Pulmonary atresia (PA)	–	–	–	–	–	+	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/49)
Positional anomaly of valsalva sinus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	2% (1/49)
Functional abnormalities																																																			
Pulmonary hypertension (PH)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	8% (4/49)
Left ventricular noncompaction (LVNC)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	+	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	10% (5/49)
Dilated cardiomyopathy	–	–	–	–	–	–	–	–	+	–	+	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4% (2/49)
5. Sensory organs																																																			
Ears																																																			
Hearing problems	–	+	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	+	+	–	–	+	–	–	–	+	+	–	–	+	–	–	NA	+	–	+	+	+	–	–	+	+	+	+	+	–	–	–	+	–	39% (19/49)
Eyes																																																			
Strabismus	–	–	+	–	–	–	+	NA	–	–	+	–	+	–	–	–	+	+	–	–	+	–	–	+	–	–	–	–	+	NA	–	–	NA	+	–	–	+	–	–	+	+	–	NA	–	–	+	–	–	–	+	33% (15/46)
Ametropia	–	–	+	–	–	–	–	NA	–	–	+	–	–	+	–	–	+	+	+	–	+	–	+	–	+	–	–	–	–	NA	–	–	NA	–	–	–	–	–	–	+	–	–	NA	–	–	+	–	+	–	–	26% (12/46)
Nystagmus	–	–	–	–	–	–	–	NA	+	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	NA	–	–	NA	+	–	–	–	–	–	+	–	–	NA	+	+	–	+	–	–	–	20% (9/46)
Oculomotor disturbance	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	–	–	–	–	–	–	–	–	NA	–	–	+	–	–	–	–	2% (1/46)
Coloboma	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	–	–	+	–	–	–	–	–	NA	–	–	–	–	–	–	–	2% (1/46)
Congenital microphthalmia	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	+	–	2% (1/46)
Retinitis pigmentosa	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	NA	–	–	NA	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	2% (1/46)
6. Urogenital abnormalities																																																			
Renal abnormalities																																																			
Renal hypoplasia	–	–	NA	–	–	–	NA	NA	–	+	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	NA	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/43)
Hydronephrosis	–	–	NA	–	–	–	NA	NA	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	+	–	NA	–	–	–	NA	–	NA	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5% (2/43)
Pyelectasis	–	–	NA	–	–	–	NA	NA	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	NA	+	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/43)
Nephronophthisis	–	–	NA	–	–	–	NA	NA	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	+	–	NA	–	–	–	NA	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/43)
Extragenital abnormalities																																																			
Ectopic kidney	–	–	NA		–	–	NA	NA	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	+	–	NA	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/44)
Ambiguous external genitalia	–	–	NA	–	–	–	NA	NA	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	NA	+	–	–	–	–	–	–	–	–	+	+	–	–	NA	–	–	7% (3/43)
Cryptorchidism	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	–	NA	NA	NA	+	NA	NA	+	NA	NA	+	NA	NA	NA	NA	NA	+	–	NA	–	+	NA	NA	+	+	NA	NA	–	NA	+	NA	NA	NA	NA	NA	64% (9/14)
Scrotal hypoplasia	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	NA	–	NA	NA	NA	–	NA	NA	–	NA	NA	–	NA	NA	NA	NA	NA	–	–	NA	+	–	NA	NA	–	–	NA	NA	–	NA	+	NA	NA	NA	NA	NA	14% (2/14)
7. Endocrinology and nutrition																																																			
Hypothyroidism	–	–	NA	–	–	–	NA	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	NA	+	–	–	+	+	NA	–	–	–	+	–	–	–	NA	–	–	16% (7/45)
Obesity	–	–	NA	–	NA	–	–	+	–	+	+	–	+	–	–	–	–	–	–	NA	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	11% (5/46)
8. Other complications																																																			
Respiratory tract related																																																			
Stenosis of nasal cavity	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	
Laryngomalacia and/or tracheomalacia	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Adenoidal hypertrophy and/or enlarged tonsil	NA	+	NA	–	NA	NA	NA	+	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Obstructive sleep apnea	NA	NA	NA	–	NA	NA	NA	+	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Allergy related																																																			
Atopic dermatitis	NA	NA	NA	–	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	+	NA	NA	–	–	NA	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Food allergy	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Bronchial asthma	NA	+	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Related to secondary																																																			
Precocious puberty	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Breast enlargement	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Others																																																			
Atresia of external acoustic foramen	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	
Dental abnormalities	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	
Non alcoholic steatohepatitis (NASH)	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Constipation	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	–	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	

NA, not available; S, severe; B, borderline; M, moderate.

