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Significant correlation of the SCN1A mutations and severe myoclonic epilepsy in infancy☆

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Abstract

To investigate the possible correlation between genotype and phenotype of epilepsy, we analyzed the voltage-gated sodium channel α1-subunit (SCN1A) gene, β1-subunit (SCN1B) gene, and γ-aminobutyric $acid_A$ receptor γ2-subunit (GABRG2) gene in DNAs from peripheral blood cells of 29 patients with severe myoclonic epilepsy in infancy (SME) and 11 patients with other types of epilepsy. Mutations of the SCN1A gene were detected in 24 of the 29 patients (82.7%) with SME, although none with other types of epilepsy. The mutations included deletion, insertion, missense, and nonsense mutations. We could not find any mutations of the SCN1B and GABRG2 genes in all patients. Our data suggested that the SCN1A mutations were significantly correlated with SME ($p < .0001$). As we could not find SCN1A mutations in their parents, one of critical causes of SME may be de novo mutation of the SCN1A gene occurred in the course of meiosis in the parents.

Keywords: Neuronal voltage-gated sodium channel; SCN1A; SCN1B; GABRG2; Generalized epilepsy with febrile seizures plus; Sever myoclonic epilepsy in infancy

Generalized epilepsy with febrile seizures plus (GEFS+) is a benign childhood-onset epileptic syndrome with autosomal dominant inheritance [1,2]. This syndrome is characterized by febrile seizures that persist beyond 6 years of age and by an appearance of other types of afebrile seizures. In the GEFS+ family members, phenotypic and genetic heterogeneities are seen. GEFS+ includes childhood absence epilepsy, myoclonic-astatic epilepsy (MAE), severe myoclonic epilepsy in infancy (SME) [3], idiopathic generalized epilepsy (IGE), and localization-related epilepsy (LRE). Among these epileptic syndromes, SME is considered to be the most severe phenotype within the GEFS+ spectrum [4]. SME is a malignant epileptic syndrome, while GEFS+ is usually benign. It is very important to elucidate the molecular genetic basis of SME.

Epileptic seizures are induced by abnormal electrical discharges within the brain. Since ion channels are fundamental in membrane potential generation, mutations of genes encoding some types of ion channels may cause epilepsy. Recent molecular genetic studies have revealed that mutations of the voltage-gated sodium channel α1-subunit (SCN1A) gene [5–10], α2-subunit (SCN2A) gene [11], β1-subunit (SCN1B) gene [12], and γ-aminobutyric acid $(GABA)_A$ receptor γ2-subunit (GABRG2) gene [13,14] were detected in patients with GEFS+, while there have been only a few reports of mutation analysis in the patients with SME [15–17].

To find the causing genes of SME and clarify the possible correlation between the genotype and phenotype of epilepsy, we performed mutation analysis of the SCN1A, SCN1B, and GABRG2 genes in the patients with SME and other types of epilepsy.

☆ *Abbreviations:* SME, severe myoclonic epilepsy in infancy; SCN1A, sodium channel α1-subunit; SCN1B, sodium channel β1-subunit; GABRG2, γ-aminobutyric $acid_A$ receptor γ2-subunit; GEFS+, generalized epilepsy with febrile seizures plus; MAE, myoclonic-astatic epilepsy; LRE, localization-related epilepsy; IGE, idiopathic generalized epilepsy; bp, base pair.

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Materials and methods

Patients. Twenty-nine patients with SME and eleven patients with other types of epilepsy including MAE, IGE, and LRE were recruited who were seen at Okayama University Hospital from 1969 to 2001. Epileptic syndromes were classified according to the diagnostic criteria of the International League against Epilepsy classification [18]. Clinical features of patients were presented in Table 1.

DNA preparation. Peripheral blood was acquired from the patients and their parents after written informed consent was obtained from all participants. Genomic DNA was extracted from peripheral blood leukocytes by DNA extract WB Kit (Nippon gene, Tokyo, Japan).

