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SCN1A mutational analysis in Korean patients with Dravet syndrome

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ABSTRACT

Objective: The aim of this study was to characterize the *SCN1A* mutation spectrum in Korean patients with Dravet syndrome.
Methods: Twenty-nine patients diagnosed with Dravet syndrome at the Seoul National University Children's Hospital were included in the study. Direct sequencing and multiplex ligation-dependent probe amplification (MLPA) were used to identify *SCN1A* mutations. Mutations were classified as either truncation (nonsense and frameshift) or missense mutations.
Results: Nineteen pathogenic mutations (19/29; 66%) and three unclassified variants were identified. One large deletion mutation spanning exons 1–20 was detected using MLPA. Fifteen of these 19 *SCN1A* mutations were novel. Eleven mutations were classified as truncations (seven frameshift and four nonsense mutations) and seven were classified as missense mutations. Truncating mutations spanned the whole span of subunits of the SCN1A protein, whereas all missense mutations were localized at either the voltage sensor (S4) or the ion pore (S5–S6) regions. Analysis according to clinical phenotype revealed that *SCN1A* mutations were more frequent in the classic group than in the borderline group (78% vs. 45%).
Conclusions: *SCN1A* mutational analysis of Korean Dravet syndrome patients resulted in the identification of 15 novel mutations, which could expand the spectrum of *SCN1A* mutations and confirms the current understanding of genotype–phenotype correlations.
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1. Introduction

Dravet syndrome (OMIM 607208) is an epileptic encephalopathy with typical clinical features, including seizure onset at infancy, prolonged convulsive seizures usually triggered by fever, later occurrence of polymorphic afebrile seizures, ataxia, cognitive decline, and medical intractability.[1] Since *SCN1A* was identified as a major causative gene for Dravet syndrome, more than 600 *SCN1A* mutations relevant to the disease have been reported.[2,3] In addition, animal models of *SCN1A* mutants recapitulate the human disease successfully, demonstrating that interneurons are the cellular substrate of Dravet syndrome, which results in decreased inhibition in the presence of *SCN1A* mutation.[4,5] Truncating (nonsense and frameshift) and missense mutations are the major mutation types in Dravet syndrome and their relative frequency in patients with this disease was estimated as being roughly similar to that reported in the large *SCN1A* database.[5] Loss of function was postulated as the mechanism underlying truncating mutations in *SCN1A*, whereas missense mutations may involve more complex mechanisms and are possibly affected by other modifier genes or environmental factors.[6] Recent reports have provided additional evidence that supports this concept: first, several genetic modifiers, such as *SCN8A*, *SCN9A*, and *CACNB4*, have been described[7–9]; second, the presence of mosaicism of truncating mutations in parents is responsible for a milder phenotype and for the familial occurrence of Dravet syndrome[10]; third, missense mutations in cases with severe phenotype are clustered in the voltage sensor and ion pore regions of SCN1A.[11,12]

In the present study, an *SCN1A* mutational analysis was conducted in 29 Korean patients with Dravet syndrome and their phenotypic variability was evaluated according to mutation type and location in the subunits of the protein.

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2. Methods

2.1. Patients and clinical data

The Institutional Review Board of the Seoul National University Hospital approved the study protocol. The study subjects were 29 patients diagnosed and followed at the Seoul National University Children's Hospital. The inclusion criteria for Dravet syndrome were those described in a previous study[13]: normal development before seizure onset, onset of the seizures before 1 year of age, seizures mainly triggered by fever, prolonged convulsive seizures (>15 min), later occurrence of seizures of various types, and later cognitive regression. A diagnosis of classic Dravet syndrome was established if either myoclonic or atypical absence seizures was present. Seizure type was determined based either on description provided by the parents or on video electroencephalographic (EEG) monitoring results. Myoclonic seizures detected using video EEG monitoring were further classified into overt or subtle myoclonic types. Subtle myoclonic seizures consist of brief subtle successive myoclonic twitching including rhythmic retropulsion of the head accompanied by irregular generalized spike waves of brief duration (2–3 s). These subtle myoclonic seizures cannot be clearly differentiated from atypical absence seizures, as suggested by Oguni et al.[14] Family history of febrile seizures or epilepsy in first- or second-degree relatives was investigated. Rufinamide was administered to five selected patients. In these patients, all seizures were intractable to multiple (≥3) antiepileptic drugs. Baseline seizure frequency was counted over a period of 1 month before rufinamide administration. Treatment response was estimated based on seizure frequency reduction compared with the baseline at 3 months after the treatment.

