Table 1
Clinical characteristics of patients with acute encephalopathy in children taking theophylline (AET).

	Age, sex	History of febrile seizures		Blood concentration of theophylline	Initial seizure (duration) / intravenous barbiturate / biphasic seizures	Cranial CT/MRI		Diagnosis of AESD	Outcome	
		Past	Family			Subacute period	Convalescence		Intellectual disabilities	Motor disabilities
1	2y1m, M	+	–	NR	<15 min/–/+	Delayed cerebral edema	Diffuse cerebral atrophy	Definite	Severe	Severe
2	2y3m, F	–	+	NR	>15 min/+/+	NR (Normal on day 2)	Diffuse cerebral atrophy, CS	Definite	Severe	Severe
3	4y0m, F	–	–	Therapeutic range	>30 min/–/+	Not available	Diffuse cerebral atrophy	Probable	Severe	Severe
4	2y7m, M	–	–	NR	>15 min/+/–	Mild cerebral edema	Diffuse cerebral atrophy	Possible	Severe	Severe
5	2y2m, M	–	–	13.4 μg/ml	>30 min/+/+	Delayed cerebral edema	Diffuse cerebral atrophy	Definite	Severe	Severe
6	1y0m, M	NR	–	NR	<15 min/–/+	Delayed cerebral edema, BTA, CS	Bilateral frontal atrophy	Definite	Moderate	Full recovery
7	3y5m, M	+	–	NR	>30 min/–[#]/–	Left temporal subcortical edema	Diffuse cerebral atrophy	Probable	Severe	Severe
8	2y4m, F	+	–	NR	>30 min /+/+	Delayed cerebral edema, right parietal dominant	Diffuse cerebral atrophy	Definite	Severe	Mild
9	3y3m, M	–	–	NR	>15 min/+/–	Delayed cerebral edema, bilateral parietal dominant	Diffuse cerebral atrophy	Probable	Severe	Mild
10	4y0m, F	–	–	5.8 μg/ml	>30 min/–/–	NR (Mild cortical edema on day 2)	Diffuse cerebral atrophy	Possible	Severe	Mild
11	1y11m,M	–	+	NR	>15 min/+/+	BTA, left temporal dominant	Left temporal atrophy	Definite	Mild	Full recovery
12	2y7m, F	–	–	3.9 μg/ml	>15 min/+/–	Early cerebral edema	–	Unlikely	Death	
13	2y6m, F	–	–	NR	>15 min/–/+	Delayed cerebral edema, bilateral frontal dominant	Diffuse cerebral atrophy, bilateral frontal dominant	Definite	Severe	Mild
14	0y6m, M	–	–	NR	>15 min/+/+	Normal	Bilateral hippocampal sclerosis	Possible	Moderate	Full recovery
15	2y10m, M	–	–	5.6 μg/ml	>15 min/–/+	Hemispheric cortical edema	Hemispheric atrophy	Definite	Mild	Mild
16	4y4m, M	NR	–	NR	>15 min/–/+	Delayed cerebral edema	Diffuse cerebral atrophy bilateral frontal dominant	Definite	Severe	Full recovery

NR, not recorded; BTA, bright tree appearance; CS, central sparing.
[#] Continuous intravenous midazolam administration.

