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IV. 研究成果の刊行物・別刷

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Original article

A severity score for acute necrotizing encephalopathy

Hiroyuki Yamamoto [a,*], Akihisa Okumura [b], Jun Natsume [a], Seiji Kojima [a], Masashi Mizuguchi [c]

[a] *Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[b] *Department of Pediatrics, Juntendo University Faculty of Medicine, Tokyo, Japan*
[c] *Department of Developmental Medical Sciences, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*

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Abstract

Objective: To develop a score that predicts the prognosis of children with acute necrotizing encephalopathy (ANE).

Method: We retrospectively evaluated clinical variables and neurological outcome in two cohorts of children with ANE. Firstly, we developed the ANE severity score (ANE-SS) according to the clinical variables that correlated with neurological outcome in 41 children who were included in our previous reports in 2009. We then applied the scoring system to a second cohort of 32 patients who were newly collected in 2011. We investigated the correlation between the ANE-SS and neurological outcome in all 73 patients.

Results: In the first cohort, brain stem lesions on MRI and state of shock at onset were significantly correlated with outcome. Age over 48 months, elevated CSF protein, and low platelet counts tended to be correlated with outcome. No types of treatment were correlated with outcome. The developed ANE-SS ranged from 0 to 9 points, with 3 points for existence of shock, 2 points for brain stem lesions, 2 points for age over 48 months, 1 point for platelet count below 100,000/μL, and 1 point for CSF protein above 60 mg/dl. Patients were classed as low risk (ANE-SS 0–1 points), medium risk (ANE-SS 2–4 points), or high risk (ANE-SS 5–9 points). ANE-SS was significantly correlated with outcome in the group of 73 patients.

Conclusion: ANE-SS can be used to predict outcome in patients with ANE. More effective treatments need to be developed for high-risk patients.

Keywords: Acute necrotizing encephalopathy; ANE; Severity score; Prognosis; Risk

1. Introduction

Acute necrotizing encephalopathy (ANE) is a serious subtype of acute encephalopathy in children, first described by Mizuguchi et al. [1–3] ANE is characterized by symmetric lesions in bilateral thalami, and is often associated with lesions in the cerebral white matter, internal capsule, putamen, brainstem and cerebellum [3]. The exact etiology is not understood, although some studies have reported increased levels of cytokines such as interleukin-6 and tumor necrosis factor-α in patients with ANE and have postulated that a "cytokine storm" may be involved in the pathogenesis of this disease [4–9]. Neurological outcome of ANE is very poor and the mortality and morbidity rates are high [5,10]. Some immunomodulation therapies and hypothermia have been tried for children with ANE [10–12].

* Corresponding author. Address: Department of Pediatrics, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan. Tel.: +81 52 744 2294; fax: +81 52 744 2974.

E-mail address: h-yamamoto@med.nagoya-u.ac.jp (H. Yamamoto).

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The occurrence of ANE is rare. Hoshino et al. reported that ANE accounted for only 39 (4.0%) of 983 children with acute encephalopathy during a 3-year period in Japan [13]. Therefore, a randomized clinical trial is difficult to perform. In addition, the poor prognosis of ANE makes randomization of patients difficult because of ethical problems, and the treatment for ANE is different among hospitals because there is no standard regimen. In order to determine the efficacy of treatment and to establish a standard regimen, prognostication of children with ANE is necessary. Some previous studies have reported that neurological symptoms, abnormal laboratory data, and neuroimaging findings indicate poor prognosis in children with ANE [3,10,14,15]. However, the method for prognostication of ANE has been unclear.

The aim of this study is to establish a severity score for ANE that can predict the prognosis of children with ANE at the onset of the illness. When we can establish a well-applicable score, it will be useful to determine the efficacy of the treatment for ANE and to establish efficacious treatment regimen. For this purpose, we explored items that can be measured at onset and that correlate with outcome in children with ANE. We then combined these items into a scoring system that can be used for prognostication of children with ANE.

2. Patients and methods

We retrospectively evaluated the clinical manifestation, laboratory data, neuroimaging findings, treatment, and outcome of two groups of children with ANE. The first group comprised 41 children with ANE who had been admitted to 17 hospitals. (Supplementary Table 1) This cohort was derived from our previous report examining the relation between outcome and treatment [5,10,16]. The data were collected from the hospitals all over Japan, where the two senior authors (AO and MM) are collaborating for clinical studies on acute encephalopathy. All of them are tertiary medical centers. Data were collected during the 2006/07 winter season. The second group consisted of 32 children with ANE who were newly recruited from 27 hospitals in December, 2010. (Supplementary Table 2) This cohort was derived from a nationwide survey on the epidemiology of acute encephalopathy in Japan [13]. Thirty-nine children with ANE was identified during the first survey and data for this study were available in 32 of them. In both cohort, clinical data were obtained using a structured research form anonymously. This study was approved by the Research Ethics Commitee of the University of Tokyo (No.2116). In both groups, the diagnosis of ANE was made by the attending pediatric neurologists on the basis of neuroradiological findings according to the criteria proposed by Mizuguchi et al. [2,3] (Fig. 1). We included patients with acute encephalopathy who had multiple focal lesions that were symmetrically distributed in the bilateral thalami and other brain regions such as the putamina, cerebral and cerebellar white matter, and brainstem tegmentum [2,3,17]. We excluded patients with marked metabolic derangement indicated by elevated lactate, pyruvate, amino acid or organic acid levels.

