

Genetic Landscape of *SCN1A* Variants in a Turkish Cohort with GEFS+ Spectrum and Dravet Syndrome

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Keywords

Dravet syndrome · Epilepsy · GEFS+ spectrum · Novel variant · *SCN1A*

Abstract

Introduction: The α subunit of voltage-gated sodium channels in mammals is encoded by 9 different genes, and variations in the *SCN1A*, *SCN2A*, *SCN3A*, and *SCN8A* genes highly expressed in the CNS have been associated with epilepsy phenotypes. This study aimed at investigating the frequency of *SCN1A* gene variations in Dravet syndrome (DS) and GEFS+ spectrum phenotype cases and discussing the molecular results in the context of genotype-phenotype correlation.

Methods: Fifteen patients diagnosed with DS and 54 patients meeting the GEFS+ spectrum criteria were included in this study. All patients were evaluated by next-generation sequencing and multiplex ligation-dependent probe amplification using an *SCN1A* gene commercial kit. **Results:** A total of 17 different variants were detected in 18 index cases (26%), of which 7 were novel variations (p.M1R, p.M147T, p.I767L, p.N1391Ifs*5, p.R1886G, p.E1915G, p.R1933Q). Of the 18 cases with variation in the *SCN1A* gene, 12 had DS and

6 had GEFS+ phenotype. The variations were de novo in all DS cases and in 1 case with a GEFS+ phenotype; in 5 GEFS+ cases, the variant was inherited from the affected parent.

Discussion: This study contributes to the variation spectrum in cases with DS and GEFS+ phenotype with the novel variants detected. *SCN1A* genetic analysis can help in determining whether antiseizure medication should be selected or avoided in cases with variations. The elucidation of the molecular etiology makes it possible to provide the family with effective genetic counseling for future pregnancies.

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Introduction

Voltage-gated sodium channels which are highly expressed in the central nervous system (CNS) regulate the electrical activity of neuronal cells, and variations in these channels lead to the epilepsy phenotype [Escayg and Goldin, 2010]. Voltage-gated sodium channels have α subunits of approximately 260 kDa, and these structures comprise 4 homologous domains (termed DI–IV), each containing 6 transmembrane segments (termed S1–6)

[Trimmer and Rhodes, 2004]. The α subunits of voltage-gated sodium channels in mammals are encoded by 9 different genes, and the variants detected in *SCN1A*, *SCN2A*, *SCN3A*, and *SCN8A* genes which are highly expressed in the CNS have been associated with epilepsy phenotypes [Fujiwara, 2006; Shi et al., 2009; Larsen et al., 2015; Zaman et al., 2020]. The prototype of this epilepsy group, known as channelopathies, are the *SCN1A* gene-related phenotypes. *SCN1A*-associated phenotypes show a broad clinical spectrum ranging from familial febrile seizures (FS) and genetic epilepsy with febrile seizures plus (GEFS+; OMIM #604403) to Dravet syndrome (DS; OMIM #607208) [Scheffer and Nabbout, 2019]. FS, FS+, and GEFS+ definitions are given in the methods section. Furthermore, rare phenotypes with *SCN1A* variants include familial hemiplegic migraine (FHM; OMIM #609634), West syndrome, Panayiotopoulos syndrome, myoclonic-atic epilepsy, epilepsy of infancy with migrating focal seizures, and sudden unexpected death in epilepsy [Wallace et al., 2003; Harkin et al., 2007; Vahedi et al., 2009; Kivity et al., 2017; Scheffer and Nabbout, 2019; Rochtus et al., 2020].

DS is the most severe phenotype in the clinical spectrum associated with *SCN1A*, and its frequency is approximately 1 in 20,000–40,000 live births [Bayat et al., 2015; Wu et al., 2015]. DS is the prototype of developmental and epileptic encephalopathy group diseases that starts with hemiclonic or generalized tonic-clonic prolonged febrile seizures at a mean age of 6 months. Partial seizures, absence seizures, myoclonic seizures, and convulsive and nonconvulsive status epilepticus episodes are added to the patients' clinical phenotype between the age of 1 and 5. Usually, electroencephalography (EEG) is normal for the first 1–2 years, and then generalized spike-wave, poly spike-wave, and multifocal discharges are observed [Scheffer and Nabbout, 2019]. Generally, seizures are triggered by fever and are not totally responsive or controlled by antiseizure medications, especially in the first years of life [Dravet et al., 2005]. Myoclonic symptoms do not develop in certain cases in DS, and clinical symptoms may be milder [Guerrini and Oguni, 2011]. Non-epileptic symptoms such as intellectual disability start around age 2 years, and ataxia is seen in childhood [Scheffer and Nabbout, 2019].

GEFS+, defined by Scheffer and Berkovic [1997], is a familial epilepsy syndrome in which affected individuals show different epilepsy phenotypes. In the GEFS+ spectrum, the most common phenotype is FS, followed by FS+. In addition to FS and FS+ phenotypes, the GEFS+ spectrum includes generalized seizure types such as ab-

sence, myoclonic, and atonic seizures as well as focal seizures [Zhang et al., 2017]. In most cases, patients have easily treatable epilepsy and normal cognitive development [Myers KA et al., 2018].

The *SCN1A* gene encoding the Nav1.1 receptor is located in chromosome 2q24.3 and comprises 26 exons with an approximate size of 159.8 kb [Malo et al., 1994]. In the CNS, it regulates neuronal excitation via inhibitory GABAergic interneurons, and experimental studies have shown that its variations lead to the epilepsy phenotype associated with disruption of this balance [Rubinstein et al., 2015]. It has been shown in mouse experiments that dysfunction in cerebellar GABAergic and inhibitory Purkinje cells may be the underlying cause of ataxia and intellectual disability in these cases [Kalume et al., 2007]. To date, at least 2,069 variants in the *SCN1A* gene identified in the HGMD Professional database have been reported, 58% of which are missense/nonsense, 9% splice-site, 18% small deletions, 6% small insertions, 1.5% small indels, 6% gross deletions, 1% gross insertions, and 0.5% complex rearrangements [Stenson et al., 2020]. Note that >80% of the cases diagnosed with DS have pathogenic variants in the *SCN1A* gene, of which 95% are de novo. Half of the DS cases have truncation variants, half of them have missense variants, and ~3% of the cases have gross deletions/duplications in *SCN1A* [Claes et al., 2001]. In about 20% of GEFS+ spectrum families, there is a variant in the *SCN1A* gene, and de novo variants have been reported in certain cases of sporadic GEFS+ phenotype [Myers et al., 2017; Zhang et al., 2017; Bayat et al., 2021]. In GEFS+ families, variable expressivity and incomplete penetrance are frequent, and monoallelic missense *SCN1A* gene variations are common [Myers KA et al., 2018].