2.2. SCN1A mutational analysis

2.2.1. Direct sequencing

After obtaining informed consent from the parents of patients, genomic DNA was extracted from peripheral blood leukocytes using a QIAamp® DNA Blood Midi Kit, according to the manufacturers instructions (Qiagen, Valencia, CA, USA). Direct sequencing of all coding exons and flanking intronic sequences of the *SCN1A* gene was performed using primer pairs designed by the authors (available upon request). Polymerase chain reaction amplification was performed in a thermal cycler (Model 9700; Applied Biosystems, Foster City, CA, USA) and cycle sequencing was performed on an ABI Prism 3730xl DNA Analyzer using the BigDye Terminator Sequencing Ready Reaction Kit (Applied Biosystems). Sequence variations were analyzed via comparison with the wild-type sequence (transcript reference AB093548). The significance of novel missense variations was evaluated using the following methods: (1) allele frequencies were screened in 100 ethnically matched normal subjects; and (2) segregation patterns were analyzed among the family members available. All candidate variants were searched in the *SCN1A* variant database, to confirm their novelty.[3]

2.2.2. Multiple ligation-dependent probe amplification (MLPA)

MLPA analysis was performed using the SALSA MLPA kit P137-A2 SCN1A (MRC Holland, Amsterdam, the Netherlands), according to the manufacturers instructions. The MLPA samples consisted of 50–100 ng of genomic DNA. Ligation and amplification were performed using a PTC-200 thermal cycler (MJ Research, Waltham, MA, USA). All amplified fragments were separated using capillary electrophoresis on an ABI 3130xl Genetic Analyzer (Applied Biosystems). The area under the peak for each amplified fragment was measured and normalized to the peak areas of normal control individuals using the GeneMarker software, version 1.6 (SoftGenetics, State College, PA, USA). The reference range was set at 0.75–1.3.

2.2.3. In silico analysis

Mutational properties of intronic variations were predicted using automated splicing mutation analysis,[15] which is a web-oriented tool based on information theory (http://splice.cmh.edu).

Table 1
Mutation and unclassified variant profiles of 22 patients.

Patient number	Phenotype	cDNA	Protein	Subunit location	Family study	Previous report	100 normal controls
Truncation mutations							
8	Classic	c.7C>T	p.Gln3X	N-terminal	*De novo*	Dravet syndrome	Not evaluated
19	Borderline	c.1630delA	p.Thr544HisfsX14	DI-DII	*De novo*	Novel	Not evaluated
6	Classic	c.2088_2091delTTTC	p.Ser696SerfsX8	DI-DII	*De novo*	Novel	Not evaluated
2	Classic	c.2593C>T	p.Arg865X	DII S4	Not available	Dravet syndrome	Not evaluated
3	Classic	c.3160C>T	p.Gln1036X	DII-DIII	*De novo*[a]	Novel	Not evaluated
20	Borderline	c.3384delC	p.Asn1128LysfsX18	DII-DIII	*De novo*	Novel	Not evaluated
7	Classic	c.4127_4128delGT	p.Cys1376TyrfsX2	DIII S5	*De novo*	Novel	Not evaluated
1	Classic	c.4296delA	p.Lys1432LysfsX6	DIII S5–S6	*De novo*[b]	Novel	Not evaluated
5	Classic	c.4488delA	p.Asn1554LysfsX5	DIII-DIV	*De novo*	Novel	Not evaluated
9	Classic	c.4662delC	p.Asn1554LysfsX4	DIV S1	*De novo*	Novel	Not evaluated
4	Classic	c.4906C>T	p.Arg1636X	DIV S4	*De novo*	Novel	Not evaluated
Missense mutations							
10	Classic	c.1177C>T	p.Arg393Cys	DI S5–S6	Not available	Dravet syndrome, MAE	Not evaluated
14	Classic	c.1187G>A	p.Gly396Glu	DI S5–S6	*De novo*	Novel	Not found
12	Classic	c.2854T>C	p.Trp952Arg	DII S5–S6	*De novo*	Novel	Not found
21	Borderline	c.3946A>T	p.Arg1316Trp	DIII S4	*De novo*	Novel	Not found
22	Borderline	c.3968C>G	p.Pro1323Arg	DIII S4	*De novo*	Novel	Not found
13	Classic	c.4216G>A	p.Ala1406Thr	DIII S5–S6	Father (+)	Novel	Not found
11	Classic	c.5029C>T	p.Leu1677Phe	DIV S5	Not available	Dravet syndrome	Not evaluated
Large deletion mutation							
23	Borderline	Exon 1–20 deletion			Not available	Novel	Not evaluated
Unclassified or undetermined variants							
24	Borderline	c.602+5delG			*De novo*	Novel	Not found
26	Borderline	c.4723C>T	p.Arg1575Cys	DIV S2	Mother (+)	Rasmussen encephalitis	Not found
25	Borderline	c.4284+4A>T			*De novo*	Novel	Not found