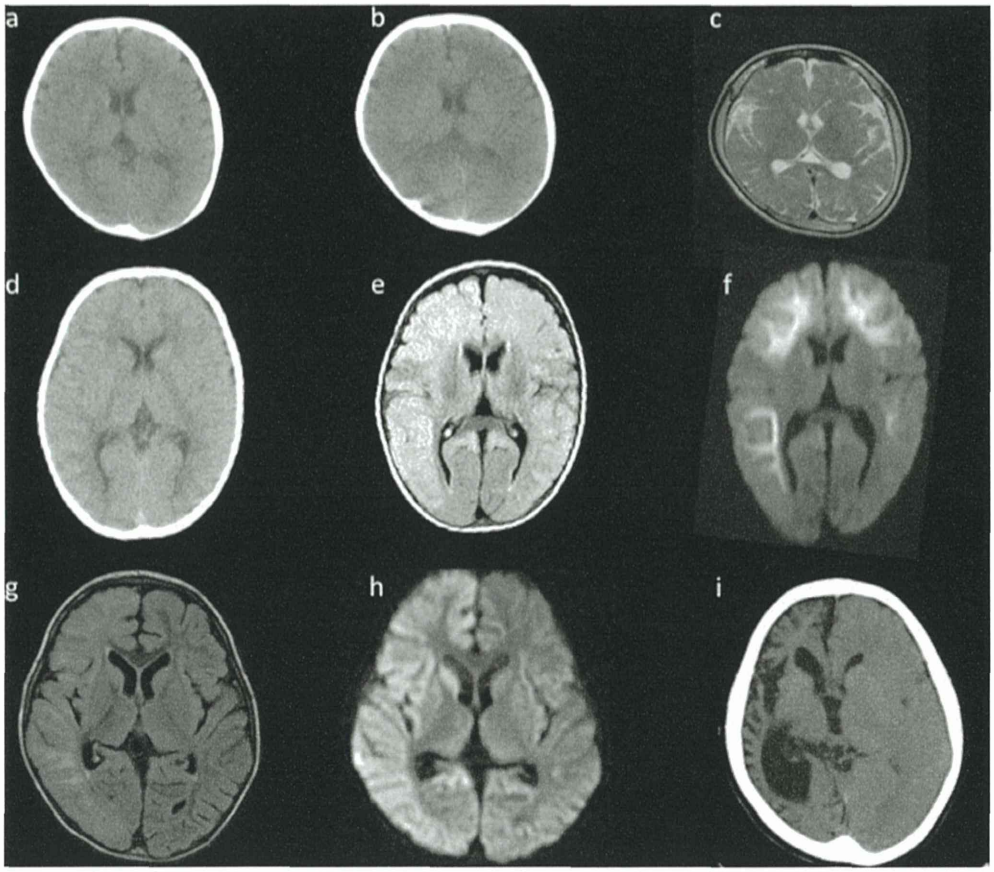

Fig. 1. Cranial CT/MRI findings in acute encephalopathy in children taking theophylline (AET). In Case 1, cranial CT on day 3 showed slight narrowing of the cerebrospinal fluid space, but no clear evidence of cerebral edema (a). On day 7, however, CT showed mild narrowing of the cerebrospinal fluid space and hypodensity of the white matter, indicating delayed cerebral edema (b). Seven years later, MRI (T2-weighted imaging) demonstrated diffuse cerebral atrophy with bilateral subdural effusion (c). In Case 6, CT on day 1 was normal (d). MRI on day 7 showed narrowing of the cerebrospinal fluid space and hyperintensity of the bilateral frontal and temporal cortex on fluid-attenuated inversion recovery (FLAIR) imaging, indicating delayed cerebral edema (e). Diffusion-weighted imaging visualized restricted diffusion in the subcortical white matter (bright tree appearance), with sparing of bilateral peri-Rolandic regions (f). In Case 15, MRI in the subacute period (day 28) showed T1/T2 prolongation of the right cerebral cortex ((g) T1-weighted imaging, (h) FLAIR imaging). Two months later, CT showed atrophy of the right hemisphere (i).

Table 2
Genetic Background of ATE.

Patient No.	*CPT2* diplotype[a]	*ADORA2A* diplotype[b]	*SCN1A* mutation	*SCN2A* mutation
1	FC	AB	No	No
2	FF	AB	V982L	No
3	FF	BB	No	No
4	CC	AB	No	No
5	FF	AA	No	No
6	FC	AB	No	No
7	FF	AA	No	No
8	FC	AB	No	No
9	FC	AA	No	No
10	FC	AB	No	No
11	FC	AB	No	No
12	FC	AB	No	No
13	FF	AB	No	No
14	FF	AB	No	F328V
15	FF	BB	No	No
16	FF	AB	No	No

[a] F352C polymorphism. Allele C is thermolabile variation.
[b] Combination of four SNPs. Haplotype A is associated with high expression of ADORA2A.

types of sodium channels. This mutation was previously reported in a patient with Dravet syndrome without myoclonic seizures and ataxia [18]. Case 2 with V982L of *SCN1A* had typical AESD ("definite" AESD in this study). The clinical course of this case was reported previously [14].

3.2.4. *SCN2A*

We found in one case (Case 14) a missense mutation, F328V (Fig. 2). The phenylalanine 328 residue is located on the loop between the transmembrane segments 5 and 6, domain I of SCN2A ($Na_v1.2$) protein (Fig. 2). The F328V mutation had previously been reported in a patient with Dravet syndrome [19]. Case 14 with F328V of *SCN2A* was born to a family with no history of epilepsy and seizure disorders. He had no seizures during the neonatal period. At 6 months old, he had acute bronchiolitis and took theophylline for 4 days. He then developed prolonged generalized tonic convulsions with the eyes deviated to the right. Status