We investigated the following items: age, sex, existence of shock on admission, laboratory data on admission (platelet count, serum levels of aspartate transaminase, alanine aminotransferase, lactate dehydrogenase, and creatine kinase, and CSF protein level), existence of brainstem lesions on CT or MRI, treatment (methylprednisolone pulse therapy, intravenous immunoglobulin, plasma exchange, hypothermia and antithrombin III) and outcome (normal, mild sequelae,

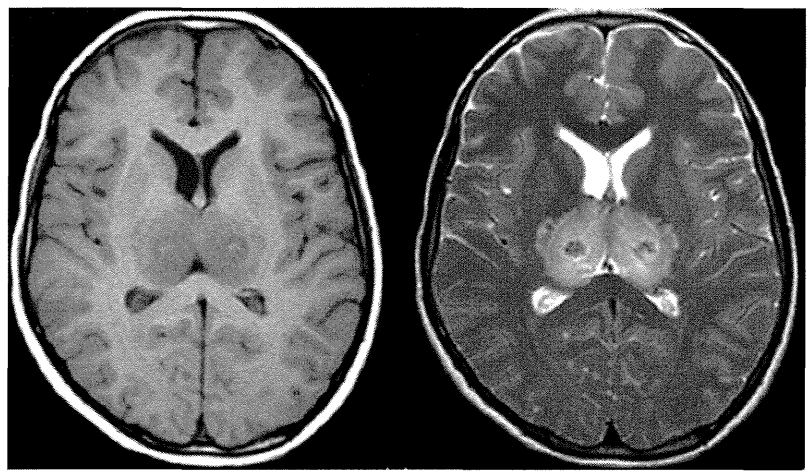

Fig. 1. MRI from a 12-year-old boy showing typical findings in ANE. The T1WI (left) and T2WI (right) show concentric lesions in the bilateral thalami and lentiform nuclei. The center and periphery of the thalamic and lenticular lesions show hyperintensity on T1WI (left) and hypointensity on T2WI (right), suggestive of hemorrhagic change.

moderate sequelae, severe sequelae, or death). These data were obtained using a structured research form that was completed by the attending pediatric neurologist in each hospital. Outcome of surviving children was determined according to both motor and cognitive impairments. Motor impairment was categorized as none, mild (walking with or without support), moderate (sitting with or without support), or severe (required support to sit). Cognitive impairment was categorized according to a developmental (DQ) or intelligence quotient (IQ) as none (DQ/IQ $\geqslant$ 70), mild (50 $\leqslant$ DQ/IQ $<$ 70), moderate (30 $\leqslant$ DQ/IQ $<$ 50), or severe (DQ/IQ $<$ 30). The severest of the motor and cognitive impairment categories was used as the grade of neurological outcome. For example, neurological outcome was severe in a child with mild motor impairment and severe cognitive impairment. Neurological outcome was determined one year or longer after the onset of ANE, whereas the duration of follow-up was not collected for this study.

To select items for inclusion in the ANE severity score (ANE-SS), we analyzed the correlation between each item and neurological outcome in the first cohort. For the purpose of statistical analyses, neurological outcome was expressed as 1 for normal, 2 for mild, 3 for moderate, 4 for severe, and 5 for death. Numerical and categorical variables were analyzed using Pearson's correlation test and Spearman's rank correlation test, respectively, and the correlation coefficient was calculated for each variable. The Mann–Whitney U test was performed to determine the degree of contribution for some candidate items. The ANE-SS was constructed by adopting the items that had a significant correlation with neurological outcome. Each item was weighted according to the degree of correlation. The adequacy of this score was evaluated in the first cohort and in a combined group of the first and second cohorts by Spearman's rank correlation test. In all statistical analyses, a p value < 0.05 was determined as statistically significant.

3. Results

3.1. Correlation between each item and outcome in the first cohort

Among the 41 patients in the first cohort, 10 of 11 patients over 48 months of age had severe sequelae or death (Fig. 2A) and patients with elevated CSF protein or low platelet count tended to have severe sequelae (Fig. 2B and C). Age, CSF protein level, existence of shock on admission, and brain stem lesions on CT or MRI were significantly correlated with outcome, but sex, serum levels of aspartate transaminase aspartate