PCR amplification and DNA sequencing. Twenty-six exons of the SCN1A gene, five exons of the SCN1B gene, and nine exons of the GABRG2 gene were amplified with the intronic primers [12,17]. The SCN1A gene was amplified with a pair of primers as follows: exon 1 sense 5′-TCATGGCACAGTTCCTGTATC and antisense 5′-GCAGT AGGCAATTAGCAGCAA, exon 2 sense 5′-TGGGGCACTTTAG AAATTGTG and antisense 5′-TGACAAAGATGCAAAATGAG AG, exon 3 sense 5′-GCAGTTTGGGCTTTTCAATG and antisense 5′-TGAGCATTGTCCTCTTGCTG, exon 4 sense 5′-AGGGCTACG TTTCATTTGTATG and antisense 5′-TGTGCTAAATTGAAATC CAGAG, exon 5 sense 5′-CAGCTCTTCGCACTTTCAGA and antisense 5′-TCAAGCAGAGAAGGATGCTGA, exon 6 sense 5′-AGC GTTGCAAACATTCTTGG and antisense 5′-GGGATATCCAG CCCCTCAAG, exon 7 sense 5′-GACAAATACTTGTGCCTTTGAA TG and antisense 5′-ACATAATCTCATACTTTA TCAAAAACC, exon 8 sense 5′-GAAATGGAGGTGTTGAAAA TGC and antisense 5′-AATCCTTGGCATCACTCTGC, exon 9 sense 5′-AGTACAGGG TGCTATGACCAAC and antisense 5′-TCCTCATACAACCACC TGCTC, exon 10 sense 5′-TCTCCAAAAGCCTTCATTAGG and antisense 5′-TTCTAATTCTCCCCCTCTCTCC, exon 11 sense 5′-TC CTCATTCTTTAATCCCAAGG and antisense 5′-GCCGTTCTGT AGAAACACTGG, exon 12 sense 5′-GTCA GAAATATCTGCCAT CACC and antisense 5′-GAATGCACTATT CCCAACTCAC, exon 13 sense 5′-TGGGCTCTATGTGTGTGTCTG and antisense 5′-GGA

Table 1
Summary of the clinical features of patients

Patients no.	Type of epilepsy	Sex	Age at onset	Family history of onset convulsive disorders
1	SME	M	5 m	Paternal aunt and paternal GF:FS
2	SME	M	7 m	—
3	SME	M	3 m	Father:FS
4	SME	F	4 m	—
5	SME	F	4 m	—
6	SME	F	2 m	Father and paternal:FS
7	SME	F	3 m	Brother:FS
8	SME	M	5 m	Brother:FS
9	SME	M	5 m	—
10	SME	F	6 m	—
11	SME	M	4 m	Paternal uncle:FS
12	SME	M	5 m	Paternal aunt:FS
13	SME	M	7 m	—
14	SME	F	6 m	—
15	SME	F	5 m	Maternal cousin:FS
16	SME	M	6 m	Father and sister:FS
17	SME	F	7 m	—
18	SME	M	7 m	Sister and paternal GF:FS
19	SME	F	4 m	—
20	SME	M	8 m	—
21	SME	F	3 m	Paternal cousin:FS
22	SME	F	5 m	Mother:Ep, sister:FS
23	SME	F	6 m	—
24	SME	F	7 m	—
25	SME	F	4 m	Father and paternal GF:FS
26	SME	F	4 m	Brother:FS
27	SME	F	6 m	Paternal GF and brother:Ep
28	SME	F	6 m	—
29	SME	M	4 m	—
30	MAE	M	8 m	Maternal cousin:FS
31	MAE	M	2 y 10 m	—
32	MAE	F	2 y 9 m	Maternal cousin:FS
33	MAE	M	7 m	—
34	I GE	F	8 m	Father:FS
35	I GE	M	2 y 10 m	Father and paternal cousin:FS
36	I GE	F	1 y 3 m	—
37	I GE	M	5 y 6 m	—
38	I GE	M	1 y 0 m	Father:FS
39	LRE	M	1 y 2 m	—
40	LRE	M	3 y 1 m	—

Note. SME, severe myoclonic epilepsy in infancy; MAE, myoclonic-astatic epilepsy; IGE, idiopathic generalized epilepsy; LRE, localization-related epilepsy; M, male; F, female; m, months; y, years; GF, grandfather; FS, febrile seizure; Ep, epilepsy.