Fig. 1. Facial features of the patients with variably sized 1p36 deletions. Pt 1 (a; at 14 years of age) shows edematous eyelids rather than deep-set eyes. Pt 3 (b; 6 years), 6 (c; 5 years), and 14 (d; 15 years) share characteristic features, including deep-set eyes, hypotelorism, and pointed chins. Pt 47 (e; 4 years) and 48 (f; 8 years) do not exhibit such characteristic features, with round faces rather than hypotelorism and pointed chins. Pt 50 (g; 3 years) exhibits distinctive features with arched eyebrows and hypertelorism. Written informed consent to publish patient photos was obtained from all the patient families.

involved in chromatin remodeling and gene transcription, regulating the expression of neuronal genes [29]. Thus, *CHD5* also may be a modifier gene for severe ID.

It has been suggested that two genes, gamma-aminobutyric acid (GABA) A receptor delta (*GABRD*; chr1: 1,950,768–1,962,192), and *KCNAB2* (chr1: 6,105,981–6,161,253), are associated with the manifestations of epilepsy [27]. This is also been suggested by our present study, as there was no history of epilepsy in a patient (Pt 2) with a 1.8 Mb terminal deletion and a patient (Pt 50) with a 10.0 Mb interstitial deletion; both of the deletions includes neither *GABRD* nor *KCNAB2* (Fig. 2). The incidence of epilepsy was higher in the patients with severe ID (30/38; 79%) than in the patients with moderate ID (4/8; 50%). Thus, the severity of ID was associated with the incidence of epilepsy and the same gene/set of genes may be involved in both of these neurological manifestations.

Several case reports have suggested an association between periventricular nodular heterotopia (PVNH) and 1p36 deletion [16,30–32], and the candidate region for polymicrogyria has been mapped to the distal 4.8 Mb region [33]. As the smallest deletion among the patients with abnormal neuronal migration was 3.0 Mb (Pt 8), the gene(s) responsible for this phenotype may be narrowed down to the distal 3.0 Mb region (Fig. 2; region D). Chiari malformation type II was identified only in Pt 34, who showed an unbalanced translocation with chromosome 4. Thus, this rare feature may be attributable to the partial trisomy of chromosome 4.

4.4. Cardiac abnormality

Previously, the genetic region responsible for left ventricular noncompaction (LVNC) was assigned to the 1.9–3.4 Mb region [34–36]. On the other hand, there are many reports which show an association between Ebstein anomaly and 1p36 deletion [7,37–40]. The genomic region responsible for Ebstein anomaly was assigned to the 2.9–3.8 Mb region [39,40]. In 2005, Sinkovec et al. reported two patients with LVNC associated with Ebstein anomaly [41]. In this study, we identified a patient (Pt 24) who showed both LVNC and Ebstein anomalies. Given this perspective, it might be reasonable to conclude that the critical regions involved in LVNC and Ebstein anomaly are relatively close. As mentioned above *PRDM16* located on chr1: 2,985,742–3,355,185 was reported as a gene responsible for cardiomyopathy and LVNC [28]. This is in agreement with our study, as the smallest deletion identified in a patient (Pt 9) with DCM was 3.1 Mb in size. It is possible that *PRDM16* may also be related not only to LVNC but also to the Ebstein anomaly.