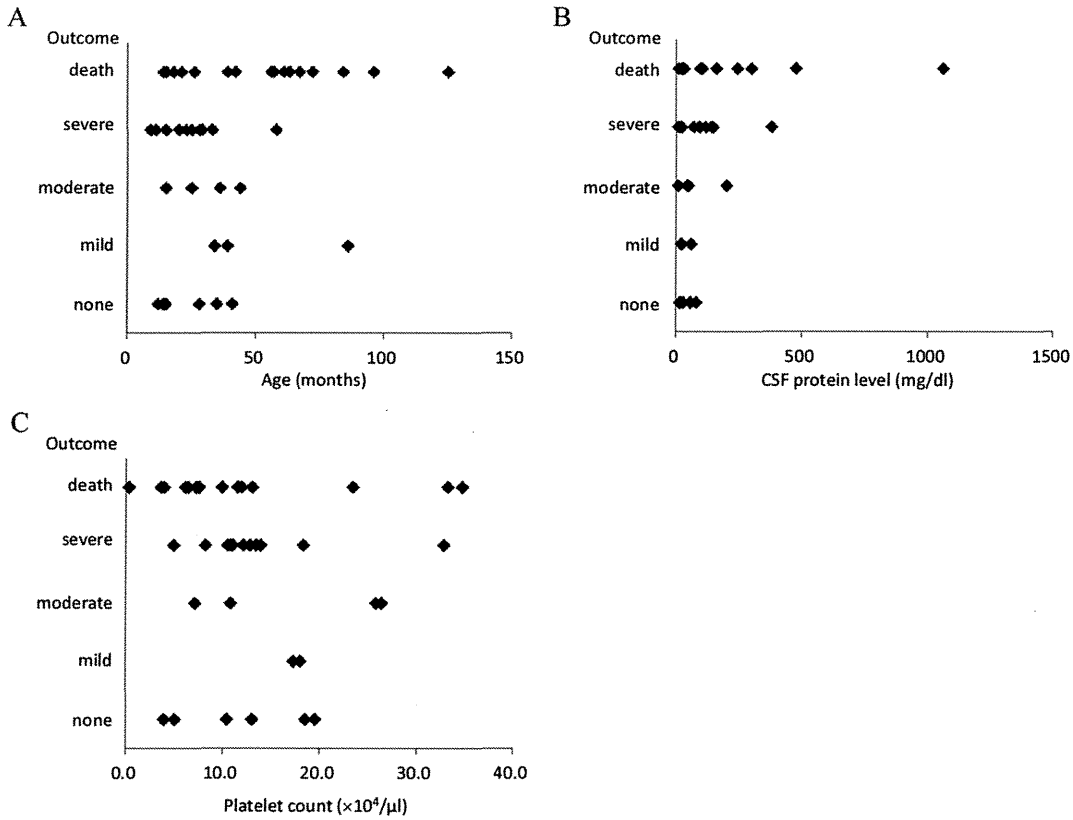

Fig. 2. Scatter diagram of outcome and age (A), CSF protein level (B) and platelet count (C). Ten of 11 patients over 48 months of age had severe sequelae or death, and patients with elevated CSF protein or low platelet count tended to have severer sequelae.

transaminase, alanine aminotransferase, lactase dehydrogenase, and creatine kinase, platelet count, and treatment types were not significantly correlated with outcome (Table 1). Comparison analyses also showed that shock, brainstem lesions, and age > 48 months were significantly correlated with poor outcome, whereas CSF protein > 60 mg/dL showed a marginal result. (Table 2) Although platelet count did not have a significant correlation with outcome, we included this in the ANE-SS because of clinical importance.

3.2. Ane-ss

The weight of each item in the ANE-SS was determined according to the strength of correlation with outcome. Because shock on admission was most closely related to outcome, it accounted for 3 points in the ANE-SS. Age > 48 months and existence of brainstem lesions were also closely correlated with outcome and accounted for 2 points each. Platelet count <100,000/μL and CSF protein > 60 mg/dl were weakly correlated with outcome and accounted for 1 point each. Among 41 patients in the first cohort, 30 had sufficient information to determine ANE-SS. In these 30 patients there was a significant correlation between ANE-SS and outcome (Spearman's $r = 0.781$, $p < 0.001$; Table 3). Sixteen of 32 patients in the second cohort had sufficient information to determine ANE-SS. In the combined group of 46 patients from the first and second cohorts there was a significant correlation between ANE-SS and outcome (Spearman's $r = 0.627$, $p < 0.001$; Table 4).

We divided patients into three groups according to ANE-SS: low risk (0–1 points, $n = 11$), medium risk (2–4 points, $n = 18$), and high risk (5–9 points, $n = 17$). There was a significant correlation between risk classification and outcome (Spearman's $r = 0.518$,

Table 1
Correlations between clinical information and prognosis (continuous variable).

	p Value	Correlation coefficient
Patient characteristics		
Age*	0.043	0.318
Sex**	0.253	0.183
Shock on admission**	<0.001	0.627
Brainstem lesions**	0.004	0.441
Laboratory data		
AST (IU/L)*	0.183	0.212
ALT (IU/L)*	0.333	0.155
LDH (IU/L)*	0.114	0.254
CK (IU/L)*	0.152	0.237
Platelet count* ($\times 10^4/\mu L$)	0.211	0.208
CSF protein* (mg/dL)	0.041	0.347
Treatment		
mPSL pulse**	0.138	0.236
Intravenous immunoglobulin**	0.769	0.047
Plasma exchange**	0.633	0.077
Hypothermia**	0.951	0.010
Anti-thrombin III**	0.951	0.010

AST: aspartate transaminase, ALT: alanine aminotransferase, LD: lactase dehydrogenase, CK: creatine kinase, CSF: cerebrospinal fluid, mPSL: methylprednisolone.
* Analyzed by Pearson's correlation test.
** Analyzed by Spearman's rank correlation test.