AGCATGAAGGATGGTTG, exon 14 sense 5′-TACTTCGCGTTT CCACAAGG and antisense 5′-GCTATGCAAGAACCCTGATTG, exon 15 sense 5′-ATGAGCCTGA GACGGTTAGG and antisense 5′-ATACATGTGCCATGCTGGTG, exon 16 sense 5′-TGCTGTG GTGTTTCCTTCTC and antisense 5′-TGTATTCATACCTTCCC ACACC, exon 17 sense 5′-AAAAGGGTTAGCACAGACAATG and antisense 5′-ATTGGGCAGATATAATCAAAGC, exon 18 sense 5′-C ACACAGCTGATGAATGTGC and antisense 5′-TG AAGGGCTA CACTTTCTGG, exon 19 sense 5′-TCTGCCCTCCTATTCCAATG and antisense 5′-GCCCTTGTCTTCCAGAAATG, exon 20 sense 5′-AAAAATTACATCCTTTACATCAAACTG and antisense 5′-TT TTGCATGCATAGATTTTCC, exon 21 sense 5′-TGAACCTTGC TTTTACATATCC and antisense 5′-ACCCATCTGGG CTCATA AAC, exon 22 sense 5′-TGTCTTGGTCCAAAATCTGTG and antisense 5′-TTGGTCGTTTATGCTTTATTCG, exon 23 sense 5′-CCC TAAAGGCCAATTTCAGG and antisense 5′-ATTTGGCAGA GAAAACACTCC, exon 24 sense 5′-GAGATTTGGGGGTGTT TGTC and antisense 5′-GGATT GTAATGGGGTGCTTC, exon 25 sense 5′-CAAAAATCAGGGCC AATGAC and antisense 5′-TG ATTGCTGGGATGATCTTG, exon 26(1) sense 5′-AGGACTCTG AACCTTACCTTGG and antisense 5′-CCATGAATCGCTCTTCC ATC, exon 26(2) sense 5′-TGTGGGAA CCCATCTGTTG and antisense 5′-GTTTGCTGACAAGGGGTCAC. PCR was performed in 25 μl reaction volume containing 50 ng of genomic DNA, 20 pmol of each primer, 0.8 mM dNTPs, 1× reaction buffer, 1.5 mM $MgCl_2$, and 0.7 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA). All PCR products were purified with a PCR products pre-sequencing kit (Amersham Biosciences, Little Chalfont, Buckinghamshire, England), reacted with the Big Dye Terminator FS ready-reaction kit (Applied Biosystems), and analyzed on an ABI PRISM3100 sequencer (Applied Biosystems). Mutations we found were confirmed twice by independent PCR amplification and sequencing.

Statistical analysis. Fisher's exact test was used for the statistical analysis to clarify the correlation between the mutations and subgroup of epilepsy.

Results

Mutation analysis

The sodium channels are composed of a pore-forming α-subunit and one or more smaller β-subunit(s). The β-subunit enhances the Na^+ current amplitude and modifies its properties [19]. We analyzed the SCN1A, SCN1B, and GABRG2 genes in DNAs from peripheral blood cells of 29 patients with SME and 11 patients with other types of epilepsy within GEFS+ spectrum. The clinical features of patients are shown in Table 1. Interestingly, mutations of the SCN1A gene were detected in 24 of the 29 patients (82.7%) with SME, while there was no mutation in the patients with other types of epilepsy (Table 2). Various types of mutations including missense and nonsense mutations and frameshift mutation by insertion or deletion were detected. Representative data of missense, nonsense, and frameshift mutations were shown in Fig. 1A. Location of the all mutations on the sodium channel α1-subunit was schematically presented in Fig. 1B, in which the mutations leading the premature termination by nonsense and frameshift mutations are distinctly shown to the mutations including missense mutation and 3-bp deletion. The protein has four homologous repeats (domain 1–4), and a domain and the neighbor one are linked with a loop structure. Each of these domains contains six transmembrane segments (S1–S6). The mutations were mainly located in the intracellular loop 1, loop 2, and S5–S6 pore regions. In Fig. 2, position of the representative missense mutations is indicated on amino acid sequence similarity of sodium channel α-subunit gene family. The amino acids that were substituted by missense mutation were highly conserved in the wide species including Drosophila, not only in these three cases shown in Fig. 2 but also in the others (data not shown). No mutations were detected in the patients' parents we tested (Table 2). The substitution of threonine with alanine at codon 1057 in the patients 1, 6, 15, 21, and their parents seems to be a single nucleotide polymorphism (SNP), because Escayg et al. reported that the rate of alanine allele on this SNP is 0.33 in the normal population [6]. We could not find any mutations of the SCN1B and GABRG2 genes in all patients (data not shown).