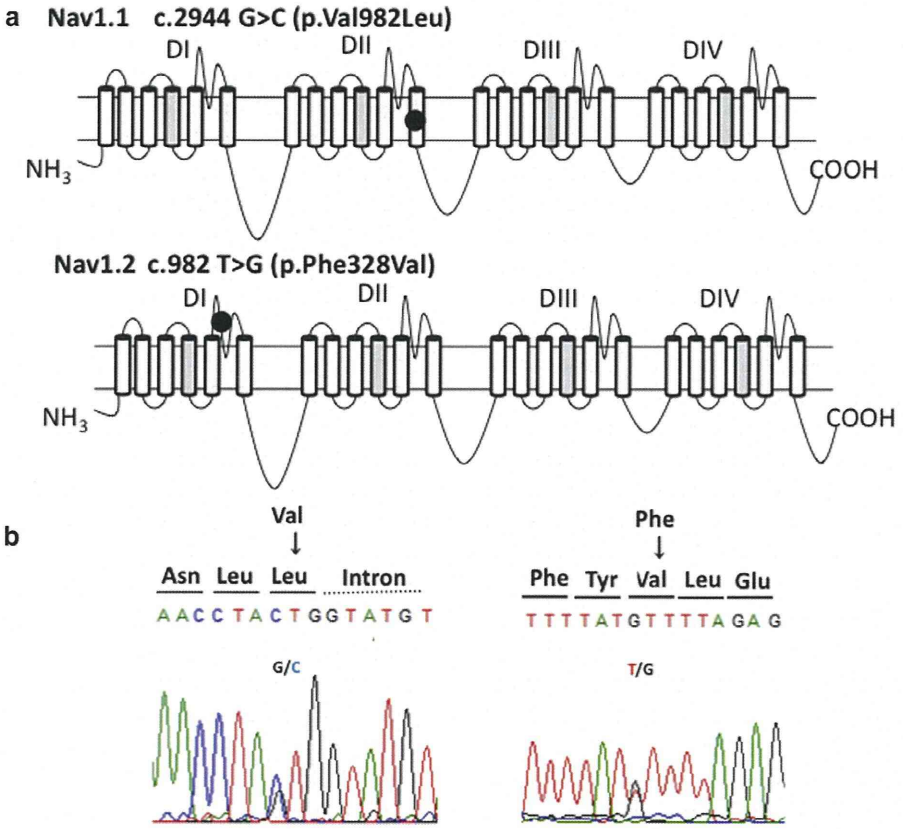

Fig. 2. Cases with *SCN1A* and *SCN2A* mutations in AET. (a) Structure of SCN1A ($Na_V1.1$) and SCN2A ($Na_V1.2$) with localization of the mutation (closed circle). c.2944 G>C (p.Val982Leu) is localized in the transmembrane segment 6 of domain II in $Na_V1.1$, whereas c.982 T>G (p.Phe328Val) is localized in the loop between S5 and S6 of segment I in $Na_V1.2$. (b) Electropherogram of the mutations. Substitution of G with C at nucleotide position c.2944 of *SCN1A* resulted in a change from valine to leucine (left), whereas substitution of T with G at nucleotide position c.982 caused a change from phenylalanine to valine, Accession numbers of *SCN2A* and *SCN2A* are AF117907.1 and Q99250, respectively.

epilepticus was refractory to anticonvulsants and lasted more than 15 min, requiring general anesthesia and mechanical ventilation for 6 days. Two days after extubation, he had a cluster of seizures presenting with apnea, staring, and bradycardia. At the age of 3, he started to take carbamazepine for complex partial seizures. Although cranial CT was normal in the acute phase, bilateral hippocampal sclerosis was revealed at follow-up cranial MRI imaging at 4 years and 1 month. He was eventually left with pervasive developmental disorders, mental deficiency and complex partial seizures. Neither myoclonic seizures nor generalized spike-wave discharges were noted during the follow-up period. Despite the presence of compatible clinical signs (biphasic seizures), the atypical CT/MRI findings rendered the diagnosis of AESD equivocal ("possible" AESD) in this case.

4. Discussion

This study elucidated the relationship between AET and AESD from both clinical and genetic viewpoints. The clinical picture of AESD has recently been well delineated [8,10]. The initial manifestation of AESD is a prolonged convulsive seizure triggered by acute febrile infection. In typical cases, the seizure is followed by post-ictal coma on day 1, and by recovery of consciousness on day 2. Cranial CT/MRI findings are normal at this stage. On day 3–9, however, there is a cluster of brief partial seizures, followed by a second coma. CT/MRI studies at this stage disclose cerebral cortical edema. Although the topography of cerebral lesions varies among patients, many of them show lobar (e.g. bilateral frontal) or hemispheric distribution. The peri-Rolandic regions (pre- and post-central gyri) are spared in many cases. The lesions are hypodense on CT and hyperintense on T2-weighted images of MRI. The most sensitive sequence is diffusion-weighted imaging, which visualizes restricted diffusion of the subcortical white matter, a characteristic pattern called a bright tree appearance. This finding provides strong evidence for the diagnosis of AESD. After the second coma, there are signs of cerebral cortical dysfunction, such as intellectual deficits, motor paralysis and epileptic seizures.

In convalescence, CT/MRI shows atrophy of the affected cortical regions. As many as 66% of patients are eventually left with neurological sequelae, in contrast to the low fatality of 1% [16].