The phenotypes associated with the *SCN1A* gene vary depending on the effect of the detected variation in the gene. Loss-of-function (LOF) variations resulting in total loss of sodium channel activity result in the DS phenotype, and LOF variations resulting in partial loss lead to the GEFS+ phenotype [Scheffer and Nabbout, 2019]. On the other hand, gain-of-function (GOF) variations result in the GEFS+, FHM, and “developmental and epileptic encephalopathy 6B, non-Dravet” (OMIM #619317) phenotypes [Dichgans et al., 2005; Fan et al., 2016]. Cases with LOF variations resulting in total loss of sodium channel activity exhibit the DS phenotype (presenting with hemiclonic or generalized tonic-clonic febrile seizures at a mean age of 6 months, partial seizures between 1 and 5 years of age, absence seizures, myoclonic seizures, seizures triggered with fever, and intellectual disability and ataxia in later stages). On the other hand, cases with

partial LOF variations exhibit generalized seizures, such as myoclonic and atonic seizures, and focal seizures that can be controlled with antiepileptic therapy, in addition to FS, FS+ phenotypes compatible with the GEFS+ spectrum without intellectual disability, and ataxia [Scheffer and Berkovic, 1997; Dravet et al., 2005]. Epileptic spasms beginning in the first 3 months of life, severe developmental impairment, and movement disorders beginning in the infantile period (choreoathetosis, dystonia, and perioral hyperkinesia), all of which are more severe than the DS phenotype, are observed in some of the cases with GOF variation, whereas others demonstrate the FHM phenotype accompanied by migraine attacks with and without aura and hemiplegia/hemiparesis [Dichgans et al., 2005; Fan et al., 2016].

This study aimed at investigating the frequency of *SCN1A* gene variations in DS and GEFS+ spectrum phenotype cases and discussing the molecular results in the context of genotype-phenotype correlation.

Materials and Methods

All patients have given their informed consent for participation in the research study. Patients followed by 2 different centers (Giresun University Department of Pediatric Neurology and Erzurum Regional Training and Research Hospital Clinics of Pediatrics and Medical Genetics) between June 2012 and January 2021 were included in this study. Note that 15 patients diagnosed with DS according to the International League Against Epilepsy (ILAE) criteria and 54 patients meeting the GEFS+ spectrum criteria were included in the study [Scheffer et al., 2017; Myers KA et al., 2018]. Patients classified as belonging to the DS phenotype demonstrated the following symptoms: (1) frequent occurrence of convulsive seizures in the first year of life with an onset of within 5 to 8 months, and/or convulsive seizures triggered by vaccines; and (2) occurrence of febrile and afebrile seizures at an average of 2 weeks to 2 months after the first seizure, developmental delay, gait anomalies, and occurrence of different types of seizures between 1 and 4 years of age [Dravet, 2011]. For the DS population, a definite delay may not be found until around age 2 years. For this reason, all DS cases were evaluated after 2 years of age in terms of language and developmental delay. All but one of the DS cases were followed up to at least 2.5 years of age. The cases with findings consistent with the DS phenotype were classified after 2.5 years of age. The seizure type, age of onset, family history, EEG results, and antiseizure medication responses of the patients were evaluated and noted. Tonic-clonic seizures associated with fever $\geq 38^{\circ}\text{C}$ in the age group of 3 months to 6 years were considered as FS [Myers KA et al., 2018]. FS+ is defined as one or both of the following: (1) febrile tonic-clonic seizures that begin before the age of 3 months and/or continue after the age of 6 years and (2) coexistence of febrile and afebrile generalized tonic-clonic seizures [Myers KA et al., 2018]. The following conditions are considered as complex GEFS+ phenotypes: (1) combination of FS and FS+ with various seizure types (absence, myoclonic or atonic seizures), (2) focal seizures without

FS (indicative of temporal and frontal lobe semiology), and (3) only afebrile generalized tonic-clonic seizures without FS [Myers KA et al., 2018]. All cases were evaluated with brain magnetic resonance imaging (MRI) to exclude epilepsies secondary to structural brain anomalies, and cases with anomalies were excluded from the study. The patients included in this study had not previously undergone any genetic testing.

Molecular Analysis

To investigate the molecular etiology of the diagnosis of DS and GEFS+ patients, genomic DNA was isolated from the peripheral blood of patients using the QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany) as per the manufacturer's protocols. Samples from patients were evaluated by next-generation sequencing (NGS) using an *SCN1A* gene (sequence reference: NM_001165963, NP_001159435) commercial kit (Blueprint Genetics, Seattle, WA, USA). The NGS process was performed using the Illumina Miseq platform (Illumina, San Diego, CA, USA), and the raw data were analyzed through the Illumina BaseSpace Variant Interpreter bioinformatics program. The raw data were visualized via Integrated Genom Viewer. In the second step, 51 cases in which variations could not be detected were evaluated by multiplex ligation-dependent probe amplification (MLPA) using the SALSA MLPA Kit P-137 Probemix (MRC-Holland, The Netherlands). To evaluate the pathogenicity of novel variants, *in silico* prediction tools (MutationTaster, SIFT, Provean, PolyPhen2, CADD, Varsome), allele frequencies in population studies (1000 G, gnomAD, ExAC), ClinVar, and Human Gene Mutation Database (HGMD) and American College of Medical Genetics and Genomics (ACMG) criteria were used [Richards et al., 2015; Kopanos et al., 2019]. Variations detected in index cases were investigated in parents by Sanger sequencing. When the *de novo* variant was detected, the paternity status was confirmed by fragment analysis.

The functional variant prediction in Na_vs and Ca_vs ion channels (funNCion) online tool was used for the analysis of pathogenicity and functional predictions of novel missense variations evaluated as variants of uncertain significance (VUS) according to ACMG criteria (<https://funnc.shinyapps.io/shinyappweb/>). These web based tools can help researchers and clinicians to interpret their variants [Heyne et al., 2020].

Statistical Analysis

Results were presented as median (min–max), number (*n*), and percentage (%). Statistical analyses were performed using SPSS version 25 (IBM, Inc., Chicago, IL, USA). Statistical comparisons between groups were performed using Fisher's exact test and Mann-Whitney U-test, and $p < 0.05$ was considered statistically significant.