Table 2
Correlations between clinical information and prognosis (categorical variables).

	Mean neurological outcome class (range)		
	Yes	No	*p* Value
Age > 48 months	5 (2–5)	4 (1–5)	0.007
Shock at onset	5 (3–5)	3.5 (1–5)	<0.001
Brainstem lesions	5 (2–5)	4 (1–5)	0.006
Platelet count < $10 \times 10^4/\mu L$	5 (1–5)	4 (1–5)	0.059
CSF protein > 60 mg/dL	4 (1–5)	3 (1–5)	0.071

Analyzed by Mann–Whitney U test.

Table 3
Acute necrotizing encephalopathy severity score (ANE-SS) and outcome in the first cohort ($n = 30$).

	Outcome				
ANE-SS	No sequelae	Mild sequelae	Moderate sequelae	Severe sequelae	Death
0	3	0	0	1	0
1	3	0	0	2	0
2	0	1	3	1	1
3	0	0	0	3	0
4	0	0	0	1	1
5	0	0	1	1	2
6	0	0	0	0	2
7	0	0	0	0	1
8	0	0	0	0	0
9	0	0	0	0	3

Spearman's rank test: correlation coefficient 0.781 ($p < 0.001$).

Table 4
Acute necrotizing encephalopathy severity score (ANE-SS) and outcome in the first and second cohorts combined ($n = 46$).

	Outcome				
ANE-SS	No sequelae	Mild sequelae	Moderate sequelae	Severe sequelae	Death
0	3	1	0	1	0
1	4	0	0	2	0
2	0	1	3	2	1
3	0	0	1	6	0
4	0	1	0	1	2
5	0	0	1	1	2
6	0	0	0	2	4
7	0	0	0	1	1
8	0	0	0	0	0
9	1	0	0	1	3

Spearman's rank test: correlation coefficient 0.627 ($p < 0.001$).

Table 5
Risk classification and outcome in the first and second cohorts combined ($n = 46$).

	No or mild sequelae	Moderate sequelae	Severe sequelae or death
Low risk (ANE-SS 0–1)	8	0	3
Medium risk (ANE-SS 2–4)	2	4	12
High risk (ANE-SS 5–9)	1	1	15

Spearman's rank test: correlation coefficient 0.518 ($p < 0.001$).
ANE-SS: Acute necrotizing encephalopathy severity score.

$p < 0.001$; Table 5). Of the 11 patients classified as low risk, eight had no or mild sequelae. Of the 18 patients classified as medium risk, two had no or mild sequelae, four had moderate sequelae, and 12 had severe sequelae or death. Of the 17 patients classified as high risk, 15 had severe sequelae or death.

4. Discussion

In this study we developed the ANE-SS, which will be useful for presuming the prognosis of children with ANE. We verified that there was a significant correlation between the ANE-SS and outcome in a combined cohort of patients. There are no previous reports of a score that indicates the severity of ANE. This is attributable to several factors including low incidence of ANE and wide differences in treatment across different hospitals. We addressed these problems by accumulating patients from many hospitals in Japan.

The items used in the ANE-SS were closely related to pathogenesis and clinical features of ANE. Shock and reduced platelet count are signs of multiorgan failure and/or disseminated intravascular coagulation resulting from systemic hypercytokinemia which is considered to be the main pathomechanism of ANE [4,18]. Elevated CSF protein level is ascribed to an increased permeability of blood–brain barrier and destruction of brain parenchyma [19]. For these reasons, a decrease in platelet count and an increase in CSF protein were included in the ANE-SS despite relatively weak correlations with outcome. On the other hand, the existence of brainstem lesions and age > 48 months were also selected as the component of ANE-SS, primarily because of their robust statistical correlation with outcome. Brainstem lesions were shown to be a strong indicator of poor outcome probably due to the functional importance. [14] The reason why the older patients had worse outcome is unclear, and requires further analysis.

An important feature of the ANE-SS is its simplicity. The score consists of only five items, which can all be determined within a few hours after presentation. None of the five items is difficult to judge or necessitates additional load to patients or physicians. Thus, we expect the ANE-SS to be widely used for clinical as well as research purposes. Another important feature of the ANE-SS is the weighting of items according to the strength of their relation with outcome. This will increase the accuracy of ANE-SS in prognostication and result in a greater accuracy compared to a simple summation of items. Because of these advantages, ANE-SS can be used for prognostication of children with new-onset ANE and can contribute to the evaluation of treatment efficacy. In Japan, an increasing number of patients with ANE have recently been treated with early application of hypothermia, which is expected to be effective for ANE [11]. ANE-SS will be useful in evaluating the efficacy of hypothermia or other novel treatments for ANE.