High correlation of the SCN1A mutation and SME

When statistical analysis was performed on SME and other types of epilepsy, we found a significant correlation between the SCN1A mutation and SME ($p < .0001$). As regards clinical manifestations, all patients with SME had intractable epileptic seizures. Various degrees of ataxia were observed in all except for patients 8, 13, 17, 23, and 24. All patients had slight to severe mental retardation. Patients 8, 17, 23, and 24 showed less severe mental impairment than the other patients did. We could not find significant correlation between the type of the SCN1A mutations and clinical severity.

Discussion

There have been only a few reports of mutation analysis in the patients with SME. Claes et al. first described seven mutations of the SCN1A gene [15] and Sugawara et al. recently reported 10 mutations of the SCN1A gene in 14 patients with SME [16]. Only one GABRG2 mutation was found in a patient with SME in a family with GEFS+ [17]. The present study is the largest series of comprehensive mutation screening in the patients with SME and other types of epilepsy. By direct sequencing of all coding exons of the SCN1A, SCN1B, and GABRG2 genes, we detected 24 mutations of the SCN1A gene in the 29 patients with SME (82.7%) while none with other types of epilepsy. Our data suggested that the SCN1A mutation was significantly correlated with SME ($p < .0001$), although the number of other types of epilepsy was not so many. Escayg et al.

Table 2
Mutations in the SCN1A gene

Patients no.	Type of epilepsy	Exon	Nucleotide change[a]	Amino acid change[b]	Mutation type	Position	The same mutations in	
							Father	Mother
1	SME	Exon 10	G1502del	R501fsX543	Frameshift	loop1	–	–
		Exon 16	A3169G	T1057A	Polymorphism	loop2	+	–
2	SME	Exon 10	1641insA	K547fsX570	Frameshift	loop1	–	–
3	SME	Exon 11	C1702T	R568X	Nonsense	loop1	–	–
4	SME	Exon 11	C1820del	S607fsX622	Frameshift	loop1	NE	–
5	SME	Exon 12	C2087del	P696fsX703	Frameshift	loop1	–	–
6	SME	Exon 12	C2101T	R701X	Nonsense	loop1	–	–
		Exon 16	A3169G	T1057A	Polymorphism	loop2	–	+
7	SME	Exon 15	C2560T	R854X	Nonsense	DIIS3-S4loop	–	–
8	SME	Exon 15	T2672G	F891C	Missense	DIIS4-S5 loop	NE	–
9	SME	Exon 15	C2761T	R921C	Missense	DIIS5-S6 pore	–	–
10	SME	Exon 15	C2761T	R921C	Missense	DIIS5-S6 pore	–	–
11	SME	Exon 16	C2976del	A992fsX999	Frameshift	DIIS6	–	–
12	SME	Exon 16	A3049T	K1017X	Nonsense	loop2	–	–
13	SME	Exon 16	C3215del	T1072fsX1077	Frameshift	loop2	–	–
14	SME	Exon 19	T3764C	L1255P	Missense	DIIIS2	–	–
15	SME	Exon 19	G3782A	W1261X	Nonsense	DIIIS2	NE	NE
		Exon 16	A3169G	T1057A	Polymorphism	loop2	NE	NE
16	SME	Exon 19	CTT3837-3839del	F1279del	Deletion	DIIIS2-S3 loop	NE	NE
17	SME	Exon 21	A3169G	V1380M	Missense	DIIIS5-S6 pore	NE	NE
18	SME	Exon 22	4256-4260 del(CCACA) ins(ATGTCC)	A1419fsX1433	Frameshift	DIIIS5-S6 pore	NE	NE
19	SME	Exon 22	T4270C	W1424R	Missense	DIIIS5-S6 pore	NE	NE
20	SME	Exon 23	A4319G	Q1440R	Missense	DIIIS5-S6 pore	–	–
21	SME	Exon 26	C4912T	R1638C	Missense	DIVS4	NE	–
		Exon 16	A3169G	T1057A	Polymorphism	loop2	NE	–
22	SME	Exon 26	G4990C	G1664R	Missense	DIVS4-S5 loop	–	–
23	SME	Exon 26	5610-5615 del(AGAGAT) ins(CTAGAGTA)	G1870fsX1871	Frameshift	C-terminal	NE	NE
24	SME	Exon 26	C5696T	T1899I	Missense	C-terminal	NE	NE
25	SME	–	–				NE	NE
26	SME	–	–				NE	NE
27	SME	–	–				NE	NE
28	SME	–	–				NE	NE
29	SME	–	–				NE	NE
30	MAE	–	–				NE	NE
31	MAE	–	–				NE	NE
32	MAE	–	–				NE	NE
33	MAE	–	–				NE	NE
34	I GE	–	–				NE	NE
35	I GE	–	–				NE	NE
36	I GE	–	–				NE	NE
37	I GE	–	–				NE	NE
38	I GE	–	–				NE	NE
39	LRE	–	–				NE	NE
40	LRE	–	–				NE	NE