MAE, myoclonic astatic epilepsy.
[a] Elder sister of the patient had childhood absence epilepsy but did not carry the mutation identified in the patient.
[b] Mosaicism of the mutation identified in the patient was suspected in the patient's father, who experienced several febrile seizures in his childhood.

3. Results

3.1. SCN1A mutational analysis of 29 patients with Dravet syndrome

Direct sequencing of *SCN1A* in 29 patients with Dravet syndrome revealed the presence of 11 truncating mutations (four nonsense and seven short deletion mutations ranging from one to four base pairs), seven missense mutations, and three unclassified variants (Table 1). One large deletion mutation spanning exons 1–20 was identified using MLPA. Thus, 19 *SCN1A* mutations, including 15 novel mutations, were confirmed (19/29; 66%). Truncating mutations spanned the whole span of subunits of the SCN1A protein, whereas all missense mutations localized to either the voltage sensor (S4) or the ion pore (S5–S6) regions. Family studies revealed that most of the mutations occurred *de novo* (14 out of 17 mutations tested). Automated splicing mutation analysis predicted that two novel intronic variants (c.602+5delG and c.4284+4A>T) caused a leaky splice site (Supplementary Figs. 1 and 2). However, they were not classified as pathogenic mutations because confirmatory tests were not performed. One missense variant (p.Arg1575Cys) was also classified as undetermined because of the conflicting results of previous reports.[13,16]

3.2. Clinical features according to SCN1A mutation types

The phenotypes of the 29 patients were classified as either classic or borderline, according to the criteria described in Section 2. The *SCN1A* mutation status (truncating or missense), seizure onset, presence of myoclonic/absence seizures, video EEG monitoring results, family history, and rufinamide treatment for these patients are summarized in Table 2. *SCN1A* mutations were more frequent in the classic group than in the borderline group (78% vs. 45%). Most of the truncating mutations (9/11) were found in the classic group. Video EEG monitoring was performed in 15 patients. Various seizure types were observed during the monitoring, with subtle myoclonic seizure identified most frequently (seven patients). Family history of febrile seizure or epilepsy was reported in six families (6/29; 21%). The missense mutation detected in patient 13 was also found in her father, who experienced several febrile seizures in early childhood and generalized tonic–clonic seizures during adolescence (Fig. 1A). The parents of two patients with truncating mutations (patients 1 and 18) were mildly affected. A mosaic state of the truncating mutation was suspected in the father of patient 1 (Fig. 1B), although a confirmatory test was not performed. Patient 3, who harbored a truncating mutation, had a sister affected with childhood absence epilepsy, although the sister did not carry an *SCN1A* mutation (Fig. 1C). Seizure reduction was observed in one patient among the five patients that received rufinamide treatment (Table 3). Although no specific acute adverse events occurred, three patients discontinued the treatment because of aggravation of myoclonic or generalized tonic–clonic seizures.

4. Discussion

Although the *SCN1A* mutation detection rate in patients with Dravet syndrome was below 50% in one previous study,[17] recent studies reported a uniform detection rate, ranging from 70% to 80%.[13,18,19] Some authors speculated that this discrepancy may have stemmed from the use of different inclusion criteria,[13] although most studies reported using criteria adapted from those proposed by the International League Against Epilepsy (2001). The diagnosis of classic Dravet syndrome is sometimes difficult in young patients, as characteristic features, such as EEG changes, seizure evolution, psychomotor regression, and presence of ataxia, require a longer period of clinical observation. In addition, the definition of borderline cases may be more problematic. Considering this limitation in defining the diagnosis of study subjects, we

Table 2
Clinical features and genotype–phenotype correlations of 29 patients.