In typical cases of AESD, the diagnosis is made easily, based on the characteristic clinical course (biphasic seizures) and MRI findings (bright tree appearance). In very severe cases, however, the diagnosis is often difficult for the following reasons. At onset, these cases usually have very persistent (more than 1 h) and intractable status epilepticus, and undergo intensive treatment, including continuous infusion of a large dose of intravenous barbiturate and brain hypothermia. There is neither recovery of consciousness on day 2 nor recurrence of partial seizures on day 3–9. Thus, the biphasic clinical course is not recognized. In addition, the critical condition of patients, as well as multiple lines for monitoring and tubes for ventilation and infusion, often renders MRI studies difficult and unsafe. Even in such cases, diagnosis of AESD may be made on the basis of CT findings, such as delayed cerebral edema, lobar or hemispheric involvement and peri-Rolandic sparing. Occasionally, the latter two features are first recognized by follow-up MRI during convalescence.

In this study on AET, we often encountered the same diagnostic problems. Nevertheless, we could make a diagnosis of AESD in 12 out of 16 cases (definite in 9 and probable in 3), by identifying either or both of the neurological and imaging features (Table 1, Fig. 1). The diagnosis of AESD was equivocal in 3 cases (possible AESD), and unlikely in one case (Case 12) that showed early cerebral edema (on day 1), multiorgan failure and fatal outcome. All these findings are very rare in AESD.

In this study, we revealed for the first time the genetic background of AET, focusing on the genes associated with AESD: *CPT2*, *ADORA2A*, *SCN1A* and *SCN2A*. Fifteen out of 16 patients had at least one of the following genotypes: polymorphism of *CPT2* (352C) and *ADORA2A* (hapolotype A), and mutation of *SCN1A* and *SCN2A*.

CPT2 is a mitochondrial enzyme essential for the metabolism of fatty acids and the resultant production of ATP. Certain polymorphisms of the *CPT2* gene cause thermolability, a sharp decline in enzymatic activity at high body temperature (e.g. 41 °C). Previous studies in Japan have demonstrated that CPT2 thermolabile variations predispose children to influenza-associated encephalopathy [20]. In particular, F352C, a typical variation, is a risk factor for AESD [12]. Interestingly, the [1055T>G/F352C] substitution has been reported only in East Asians and not in Caucasians (rs2229291 on NCBI, http://www.sanger.ac.uk/), which partially accounts for the high incidence of AESD in Japanese. In this study, we found that half of the AET cases (8 out of 16) had F352C, suggesting the role of CPT2 thermolability in the pathogenesis of AET.

ADORA2A is a receptor coupled to a stimulatory G protein. On adenosine binding, ADORA2A stimulates adenylate cyclase to produce cyclic adenosine monophosphate (cAMP), which in turn facilitates calcium ion influx, glutamate release and neuronal excitation. Genetic variation of the *ADORA2A* gene is associated not only with caffeine sensitivity [21], but also with AESD. Haplotype A, a predisposing factor of AESD, causes high expression of the *ADORA2A* mRNA and ADORA2A protein, as well as high production of cAMP in response to adenosine, in an additive manner (diplotype AA>AB>BB) [13]. Thus, in the presence of haplotype A, the balance between inhibitory ADORA1 and stimulatory ADORA2A may shift to favor the latter. When combined with the non-selective inhibitory effects of theophylline for both the receptors [22], this altered balance may lead to the onset of acute encephalopathy. Indeed, this study found that the vast majority of cases (14 out of 16) had at least one haplotype A. Interestingly, two patients with diplotype AA had no other risk genotypes (regarding *CPT2*, *SCN1A* and *SCN2A*), whereas 10 out of the 12 patients with diplotype AB had another risk genotype. This study failed to show a statistically significant difference in the genotype distribution of *CPT2* and *ADORA2A* between AET and controls because of the small number of cases. A study involving a larger number of AET patients is necessary to further elucidate the genetic background.

SCN1A and SCN2A are voltage-gated sodium ion channels on the cell membrane of CNS neurons. Mutations of the *SCN1A* and *SCN2A* genes cause familial epileptic syndromes, such as Dravet syndrome and generalized epilepsy with febrile seizures plus (GEFS plus). Recently, the clinical spectrum of these mutations has widened considerably. We and other investigators have reported cases presenting clinically with syndromes of acute encephalopathy, such as AESD and AERRPS, but not with Dravet's syndrome or GEFS plus [14,15]. In this study of AET, we found two patients: Case 2 with V982L of *SCN1A*, and Case 14 with F328V of *SCN2A*. The former patient had typical AESD, whereas the latter showed bilateral hippocampal sclerosis, an MRI finding atypical for AESD. In this context, a recent animal experiment has shown that aminophylline at the usual doses aggravates hypoxia-induced injury of hippocampal neurons [23]. It is plausible that mutations of the *SCN1A* and *SCN2A* genes, when combined with the multiple effects of theophylline, lead to variable neurological phenotypes, including AESD and other encephalopathies.

In summary, our clinical and genetic studies of Japanese patients with AET revealed that AET overlaps with AESD. Of the 16 AET cases, 12 met the diagnostic criteria of AESD, and 14 had at least one gene polymorphism or mutation previously known as genetic risk factors of AESD.

