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.

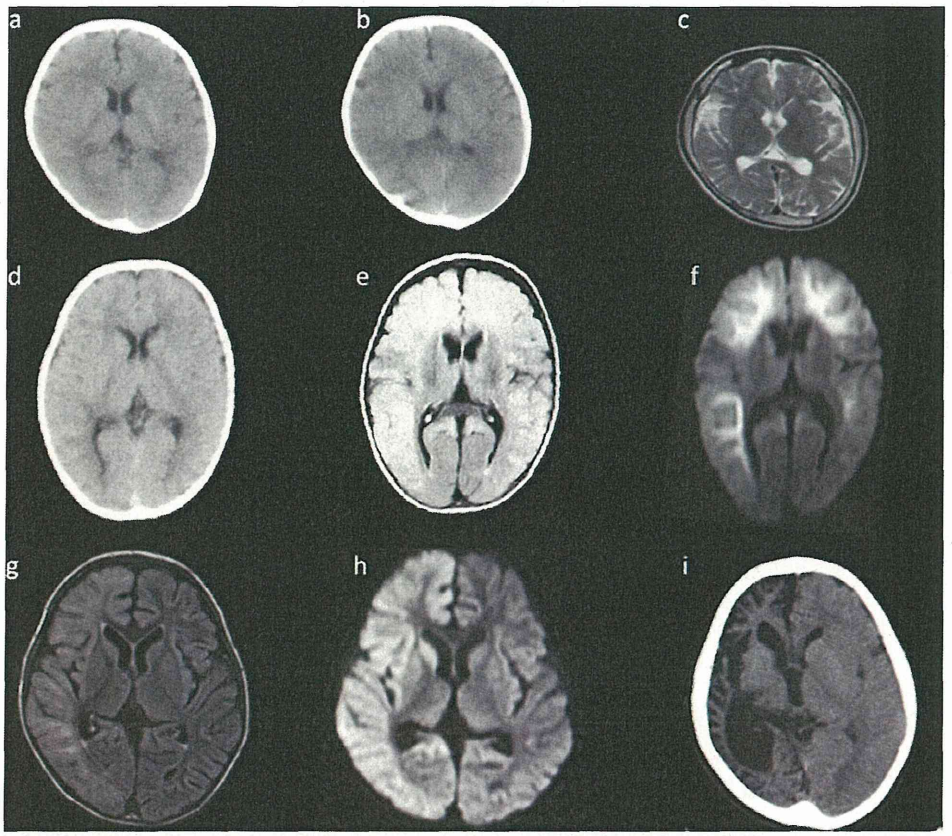


Fig. 1. Cranial CT/MRI findings in acute encephalopathy in children taking theophylline (AET). In Case 1, cranial CT on day 3 showed slight narrowing of the cerebrospinal fluid space, but no clear evidence of cerebral edema (a). On day 7, however, CT showed mild narrowing of the cerebrospinal fluid space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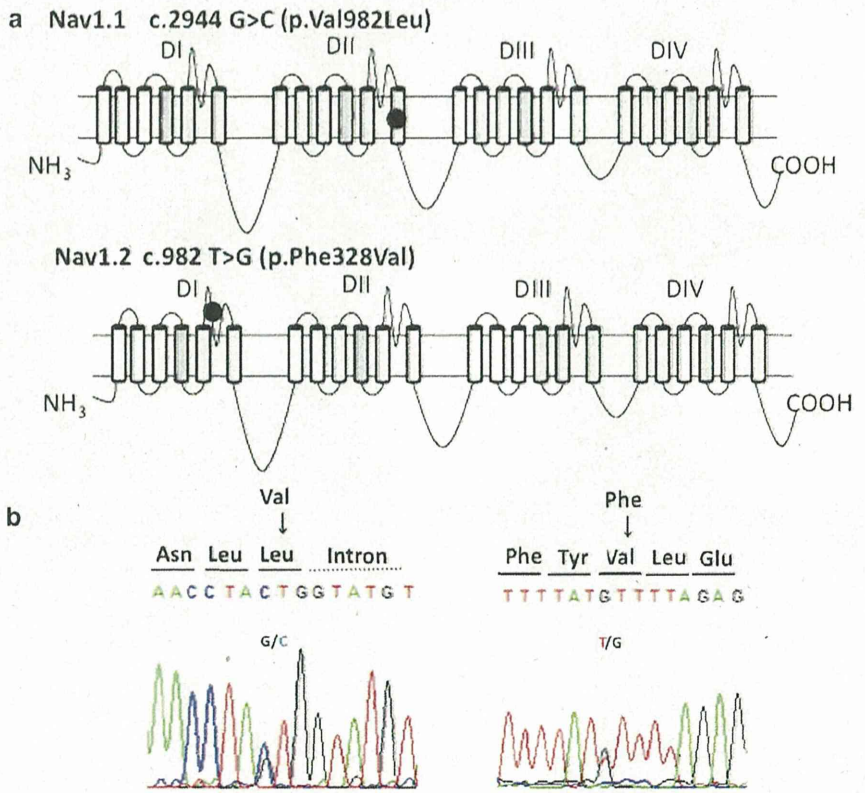