Patient number	Phenotype	*SCN1A* mutation	Onset (months)	Last follow-up (years)	Myoclonic/atypical absence seizure	Video EEG monitoring	Family history	Rufinamide
1	Classic	Truncation	5	1.3	+/+	Subtle myoclonic	Father FS	–
2	Classic	Truncation	4	9.8	+/–	GTC, myoclonic, CPS	–	+
3	Classic	Truncation	8	5.0	+/+	Subtle myoclonic	Sister CAE	–
4	Classic	Truncation	3	4.1	+/–	Subtle myoclonic	–	+
5	Classic	Truncation	6	3.1	+/+	Subtle myoclonic	–	–
6	Classic	Truncation	3	11.1	+/–	GTC, myoclonic, SPS	–	–
7	Classic	Truncation	3	2.5	+/–		–	–
8	Classic	Truncation	5	7.6	+/+	Myoclonic	Uncle FS	+
9	Classic	Truncation	2	1.5	+/–		–	–
10	Classic	Missense	3	14.4	+/+		–	+
11	Classic	Missense	4	1.6	+/–		–	–
12	Classic	Missense	7	2.8	–/+	CPS, atypical absence	–	–
13	Classic	Missense	9	3.0	+/+	Subtle myoclonic	Father IGE	–
14	Classic	Missense	3	1.9	+/+	Subtle myoclonic	–	–
15	Classic	Unclassified	4	12	–/+	Atypical absence	–	–
16	Classic	Negative	8	15.6	+/–		–	+
17	Classic	Negative	11	11.1	+/–		–	–
18	Classic	Negative	10	2.9	+/–	Subtle myoclonic	Uncle FS	–
19	Borderline	Truncation	6	2.1	–/–		Mother FS	–
20	Borderline	Truncation	7	2.5	–/–		–	–
21	Borderline	Missense	6	1.8	–/–		–	–
22	Borderline	Missense	9	3.3	–/–	CPS	–	–
23	Borderline	Large deletion	3	8.6	–/–		–	–
24	Borderline	Unclassified	5	4.8	–/–		–	–
25	Borderline	Unclassified	6	2.4	–/–	GTC	–	–
26	Borderline	Unclassified	9	1.9	–/–		–	–
27	Borderline	Negative	4	2.8	–/–		–	–
28	Borderline	Negative	6	1.9	–/–		–	
29	Borderline	Negative	6	6.5	–/–	GTC	–	

GTC, generalized tonic–clonic seizure; SPS, simple partial seizure; CPS, complex partial seizure; IGE, idiopathic generalized epilepsy; FS, febrile seizure; and CAE, childhood absence epilepsy.