There are several limitations in this study. Prognostication of the medium risk group was insufficient compared with that of low and high risk groups. The majority of patients in the medium risk group had severe sequelae or death. Further revision will be necessary to differentiate between medium- and high-risk groups. The timing of the laboratory examination may influence the result, because symptoms of ANE often show rapid deterioration. Thus, a few hours' difference in the timing of neuroimaging, blood sampling, or spinal tap may have a large effect on the results. For precise prognostication, it may be necessary to consider the timing of examination. The patients included in this study received different treatments over different time scales, according to the hospital at which they were treated. (Supplementary Tables 1 and 2) There is a possibility that some treatments affected the outcome, even though statistical analyses did not show a significant difference in the outcome according to treatment. It is also problematic that some patients were omitted from the study because of insufficient data to calculate ANE-SS. CSF protein levels were often unavailable because lumbar tap is not always necessary for diagnosis of ANE and may not be performed due to impending brain herniation. We tried to modify the ANE-SS by eliminating CSF protein levels, but the accuracy of the modified score was clearly reduced (data not shown).

In conclusion, we developed the ANE-SS for prognostication of children with ANE. This score is simple and easy to apply. The correlation between ANE-SS and outcome was significant in the cohort studied. We consider that the ANE-SS will be useful for evaluation of novel treatments for new-onset ANE and will contribute to evaluation of the optimal treatment.

Acknowledgements

Hiroyuki Yamamoto designed the study and drafted the initial manuscript, and approved the final manuscript as submitted. Akihisa Okumura originated the

idea for this study, supervised data collection and interpretation, critically reviewed and revised manuscript, and approved the final manuscript as submitted. Jun Natsume and Seiji Kojima interpreted the data and reviewed and revised manuscript, and approved the final manuscript as submitted. Masashi Mizuguch critically supervised data collection and interpretation, reviewed and revised manuscript, and approved the final manuscript as submitted.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.braindev.2014.05.007.

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Original article

Clinical and genetic features of acute encephalopathy in children taking theophylline

Makiko Saitoh [a,*], Mayu Shinohara [a], Atsushi Ishii [b], Yukiko Ihara [b], Shinichi Hirose [b], Masashi Shiomi [c], Hisashi Kawawaki [d], Masaya Kubota [e], Takanori Yamagata [f], Akie Miyamoto [g], Gaku Yamanaka [h], Kaoru Amemiya [i], Kenjiro Kikuchi [j], Atsushi Kamei [k], Manami Akasaka [k], Yuki Anzai [l], Masashi Mizuguchi [a]

[a] *Department of Developmental Medical Sciences, Graduate School of Medicine, The University of Tokyo, Japan*
[b] *Department of Pediatrics, Fukuoka University, Japan*
[c] *Department of Pediatrics, Child Medical Center, Osaka City General Hospital, Japan*
[d] *Department of Pediatric Neurology, Child Medical Center, Osaka City General Hospital, Japan*
[e] *Department of Neurology, National Center for Child Health and Development, Japan*
[f] *Department of Pediatrics, Jichi Medical University, Japan*
[g] *Department of Pediatrics, Asahikawa Habilitation Center for Disabled Children, Japan*
[h] *Department of Pediatrics, Tokyo Medical University, Japan*
[i] *Department of Neurology, Tokyo Metropolitan Hachioji Children's Hospital, Japan*
[j] *Division of Neurology, Saitama Children's Medical Center, Japan*
[k] *Department of Pediatrics, Iwate Medical University, Japan*
[l] *Department of Pediatrics, Saiseikai Yokohamashi Tobu Hospital, Japan*

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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.

* Corresponding author. Address: Department of Developmental Medical Sciences, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-0033, Japan. Tel.: +81 3 5841 3615; fax: +81 3 5841 3628.
E-mail address: makisaito-tky@umin.ac.jp (M. Saitoh).

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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

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

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.