Fig. 2. Cases with *SCN1A* and *SCN2A* mutations in AET. (a) Structure of SCN1A ($Na_V1.1$) and SCN2A ($Na_V1.2$) with localization of the mutation (closed circle). c.2944 G>C (p.Val982Leu) is localized in the transmembrane segment 6 of domain II in $Na_V1.1$, whereas c.982 T>G (p.Phe328Val) is localized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

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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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## References

[1] Tsiu SJ, Self TH, Burns R. Theophylline toxicity: update. Ann Allergy 1990;64:241–57.
[2] Miura T, Kimura K. Theophylline-induced convulsions in children with epilepsy. Pediatrics 2000;105:920.
[3] Dunwiddie TV. Endogenously released adenosine regulates excitability in the in vitro hippocampus. Epilepsia 1980;21:541–8.
[4] Dunwiddie TV. Adenosine and suppression of seizures. Adv Neurol 1999;79:1001–10.
[5] Korematsu S, Miyahara H, Nagakura T, Suenobu S, Izumi T. Theophylline-associated seizures and their clinical characterizations. Pediatr Int 2008;50:95–8.
[6] Nakada T, Kwee IL, Lerner AM, Remler MP. Theophylline-induced seizures: clinical and pathophysiologic aspects. West J Med 1983;138:371–4.
[7] Yoshikawa H. First-line therapy for theophylline-associated seizures. Acta Neurol Scand (Suppl) 2007;186:57–61.
[8] Mizuguchi M, Yamanouchi H, Ichiyama T, Shiomi M. Acute encephalopathy associated with influenza and other viral infections. Acta Neurol Scand (Suppl) 2007;186:45–56.
[9] Nabbout R, Vezzani A, Dulac O, Chiron C. Acute encephalopathy with inflammation-mediated status epilepticus. Lancet Neurol. 2011;10:99–108.
[10] Takanashi J, Oba H, Barkovich AJ, Tada H, Tanabe Y, Yamanouchi H, et al. Diffusion MRI abnormalities after prolonged febrile seizures with encephalopathy. Neurology 2006;66:1304–9.
[11] Shiomi M. A proposal of the clinical classification of influenza encephalopathy. (in Japanese). Jpn J Pediatr 2000;53:1739–46.
[12] Shinohara M, Saitoh M, Takanashi J, Yamanouchi H, Kubota M, Goto T, et al. Carnitine palmitoyl transferase II polymorphism is associated with multiple syndromes of acute encephalopathy with various infectious diseases. Brain Dev 2011;33:512–7.
[13] Shinohara M, Saitoh M, Nishizawa D, Ikeda K, Hirose S, Takanashi J, et al. ADORA2A polymorphism predisposes children to encephalopathy with febrile status epilepticus. Neurology 2013;80:1571–6.
[14] Saitoh M, Shinohara M, Hoshino H, Kubota M, Amemiya K, Takanashi J, et al. Mutations of the SCN1A gene in acute encephalopathy. Epilepsia 2012;53:558–64.
[15] Kobayashi K, Ohzono H, Shinohara M, Saitoh M, Ohmori I, Ohtsuka Y, et al. Acute encephalopathy with a novel point mutation in the SCN2A gene. Epilepsy Res 2012;102:109–12.
[16] Hoshino A, Saitoh M, Oka A, Okumura A, Kubota M, Saito Y, et al. Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes. Brain Dev 2012;34:337–43.
[17] Mizuguchi M. Influenza encephalopathy and related neuropsychiatric syndromes. Influenza Other Respir Viruses 2013;7:67–71.