Results

The mean age of onset of the first seizure was 17.2 ± 3.6 months in the patients with the GEFS+ phenotype (54 cases) included in the study; however, the mean age of onset of the first seizures in the patients with the DS phenotype (15 cases) was 4.6 ± 1.7 months. In 65% (45/69) of the cases, there was a history of febrile or afebrile seizures

Table 1. Summary of clinical features in the Dravet syndrome study population

Patient characteristics	Patients, n
Sex	
Male	8
Female	7
Age of onset	
≤6 months	11
7–12 months	4
>12 months	0
Age of evaluation	
≤2 years	1
2–6 years	13
>6 years	1
History of febrile seizure	15
Family history	1
Age at first afebrile seizure	
<12 months	6
≥12 months	9
None	0
Type of first seizure	
Febrile seizure	15
Afebrile seizure	0
Fever sensitivity	15
Vaccine-associated	6
Developmental delay	14
EEG abnormalities	
Slow background activity	1
Focal epileptiform discharges	2
Multifocal epileptiform discharges	1
Diffuse epileptiform discharges	2
Subclinical epileptiform discharges	0
Normal	9
Treatment	
Single ASM	0
2 ASMs	14
≥3 ASMs	1
None	0

EEG, electroencephalogram; ASM, antiseizure medication.

in their first and/or second degree relatives. There was no family history of seizures in all but one of the 15 patients with the DS phenotype. Early development was normal in all cases. Delay in language development was detected in 14 cases with DS phenotype. Developmental delay was reported in 93% (14/15) and 7% (4/54) of the DS and GEFS+ patients, respectively. Ataxia was detected in 73% (11/15) of the DS patients and none of the GEFS+ patients. Note that 93% (14/15) of DS patients and 5% (3/54) of GEFS+ patients who received antiseizure medication were resistant or not totally responsive to treatment. Table 1 and Table 2 represent the distribution of DS and

Table 2. Summary of clinical features in the GEFS+ study population

Patient characteristics	Patients, n
Sex	
Male	32
Female	22
Age of onset	
≤6 months	3
7–12 months	9
>12 months	42
Age of evaluation	
≤2 years	8
2–6 years	39
>6 years	7
History of febrile seizure	21
Family history	44
Age of first afebrile seizure	
<12 months	0
≥12 months	31
None	23
Type of first seizure	
Febrile seizure	21
Afebrile seizure	33
Fever sensitivity	29
Vaccine-associated	1
Developmental delay	4
EEG abnormalities	
Focal epileptiform discharges	25
Central region discharges	17
Non-central region discharges	8
Generalized discharges (2–4 Hz) and normal background activity	10
Normal	19
Treatment	
Single ASM	31
2 ASMs	5
≥3 ASMs	0
None	18

GEFS+, genetic epilepsy with febrile seizures plus; EEG, electroencephalogram; ASM, antiseizure medication.

GEFS+ patients by seizure types, clinical, and EEG findings, respectively.

Statistical analysis was performed to determine whether there was a difference in clinical findings of *SCN1A*-positive and *SCN1A*-negative patients in the DS and GEFS+ patient cohorts. None of the aforementioned findings were statistically significant (Tables 3, 4).

A total of 17 different variants were detected in 18 index cases (26%) and 5 symptomatic relatives as a result of sequencing of the *SCN1A* gene. Note that 51 cases with no variant detected in the *SCN1A* gene sequencing analysis were included in MLPA analysis to evaluate gross de-

Table 3. Comparison of the examination findings in Dravet syndrome patients with and without *SCN1A* variations

Variable	<i>SCN1A</i> -positive (n = 12)	<i>SCN1A</i> -negative (n = 3)	Test	p value
Age of onset of febrile seizure, months (median, range)	4.75 (3–9)	5.0 (4–7)	Z = 0.591	0.555
Age of onset of afebrile seizure, months (median, range)	6.0 (4–10)	6.0 (5–8)	Z = 0.221	0.825
EEG initial abnormalities, % (n)	50 (6)	0 (0)	$\chi^2 = 2.500$	0.229
Present focal seizures, % (n)	25 (3)	33.3 (1)	$\chi^2 = 0.085$	1.000
Fever-sensitive seizures, % (n)	83.3 (10)	66.7 (2)	$\chi^2 = 0.417$	0.516
Vaccination-related seizures, % (n)	33.3 (4)	0 (0)	$\chi^2 = 1.364$	0.516
Status epilepticus, % (n)	25 (3)	33.3 (1)	$\chi^2 = 0.085$	1.000

EEG, electroencephalogram; Z, Mann-Whitney U-test value. χ^2 , Fisher's exact test.**Table 4.** Comparison of the examination findings in GEFS+ spectrum patients with and without *SCN1A* variations

Variable	<i>SCN1A</i> -positive (n = 6)	<i>SCN1A</i> -negative (n = 48)	Test	p value
Age of onset of febrile seizure, months (median, range)	15 (3–30)	12 (3–48)	Z = 0.654	0.907
Age of onset of afebrile seizure, months (median, range)	24 (9–38)	29 (8–144)	Z = 0.964	0.335
EEG initial abnormalities, % (n)	50 (3)	39.6 (19)	$\chi^2 = 0.240$	0.678
Present focal seizures, % (n)	33.3 (2)	39.6 (19)	$\chi^2 = 0.088$	1.000
EEG findings (central discharges), % (n)	33.3 (2)	31.3% (15)	$\chi^2 = 0.011$	1.000

EEG, electroencephalogram; Z, Mann-Whitney U-test value; χ^2 , Fisher's exact test.

letion/duplications; however, no pathogenic variation was reported in this study. Of the 17 different variations identified, 7 were novel and 10 were previously reported in the literature. Furthermore, 56% of the variations were missense, 17% nonsense, 17% splice-site, 5% frameshift, and 5% start loss variations. According to ACMG criteria, 3 of the novel variations were evaluated as likely pathogenic, 2 as pathogenic, and 2 as VUS (Table 5). The previously reported c.3284A>G (p.Y1095C) variation, which was evaluated as VUS according to ACMG criteria, was predicted as neutral variation with no functional effect according to the “FunNCion” tool analysis. The novel missense c.5798G>A (p.R1933Q) and c.5656C>G (p.R1886G) variants evaluated as VUS according to ACMG criteria were predicted as neutral and pathogenic variations, respectively. It was also predicted that the c.5656C>G (p.R1886G) variation could be a LOF variant. Of the 18 cases with variation in the *SCN1A* gene, 12 had DS and 6 had GEFS+ phenotype. The variations were de novo in all DS cases and in 1 case with a GEFS+ phenotype, and were inherited from an affected parent in 5 GEFS+ cases. All cases with a truncating variation caus-

ing premature termination of protein synthesis were in the DS group.

In the DS patients with a variation in the *SCN1A* gene, 4/12 cases were novel variations and 8/12 cases had variations previously defined in the literature or in the ClinVar database [Claes et al., 2001; Fukuma et al., 2004; Zucca et al., 2008; Depienne et al., 2009; Cho et al., 2018]. In DS cases, febrile status epilepticus was observed in 33% (4/12), EEG anomalies (1 slow background activity, 2 focal, 1 multifocal, 2 diffuse epileptiform discharges) in 50% (6/12), vaccine-associated seizure (seizure occurring within 72 h of vaccination) in 33% (4/12), and ataxia in 75% (9/12). Moreover, 2 of the novel variations detected in DS cases were missense (p.I767L, p.E1915G), one was frameshift (p.N1391Ifs*5), and one was start loss (p.M1R). Patient 12, with the novel missense c.5744A>G (p.E1915G) variant, was a 14-year-old male patient whose first seizure started as a febrile seizure at the age of 9 months. This patient, who had a history of multiple febrile seizures, had ataxia and developmental delay. In his family history, his mother had a febrile convulsion in childhood and her cousin died because of sudden unexpected death.