Although double-outlet right ventricle (DORV) has never been reported in individuals with 1p36 deletions, we found DORV in two patients. We found a relatively small deletion (2.5 Mb) in a patient (Pt 6) with DORV (Fig. 2; region D). There is a possibility that the protein kinase C zeta gene (*PRKCZ*; chr1 chr1: 1,981,909–2,116,834) is related to cardiac abnormalities, because this gene had been implicated in a variety of process including cardiac muscle function [42,43]. The positional

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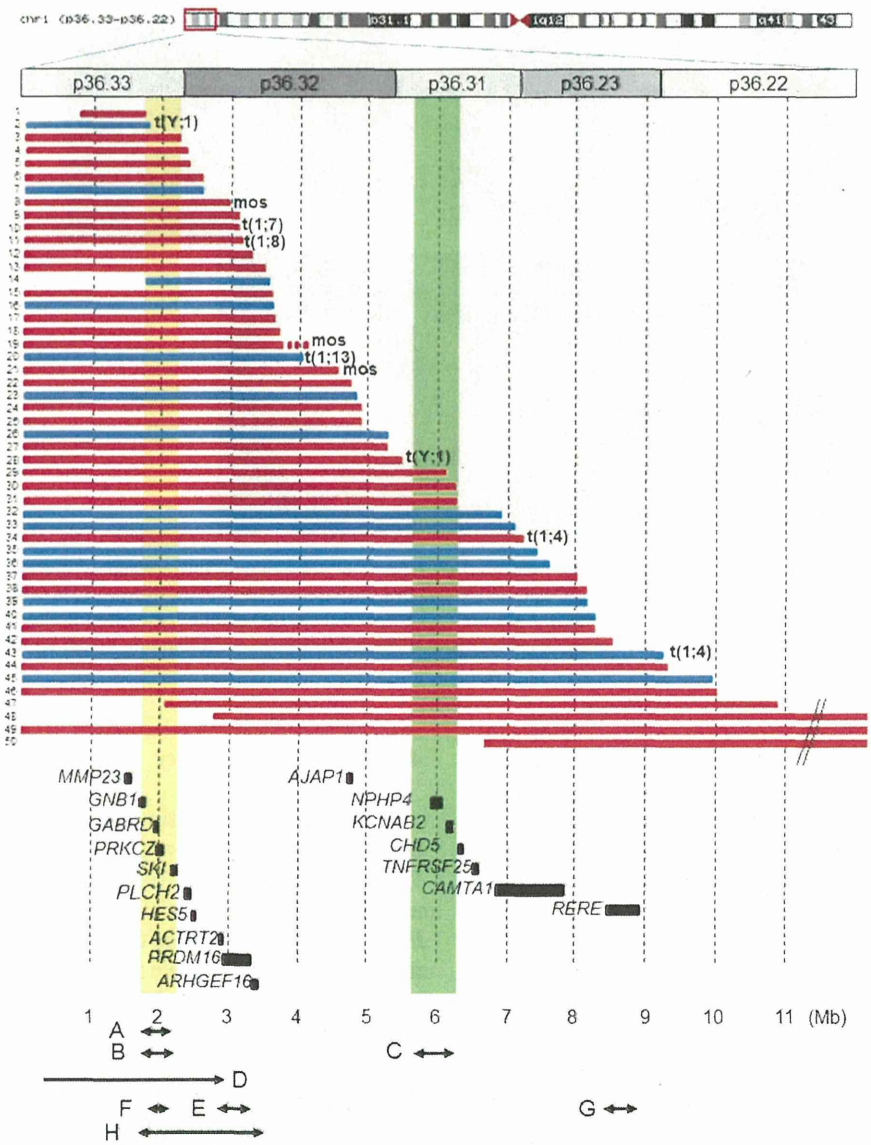

Fig. 2. Result of chromosomal microarray testing depicted in a genome map of the 1p36 region. The scheme of chromosome 1 (top) is downloaded from the UCSC genome browser. Red and blue bars indicate the deletion regions identified in female and male patients, respectively. Black bars indicate the locations of the genes, discussed in the text. The numbers depicted on the left side of each bar indicate patients' numbering. "t" and "mos" indicate unbalanced translocations and mosaicism, respectively. Yellow and green translucent vertical lines emphasize the proposed responsible regions for ID. Proposed responsible regions for each phenotype; A, distinctive craniofacial findings; B, ID; C, modifier effect for ID; D, LVNC and Ebstein anomaly; E, DORV; F, cardiac anomalies; G, cryptochidisms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

effects for *PRDM16* may be another possibility in this case.