[18] Singh NA, Pappas C, Dahle EJ, Claes LR, Pruess TH, De Jonghe P, et al. A role of SCN9A in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. PLoS Genet 2009;5:e1000649.
[19] Shi X, Yasumoto S, Nakagawa E, Fukasawa T, Uchiya S, Hirose S. Missense mutation of the sodium channel gene SCN2A causes Dravet syndrome. Brain Dev 2009;31:758–62.
[20] Chen Y, Mizuguchi H, Yao D, Ide M, Kuroda Y, Shigematsu Y, et al. Thermolabile phenotype of carnitine palmitoyltransferase II variations as a predisposing factor for influenza-associated encephalopathy. FEBS Lett 2005;579:2040–4.
[21] Rogers PJ, Hohoff C, Heartherley SV, Mullings EL, Maxfield PJ, Evershed RP, et al. Association of the anxiogenic and alerting effects of caffeine with ADORA2A and ADORA1 polymorphisms and habitual level of caffeine consumption. Neuropsychopharmacology 2010;35:1973–83.
[22] Fukuda M, Suzuki Y, Hino H, Kuzume K, Morimoto T, Ishii E. Adenosine A1 receptor blockage mediates theophylline-associated seizures. Epilepsia 2010;51:483–7.
[23] Somekawa-Kondo T, Yamaguchi K, Ishitsuka Y, Ito S, Tanaka K, Irikura M, et al. Aminophylline, administered at usual doses for rodents in pharmacological studies, induces hippocampal neuronal cell injury under low tidal volume hypoxic conditions in guinea-pigs. J Pharm Pharmacol 2013;65:102–14.

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ORIGINAL ARTICLE

# Extracorporeal membrane oxygenation for 2009 influenza A(H1N1) severe respiratory failure in Japan

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**Abstract**
*Purpose* To evaluate procedures and outcomes of extracorporeal membrane oxygenation (ECMO) therapy applied to 2009 influenza A(H1N1) severe respiratory failure patients in Japan.
*Methods* This observational study used database information about adults who received ECMO therapy for H1N1-related severe respiratory failure from April 1, 2010 to March 31, 2011.
*Results* Fourteen patients from 12 facilities were enrolled. Anti-influenza drugs were used in all cases. Before the start of ECMO, the lowest $PaO_2/FiO_2$ was median (interquartile) of 50 (40–55) mmHg, the highest peak inspiratory pressure was 30 (29–35) $cmH_2O$, and mechanical ventilation had

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been applied for at least 7 days in 5 patients. None of the facilities had extensive experience with ECMO for respiratory failure (6 facilities, no previous experience; 5 facilities, one or two cases annually). The blood drainage cannula was smaller than 20 Fr. in 10 patients (71.4 %). The duration of ECMO was 8.5 (4.0–10.8) days. The duration of each circuit was only 4.0 (3.2–5.3) days, and the ECMO circuit had to be renewed 19 times (10 cases). Thirteen patients (92.9 %) developed adverse events associated with ECMO, such as oxygenator failure, massive bleeding, and disseminated intravascular coagulation. The survival rate was 35.7 % (5 patients).