Table 5. Genetic features of patients with *SCN1A* variants

Pa- tient	Sex	Pheno- type	Genomic coordinates (NC_000002.11)	Nucleotide (c.DNA) (NM_006920.6)	Protein (NP_008851.3)	Exon	Variant type	Protein domain	Inherit- ance	Family history	gnomAD v2.1.1 frequency	ACMG	Reported
1	M	GEFS+	g.166915152G>T	c.311C>A	p.Ala104Asp	2	Missense	N-terminus	De novo	–	–	Likely pathogenic (PM1, PM2, PP2, PP3)	Yes (ClinVar ID: 567303)
2	F	DS	g.166930130A>C	c.2>G	p.Met1?	1	Initiation codon variant	N-terminus	De novo	–	–	Pathogenic (PV51, PM2, PP3)	This paper
3	F	DS	g.166909392G>A	c.664C>T	p.Arg222*	5	Nonsense	DI-S4	De novo	–	–	Pathogenic (PV51, PM2, PP3, PP5)	Yes (ClinVar ID: 12889)
4	F	DS	g.166909392G>A	c.664C>T	p.Arg222*	5	Nonsense	DI-S4	De novo	–	–	Pathogenic (PV51, PM2, PP3, PP5)	Yes (ClinVar ID: 12889)
5	F	GEFS+	g.166903464G>A	c.1193C>T	p.Thr398Met	9	Missense	DI-S5S6 loop	Paternal	Father: FS+, behavioral disorder	0.00002124	Likely pathogenic (PM1, PM2, PP2, PP3)	Yes (ClinVar ID: 206762)
6	M	DS	g.166897824T>G	c.2299A>C	p.Ile767Leu	13	Missense	DI-DII linker	De novo	–	–	Likely pathogenic (PM1, PM2, PP2, PP3)	This paper
7	F	DS	g.166897740C>T	c.2415+1G>A			Splice-site	DII-S2	De novo	–	–	Pathogenic (PV51, PM2, PP3, PP5)	Yes (ClinVar ID: 801805)
8	F	DS	g.166894396G>A	c.2803C>T	p.Arg935Cys	15	Missense	DII-S5S6 loop	De novo	Brother: SGS	–	Pathogenic (PM1, PM2, PM5, PP2, PP3, PP5)	Yes (ClinVar ID: 68604)
9	F	GEFS+	g.166892703T>C	c.3284A>G	p.Tyr1095Cys	16	Missense	DII-DIII linker	Maternal	Mother: FS	0.000003978	VUS (PM2, PP2, PP3)	Yes (ClinVar ID: 964594)
10	F	DS	g.166866246G>A	c.3985C>T	p.Arg1329*	20	Nonsense	DIII-S4	De novo	–	–	Pathogenic (PV51, PM2, PP3, PP5)	Yes (ClinVar ID: 206816)
11	F	DS	g.166859094T>C	c.4172A>G	p.Asn1391Ser	21	Missense	DIII-S5S6 loop	De novo	–	–	Likely pathogenic (PM1, PM2, PM5, PP2)	Yes (ClinVar ID: 449374)
12	M	DS	g.166848041T>C	c.5744A>G	p.Glu1915Gly	26	Missense	C-terminus	De novo	–	–	Likely pathogenic (PM1, PM2, PM5, PP2, PP3)	This paper
13	F	GEFS+	g.166847987C>T	c.5798G>A	p.Arg1933Gln	26	Missense	C-terminus	Maternal	Mother: FS	0.000007982	VUS (PM1, PM2, PP2)	This paper
14	M	DS	g.166854548C>T	c.4476G>A	p.Lys1492Lys	23	Synonymous (splice-site)	DIII-DIV linker	De novo	–	–	Likely pathogenic (PV51, PM2, BP4)	Yes (ClinVar ID: 426654)
15	M	GEFS+	g.166848129G>C	c.5656C>G	p.Arg1886Gly	26	Missense	C-terminus	Paternal	Father: FS	–	VUS (PM2, PP2, PP3)	This paper
16	M	DS	g.166859091 TTATTCACG>CA	c.4167_4175del- CGTGAATAAinsTG	p.Asn1391Ilefs*5	21	Frameshift	DIII-S5S6 loop	De novo	–	–	Pathogenic (PV51, PM2, PP3)	This paper
17	M	DS	g.166904136C>T	c.1170+1G>A			Splice-site	DI-S5S6 loop	De novo	–	–	Pathogenic (PV51, PM2, PP3, PP5)	Yes (ClinVar ID: 381570)
18	F	GEFS+	g.166912954A>G	c.440T>C	p.Met147Thr	3	Missense	DI-S1	Maternal	Mother: FS	–	Likely pathogenic (PM1, PM2, PP2, PP3)	This paper

DS, Dravet syndrome; GEFS+, genetic epilepsy with febrile seizures plus; FS, febrile seizure; FS+, febrile seizure plus; SGS, secondary generalized seizure; ACMG, American College of Medical Genetics and Genomics; VUS, variant of uncertain significance; gnomAD, genome aggregation database.

Table 6. Clinical features of patients with *SCN1A* variants

Patient	Sex	Age of onset	Clinical onset	All types of seizures	EEG features	Fever sensitivity	Vaccine-associated	Developmental delay	Other symptoms	Follow-up period	All types of ASMs used	Diagnosis
1	M	9 months	FS	FS+ SGS	N	+	–	–	Language delay	18 months	VPA	GEFS+
2	F	4 months	FMS	FMS MS	N	+	–	+	Language delay	24 months	LEV, PB	DS
3	F	5 months	FS	FS MS	N	+	+	–	Language delay	5 years	LEV, PB	DS
4	F	4.5 months	FS	FS MS	N	+	+	–	Language delay	6 years	LEV, PB	DS
5	F	9 days	FMS	FS FIAS	Right centrottemporal	+	–	–	–	4 years	VPA	GEFS+
6	M	3 months	FS	Febrile SE SGS MS	Slow background multifocal	+	+	+	Ataxia	5 years	VPA, CLB STP	DS
7	F	3 months	FS	Febrile SE SGS MS	Slow background multifocal	+	–	+	Ataxia	3 years	VPA, CLB	DS
8	F	6 months	FMS	Febrile SE SGS MS	N	+	+	+	Ataxia	3 years	VPA, CLB	DS
9	F	12 months	FS	Febrile SE SGS	Bilateral centrottemporal	+	–	–	Language delay	12 months	VPA	GEFS+
10	F	5 months	FS	FS SGS	N	+	–	+	Ataxia	3 years	VPA, CLB	DS
11	F	3 months	FMS	FS SGS FMS	Slow background multifocal	+	–	+	Ataxia	3 years	VPA, CLB	DS
12	M	9 months	FS	FS MS	N	+	–	+	Ataxia	4 years	VPA	DS
13	F	2.5 years	FS	FS SGS	Bifrontal generalized	–	–	–	–	1.5 years	VPA	GEFS+
14	M	5 months	FS	FS SGS	Right centrottemporal	+	–	+	Ataxia	4 years	VPA, LEV, STP, CLB	DS
15	M	2.5 years	FS	FS+ MS	N	+	–	–	–	5 years	PB	GEFS+
16	M	3 months	FS	FS SGS	Right centroparietal	+	–	+	Language delay, ataxia	6 years	PB	DS
17	M	6 months	FS	Febrile SE SGS MS	Left centro-parietal occipital	+	–	+	Ataxia	9 years	VPA, CLB	DS
18	F	1.5 years	FS	FS+ SGS	N	+	–	–	–	4 years	VPA	GEFS+