[a] Nucleic acid numbering started from A of the intiating ATG.
[b] Amino acid numbering started from the intiating ATG codon. del, deletion; ins, insertion; fs, frameshift; X, stop codon; DII, Domain 2; DIII, Domain 3; DIV, Domain 4; NE, not examined; –, negative; +, positive.

reported that the rate of the SCN1A mutation in GEFS+ was 5.6% [6]. In their study, the subjects included mainly juvenile myoclonic epilepsy and childhood absence epilepsy except SME. The prominent clinical feature of SME is the frequent occurrence of convulsions associated with fever, which sometimes develop to status epilepticus. The Japanese authors reported that hot baths could also provoke epileptic seizures [20,21]. These clinical manifestations are very rare among other epileptic syndromes. Since the SCN1A mutations were frequently detected in SME but not in other types of epilepsy, this channel may be related to a dysfunction of the brain neurons exacerbated by high body temperature.

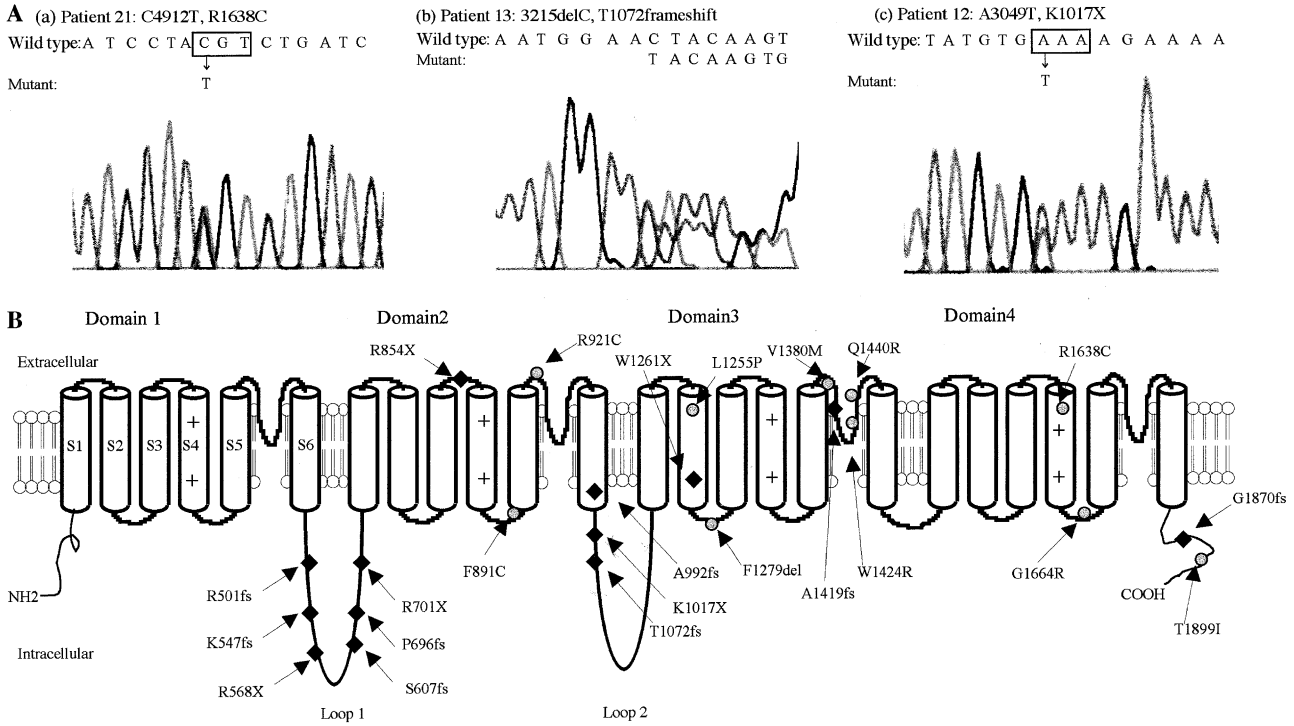

Fig. 1. Mutations in the SCN1A gene detected in the patients with SME. (A) Representative sequencing data of the SCN1A mutations. (a) Substitution of C with T at nucleotide position 4912 results in a change from arginine to cysteine at codon 1638. (b) One nucleotide deletion at nucleotide position 3215 results in frameshift at codon 1072, leading to a premature termination at codon 1077. (c) Substitution of A with T at nucleotide position 3049 leads to a change from lysine to termination codon at codon 1017. (B) Location of the mutations on the sodium channel α1-subunit. S1–S6 indicate transmembrane segments 1–6. + shows a positively charged arginine or lysine residue. Closed diamonds indicate truncation type-mutation (nonsense and frameshift mutations) and gray circles indicate missense mutation and 3-bp deletion. Each of the mutations is indicated by arrow.