The arginine-glutamic acid dipeptide (RE) repeats gene (*RERE*; chr1: 8,412,464–8,877,699) has been reported to play a critical role in early cardiovascular development [44]. In this study, all patients with deletions larger than 8.4 Mb, which involve *RERE*, showed cardiac anomalies. Thus, *RERE* may be involved in the pathogenesis of congenital heart defects (Fig. 2; region G).

Only Pt 20, with an unbalanced translocation between 13q32.3, showed hypoplasia of the left ventricle (HLHS) in this study. HLHS accounts for 2–3% of all congenital heart defects, and a minority of HLHS cases have been associated with congenital anomaly syndromes, e.g., the Jacobsen, Turner, and Potocki–Lupski syndromes, respectively [45–47]. As 13q duplication has been reported to be associated with this manifestation, the findings of HLHS found in Pt 20 may be due to a partial trisomy of 13q [48].

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4.5. Other complications

In patients with 1p36 monosomy, a Prader–Willi syndrome (PWS)-like phenotype has been described [6,13,49]. The clinical features that overlap between the 1p36 deletion syndrome and PWS are ID, neonatal hypotonia, obesity, craniofacial anomalies, hyperphagia, short stature, and behavior problems. D'Angelo et al. described a patient with a 2.5 Mb deletion within the chromosome region 1p36.33–1p36.32 [13]. Tsuyusaki et al. hypothesized that the critical region for the PWS-like phenotype was within 4 Mb from 1pter [49]. Rosenfeld et al. suggested a critical region for the PWS-like phenotype in the 1.7–2.3 Mb region [12]. In this study, all five patients with obesity (Pt 8, 10, 11, 13, and 21) were female, and acquired ambulatory ability within the ages of 2–8 years. Two of the patients (Pt 8 and 21) showed mosaic deletions [17]. From these perspectives, we speculate that female patients who showed 1p36 deletions involving the critical region and who acquired ambulatory ability are likely to be at risk for obesity.

5. Conclusion

In this study, we successfully accumulated the genotype–phenotype data of 50 patients with the deletions of 1p36 regions. As hypotelorism was commonly observed in patients, it may be characteristic of Asian patients. The genotype–phenotype correlation analysis narrowed down the regions responsible for distinctive craniofacial features and ID to the 1.8–2.1 and 1.8–2.2 Mb regions, respectively. Patients with deletions larger than 6.2 Mb showed no ambulation, indicating that severe neurodevelopmental prognosis may be modified by haploinsufficiencies of *KCNAB2* and/or *CHD5*, located 6.2 Mb away from the telomere. Although the genotype–phenotype correlation for the cardiac abnormalities is unclear, *PRDM16*, *PRKCZ*, and *RERE* may be related to this complication. One more finding revealed by this study for the first time, is that female patients who acquired ambulatory ability are likely to be at a risk for obesity.

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Original article

Clinical and genetic features of acute encephalopathy in children taking theophylline

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Abstract

Background: Theophylline has recently been suspected as a risk factor of acute encephalopathy with biphasic seizures and late reduced diffusion (AESD), although there has been no systematic study on the relationship between acute encephalopathy in children taking theophylline (AET) and AESD.

Methods: We recruited 16 Japanese patients (11 male and 5 female, median age of 2 years and 7 months) with AET from 2008 to 2013. We evaluated their clinical features, such as the duration of first seizure, biphasic clinical course and cranial CT/MRI imaging and compared them with those of AESD. We analyzed the polymorphisms or mutations of genes which are associated with AESD.