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Original article

Effect of CYP2C19 polymorphisms on stiripentol administration in Japanese cases of Dravet syndrome

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Abstract

Objective: The objective of this study was to investigate stiripentol (STP) administration in cases of Dravet syndrome (DS) by comparing CYP2C19 allelic polymorphisms with the clinical effects of STP, including plasma concentrations of concomitant drugs and adverse effects of STP.

Materials and methods: Eleven cases of DS cases were included. Demographic and clinical characteristics of the cases (age at the study period, body weight, mean dose and plasma concentration of valproate acid (VPA)/clobazam (CLB) off and on STP, mean plasma concentration of norclobazam (N-CLB) off and on STP, degree of seizure reduction, and adverse effects of STP) were examined with each CYP2C19 polymorphism.

Results: There were 3 cases of DS with wild type, 6 with intermediate type, and 2 with poor type of CYP2C19 polymorphisms. The N-CLB concentration/CLB dose ratio and N-CLB/CLB concentration ratio off STP were significantly higher in poor metabolizers. Three (37%) of 8 cases showed no effectiveness of STP regardless of the N-CLB concentration increase, and 1 (33%) of 3 cases showed effectiveness of STP regardless of N-CLB concentration decrease. In total, 6 (54%) of 11 cases with DS had >50% reduction in seizure frequency without significant differences in CYP2C19 polymorphisms.

Conclusion: This study demonstrated an effect of CYP2C19 polymorphisms on STP administration in Japanese cases of DS. There were cases of seizure reduction regardless of N-CLB concentration decrease on STP, which suggests a significant anti-convulsant action of STP. N-CLB concentration decrease on STP was observed in 1 case with ketogenic diet and 2 cases with *3 allelic polymorphisms of CYP2C19.

Keywords: Dravet syndrome; CYP2C19 polymorphisms; Stiripentol; Clobazam; Norclobazam

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1. Introduction

Dravet syndrome (DS) is an intractable form of epilepsy characterized by the recurring presence of prolonged febrile and afebrile seizures in early childhood. DS is one of the most pharmacoresistant form of epilepsy. The efficacy of stiripentol (STP) in Dravet Syndrome was first identified in an exploratory study of pediatric pharmacoresistant epilepsies [1]. In addition, STP is the only compound that showed efficacy in DS through randomized placebo-controlled trials [2,3]. STP is an inhibitor of cytochrome P450 and increases plasma concentrations of concomitant antiepileptic drugs (AEDs). An increase in blood concentration levels of clobazam (CLB) and norclobazam (N-CLB) had been attributed to the antiepileptic efficacy of STP in DS [3]. However, direct anti-convulsant activities of STP have been proposed, such as the allosteric modulation of γ-aminobutyric acid (GABA) receptors through different binding sites from benzodiazepines [1].

STP inhibits moderately the formation of norclobazam (N-CLB) from CLB by CYP3A (and partly CYP2C19), but its inhibitory potency toward N-CLB hydroxylation by CYP2C19 to 4′-hydroxynorclobazam (OH-NCLB) is much higher [1]. N-CLB, the major metabolite of CLB, is known to contribute to the therapeutic and adverse effects to a greater degree than CLB in epileptic patients [4].

CYP2C19, the metabolic target of STP, has two main allelic polymorphisms, CYP2C19*2 and CYP2C19*3, in addition to the wild genotype. The allelic frequencies of CYP2C19*2 and CYP2C19*3 are 23% and 10%, respectively, in the Japanese population [1,5], which are greater than the 13% and 0.3%, respectively, in European-Caucasians [6]. The allelic defects lead to a non-functional allele through a splicing defect in exon 5 (CYP2C19*2) and a stop codon in exon 4 (CYP2C19*3).

The one allele defect of CYP2C19 induces mild inhibition of CLB metabolism, and results in a modest increase in the concentration of N-CLB. If both alleles are deficient, the concentration of N-CLB increases dramatically [7]. It has been hypothesized that, in people carrying deficient CYP2C19 at both alleles, the addition of STP to an initial CLB treatment would have almost no effect on the N-CLB concentrations [4]. This may abolish the efficacy of STP if a pharmacological property of STP relies on an increase in the levels of N-CLB.

The objective of the present study was to examine STP administration in cases with DS by comparing CYP2C19 allelic defects with the clinical effects of STP, the plasma concentrations of the concomitant drugs, and adverse effects of STP. We compared our results with previous studies, and implicated unique effects of CYP2C19*3 polymorphisms on STP administration.

2. Materials and methods

2.1. Subjects

This is a retrospective study that was conducted at Kanagawa Children's Medical Center. Eleven cases of DS (age range, 1.2–12.1 years; 6 males) were included. They were diagnosed with DS by the pathognomonical clinical course, such as normal development before seizure onset, the occurrence of seizures during the first year of life, and seizures frequently provoked by fever or hot water. The mean age of seizure onset was 5.1 months (range, 2–9 months). All patients in this study fulfilled the criteria by Hattori et al. [8]. SCN1A mutations were confirmed in all 5 patients who underwent sequence analysis. PCR conditions and primers used for analysis were described in previous articles [9,10].