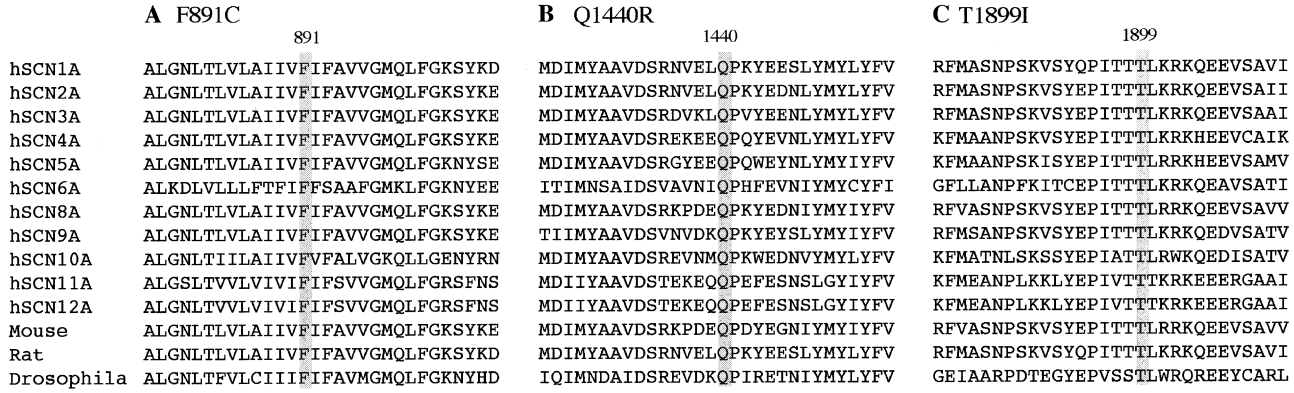

Fig. 2. Alignments of amino acid sequence in sodium channel α-subunit gene family members. Amino acids changed from phenylalanine to cysteine at codon 891 (A), from glutamine to arginine at codon 1440 (B) and from threonine to isoleucine at codon 1899 (C) are shown. GenBank Accession Nos., from top to bottom, are AF225985, M94055, AF225987, M81758, AY038064, M91556, AF225988, NM_002977, AF117907, AF188679, AF109737, 2203417A, A25019, and P35500.

As regards mutation type of the SCN1A gene in the patients with SME, 14 cases of 24 mutations were nonsense and frameshift mutations which led to some truncated proteins. It has been reported that truncation type-mutations of the SCN1A gene is related to severe phenotype in SME because all mutations in GEFS+ were missense mutations [5–10] and all but one mutations in SME were truncation type-mutation [15,16]. In our study, however, not only truncation type-mutations but also missense mutations were identified. The type of the SCN1A mutation could not be linked to the clinical severity of each patient with epilepsy.

Six missense mutations were located in the S5–S6 pore regions of domain 2 or 3 in the sodium channel α1-subunit. The substitution of arginine with cysteine at codon 1638 was located in the S4 segment of domain 4. These mutations were located on important regions which play a role to generate action potentials. The part of extracellular loop which links S5 and S6 segments dips down into the membrane to form the ion pore. Some positively charged arginine residues in the S4 segment are known to be essential for the S4 segment to act as the voltage sensor, and the importance was verified by the experiment in which each of the arginine residues in the S4 segment was replaced in turn by a cysteine [22]. In addition to most missense mutations on the regions which have an important function in the electrophysiological properties of the channel [19], we found a missense mutation at codon 1899 of an intracellular C terminal region. The region may also have a basic role in spite of a distal region on the molecule, because this amino acid is highly conserved in α-subunit gene family shown in Fig. 2. In fact, patients 23 and 24 who have the mutation in C terminal region showed slight mental impairment and absence of ataxia, suggesting that location of the