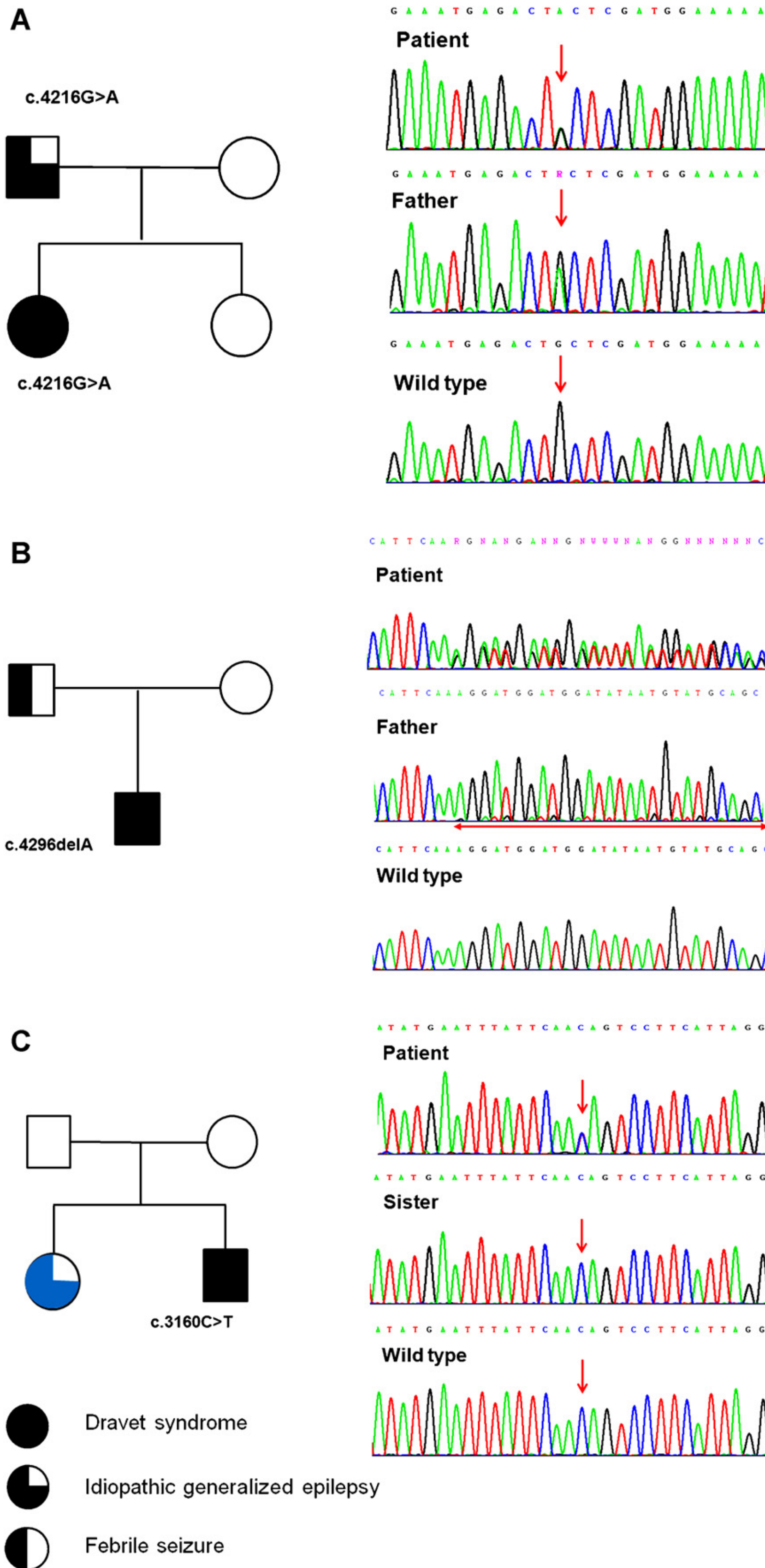

Fig. 1. Family study of three patients with novel *SCN1A* mutations. (A) c.4216G>A (p.Ala1406Thr) mutation in patient 13. The father, who exhibited clinical features of idiopathic generalized epilepsy, harbored the same mutation. (B) c.4296delA (p.Phe1432LeufsX6) mutation in patient 1. The mildly affected father may be a mosaic for the same mutation found in the patient. (C) c.3160C>T (p.Gln1036X) mutation in patient 3. The elder sister was diagnosed with childhood absence epilepsy; however, she did not carry a mutation in *SCN1A*.

Table 3
Clinical features of five patients treated with rufinamide.

Patient number	Phenotype	*SCN1A* mutation type	Seizure aggravation by previous AEDs	Main seizure types at RFN start	Concomitant AEDs at RFN start	Age of RFN start (years)	RFN dose (mg/day/mg/kg/day)	Response
10	Classic	Missense	Not definite	GTC	TPM, LEV, CLB	14.1	400/8	Seizure reduction of 50–99%
2	Classic	Missense	OXC	GTC, absence	VPA, LTG, LEV	9.3	600/23	No change
4	Classic	Truncation	Not definite	GTC	LEV, CNZ, LTG	3.6	200/16	Seizure aggravation
8	Classic	Truncation	Not definite	GTC, myoclonic	ZNS, CLB, LTG	7.1	800/40	Seizure (GTC + myoclonic) aggravation
16	Classic	Negative	Not definite	GTC, myoclonic	VPA, LTG	15.1	800/16	Myoclonic seizure aggravation

AED, antiepileptic drug; RFN, rufinamide; OXC, oxcarbazepine; TPM, topiramate; LEV, levetiracetam, CLB, clobazam; VPA, valproic acid; LTG, lamotrigine; CNZ, clonazepam; ZNS, zonisamide; and GTC, generalized tonic–clonic seizure.