*Conclusion* ECMO therapy for H1N1-related severe respiratory failure in Japan has very poor outcomes, and most patients developed adverse events. However, this result does not refute the effectiveness of ECMO. One possible cause of these poor outcomes is the lack of satisfactory equipment, therapeutic guidelines, and systems for patient transfer to central facilities.

**Keywords** ECMO · Influenza · Respiratory failure · Mortality

## Introduction

The World Health Organization reported individuals infected with a novel swine-origin influenza virus 2009 influenza A(H1N1) in Mexico and the United States in April 2009 [1]. This report was quickly followed by a worldwide pandemic. The severity of infection was the same as that of seasonal influenza in many cases, but more than a few patients developed severe respiratory failure of a kind that was unlikely to have resulted from conventional seasonal influenza.

Serious cases in which oxygenation could not be maintained by conventional mechanical ventilation were managed with extracorporeal membrane oxygenation (ECMO), often yielding excellent outcomes. According to reports from Australia and New Zealand, 68 patients received ECMO therapy during the 2-month period at the height of the epidemic, and the survival rate exceeded 70 % [2]. The Extracorporeal Life Support Organization (ELSO) reported a survival rate of more than 60 % for 323 patients [3]. According to a report from the United Kingdom, treatment outcomes in very severe cases were better when ECMO was applied than when only conventional mechanical ventilation was employed [4]. Utilization of the ECMO network system and transfer of patients to the ECMO center were considered to be among the factors that resulted in better treatment outcomes [4–7]. The ECMO Center Karolinska, Sweden, reported a survival rate of more than 90 % [5].

In Japan, however, no network system or center for ECMO therapy is available, and ECMO has been applied only at individual medical facilities in cases where this therapy was indicated. No data are as yet available in Japan regarding the outcomes of ECMO therapy for 2009 influenza A(H1N1). The present study was undertaken to analyze the procedures and outcomes of ECMO therapy applied to adult patients, using information on patients infected with H1N1 and admitted to intensive care units (ICUs). These data were collected by the Committee of Crisis Control, the Japanese Society of Respiratory Care Medicine and the Committee of Pandemic H1N1 Surveillance, the Japanese Society of Intensive Care Medicine.

## Methods

The study involved adults who received ECMO therapy for severe respiratory failure associated with H1N1 influenza from April 1, 2010 to March 31, 2011. A database was created using patient information that had been collected from attending physicians of the facilities participating in this study; the information was provided at the physicians' own discretion in response to a public notification (data collection on ICU patients infected with H1N1) issued by the Japanese Society of Respiratory Care Medicine and the Japanese Society of Intensive Care Medicine. Informed consent from individual patients was obtained by each reporting physician and facility. Data collection pertaining to the findings before hospitalization and upon admission included age, sex, body weight, body mass index (BMI), body temperature, Acute Physiology and Chronic Health Evaluation (APACHE) II score, underlying disease, and vaccination. During treatment, information was collected about complications, Sequential Organ Failure Assessment (SOFA) score, type of anti-influenza drug, mechanical ventilation, blood gas analysis, and continuous renal replacement therapy. In addition, data were collected about the duration of mechanical ventilation, duration of ICU stay, hospitalization period, and patient outcome.

From this database, adult patients who had received ECMO therapy were extracted for analysis. The physician in charge at each facility that provided the ECMO therapy was requested by e-mail or telephone to supply additional detailed information with regard to the following: the equipment used for ECMO therapy, cannula size, site of approach with the cannula, duration of ECMO therapy, duration of mechanical ventilation before the start of therapy, adverse events, cause of death, and previous ECMO experience.

With respect to the ECMO therapy procedures, detailed information was collected on each survey item. The

survival and non-survival groups were compared using the Mann–Whitney test, Fisher's exact test, or chi-square test. Statistical analyses were performed using SPSS II (Abacus Concepts, Berkeley, CA, USA). All values are reported as median (interquartile), and all $p$ values $<0.05$ are considered statistically significant.