DS, Dravet syndrome; GEFS+, genetic epilepsy with febrile seizures plus; EEG, electroencephalogram; ASMs, antiseizure medications; M, male; F, female; N, normal; FS, febrile seizures; MS, myoclonic seizures; FMS, focal motor seizures; SE, status epilepticus; SGS, secondary generalized seizure; FIAS, focal impaired awareness seizures; LEV, levetiracetam; VPA, valproic acid; PB, phenobarbital; CLB, clobazam; STP, stiripentol.

In the GEFS+ phenotype with a variation in the *SCN1A* gene, 3 of the 6 cases had novel variations and 3 had variations previously defined in the literature or in the ClinVar database [Kang et al., 2019]. Furthermore, 1/6 of GEFS+ cases had febrile status epilepticus, and 3/6 of GEFS+ cases had EEG anomalies (bifrontal generalized, bilateral centrottemporal and right centrottemporal epileptiform discharges). All of the novel variations detected in GEFS+ cases were missense (p.R1933Q, p.R1886G, p.M147T) variations. In all cases with novel variations, the variation was reported to be inherited from the affected parents. Tables 5 and 6 show the clinical and genetic results of all patients with variations.

In cases with a variation in the *SCN1A* gene, the phenotype is quite heterogeneous. Phenotypic variability is considered to be attributed to the type of variant, the protein domain affected, and the change in sodium channel function, modifier genes, epigenetics, environmental factors, and mosaicism [Thompson et al., 2012; Ishii et al., 2017]. In this study, all cases with nonsense, frameshift, splice-site, start-loss variations that lead to the formation of premature stop codons and loss of sodium channel function showed treatment-resistant severe epilepsy phenotype (DS phenotype). Furthermore, DS phenotype was reported in cases with missense variations in the voltage sensor segment (S4) and ion-pore region (S5-S6) of the sodium channel protein. A GEFS+ phenotype was observed in cases with missense variations outside the voltage sensor segment (S4) and ion-pore region (S5-S6). However, in 2 cases (patients 5 and 6), the variation site and the phenotype of the affected individual were reported to be incompatible with this general information. Patient 5 is a 4.5-year-old girl, and her first seizures started at the age of 9 days as focal clonic seizures in the extremities and ended in the 3rd month. The patient, who had a history of non-resistant FS in the 12th month, had seizures in the form of focal impaired awareness seizures after the age of 3. EEG performed at the age of 3 years showed slow wave discharge with normal background activity in the right centrottemporal region, and the language and motor development of the patient were compatible with her peers. Her father had a history of seizures and behavioral problems corresponding to the FS+ phenotype in childhood. This patient had a missense variation c.1193C>T (p.T398M) in the pore forming linker S5-S6 segment from domain DI which was demonstrated to be paternally inherited. The same variation was reported by Kang et al. [2019] in a 43-year-old female patient whose first seizure started as a focal seizure at the age of 29. When both cases are evaluated together, this variation

in the *SCN1A* gene is associated with focal seizures. The c.1193C>T (p.T398M) variation detected in patient 5 was evaluated as likely pathogenic according to ACMG criteria, whereas it was predicted as a neutral variation with no functional effect according to the “FunNCion” tool analysis. The elucidation of this variation’s mechanism of inducing focal seizures through in vivo experimental studies may contribute to a clearer understanding of the genotype-phenotype relationship. Patient 6 is a 10-year-old male patient whose first seizure started in the 3rd month as FS and who had febrile status epilepticus twice, followed by myoclonic seizures. The age at seizure onset, seizure types, development of ataxia and drug-resistant seizures of the patient were reported to be compatible with the DS phenotype. In this case, a novel de novo missense c.2299A>C (p.I767L) variant affecting the *SCN1A* protein DI-DII linker region was detected. The fact that this variation causes the DS phenotype, although the variation is outside the voltage sensor segment (S4) and ion-pore region (S5-S6) can be explained by the following: (1) there may be a variation in another epilepsy gene that contributes to the patient’s clinical picture of severe epilepsy; (2) the specific variant could perhaps cause a severe LOF effect and therefore cause DS phenotype; and (3) the detected variation is heterozygous at the germline level. However, the rate of mutated cells in the brain tissue may be higher (somatic mosaicism), which explains the clinical picture of severe epilepsy. Nevertheless, these 2 possibilities could not be analyzed with additional genetic analyses and experimental studies. Studies in this area can provide a clearer understanding of the molecular etio-pathogenesis of the disease.

The pathogenicity of 7 novel variations identified in this study was evaluated together with the ACMG criteria, segregation analysis results, and literature data. It is predicted that the novel start-loss p.M1? variation detected in patient 2 will disrupt full-length protein synthesis by utilization of an alternative initiation codon further downstream. This variation was evaluated as pathogenic based on ACMG criteria, and it has been shown in segregation analysis that the variation is de novo. Furthermore, in the literature, transformations to other amino acids in the same codon have been described in cases with the DS phenotype [Depienne et al., 2009; Zuberi et al., 2011]. The novel missense variations detected in patients 6 and 12 were evaluated as likely pathogenic according to ACMG criteria, and the variations were demonstrated to be de novo in segregation analysis. The novel frameshift variant detected in patient 16 and the novel missense variant detected in patient 18 were classified as pathogenic and like-

ly pathogenic, respectively, as per ACMG criteria. It has been shown that the variation in patient 16 showing the DS phenotype is de novo, and the variation in patient 18 showing the GEFS+ phenotype is maternal. This patient's mother has an FS history. The novel missense variations detected in patients 13 and 15 showing the GEFS+ phenotype were evaluated as VUS according to ACMG criteria, and in the segregation analyses it was shown that the variations were inherited from the affected parents in both cases. Evaluating the effect of novel variations on sodium channel activity with in vitro and in vivo functional studies in animals would be useful for understanding their exact effects.