used an approach similar to that reported recently for a study using a large patient group,[13] which included patients with various clinical presentations that shared the core features and focused on initial manifestations. Using these criteria, we obtained a mutation detection rate (66%) that was slightly lower than that reported in other recent studies. As two of three unclassified variants were novel and predicted to cause aberrant splicing based on in silico analysis, mutation detection rate would be over 70% when including these variants. A prospectively designed study with a genetic analysis performed in the earlier disease stage may be required to avoid possible selection or ascertainment biases. Furthermore, the accurate timing of *SCN1A* testing could also be determined using this design, which could be helpful for the management and genetic counseling of the disease.

Among the 19 pathogenic mutations detected, 15 mutations had not been reported in the literature or in the *SCN1A* variant database.[3] In our study, the proportion of truncating mutations among the point mutations (11/18; 61%) was slightly higher than the proportion of missense mutations (7/18; 39%). Regarding the location of missense mutations in the subunits of the protein, all seven missense mutations were located in the S4 or S5–S6 regions, which constitute the voltage sensor and ion pore regions, respectively. These genotype–phenotype correlations appear to be consistent with the results of recent studies.[12,19]

Somatic mosaicism may explain the variable phenotypes associated with truncating mutations.[10] The father of patient 1 experienced several febrile seizures in his early childhood. Somatic mosaicism of the same frameshift mutation identified in patient 1 was suspected on the electropherogram of the father, although exact quantification was not performed. The mother of patient 18 had a history of febrile seizures and may be in a similar situation regarding somatic mosaicism; however, confirming this status would also require further testing. Although this mechanism may be a more frequent cause of familial cases or of mild phenotypes than thought previously, the need for additional confirmatory procedures is the main obstacle to conducting more extensive research. Next-generation sequencing technology could be used for this purpose, as quantification of variants is feasible using a single platform.[20]

Among the three unclassified or undetermined variants detected here, the pathogenicity of the p.Arg1575Cys variant was interpreted differentially in two previous studies. This variant was reported as a pathogenic mutation in a patient with Rasmussen encephalitis, based on an *in vitro* functional study.[16] However, Depienne et al. suggested that this variant may be a rare polymorphism that is unrelated to Dravet syndrome.[13] The index case in the study of Depienne et al. harbored another nonsense mutation, in addition to p.Arg1575Cys. Moreover, p.Arg1575Cys was found in his asymptomatic mother, although this variant was not found in 100 Caucasian controls. In the present study, this variant was also not found in 100 Korean controls, but was found in the asymptomatic mother of the patient. Although there was insufficient evidence to ascertain the pathogenicity of this variant, the recurrence of rare neurological disorders, such as Rasmussen encephalitis and Dravet syndrome, in different ethnic groups (Caucasian and Korean populations) raises the suspicion that this variant may be an incompletely penetrant pathogenic variant or a susceptiblity allele acting in a polygenic manner. The presence of two *SCN1A* pathogenic mutations in a single Dravet syndrome patient or the presence of mildly affected transmitting parents has been reported,[13,19] suggesting the existence of modifying factors, especially in patients with missense mutations. The presence of additional modifying factors could also be suspected in the case of patient 3, whose elder sister was diagnosed with childhood absence epilepsy and did not harbor the same *SCN1A* nonsense mutation. However, despite the report of a few presumed genetic modifiers, the genes involved as a whole and their complex interaction with *SCN1A* remain poorly understood.

A recent study that evaluated the long-term efficacy and tolerability of rufinamide treatment in patients with Dravet syndrome reported a rather disappointing outcome.[21] Both low efficacy and aggravation of seizures in a significant proportion of patients (30%) contributed to a low long-term retention rate of only 5% after 34 months of treatment. The present study, although based on the results obtained from a small number of patients, also revealed that three out of five patients had seizure aggravation, which could represent supporting evidence against the use of rufinamide in patients with Dravet syndrome.

This is the first report discussing the *SCN1A* mutation spectrum in Korean patients with Dravet syndrome. Genotype–phenotype correlation was largely consistent with the previous studies of individuals with European and Japanese ancestry. Although our study presented confirmatory results stemming partly from a small cohort, the phenotypic variablity found within the affected family members will be the subject of future research.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.seizure.2011.08.002.

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