After obtaining informed consent, genomic DNA was isolated from whole white blood cells, and CYP2C19 polymorphisms were examined by sequencing CYP2C19 around the polymorphic sites. The CYP2C19*2 variant derives from a single-nucleotide polymorphism (G to A) at position 681 in exon 5 of CYP2C19, and creates an exon aberrant splice site resulting in aberrant splicing and out of frame transcription. The CYP2C19*3 variant derives from a single-nucleotide polymorphism (G to A) at position 636 in exon 4 of CYP2C19, resulting in a premature stop codon [7].

Concomitant AEDs, dose and plasma concentration of STP and VPA/CLB (off and on STP), plasma concentration of N-CLB (off and on STP), changes of seizure frequencies by STP administration, and adverse effects of STP were examined. For analysis, we used blood samples available just after adjustment of STP to a maximum dose during the study period. The interval between the latest dose adjustment of STP and sampling, the prescribed regimens of STP at the time of sampling, and the time of sampling in each case are shown in Table 1.

2.2. Statistical analyses

The demographic characteristics of the cases (age at the study period, body weight, mean dose and plasma concentration of STP and VPA/CLB before and after administration of STP (off and on STP), mean plasma concentration of N-CLB off and on STP, CLB concentration/CLB dose ratio, N-CLB concentration/CLB dose ratio, and N-CLB/CLB concentration ratio were compared in each CYP2C19 polymorphism (wild, intermediate, poor type) by the Tukey–Kramer method. Next, the clinical characteristics of the cases (degree of seizure reduction, adverse events after STP administration) were compared in each CYP2C19 polymorphism by Fisher's test corrected with Holm's method.

Table 1
Case profile and relationship between the effectiveness of STP and CLB/N-CLB concentration.

Case	SCN1A mutation	CYP2C19	Interval between the latest dose adjustment of STP and blood sampling (days)	Prescribed regimen of STP (a day)	Time of blood sampling	STP dose (mg/kg)	VPA dose (mg/kg)	CLB dose (mg/kg)	CLB concentration off STP (ng/ml)	CLB concentration on STP (ng/ml)	N-CLB concentration off STP (ng/ml)	N-CLB concentration on STP (ng/ml)	>50% seizure reduction	Concomitant drugs except VPA and CLB
1	Unconfirmed	*1/*1	11	Two times	9:18 a.m.	40.0	26.4	0.34	77	245	161	1854	–	KBr
2[#]	c.1130G>C, p.R377P	*1/*1	9	Two times	4:34 p.m.	33.0	34.4	0.06	57	15	562	132	–	KBr
3	c.2134C>T, p.R712X	*1/*1	186	Two times	3:33 p.m.	45.8	23.0	0.36	105	159	210	2714	+(seizure free)	KBr
4	Unconfirmed	*1/*2	25	Two times	11:51 a.m.	20.0	24.0	0.40	66	194	738	3379	+(seizure free)	KBr
5	Unconfirmed	*1/*2	52	Two times	4:15 p.m.	17.9	25.7	0.35	99	160	489	3332	+	KBr
6	Unconfirmed	*1/*2	23	Two times	9:00 a.m.	28.6	45.8	0.57	63	96	289	1977	+	–
7	Unconfirmed	*1/*2	49	Once (evening)	10:00 a.m.	15.6	25.0	0.53	29	183	678	2961	–	KBr
8	c.2262G > A, p.W754X	*1/*2	14	Two times	5:07 p.m.	14.3	25.0	0.36	99	125	1252	2884	+	KBr
9	Unconfirmed	*1/*3	71	Two times	3:39 p.m.	53.2	15.9	0.24	152	98	2117	1479	–	KBr
10	c.296T > C, p.I99T	*2/*2	10	Once (evening)	2:21 p.m.	6.9	19.3	0.39	143	136	5806	7236	–	KBr, CZP
11	c.2131C > T, p.Q711X	*3/*3	10	Two times	10:39 a.m.	41.0	24.6	0.25	47	109	3416	2424	+	KBr

[#] Case with ketogenic diet.

All statistical analyses were performed using Excel 2007 (Microsoft, USA) with the add-in software Statcel version 3 (OMS Ltd., Tokorozawa, Japan). In all analyses, the significance level was set at $p < 0.05$.

3. Results

3.1. CYP2C19 polymorphisms and concomitant drugs

There were 3 cases with *1/*1 (wild type), 5 cases with *1/*2, 1 case with *1/*3 (intermediate type), 1 case with *2/*2, and 1 case with *3/*3 (poor type) of CYP2C19 polymorphisms. In short, 3 extensive, 6 intermediate, and 2 poor metabolizers were analyzed. No cases were administered CYP2C19/CYP3A4 specific inhibitors or inducers. The concomitant drugs were VPA + CLB + potassium bromide (KBr) in 9 cases, VPA + CLB in 1 case, and VPA + CLB + KBr + clonazepam (CZP) in 1 case.

