). In accordance with our data, previous observations also show that out of more than 1200 reported *SCN1A* mutations, only 18% are recurrent [20]. Apart from the 30 patients with *SCN1A* point mutations, MLPA method revealed three cases of *SCN1A* gene deletion. The frequency of MLPA-detected anomalies were 9,09% in our cohort which is similar to that published in the study of Marini et al. (2009) [32,33]. Based on literature data, the average

Table 2
Novel mutations in the *SCN1A* gene.

Patient	Location	Sequence change	Amino acid change	Mutation type	Phenotype	ACMG-based classification and supporting evidence
14	exon 1	c.182 T > A	p.Leu61His	missense	GEFS +	Likely pathogenic (PM2, PM6, PP2, PP3)
15	exon 2	c.364A > G	p.Ile122Val	missense	DS	Likely pathogenic (PM1, PM5, PP2, PP3)
15	exon 2	c.377 T > C	p.Val126Ala	missense	DS	Uncertain significance (PM2,PP2,PP3)
16	exon 4	c.543_545delAGA	p.Glu181del	in frame del	DS	Likely pathogenic (PM1, PM2,PM6)
17	exon 9	c.1184C > A	p.Ala395Asp	missense	DS	Likely pathogenic (PM1, PM2, PM5, PM6, PP2, PP3)
18	exon 14	c.2510delG	p.Gly837ValfsX4	frameshift	DS	Pathogenic (PVS1, PM1, PM2, PM6, PP3)
19	exon 15	c.2666C > T	p.Ala889Val	missense	DS[b]	Likely pathogenic (PM1, PM2, PM6, PP2, PP3)
20[a]	exon 18	c.3677delT	p.Phe1226SerfsX2	frameshift	DS[b]	Pathogenic (PVS1, PM1, PM2, PM6, PP3)
21/a	exon 21	c.4223 G > C	p.Trp1408Ser	missense	DS	Likely pathogenic (PM1, PM2, PP2, PP3)
21/b	exon 21	c.4223 G > C	p.Trp1408Ser	missense	FS	Likely pathogenic (PM1, PM2, PP2, PP3)
22	exon 22	c.4322C > T	p.Ala1441Val	missense	DS	Likely pathogenic (PM2, PM6, PP2, PP3)
23	exon 22	c.4328A > C	p.Asp1443Ala	missense	GEFS +	Likely pathogenic (PM2, PM6, PP2, PP3)
24/a	exon 26	c.5332 G > C	p.Val1778Leu	missense	GEFS +	Likely pathogenic (PM1, PM2, PP2, PP3)
24/b	exon 26	c.5332 G > C	p.Val1778Leu	missense	FS	Likely pathogenic (PM1, PM2, PP2, PP3)
25	exon 26	c.5399 T > G	p.Leu1800Arg	missense	DS	Likely pathogenic (PM1, PM2, PM6, PP2, PP3)
26/a	exon 26	c.5621 G > C	p.Arg1874Pro	missense	DS	Likely pathogenic (PM2, PM6, PP2, PP3)
26/b	exon 26	c.5621 G > C	p.Arg1874Pro	missense	DS	Likely pathogenic (PM2, PM6, PP2, PP3)
27	exon 26	c.5438A > C	p.Glu1813Ala	missense	DS	Likely pathogenic (PM1, PM2, PM6, PP2, PP3)

Sequence reference: NM_001165963 NP_001159435.
Patient 21/b is the mother of Patient 21/a; Patient 24/b is the mother of Patient 24/a.
[a] This patient has an already known missense mutation (c.3521C > G, p.Thr1174Ser) also.
[b] Mild type of the disease with near normal cognitive ability and satisfactory seizure-control.

Table 3
Clinical features of patients with novel mutations in the *SCN1A* gene.