Results: Clinically, 12 patients had neurological and/or radiological features of AESD. Only one patient died, whereas all 15 surviving patients were left with motor and/or intellectual deficits. Genetically, 14 patients had at least one of the following polymorphisms or mutations associated with AESD: thermolabile variation of the carnitine palmitoyltransferase 2 (*CPT2*) gene, polymorphism causing high expression of the adenosine receptor A2A (*ADORA2A*) gene, and heterozygous missense mutation of the voltage gated sodium channel 1A (*SCN1A*) and 2A (*SCN2A*) gene.

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Conclusions: Our results demonstrate that AET overlaps with AESD, and that AET is a multifactorial disorder sharing a genetic background with AESD.

Keywords: Theophylline; Adenosine receptors; Acute encephalopathy; Status epilepticus

1. Introduction

Theophylline is a methylxanthine that exerts multiple pharmacologic effects by inhibiting phosphodiesterases. Until recently, it has been commonly used in clinical practice for the treatment of bronchial asthma and acute bronchitis, especially in Japan. However, theophylline may trigger seizures in patients with or without epilepsy, even when the concentration is within the therapeutic range [1,2]. The pro-convulsive effects of theophylline are explained by its activity as a non-selective, competitive antagonist of adenosine. In the central nervous system (CNS), adenosine plays a role as an endogenous anticonvulsant [3,4], since the effects of anti-excitatory A1 receptor (ADORA1) predominate over those of pro-excitatory A2A receptor (ADORA2A). Theophylline-associated seizures (TASs) are most prevalent among children under 6 years of age and usually occur during a febrile infectious disease [5]. TASs often persist and resist first-line anticonvulsants, leading to refractory status epilepticus and a poor neurologic outcome [6,7].

When a post-ictal coma lasts for more than 24 h, the condition should be regarded as acute encephalopathy rather than a mere seizure [8]. Acute encephalopathy with inflammation-mediated status epilepticus includes multiple syndromes [9], such as fever-induced refractory epileptic encephalopathy in school-aged children (FIRES) (or its eponym, acute encephalitis with refractory, repetitive partial seizures (AERRPS)), and acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) [10] (or its eponym, acute encephalopathy with febrile convulsive status epilepticus (AEFCSE)) [11]. In a case series in a referral hospital in Japan, many children taking theophylline reportedly had clinical and radiological features of AESD or AEFCSE [12]. Thus, theophylline has recently been suspected as a risk factor of AESD [8], although there has been no systematic study on the relationship between acute encephalopathy in children taking theophylline (AET) and AESD.

In this paper, we recruited Japanese patients with AET by means of a nationwide, multi-institutional study supported by the Japanese Society of Child Neurology. We reviewed their clinical data and examined whether the findings meet the diagnostic criteria of AESD. We also conducted genetic analysis of these patients, focusing on genes that were shown to be associated with AESD in our previous studies: carnitine palmitoyltransferase 2 (*CPT2*), *ADORA2A*, and voltage-gated sodium channel subunit 1A (*SCN1A*) and 2A (*SCN2A*) [12–15]. The aim of this study was to elucidate the relationship between AET and AESD from both clinical and genetic viewpoints.

2. Methods

2.1. Patients

We defined acute encephalopathy based on the following criteria [16,17]: (1) acute onset of severe and sustained impairment of consciousness after a preceding infection, and (2) exclusion of CNS inflammation. We defined AET as acute encephalopathy with the onset with status epilepticus within several hours after administration of oral theophylline or intravenous aminophylline, and recruited patients with AET from hospitals in Japan during 2008–2012 in a retrospective manner. Sixteen Japanese patients (11 male and 5 female) aged from 6 months to 4 years and 4 months (median, 2 years and 7 months), participated in this study. One case (Case 2) had been reported previously [14]. Their clinical characteristics including the family and past history, preceding infection, serum concentration of theophylline, duration of status epilepticus, presence or absence of biphasic seizures, cranial CT and/or MRI findings, therapy and outcome, were evaluated. The diagnosis of AESD was based on the criteria described previously [16]. It was regarded as 'definite' when both the characteristic clinical course (biphasic seizures) and CT/MRI findings (delayed appearance of cerebral cortical edema, distribution of lesions showing lobar or hemispheric involvement and peri-Rolandic sparing, and restricted diffusion of the subcortical white matter (so-called bright tree appearance) were present [8,10], 'probable' when either clinical or CT/MRI features were present, and 'possible' when prolonged febrile seizures were followed by non-specific CT/MRI findings (diffuse cortical damage) and other diagnostic possibilities were unlikely. In some patients whose CT/MRI findings in the acute/subacute period were either unavailable or insufficient, distribution of lesions was inferred on the basis of those in the convalescence. Other conditions that occasionally show bright tree appearance, such as hemorrhagic shock and encephalopathy syndrome, head