mutation may influence the phenotype. In the patients 8 and 17 who also showed slight mental impairment and absence of ataxia, missense mutations of phenylalanine to cysteine and of valine to methionine were detected, respectively. Both phenylalanine and cysteine belong to an uncharged amino acid group, and both valine and methionine are also members of a non-polar and hydrophobic amino acid group. The severity of clinical manifestation might depend on the amino acid produced by missense mutation in the SCN1A gene. It is very important to investigate whether the missense mutations are gain-of-function mutants, if the truncated protein functions as a dominant negative mutant, if the remaining normal gene shows haploid insufficiency in the channel protein level, or if the remaining normal gene is a functional gene in these patients.

Spampanato et al. [23] reported functional analysis of two missense mutations of the SCN1A gene in the patients with GEFS+, in which one mutation showed hyperexcitability and the other showed hypoexcitability of the sodium channel. Their results suggested that either an increase or a decrease in sodium channel activity could result in seizures. Usage of the mutations we detected may be helpful for further analysis of the mechanism.

In only 5 patients with SME, we could not find any mutations in the SCN1A gene. One of the possible reasons is that there are some mutations in the neuronal cells of brain but not in the blood cells. Another is that an expression of the SCN1A gene is decreased or none in the neuronal cells by the mechanisms such as aberrant regulation of the promoter in the SCN1A gene. However, the possibility is still remaining because the brain tissues could not be acquired from the patients with epilepsy.

We could not find the SCN1A mutations in the parents of the patients we studied. One of critical causes of SME may be de novo mutation of the SCN1A gene occurred in the course of meiosis in the germ cells of the parents. The high rate of a family history of convulsive disorders and the presence of sibling cases [20] indicate also a possibility that some mutations of other genes may be associated with the occurrence of SME. To clarify these unsolved issues, a functional analysis of the SCN1A gene and a search for new target genes in SME are required.

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