3.2. Mean dose and plasma concentration of concomitant drugs

A comparison of each CYP2C19 polymorphism, the mean plasma concentration of VPA/CLB, and the CLB concentration/CLB dose ratio off and on STP showed no significant differences (Table 2). Poor metabolizers had a significantly higher mean plasma concentration of N-CLB, N-CLB concentration/CLB dose ratio, and N-CLB/CLB concentration ratio off STP compared with that of extensive and intermediate metabolizers. On STP, the significant difference of the N-CLB plasma concentration disappeared in each polymorphism. The N-CLB concentration/CLB dose ratio and N-CLB/CLB concentration ratio diminished between extensive and poor metabolizers. In each CYP2C19 polymorphism, there were cases of increased and decreased N-CLB concentrations after STP administration.

3.3. Relationship between the effectiveness of STP and CLB/N-CLB concentration

Three (Cases 1, 7, and 10) of 8 cases showed no effectiveness of STP regardless of N-CLB concentration increase, and 1 (Case 11) of 3 cases showed effectiveness of STP regardless of the N-CLB concentration decrease (Table 1). Three (Cases 2, 9, and 11) cases demonstrated an N-CLB concentration decrease after STP administration, and, of those cases, Case 2 had tried a ketogenic diet.

3.4. Seizure reduction and adverse events after STP administration

Six (54%) of 11 cases with DS had >50% reduction in seizure frequency with no significant differences by CYP2C19 polymorphisms (Table 3). Of those cases, 1 extensive metabolizer and 1 intermediate metabolizer became seizure free. Adverse effects

Table 2
Demographic characteristics of cases grouped according to CYP2C19 polymorphisms.

	Extensive metabolizer ($n = 3$)	Intermediate metabolizer ($n = 6$)	Poor metabolizer ($n = 2$)	Significance
Age (year)	5.0(1.5–6.6)	5.5(1.2–12.1)	7.3(2.6–12.0)	N.S.
Male/female	1/2	4/2	1/1	
Body weight[a] (kg)	15.9 ± 3.8	18.4 ± 2.9	24.1 ± 12.0	N.S.
STP dose (mg/kg per day)	39.6 ± 3.7	24.9 ± 6.0	23.9 ± 13.6	N.S.
STP concentration[a] (μg/ml)	3.5 ± 0.46	4.7 ± 2.2	6.0 ± 0	N.S.
VPA dose[a] (mg/kg per day)	27.9 ± 3.3	26.9 ± 4.0	21.9 ± 2.6	N.S.
VPA concentration off STP[a] (μg/ml)	106.8 ± 20.0	99.7 ± 11.4	115.8 ± 0.10	N.S.
VPA concentration on STP (μg/ml)[a]	86.1 ± 20.2	106.4 ± 4.2	96.1 ± 6.7	N.S.
CLB dose[a] (mg/kg per day)	0.25 ± 0.09	0.40 ± 0.05	0.32 ± 0.07	N.S.
CLB concentration off STP (ng/ml)[a]	79.6 ± 13.9	84.6 ± 17.1	95.0 ± 48.0	N.S.
CLB concentration on STP[a] (ng/ml)	105.3 ± 47.3	145.6 ± 18.9	122.5 ± 13.5	N.S.
N-CLB concentration off STP[a] (ng/ml)	311.0 ± 126.2	927.1 ± 271.9	4611.0 ± 1195.0	$p < 0.01$*
N-CLB concentration on STP[a] (ng/ml)	1387.0 ± 757.0	2683.5 ± 317.7	4830.0 ± 2406.0	N.S.
CLB concentration/CLB dose ratio off STP[a]	489.3 ± 231.1	253.5 ± 84.3	277.3 ± 89.3	N.S.
CLB concentration/CLB dose ratio on STP[a]	351.4 ± 84.1	374.2 ± 45.9	392.3 ± 43.6	N.S.
N-CLB concentration/CLB dose ratio off STP[a]	3474.4 ± 2946.2	2887.8 ± 1253.1	14275.5 ± 611.5	$p < 0.05$*
N-CLB concentration/CLB dose ratio on STP[a]	4352.4 ± 1743.3	6894.0 ± 896.7	14124.9 ± 4428.9	$p < 0.05$#
N-CLB/CLB concentration ratio off STP[a]	4.6 ± 2.6	11.7 ± 2.8	56.6 ± 16.0	$p < 0.01$*
N-CLB/CLB concentration ratio on STP[a]	11.5 ± 2.7	18.6 ± 1.3	37.7 ± 15.5	$p < 0.05$#

Significance was determined by Tukey–Kramer test.
N.S.: not significant.
[a] Mean ± SEM.
* Comparison between extensive-poor metabolizers and intermediate-poor metabolizers.
# Comparison between extensive-poor metabolizers.