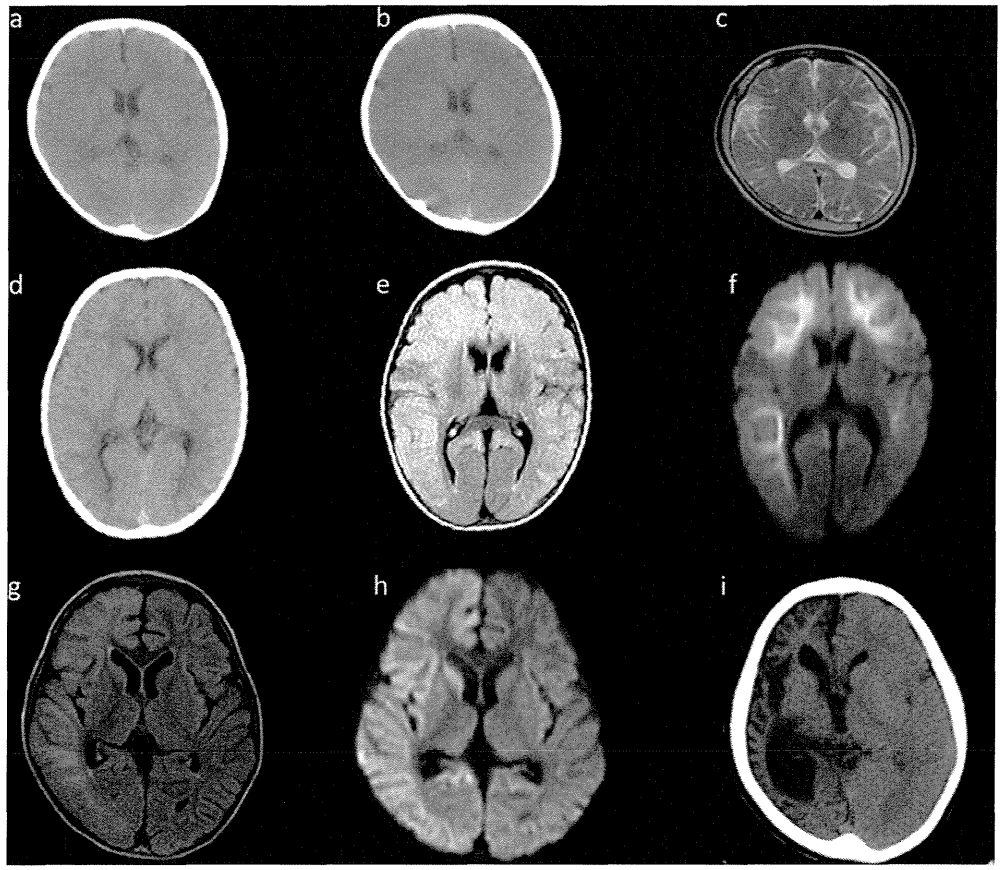

Fig. 1. Cranial CT/MRI findings in acute encephalopathy in children taking theophylline (AET). In Case 1, cranial CT on day 3 showed slight narrowing of the cerebrospinal fluid space, but no clear evidence of cerebral edema (a). On day 7, however, CT showed mild narrowing of the cerebrospinal fluid space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

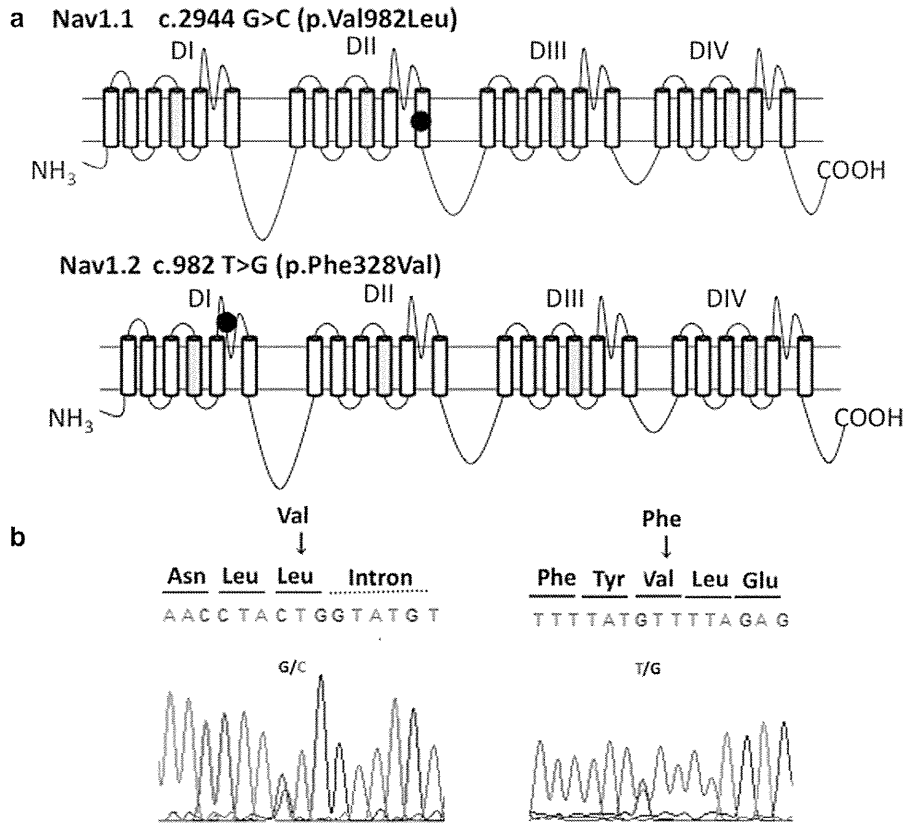

Fig. 2. Cases with *SCN1A* and *SCN2A* mutations in AET. (a) Structure of SCN1A ($Na_V1.1$) and SCN2A ($Na_V1.2$) with localization of the mutation (closed circle). c.2944 G>C (p.Val982Leu) is localized in the transmembrane segment 6 of domain II in $Na_V1.1$, whereas c.982 T>G (p.Phe328Val) is localized 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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Short communication

Clinically mild encephalitis with a reversible splenial lesion (MERS) after mumps vaccination

Jun-ichi Takanashi [a,*], Takashi Shiihara [b], Takeshi Hasegawa [c], Masaru Takayanagi [d], Munetsugu Hara [e], Akihisa Okumura [f], Masashi Mizuguchi [g]