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injury and hypoxic-ischemic encephalopathy, were excluded based on the clinical history and laboratory data.

2.2. Standard protocol approvals, registrations, and patient consent

The procedures in this study were approved by the University of Tokyo Ethics Committee. Written informed consent was obtained from all guardians of patients participating in the study.

2.3. Procedures

Peripheral blood samples were collected from all 16 patients and from 100 control subjects, namely healthy Japanese volunteers. Genomic DNA was extracted from the blood using standard protocols and was used for the analysis of *CPT2*, *ADORA1*, *ADORA2A*, *SCN1A* and *SCN2A* genes.

2.3.1. CPT2

We analyzed exon 4 and 5 of the *CPT2* gene by direct sequencing or real-time polymerase chain reaction (PCR) using the TaqMan Probe and Faststart Universal Probe Master ROX (Roche, Basel, Switzerland), as described previously [12]. In this study, we focused on the F352C genotype. We had previously found that at least one allele C in F352C is associated with AESD and other syndromes of acute encephalopathy [12].

2.3.2. ADORA1 and ADORA2A

All coding regions and intron–exon splicing sites of the *ADORA1* and *ADORA2A* genes were PCR amplified with flanking intronic primers under standard PCR conditions. PCR products of *ADORA1* and *ADORA2A* were sequenced on a 310 Genetic Analyzer, 3100 Genetic Analyzer or 3130xl Genetic Analyzer (Life Technologies, Carlsbad, CA, USA). To identify rs5751876 and rs2298383 SNPs of *ADORA2A*, the PCR-restriction fragment length polymorphism (PCR-RFLP) method was adopted. Based on the combination of four SNPs showing complete linkage disequilibrium in Japanese (human HapMap project, http://Apr2011.archive.ensemble.org), we determined whether the subjects had either haplotype A (C at rs2298383, T at rs5751876, deletion at rs35320474 and C at rs4822492) or haplotype B (T at rs2298383, C at rs5751876, T at rs35320474 and G at rs4822492). We had previously demonstrated that haplotype A is a risk factor for AESD [14].

2.3.3. SCN1A and SCN2A

The entire coding regions of the *SCN1A* and *SCN2A* genes were sequenced on a 310 Genetic Analyzer (Life Technologies) [14,15].

3. Results

3.1. Clinical findings

Clinical data were similar among the 16 patients studied (Table 1). Family history and past history were unremarkable, except for the presence of febrile seizures in two cases each. In all the cases, theophylline or aminophylline was administered temporarily for the treatment of acute asthma attacks (2 cases) and acute bronchitis (14 cases). Blood concentration of theophylline was within the therapeutic range (3.9–11.8 μg/ml) in all 5 cases examined. All patients had fever due to acute respiratory infection. The first convulsion, mostly status epilepticus, occurred within 24 h from the onset of fever. Of the 14 patients who had seizures lasting longer than 15 min, seven patients required continuous intravenous infusion of barbiturates for 2–11 days. Two underwent hypothermia. Eleven showed biphasic seizures typical for AESD. Cranial CT or MRI findings during the acute/subacute period were available in 15 cases. Ten had one of the features characteristic of AESD: delayed cerebral edema, lobar or hemispheric involvement, and bright tree appearance (Fig. 1). One of the remaining five showed, during convalescence, cerebral cortical sparing of the peri-Rolandic regions, another feature typical of AESD. Cranial CT/MRI during convalescence showed diffuse atrophy in 11 patients.