Table 3
The clinical characteristics by CYP2C19 polymorphisms.

	Extensive metabolizer ($n = 3$)	Intermediate metabolizer ($n = 6$)	Poor metabolizer ($n = 2$)	Significance
Seizure reduction (>50%)	1	4	1	N.S.
No change or aggravation of seizure	2	2	1	N.S.
With adverse events on STP	2	5	1	N.S.
Without adverse events on STP	1	1	1	N.S.

Cases are classified according to the total count of mutated allele(s).
Extensive metabolizer: CYP2C19*1/*1, intermediate metabolizer:*1/*2 or *1/*3, poor metabolizer: *2/*2 or *3/*3.
Significance are determined by Fisher's test. N.S.: not significant.

of STP administration were noticed in the 8 (72%) cases with no significance of specific CYP2C19 polymorphisms. Six (54%) cases showed drowsiness and 7 (63%) cases showed ataxia. These adverse effects were observed even in cases of decreased CLB/N-CLB plasma concentrations on STP. Two cases had the dose of concomitant drugs (dose reduction of VPA in one case, and CLB in the other case) adjusted due to strong drowsiness. Two cases discontinued STP administration due to no efficacy towards seizure control.

4. Discussion

In the present study, the mean plasma concentration of N-CLB, N-CLB concentration/CLB dose ratio, and N-CLB/CLB concentration ratio off STP were significantly high in the poor metabolizer, with no significant difference of CLB concentration/CLB dose ratio off STP. These data are consistent with those of Kosaki et al. [7]. There were no significant differences in CLB concentration/CLB dose ratio even on STP for each CYP2C19 polymorphism, which may be because CYP3A4 is the major cytochrome P450 in CLB demethylation [4,11]. Kinoshita et al. reported that the therapeutic effect of CLB may be predicted by a higher N-CLB concentration/CLB dose ratio [12]. However, several lines of evidence show that the N-CLB concentration/CLB dose ratio was not related to STP efficacy in DS [1]. In addition, Giraud et al. reported that high N-CLB plasma concentrations in epileptic patients are not sufficient to control seizures and that the addition of stiripentol to clobazam treatment improves antiepileptic efficacy [4]. This suggests that the STP action cannot be explained only by increasing blood levels of CLB/N-CLB, but by direct actions such as allosteric modulation of the GABA receptor [13,14]. STP enhances central GABA transmission through a barbiturate-like effect, suggesting that STP should possess an antiepileptic effect by itself [15]. The mean plasma concentration of VPA did not change before and after STP administration, which supports the results of Chiron et al. [3] that the plasma concentrations of VPA were not appreciably different between the STP group and the placebo group, indicated a lack of interaction between STP and VPA.

Three (37%) of 8 cases showed no effectiveness of STP regardless of N-CLB concentration increase, and 1 (Case 11) of 3 cases showed effectiveness of STP regardless of N-CLB concentration decrease. Seizure reduction despite N-CLB concentration decrease is an interesting matter. In this case, the CLB plasma concentration increased on STP, inversely to N-CLB. This may be explained, in part, by the effect of STP as a CYP3A4 inhibitor. The elevation of CLB concentration and direct anticonvulsant activities of STP to GABA receptor may contribute to seizure reduction. Strangely, including this case, 3 cases of N-CLB plasma concentration decreased on STP. One was a case of an extensive metabolizer with a ketogenic diet (Case 2), and one of an intermediate metabolizer with *3 variant (Case 9), and one of a poor metabolizer with *3/*3 variant (Case 11). Concerning Case 2, the ketogenic diet might influence the concentrations of N-CLB. It is interesting to note that both Case 9 and Case 11 had the *3 polymorphism. Although the reason for the N-CLB concentration decrease is unclear, this is the first report of the change of CLB/N-CLB concentration with the *3/*3 variant. There may be a possibility that STP prompts the metabolism from N-CLB to OH-NCLB for patients with the *3 variant. It is tempting to speculate that STP partially restores the enzyme activity of CYP2C19*3 by read through of a premature stop codon as shown in aminoglycoside [16].

Concerning the clinical effects of STP, 6 (54%: 1 case of extensive, 4 cases of intermediate, and 1 case of poor metabolizer) of 11 cases with DS had >50% reduction in seizure frequency, but showed no significant differences in CYP2C19 polymorphisms. This result was consistent with the report of Inoue et al., where CYP2C19 polymorphism analysis of 21 cases was performed [5]. STP is the only compound that proved its efficacy in DS through two independent randomized placebo-controlled trials. In one, 71% of patients with DS were responders on STP [2], and, in the other, 10 of 20 patients with DS had over 50% reduction in seizure frequency and 3 became free of seizures [3]. The rate