[a] Department of Pediatrics, Tokyo Women's Medical University, Yachiyo Medical Center, Yachiyo, Japan
[b] Department of Neurology, Gunma Children's Medical Center, Shibukawa, Japan
[c] Department of Pediatrics, Soka Municipal Hospital, Soka, Japan
[d] Division of Pediatrics, Sendai City Hospital, Sendai, Japan
[e] Department of Pediatrics & Child Health, Kurume University School of Medicine, Kurume, Japan
[f] Department of Pediatrics, Aichi Medical University, Nagakute, Japan
[g] Department of Developmental Medical Sciences, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

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ABSTRACT

We retrospectively collected three patients with clinically mild encephalitis with a reversible splenial lesion (MERS) after mumps vaccination, and reviewed five patients, including two patients previously reported. The five patients (all males, aged 1 to 9) presented with fever, vomiting, or headache as the initial symptoms (day 0), suggesting meningitis, at 13 to 21 days after mumps vaccination. Consciousness disturbance, delirious behavior, seizures, or dysarthria was observed on days 1 to 3, which had completely resolved before day 11. Hyponatremia was observed in all patients. A cerebrospinal fluid study showed pleocytosis, and confirmed the vaccine strain genome. MRI revealed reduced diffusion in the splenium of the corpus callosum on days 2 to 4, which had completely disappeared on the follow-up studies performed on days 7–15. EEG showed high voltage slow wave in three patients, which later normalized. These findings led to a diagnosis of MERS after mumps vaccination. MERS after mumps vaccination may be more common than previously considered. MERS is suspected when a male patient after mumps vaccination presents with neurological symptoms with hyponatremia, following symptoms of aseptic meningitis, and MRI would be performed to examine the splenium of the corpus callosum.

1. Introduction

Natural mumps infection causes symptomatic aseptic meningitis in 1–10%, and encephalitis in 0.1% [1]. The central nervous system (CNS) complications are much less common in mumps monovalent vaccine recipients, aseptic meningitis and encephalitis being observed in 0.05% and 0.0004% in Japan [2,3]. A reversible lesion with transiently reduced diffusion in the splenium of the corpus callosum has been reported in patients with clinically mild encephalitis/encephalopathy, leading to a new clinical–radiological syndrome, clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS) [4,5]. The reason for the transiently reduced diffusion is unknown, however, transient intramyelinic edema has been postulated as a possible mechanism [4,5]. Recently, Japanese children with MERS after mumps vaccination have been reported [6,7]. In order to better understand this condition, we evaluated the clinical and radiological features of three patients with MERS after mumps vaccination in addition to the two reported ones.

2. Methods

Information on patients with MERS after mumps vaccination was retrospectively collected by sending out a questionnaire to the members of the Annual Zao Conference on Pediatric Neurology in addition to the corresponding authors of previous reports [6,7], after approval by the institutional review board of Tokyo Women's Medical University. The diagnosis of MERS was established according to diagnostic criteria [4,8]. We reviewed the clinical charts of the patients in order to accrue information on symptoms, medication, treatment, outcome, and results of cerebrospinal fluid (CSF) analysis, magnetic resonance imaging (MRI), and electroencephalography (EEG). Mumps virus and its strain were confirmed by RT-PCR and/or isolation from CSF and sequencing of SH gene. The day of the initial symptoms, such as fever, was defined as day 0.

Abbreviations: ADEM, acute disseminated encephalomyelitis; CNS, central nervous system; CSF, cerebrospinal fluid; EEG, electroencephalography; MERS, clinically mild encephalitis/encephalopathy with a reversible splenial lesion; MRI, magnetic resonance imaging.

* Corresponding author at: Department of Pediatrics, Tokyo Women's Medical University, Yachiyo Medical Center, 477-96 Owadashinden, Yachiyo-shi 276-8524, Japan. Tel.: +81 47 450 6000; fax: +81 47 458 7047.
E-mail address: jtaka44@hotmail.co.jp (J. Takanashi).

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3. Case series and literature review

Three previously healthy Japanese patients (3 males, aged from 2 to 9 years) were enrolled in this study. Patient 3 was reported in an annual hospital report, written in Japanese [9]. The clinical and radiological records, including those of two patients previously reported (patients 4 and 5, both males aged 8 and 1), are summarized in Table 1. The five patients presented with fever (5 patients), vomiting (4), and headache (2) as the initial symptoms (day 0) at 13 to 21 days after mumps vaccination. Consciousness disturbance (Glasgow Coma Scale ≤ 13, 3 patients), delirious behavior (2), seizures (2), or dysarthria (1) was observed on days 1 to 3, which had completely resolved on days 2 to 11. A CSF study revealed pleocytosis, and confirmed mumps vaccine virus (Hoshino strain in 4 patients, Torii strain in 1). MRI revealed reduced diffusion and mild T2 prolongation in the splenium of the corpus callosum (5 patients) with the genu of the corpus callosum (1) and the symmetric white matter (3) on days 2 to 4 (Fig. 1), which had completely disappeared on follow-up scanning on days 7–15. EEG showed high voltage slow waves in 3 patients (diffuse in 1, O dominant in 2), which later normalized. Hyponatremia was observed in all patients on days 2 to 3, which normalized on days 5 to 10. Hyponatremia was due to the syndrome of inappropriate secretion of antidiuretic hormone in 4 patients. 3 patients were given steroids (3) or δ-globulin (1) as specific therapy for encephalopathy. The clinical, laboratory and radiological findings led to a diagnosis of MERS after mumps vaccination.