3.2. Genetic findings

3.2.1. CPT2

Eight out of the 16 patients had at least one allele C in F352C (Table 2). The frequency was higher in the patients (8/16, 50%) than in controls (26/100, 26%), although the difference did not reach statistical significance ($p = 0.07$).

3.2.2. ADORA1 and ADORA2A

First, we confirmed the absence of mutations in the entire coding region of *ADORA1* and *ADORA2A* in all the patients. Next, we analyzed genetic variations of *ADORA2A*. The number of homozygous/heterozygous haplotype A (AA/AB diplotype) in patients was 3 and 11, respectively. Only 2 patients had homozygous haplotype B (BB diplotype) (Table 2). The frequency of BB diplotype (2/16, 12.5%) was lower in the TAE patients than in controls (56/184, 30.4%) [13], although the difference did not reach statistical significance.

3.2.3. SCN1A

We found in one case (Case 2) a missense mutation, V982L, which was not found in the 100 control subjects. The valine 982 residue is located on transmembrane segment 6, domain II of SCN1A ($Na_v1.1$) protein, and is highly conserved among vertebrates and among other

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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.

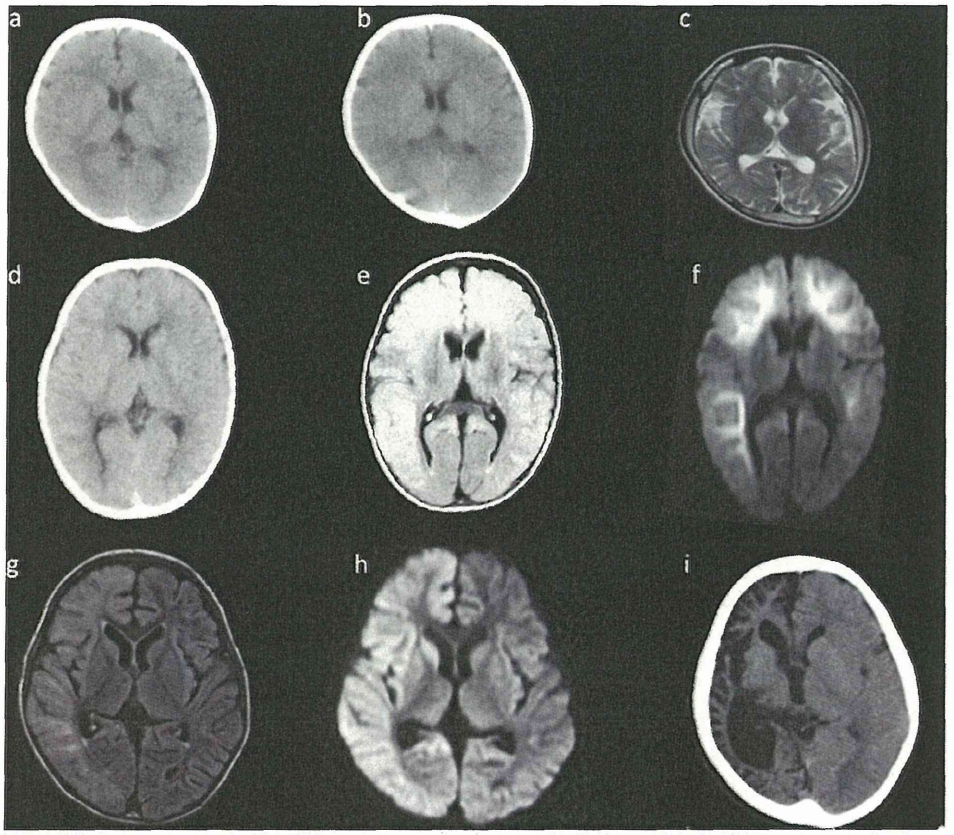

Fig. 1. Cranial CT/MRI findings in acute encephalopathy in children taking theophylline (AET). In Case 1, cranial CT on day 3 showed slight narrowing of the cerebrospinal fluid space, but no clear evidence of cerebral edema (a). On day 7, however, CT showed mild narrowing of the cerebrospinal fluid space 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

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