Discussion

Patients with *SCN1A* variations demonstrate a broad phenotypic spectrum ranging from hemiplegic migraine phenotype, one of the non-epilepsy phenotypes on the milder end, to GEFS+ phenotype on the moderate end, and the DS phenotype on the severe end [Scheffer and Nabbout, 2019]. The early diagnosis of DS, which causes additional problems such as intellectual retardation and ataxia in addition to the severe epilepsy phenotype, may be delayed because the non-epileptic clinical features only become apparent as the child gets older. There are studies reporting that early diagnosis reduces the cognitive impairment level of patients and limits the progression to epileptic encephalopathy [O'Reilly et al., 2018; Verheyen et al., 2021]. The NGS analysis methods developed in recent years allow for a faster molecular diagnosis of diseases with high genetic heterogeneity such as epilepsy.

The 18 index cases (18/69, 26%) included in this study and their 5 symptomatic parents were reported to have variations in the *SCN1A* gene. Moreover, 12 (80%) of the 15 DS phenotype patients and 6 (11%) of the 54 patients with GEFS+ phenotype included in the study were reported to have variations. The rate of variation we detected in the *SCN1A* gene (80%) in cases with the DS phenotype is similar to the literature data [Claes et al., 2001; Scheffer and Nabbout, 2019]. Molecular diagnosis rates may be increased by analyzing other epilepsy-related genes such as *PCDH19*, *GABRG2*, *CHD2*, *HCN1*, *STXBPI*, *GABRA1*, and *SCN1B*, which cause an DS-like phenotype in cases where no variation in the *SCN1A* gene is found despite having the DS phenotype [Bayat et al., 2021]. The variation rate in the *SCN1A* gene in cases with the GEFS+ phenotype ranges from 3 to 25% [Escayg et al., 2001;

Ceulemans et al., 2004; Scheffer et al., 2009; Herini et al., 2010; Tan et al., 2012]. In our study, we reported a proportion of GEFS+ patients with variation in the *SCN1A* gene similar to the studies in the literature. The fact that only 11% of the GEFS+ cases were reported to have a variant in the *SCN1A* gene in our study suggests that other genes may be responsible for the etiology in most of the cases in this phenotype. Analyzing other genes (*SCN1B*, *GABRG2*, *STX1B*, *HCN1*, *GABRD*) associated with the GEFS+ phenotype in the OMIM database may contribute to the molecular diagnosis [Bayat et al., 2021]. Moreover, there are phenotypes in the OMIM database (OMIM #609800, #612279, #613863, #613828) in which the responsible locus associated with GEFS+ has been identified; however, the responsible gene has not yet been discovered. This suggests that genes currently known to be associated with the GEFS+ phenotype represent only the tip of the iceberg.

In this study, the 17 different variations detected in *SCN1A* were distributed throughout the gene. When evaluated together with the literature data, it is not possible to talk about a hot-spot region showing frequent variations [Fujiwara et al., 2006; Escayg and Goldin, 2010; Scheffer and Nabbout, 2019]. When viewed in the context of genotype-phenotype correlation, truncating variations that usually cause early termination of the protein and missense variations in the voltage sensor segment (S4) and the ion-pore region lead to the DS phenotype, while missense variations outside the voltage sensor segment (S4) and the ion-pore region (S5-S6) cause a milder GEFS+ phenotype [Ishii et al., 2017]. Moreover, it is known that DS cases usually show de novo variations, and GEFS+ cases usually show inherited missense variations. However, there have been certain reports that do not follow this generalization, e.g., GEFS+ cases with de novo truncation variation, and DS cases with missense variation outside the voltage sensor segment (S4) and ion-pore region (S5-S6) [Jaimes et al., 2020]. Furthermore, inherited DS cases and sporadic GEFS+ cases have been described in the literature [Myers KA et al., 2017, 2018]. All these data make it difficult to correlate genotype and phenotype in cases with a variant in the *SCN1A* gene. It is not always possible to predict how the severity of the phenotypic effect will evolve, particularly in cases where genetic analysis is performed because of resistant febrile seizures in the early period and variations that were identified. In studies conducted in large families with a large number of affected individuals, the clinical severity was variable in cases with the same variant [Goldberg-Stern et al., 2014]. Variable presentations within families with the

same *SCN1A* variant have also been reported. Thus, the same variant may lead to GEFS+ or in few cases to DS [Helbig, 2015; Scheffer and Nabbout, 2019]. It has been reported that the phenotypic heterogeneity observed in cases with the same variant may be associated with modifier genes and somatic mosaicism [Goldberg-Stern et al., 2014; Myers CT et al., 2018; de Lange et al., 2020]. Whole-exome sequencing analyses performed in cases with variations in the *SCN1A* gene showed that patients had a milder clinical picture when they had variations in other epilepsy-associated genes such as *SCN8A*, *SCN9A*, *MOCS2*, *RAI1*, and *KMT2A* in addition to the variation in *SCN1A* [de Lange et al., 2020]. Seizures are a balance of neuronal excitation and inhibition. A LOF variant in *SCN1A* (on inhibitory neurons) gives increased excitation and a coexisting LOF variant in *SCN8A* on excitatory neurons may give reduced excitation. This combination could perhaps explain the milder phenotypes. Furthermore, cases with variations in *KCNQ3* and *TSC2* genes exhibited a more severe epilepsy phenotype [de Lange et al., 2020]. Moreover, the parents of DS cases with de novo truncation variants in the *SCN1A* gene have somatic mosaicism, and they show a milder phenotype [de Lange et al., 2018]. In conclusion, when interpreting the genotype-phenotype correlation in cases with variation in the *SCN1A* gene, interpretations considering only the type of variation detected and the domain affected on the protein will not be sufficient.

With the widespread use of NGS methods in recent years, the molecular genetic etiology can now be detected in epileptic patients, and treatment options change according to the affected gene and mutation type (LOF vs. GOF) [Musto et al., 2020]. In cases with LOF variation in the *SCN1A* gene, sodium channel blockers (carbamazepine, lamotrigine, oxcarbazepine, and phenytoin) are contraindicated as they worsen seizure disorder. In DS cases with LOF variation, valproic acid and clobazam are recommended as first line therapy, and stiripentol and cannabidiol, which are effective against convulsive seizures, are recommended as second line therapy. In addition, the use of fenfluramine, a serotonergic modulator, in DS cases was approved by the Food and Drug Administration (FDA) in 2020. Furthermore, Stoke Therapeutics is currently investigating the potential of antisense oligonucleotide technology, which is used in many different genetic diseases, in a phase 1/2a trial for the treatment of DS disease. In neonatal encephalopathy cases with GOF variation in the *SCN1A* gene, the most effective treatment option is sodium channel blockers. All these data point to the therapeutic importance of determining

the type of variation and its effect in patients with *SCN1A* gene-related epilepsy [Musto et al., 2020].