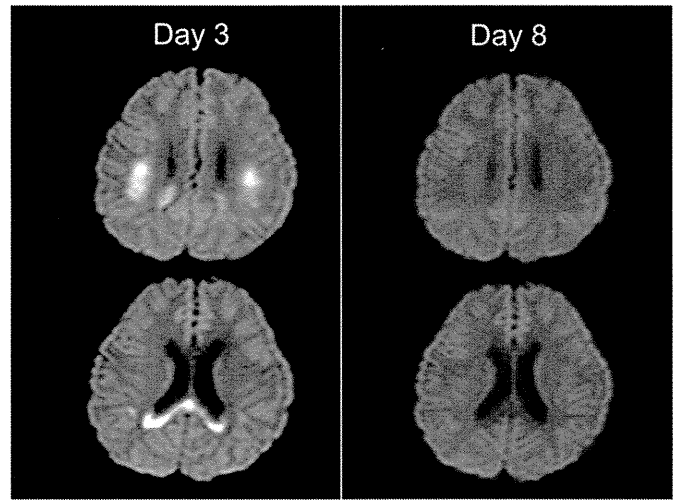

Fig. 1. MRI of patient 2. On day 3, diffusion-weighted images reveal lesions in the splenium of the corpus callosum and symmetric white matter. These lesions disappear completely on day 8, which is compatible with MERS type 2.

4. Discussion

Encephalitis is an extremely rare CNS manifestation in mumps vaccine recipients, the frequency is estimated to be 0.0004% (4/1,000,000) [3], which is much lower than that in natural mumps (0.1%) [1]. Encephalitis after mumps vaccination may include acute disseminated encephalomyelitis (ADEM) [10]. The exact frequency of ADEM is uncertain, however, it should be very rare according to the fact that possible ADEM after mumps vaccine was only reported in one patient among 1.53 million doses of mumps vaccine in Japan [11]. As far as we know, there have been five Japanese patients with MERS after mumps vaccination (Table 1) [6,7], including the present three patients, suggesting that this condition may be a more frequent adverse event after mumps vaccination than previously considered.

MERS is the mildest and the second most common infectious encephalitis/encephalopathy syndrome in Japanese children, and is characterized by mild neurological symptoms (delirious behavior in 54%, consciousness disturbance in 35%, and seizures in 33%) with complete clinical recovery, and the MRI finding of a reversible splenial lesion (type 1 MERS), sometimes associated with symmetrical white matter lesions (MERS type 2) [4,5,8]. Hyponatremia is often observed in patients with MERS [12], thus, hyponatremic cerebral edema is postulated to be a contributing factor for MERS, though the exact pathophysiology is still uncertain. The five patients with MERS after mumps vaccination showed an interval from vaccination to clinical symptoms ranging from

Table 1
Data for MERS after mumps vaccination.

Pt.	Age/sex	Initial symptoms (day 0)	Mumps vaccination	Neurological symptoms	CSF CC Mumps strain	Lowest/normalized Na mechanism
1	9/M	Fever, vomiting	D-13	Con (D2–4)	277 (M 157, P 120) (D2) Hoshino	117 (D2)/137 (D10) SIADH
2	5/M	Fever, vomiting, headache	D-20	Del (D1–2), seizure (D2)	280 (M 276, P 4) (D2) Hoshino	124 (D3)/137 (D7) SIADH
3	2/M	Fever	D-21	Con (D2), dysarthria (D2–11)	490 (M 490, P 0) (D2) Hoshino	131 (D2)/137 (D5)
4	8/M	Fever, vomiting, headache	D-20	Del (D1–6)	624 (M 618, P 6) (D4) Torii	128 (D2)/137 (D6) SIADH
5	1/M	Fever, vomiting	D-17	Con (D3–6), seizure (D3)	868 (M 854, P 14) (D3) Hoshino	125 (D3)/134 (D6) SIADH

DWI lesion	EEG results	Prognosis	Reference
Sp, genu, WM (D3) None (9)	HVS in O (D2)	CR	
Sp, WM (D3) None (D8)	HVS in P,O (D2) Normal (D8)	CR	
Sp (D3) None (D11)	Normal (D4)	CR	9
Sp, WM (D2) None (D15)	Normal (D6)	CR	6
Sp (D4) None (D7)	Diffuse HVS (D4) Normal (D11)	CR	7

Abbreviations
Pt, patient; CSF, cerebrospinal fluid; CC, cell counts; DWI, diffusion-weighted image; EEG, electroencephalography; M, male; D, day; Con, consciousness disturbance; Del, delirium; M, mononuclear leukocyte; P, polymorphonuclear leukocyte; SIADH, syndrome of inappropriate; DEX, dexamethasone; mPSL, methylprednisolone; Glo, δ-globulin; Sp, splenium; WM, white matter; HVS, high voltage slow; O, occipital; P, parietal; CR, complete recovery.