Patient	Age of onset	Febrile seizure	Afebrile seizure	Developmental delay	Intellectual disability	Prolonged seizure/Status epilepticus	Movement disorder
14	11 months	+	+	–	mild	–	–
15	4 months	+	+	–	mild	+	–
16	4 months	+	+	mild	NA	+	NA
17	5 months	+	+	–	moderate	–	–
18	6 months	+	+	–	NA	+	+
19	3 months	+	–	–	mild	–	–
20	3 months	+	+	–	mild	–	–
21/a	3 months	+	–	–	mild	+	–
21/b		+	–	–	–	–	–
22	6 months	+	–	NA	NA	NA	NA
23	1 year	+	+	–	–	–	–
24/a	1 year	+	+	–	–	–	–
24/b	childhood	+	–	–	–	–	–
25	5 months	+	+	+	NA	NA	NA
26/a	8 months	+	+	+	NA	+	–
26/b	11 months	+	+	+	NA	+	–
27	7 months	+	–	–	NA	+	–

NA: data not available.

Table 4
Patients with *SCN1A* CNVs detected by MLPA.

Patient	Genotype	Phenotype
28	heterozygous whole gene deletion	DS
29	heterozygous exon 1-17 deletion	DS[#]
30	heterozygous exon 1-2 deletion	DS

[#][#]Mild type of the disease with near normal cognitive ability and satisfactory seizure-control.

frequency of MLPA-detected deletions and duplications is approximately 10–12% among *SCN1A*-mutation negative patients; therefore we recommend this method as a second-tier of screening [34]. Our patients with confirmed gross deletions of *SCN1A* do not show any phenotypic difference compared to those with point mutations: febrile and/or afebrile seizures and intellectual disability remain the characteristic features in both groups. Despite the large heterozygous deletion, interestingly, the phenotype appeared to be a milder form of DS in one case. In fact, evidence shows that an *SCN1A* deletion combined with *SCN2A* and *SCN3A* deletions produce a more severe phenotype than DS with earlier onset and progressive microcephaly. On the other hand, disease associated with *SCN1A* and *SCN9A* deletions produces milder DS phenotypes [35].

In this paper the authors aimed to further understand the genotypic and phenotypic diversity of *SCN1A* gene-related disorders. Phenotype exhibited large variability in our patient cohort, and we could not detect any strong correlation between genotype and phenotype. As shown by Fig. 1. The detected mutations cannot be localized to a hot spot region of the gene. As Brunklaus et al. speculate, the phenotype might not necessarily be determined only by the SCN1A protein itself, but by a number of auxiliary proteins. [36]. Recently, Sadleir et al. identified a recurrent *SCN1A* missense variant in exon 5 (c.677C > T, p.Thr226Met) in 8 children with an early onset epileptic encephalopathy much more severe than DS. The disorder is characterized by an earlier age of onset, profound developmental impairment and a distinctive hyperkinetic movement disorder [37]. No patient with such a mutation was detected in our cohort.

During recent years, murine Dravet models have made a significant contribution to the better understanding of the neurobiology of the disease [38,39]. DS as a channelopathy causes widespread Nav1.1 dysfunction throughout the brain which, in turn contributes to the encephalopathy. This already-vulnerable system may be susceptible to secondary aggravating events such as status epilepticus.

The early clinical diagnosis of DS may be difficult because the typical clustering of symptoms become apparent only during follow-up. An infant with prolonged febrile seizures and a confirmed *SCN1A* mutation has an *SCN1A* gene-related disorder. Due to the lack of consistent genotype-phenotype correlations it is unpredictable whether the disorder may lead to the evolution toward GEFS + syndrome or DS.

Recently, Cetica et al. reported that age of seizure onset in individuals with *SCN1A* mutations is a stronger predictor of outcome than the type of mutation [40]. It is assumed that early recognition and treatment to control prolonged/repeated seizures in the first year of life might limit the progression to epileptic encephalopathy [40,41]. On the other hand, de Lange et al. suggest that mosaicism, caused by post-zygotic mutation, can be a major modifier of SCN1A-related diseases [42].