## Results

### Patient background and treatment course (Table 1)

Fourteen patients from 12 facilities were enrolled in this study. The survival rate was as low as 35.7 % (5 patients). Weaning from ECMO was impossible in all the patients who later died.

Most patients were male (85.7 %). The mortality rate predicted from the APACHE II score was 24.9 %, but the actual mortality rate (64.3 %) was 2.5 times higher. None of the patients had chronic respiratory failure, chronic heart failure, or immunological diseases as underlying disorders. Anti-influenza drugs were used in all cases: peramivir in 78.6 %, oseltamivir in 42.9 %, and zanamivir in 7.1 %; two drugs were used in each of four cases.

All patients received mechanical ventilation with endotracheal intubation. Airway pressure release ventilation (APRV) was used for mechanical ventilation in 92.9 % of cases. The causes of death were multiple organ failure (MOF) in four cases, respiratory failure in three cases, MOF and uncontrollable bleeding in one case, and concomitant respiratory and circulatory failures in one case. One of the discharged patients had respiratory sequelae.

### ECMO equipment and cannula (Tables 2, 3)

ECMO equipment manufactured by Terumo Corporation (Tokyo, Japan) was used in 11 patients (78.6 %). This equipment consists of a console, circuit, oxygenator, and centrifugal pump. The blood drainage cannula was smaller than 20 Fr. in 10 patients (71.4 %), and the maximum size was 21.5 Fr.

### ECMO therapy (Table 4)

All patients received venovenous ECMO therapy, and none required a switch to venoarterial ECMO therapy. None of the facilities had extensive experience with ECMO therapy for respiratory failure. At five facilities, ECMO was used for the first time. Six facilities had previously applied this therapy to one or two cases per year. One facility had used ECMO in at least five cases a year.

Before the start of ECMO therapy, mechanical ventilation had been applied for more than 7 days in two cases from the survival group and three cases from the non-survival group (13, 15, and 20 days, respectivley). The duration of ECMO therapy was 8.5 (4.0–10.8) days, ranging from 1 day (outcome, death) to 39 days (outcome, death).

The duration of each ECMO circuit was only 4.0 (3.2–5.3) days. The ECMO circuit was renewed a total of 19 times among 10 cases. The reasons for renewal were reduced oxygenating capability owing to oxygenator failure (nine times), thrombus attachment to the oxygenator (three times), circuit obstruction with thrombus (three times), poor blood drainage flow (twice), pump head trouble (once), and hemolysis (once). The duration of ECMO therapy for the four cases that did not require circuit renewal was 6 days (outcome, survival), 4 days (death), 4 days (death), and 1 day (death).

### Adverse events associated with ECMO therapy (Table 5)

Excluding 1 patient who died on the first day of ECMO therapy, all patients developed adverse events associated with ECMO (92.9 %). Direct adverse events developed in 11 patients (78.6 %); reduced oxygenating capability owing to oxygenator failure (50 %) was the most frequent. Indirect adverse events developed in 12 patients (85.7 %). The most frequent complication was disseminated intravascular coagulation (DIC, 71.4 %). All these adverse events were associated with the coagulation and fibrinolytic system (DIC, massive bleeding, thrombus, etc.). One patient underwent a surgical procedure to achieve hemostasis.

## Discussion

During the 2010–2011 season in Japan, the survival rate of patients with 2009 influenza A(H1N1) severe respiratory failure following ECMO therapy was only 35.7 %. Most patients developed adverse events associated with this therapy.