This study has certain limitations: firstly, the small number of patients and the proportionally low number of severe clinical DS cases in the study population. The low number of patients and the proportionally low number of patients with severe epilepsy phenotype make it difficult to interpret variant detection rates. Secondly, all parents could be included in the family screening, but more distant relatives with a history of seizures could not be screened for variations. Thirdly, it has not been possible to analyze the novel variations through in vitro or in vivo functional studies. Fourthly, since cases with anomalies detected in brain MRI were not included in the study, some of the patients that were excluded could in fact have had a causative *SCN1A* disorder. Fifthly, noncoding and alternative exon regions of the *SCN1A* gene were not analyzed in this study. For this reason, cases with variation in these regions may not be detected. For subsequent studies, the plan is to investigate the functional effects of novel variations through experimental studies.

Conclusion

With the novel variations detected in cases with DS and GEFS+ phenotype, this study contributes to the variation spectrum. The detected variations were discussed in the context of genotype-phenotype correlation, and possible molecular mechanisms in atypical cases were interpreted together with the literature data. The epilepsy phenotypes of the patients, their clinical outcomes and drug responses in their follow-up were examined in the context of their genotypes. *SCN1A* genetic analysis can make it possible to determine antiseizure medication to be selected or avoided in cases with variations. Elucidation of the molecular etiology can help in providing the family with effective genetic counseling for future pregnancies.

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Statement of Ethics

Informed consents were obtained from all parents in the outpatient clinic. This study was approved by the institutional ethics committee (Erzurum Training and Research Hospital, approval

number: 2020/23–218). All procedures followed were in accordance with the University of Sydney Human Research Ethics Committee and with the Helsinki Declaration of 1975, as revised in 2000.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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References

Bayat A, Hjalgrim H, Møller RS. The incidence of SCN1A-related Dravet syndrome in Denmark is 1:22,000: a population-based study from 2004 to 2009. *Epilepsia*. 2015;56:e36–9.

Bayat A, Bayat M, Rubboli G, Møller RS. Epilepsy Syndromes in the First Year of Life and Usefulness of Genetic Testing for Precision Therapy. *Genes (Basel)*. 2021;12(7):1051.

Ceulemans BP, Claes LR, Lagae LG. Clinical correlations of mutations in the SCN1A gene: from febrile seizures to severe myoclonic epilepsy in infancy. *Pediatr Neurol*. 2004;30:236–43.

Cho MJ, Kwon SS, Ko A, Lee ST, Lee YM, Kim HD, et al. Efficacy of Stiripentol in Dravet Syndrome with or without SCN1A Mutations. *J Clin Neurol*. 2018;14(1):22–8.

Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Hum Genet*. 2001;68:1327–32.

de Lange IM, Koudijs MJ, van 't Slot R, Gunning B, Sonsma ACM, van Gemert LJJM, et al. Mosaicism of de novo pathogenic SCN1A variants in epilepsy is a frequent phenomenon that correlates with variable phenotypes. *Epilepsia*. 2018;59(3):690–703.

de Lange IM, Mulder F, van 't Slot R, Sonsma ACM, van Kempen MJA, Nijman IJ, et al. Modifier genes in SCN1A-related epilepsy syndromes. *Mol Genet Genomic Med*. 2020;8(4):e1103.

Depienne C, Trouillard O, Saint-Martin C, Gourfinkel-An I, Bouteiller D, Carpentier W, et al. Spectrum of SCN1A gene mutations associated with Dravet syndrome: analysis of 333 patients. *J Med Genet*. 2009;46(3):183–91.

Dichgans M, Freilinger T, Eckstein G, Babini E, Lorenz-Depiereux B, Biskup S, et al. Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. *Lancet*. 2005;366(9483):371–7.

Dravet C. The core Dravet syndrome phenotype. *Epilepsia*. 2011;52(Suppl 2):3–9.

Dravet C, Bureau M, Oguni H, Fukuyama Y, Cokar O. Severe myoclonic epilepsy in infancy: Dravet syndrome. *Adv Neurol*. 2005;95:71–102.

Escayg A, Goldin AL. Sodium channel SCN1A and epilepsy: mutations and mechanisms. *Epilepsia*. 2010;51:1650–8.

Escayg A, Heils A, MacDonald BT, Haug K, Sander T, Meisler MH. A novel SCN1A mutation associated with generalized epilepsy with febrile seizures plus -- and prevalence of variants in patients with epilepsy. *Am J Hum Genet*. 2001;68:866–73.

Fan C, Wolkong S, Lehmann-Horn F, Hedrich UB, Freilinger T, Lerche H, et al. Early-onset familial hemiplegic migraine due to a novel SCN1A mutation. *Cephalalgia*. 2016;36(13):1238–47.

Fujiwara T. Clinical spectrum of mutations in SCN1A gene: severe myoclonic epilepsy in infancy and related epilepsies. *Epilepsy Res*. 2006;70 Suppl 1:S223–30.

Fukuma G, Oguni H, Shirasaka Y, Watanabe K, Miyajima T, Yasumoto S, et al. Mutations of neuronal voltage-gated Na⁺ channel alpha 1 subunit gene SCN1A in core severe myoclonic epilepsy in infancy (SMEI) and in borderline SMEI (SMEB). *Epilepsia*. 2004;45(2):140–8.

Goldberg-Stern H, Aharoni S, Afawi Z, Bennett O, Appenzeller S, Pendziwiat M, et al. Broad phenotypic heterogeneity due to a novel SCN1A mutation in a family with genetic epilepsy with febrile seizures plus. *J Child Neurol*. 2014;29:221–6.

Guerrini R, Oguni H. Borderline Dravet syndrome: a useful diagnostic category? *Epilepsia*. 2011;52(Suppl 2):10–2.

Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM, et al. The spectrum of SCN1A-related infantile epileptic encephalopathies. *Brain*. 2007;130:843–52.

Helbig I. Genetic Causes of Generalized Epilepsies. *Semin Neurol*. 2015;35(3):288–92.

Herini ES, Gunadi, Harahap IS, Yusoff S, Morikawa S, Patria SY, et al. Generalized epilepsy with febrile seizures plus (GEFS+) spectrum: clinical manifestations and SCN1A mutations in Indonesian patients. *Epilepsy Res*. 2010;90(1–2):132–9.

Heyne HO, Baez-Nieto D, Iqbal S, Palmer DS, Brunklaus A, May P, et al. Predicting functional effects of missense variants in voltage-gated sodium and calcium channels. *Sci Transl Med*. 2020;12(556):eaay6848.

Ishii A, Watkins JC, Chen D, Hirose S, Hammer MF. Clinical implications of SCN1A missense and truncation variants in a large Japanese cohort with Dravet syndrome. *Epilepsia*. 2017;58:282–90.

Jaimes A, Guerrero-López R, González-Giráldez B, Serratos JM. De novo truncating mutation in SCN1A as a cause of febrile seizures plus (FS+). *Epileptic Disord*. 2020;22:323–6.