One of the limitations of our study is that some mutations may pass undetected using conventional techniques such as Sanger sequencing. Resequencing of *SCN1A* – negative patients by a targeted next generation sequencing (NGS) panel comprising different epilepsy genes, including *SCN1A* or by whole exome sequencing (WES) could further refine our results [43,44]. Those patients without *SCN1A* mutation may have an alternative genetic diagnosis resembling *SCN1A* related diseases [45]. Using an NGS gene panel these patients could also obtain an accurate diagnosis.

In the future, we are also planning to investigate genes that may influence the severity of the GEFS + and DS phenotype. This could further advance our understanding of the genotype-phenotype correlations in patients with pathogenic *SCN1A* mutations.

We hope that better understanding of the pathophysiology of this epilepsy syndrome will allow more adequate therapy and better outcome in the future.

5. Conclusions

This work represents the first genetic analysis of the *SCN1A* gene in a large Hungarian cohort with DS or GEFS + syndrome phenotype. Fifteen novel point mutations of *SCN1A* gene were identified among the 12 previously described mutations. Some cases proved to be familial. Three patients with DS phenotype harboured gross gene deletion of *SCN1A*.

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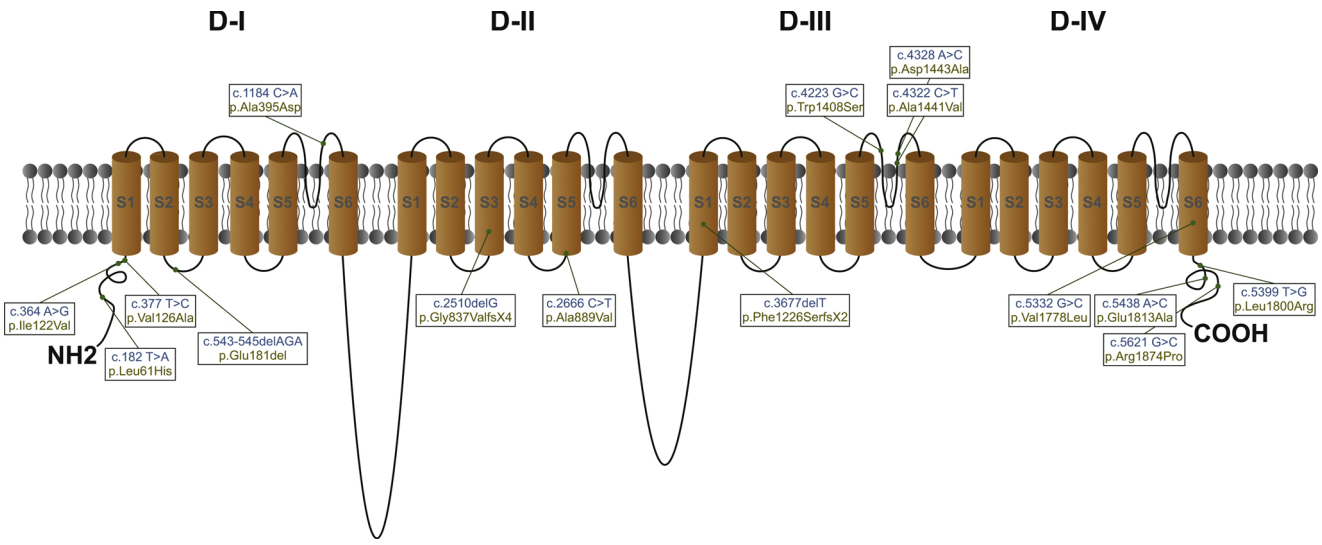

Fig. 1. The locations of the novel mutations in the SCN1A protein.

Conflicts of interest

None declared.

Ethical approval

According the Hungarian legislation, the genetic examination process was done by the guidelines of the XXI/2008; the study design was approved by the HRB National Ethics Committee. Approval for the study was also provided by the Regional Research Ethics Committee of the Medical School University of Pécs. Record number: 7473-2018PTE.

Author contributions

KH designed the study. JZ, AS and JB carried out the analysis. ÁT, KH and MC collected the data and interpreted the results. ÁT and AF wrote the first version of the article. BM and KH critically revised the manuscript. All authors reviewed and edited the manuscript and approved the final version.

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