Several reports have demonstrated the effectiveness of ECMO therapy for H1N1-related severe respiratory failure [2, 3, 5]. According to a report from the United Kingdom, the survival rate following ECMO therapy was 76 %, which is significantly higher than that following conventional mechanical ventilation (48 %), and thus demonstrates the effectiveness of ECMO [4]. In the present study in Japan, the survival rate was only 35.7 %, and the mortality rate was 2.5 times that predicted from the APACHE II score. The survival rate was 64 % in H1N1-related

**Table 1** Patient background and treatment course

| | All cases (14 patients) | Survival group (5 patients) | Non-survival group (9 patients) |
|---|---|---|---|
| Age (years) | 54 (43–60) | 54 (35–58) | 54 (41–62) |
| Sex (male/female) | 12/2 | 4/1 | 8/1 |
| Weight (kg) | 70 (64–80) | 69 (54–86) | 70 (64–80) |
| Obesity | | | |
| 35 > BMI ≥ 25 | 7 | 3 | 4 |
| BMI ≥ 35 | 1 | 0 | 1 |
| Body temperature (°C) | | | |
| At first examination | 38.8 (37.1–39.1) | 38.8 (36.8–39.0) | 38.8 (37.3–39.4) |
| Maximum | 39.4 (38.7–39.8) | 39.2 (39.0–39.7) | 39.5 (38.1–39.9) |
| APACHE II score | 17 (12–25) | 16 (12–24) | 17 (12–28) |
| Predicted death rate (%) | 24.9 (14.6–54.1) | 23.5 (15.5–49.7) | 26.2 (14.6–61.5) |
| Maximum SOFA score | 15.5 (12.0–19.3) | 12.0 (10.0–15.0) | 19.0 (14.5–20.5)* |
| Underlying condition | | | |
| Drug abuse | 1 | 0 | 1 |
| Pregnancy | 1 | 1 | 0 |
| Vaccination (H1N1 + seasonal) | 1 | 0 | 1 |
| Complications | | | |
| Acute renal failure | 7 | 2 | 5 |
| Acute hepatic failure | 3 | 0 | 3 |
| Culture-confirmed infection | 4 | 2 | 2 |
| Shock | 4 | 1 | 3 |
| Medical treatment | | | |
| Peramivir | 11 | 4 | 7 |
| Oseltamivir | 6 | 2 | 4 |
| Zanamivir | 1 | 0 | 1 |
| Antibiotics | 14 | 5 | 9 |
| Steroid | | | |
| High + low dose | 6 | 3 | 3 |
| High dose | 3 | 0 | 3 |
| Low dose | 2 | 2 | 0 |
| Sivelestat | 4 | 1 | 3 |
| Vasoactive drugs | 13 | 4 | 9 |
| Rescue therapies and adjunctive therapies | | | |
| Prone position | 3 | 1 | 2 |
| APRV | 13 | 5 | 8 |
| Nitric oxide | 1 | 0 | 1 |
| CRRT | 7 | 2 | 5 |
| Respiratory severity and ventilator parameters | | | |
| Lowest $PaO_2/FiO_2$ (mmHg) | 50 (40–55) | 49 (43–53) | 50 (40–60) |
| Highest PEEP ($cmH_2O$) | 24 (17–30) | 22 (15–28) | 28 (18–30) |
| Highest PIP ($cmH_2O$) | 30 (29–35) | 29 (23–42) | 30 (30–35) |
| Ventilator days (days) | 19 (9–25) | 24 (16–37) | 10 (6–25) |
| Length of stay in ICU (days) | 17 (9–26) | 24 (16–31) | 10 (6–25) |
| Hospitalization (days) | 25 (12–53) | 69 (35–83) | 15 (6–25)** |

Data expression, median (interquartile)

*BMI* body mass index, *APACHE* Acute Physiology and Chronic Health Evaluation, *SOFA* Sequential Organ Failure Assessment, *DIC* disseminated intravascular coagulation, *APRV* airway pressure release ventilation, *CRRT* continuous renal replacement therapy, *PEEP* positive end-expiratory pressure, *PIP* peak inspiratory pressure, *ICU* intensive care unit

* $p = 0.004$ (survival group vs. non-survival group); ** $p = 0.036$ (survival group vs. non-survival group)