Kalume F, Yu FH, Westenbroek RE, Scheuer T, Catterall WA. Reduced sodium current in Purkinje neurons from Nav1.1 mutant mice: implications for ataxia in severe myoclonic epilepsy in infancy. *J Neurosci*. 2007;27:11065–74.

Kang KW, Kim W, Cho YW, Lee SK, Jung KY, Shin W, et al. Genetic characteristics of non-familial epilepsy. *PeerJ*. 2019;7:e8278.

Kivity S, Oliver KL, Afawi Z, Damiano JA, Arsov T, Bahlo M, et al. SCN1A clinical spectrum includes the self-limited focal epilepsies of childhood. *Epilepsy Res*. 2017;131:9–14.

Kopanos C, Tsiolkas V, Kouris A, Chapple CE, Albarca Aguilera M, Meyer R, et al. VarSome: the human genomic variant search engine. *Bioinformatics*. 2019;35:1978–80.

Larsen J, Carvill GL, Gardella E, Kluger G, Schmiedel G, Barisic N, et al. The phenotypic spectrum of SCN8A encephalopathy. *Neurology*. 2015;84:480–9.

- Malo MS, Blanchard BJ, Andresen JM, Srivastava K, Chen XN, Li X, et al. Localization of a putative human brain sodium channel gene (SCN1A) to chromosome band 2q24. *Cytogenet Cell Genet*. 1994;67:178–86.
- Musto E, Gardella E, Møller RS. Recent advances in treatment of epilepsy-related sodium channelopathies. *Eur J Paediatr Neurol*. 2020;24:123–8.
- Myers CT, Hollingsworth G, Muir AM, Schneider AL, Thuesmann Z, Knupp A, et al. Parental Mosaicism in "De Novo" Epileptic Encephalopathies. *N Engl J Med*. 2018;378:1646–8.
- Myers KA, Burgess R, Afawi Z, Damiano JA, Berkovic SF, Hildebrand MS, et al. De novo SCN1A pathogenic variants in the GEFS+ spectrum: Not always a familial syndrome. *Epilepsia*. 2017;58:e26–e30.
- Myers KA, Scheffer IE, Berkovic SF, ILAE Genetics Commission. Genetic literacy series: genetic epilepsy with febrile seizures plus. *Epileptic Disord*. 2018;20:232–8.
- O'Reilly H, Eltze C, Bennett K, Verhaert K, Webb R, Merrett A, et al. Cognitive outcomes following epilepsy in infancy: A longitudinal community-based study. *Epilepsia*. 2018;59:2240–8.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–24.
- Rochtus AM, Goldstein RD, Holm IA, Brownstein CA, Pérez-Palma E, Haynes R, et al. The role of sodium channels in sudden unexpected death in pediatrics. *Mol Genet Genomic Med*. 2020;8:e1309.
- Rubinstein M, Westenbroek RE, Yu FH, Jones CJ, Scheuer T, Catterall WA. Genetic background modulates impaired excitability of inhibitory neurons in a mouse model of Dravet syndrome. *Neurobiol Dis*. 2015;73:106–17.
- Scheffer IE, Berkovic SF. Generalized epilepsy with febrile seizures plus. A genetic disorder with heterogeneous clinical phenotypes. *Brain*. 1997;120(Pt 3):479–90.
- Scheffer IE, Nabbout R. SCN1A-related phenotypes: Epilepsy and beyond. *Epilepsia*. 2019;60 Suppl 3:S17–24.
- Scheffer IE, Zhang YH, Jansen FE, Dibbens L. Dravet syndrome or genetic (generalized) epilepsy with febrile seizures plus? *Brain Dev*. 2009;31:394–400.
- Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia*. 2017;58:512–21.
- Shi X, Yasumoto S, Nakagawa E, Fukasawa T, Uchiya S, Hirose S. Missense mutation of the sodium channel gene SCN2A causes Dravet syndrome. *Brain Dev*. 2009;31:758–62.
- Stenson PD, Mort M, Ball EV, Chapman M, Evans K, Azevedo L, et al. The Human Gene Mutation Database (HGMD®): optimizing its use in a clinical diagnostic or research setting. *Hum Genet*. 2020;139:1197–207.
- Tan EH, Razak SA, Abdullah JM, Mohamed Yusoff AA. De-novo mutations and genetic variation in the SCN1A gene in Malaysian patients with generalized epilepsy with febrile seizures plus (GEFS+). *Epilepsy Res*. 2012;102:210–5.
- Thompson CH, Porter JC, Kahlig KM, Daniels MA, George AL Jr. Nontruncating SCN1A mutations associated with severe myoclonic epilepsy of infancy impair cell surface expression. *J Biol Chem*. 2012;287:42001–8.
- Trimmer JS, Rhodes KJ. Localization of voltage-gated ion channels in mammalian brain. *Annu Rev Physiol*. 2004;66:477–519.
- Vahedi K, Depienne C, Le Fort D, Riant F, Chaine P, Trouillard O, et al. Elicited repetitive daily blindness: a new phenotype associated with hemiplegic migraine and SCN1A mutations. *Neurology*. 2009;72:1178–83.
- Verheyen K, Wyers L, Del Felice A, Schoonjans AS, Ceulemans B, Van de Walle P, et al. Independent walking and cognitive development in preschool children with Dravet syndrome. *Dev Med Child Neurol*. 2021;63:472–9.
- Wallace RH, Hodgson BL, Grinton BE, Gardiner RM, Robinson R, Rodriguez-Casero V, et al. Sodium channel alpha1-subunit mutations in severe myoclonic epilepsy of infancy and infantile spasms. *Neurology*. 2003;61:765–9.
- Wu YW, Sullivan J, McDaniel SS, Meisler MH, Walsh EM, Li SX, et al. Incidence of Dravet Syndrome in a US Population. *Pediatrics*. 2015;136:e1310–15.
- Zaman T, Helbig KL, Clatot J, Thompson CH, Kang SK, Stouffs K, et al. SCN3A-Related Neurodevelopmental Disorder: A Spectrum of Epilepsy and Brain Malformation. *Ann Neurol*. 2020;88:348–62.
- Zhang YH, Burgess R, Malone JP, Glubb GC, Helbig KL, Vadlamudi L, et al. Genetic epilepsy with febrile seizures plus: Refining the spectrum. *Neurology*. 2017;89:1210–9.
- Zuberi SM, Brunklaus A, Birch R, Reavey E, Duncan J, Forbes GH. Genotype-phenotype associations in SCN1A-related epilepsies. *Neurology*. 2011;76:594–600.
- Zucca C, Redaelli F, Epifanio R, Zanotta N, Romeo A, Lodi M, et al. Cryptogenic epileptic syndromes related to SCN1A: twelve novel mutations identified. *Arch Neurol*. 2008;65(4):489–94.