13 to 21 days (equal to the incubation time of mumps infection); initial symptoms of fever, headache, and vomiting (suggesting meningitis), followed by CNS symptoms within 3 days; hyponatremia; and positive vaccine strain in CSF (Table 1). These findings strongly suggest that live attenuated mumps virus directly causes aseptic meningitis first, then possibly progressing to MERS under hyponatremic conditions.

It has been reported that males more frequently present with mumps meningitis than females [1]. Actually, the male/female ratio of natural mumps meningitis in Japan between 2009 and 2011 was 2.32 (1764/759) [13]. Meningitis after mumps vaccination also occurs more predominantly in males than in females, i.e., nine of 10 patients with meningitis after mumps vaccination were males [2]. Interestingly, the five patients with MERS after mumps vaccination were all males. The number of MERS after mumps vaccination is too small to estimate the statistical significance; male might be a risk factor for developing MERS after mumps vaccination.

In Japan, methylprednisolone pulse therapy and intravenous immunoglobulin are recommended for patients with infectious encephalopathy regardless of the pathogen or the clinicoradiological syndromes [14]. These treatments suppress inflammatory cytokines, and may be effective for those caused by cytokine storm, such as acute necrotizing encephalopathy (ANE) [14]. However, there is no evidence of their efficacy in MERS. As a treatment, steroids and intravenous immunoglobulin were given in 16 and 8 of the 54 patients with MERS in Japan [4], respectively. On the other hand, 19 patients received no such treatment. All 54 patients with MERS clinically recovered completely, irrespective of the treatments, suggesting that steroids or intravenous immunoglobulin is not always necessary. This is also the case for MERS after mumps vaccination, the five patients recovering completely, irrespective of the treatment, which might be related with normalization of sodium. In any case, MERS after mumps vaccination is expected to recover completely.

Unlike ANE or acute encephalopathy with biphasic seizures and late reduced diffusion, which are by far more common in East Asia than in the rest of the world [4,14], MERS has been reported all over the world [15]. MERS after mumps vaccination, however, has never been reported in a country other than Japan. Because of the relatively high incidence of influenza encephalopathy in Japan, we usually perform brain MRI in children with relatively mild CNS symptoms. This may explain why MERS after mumps vaccination has been reported only in Japan, that is, this might be underestimated.

This study has some limitations, mainly based on retrospective nature. Because we had no protocol for MRI, there might be some patients whose splenial lesion had already disappeared before MRI studies, which may lead to underestimation of this condition. The number of patient is too small to make statistical analysis, including whether there is male predominance. Further study with MRI protocol may lead to a better understanding of this condition.

In conclusion, it should be emphasized that MERS after mumps vaccination may be more common than previously considered, and the prognosis is excellent. One should suspect MERS when a male patient after mumps vaccination presents with neurological symptoms (delirious behavior, consciousness disturbance, and seizures) with hyponatremia, following symptoms of aseptic meningitis (fever, headache and vomiting), and MRI should be performed to evaluate the splenium of the corpus callosum.

Conflict of interest

All authors declare no conflict of interest.

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Missense mutations in sodium channel *SCN1A* and *SCN2A* predispose children to encephalopathy with severe febrile seizures

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ABSTRACT

Objective: Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is a childhood encephalopathy following severe febrile seizures. The pathogenesis of AESD is considered to be fever-induced seizure susceptibility and excitotoxicity, which may be caused by sodium channel dysfunction in some cases. Here we studied whether mutations in genes encoding sodium channels, *SCN1A* and *SCN2A*, predispose children to AESD.

Methods: We recruited 92 AESD patients in a nationwide survey of acute encephalopathy in Japan from 2008 to 2011. We collected their genomic DNA samples, and sequenced the entire coding region of *SCN1A* and *SCN2A*.

Results: Five out of 92 patients (5.4%) had missense mutations either in *SCN1A* or *SCN2A*. After a preceding infection with fever, all the patients showed status epilepticus at the onset. Hemiconvulsion–hemiplegia was recognized in three patients during the acute/subacute phase. One patient had taken theophylline for the treatment of bronchial asthma just before the onset of AESD. Familial history was not remarkable except one patient with a *SCN1A* mutation (G1647S) whose mother had a similar episode of AESD in her childhood. A different substitution (G1674R) at the same amino acid position, as well as two other *SCN1A* mutations found in this study, had previously been reported in Dravet syndrome. Another *SCN1A* mutation (R1575C) had been detected in other types of acute encephahlitis/encephalopathy. One patient had *SCN2A* mutation, F328V, which had previously been reported in Dravet syndrome. Another *SCN2A* mutation, I172V, was novel. None of the patients were diagnosed with Dravet syndrome or genetic (generalized) epilepsy with febrile seizure plus in the following-up period.

Conclusions: Mutations in *SCN1A* and *SCN2A* are a predisposing factor of AESD. Altered channel activity caused by these mutations may provoke seizures and excitotoxic brain damage.

Introduction

During the acute phase of febrile diseases, some children develop repetitive or prolonged seizures, followed by severe impairment of consciousness. Several distinct syndromes have been described and characterized: fever-induced refractory epileptic encephalopathy in school-aged children (FIRES), idiopathic hemiconvulsion–hemiplegia syndrome (IHHS), and acute encephalopathy with biphasic seizures and late reduced diffusion (AESD). As a generic term to encompass these conditions, Nabbout proposed the term acute encephalopathy with

Abbreviations: AEIMSE, acute encephalopathy with inflammation-mediated status epilepticus; AESD, acute encephalopathy with biphasic seizures and late reduced diffusion; ANE, acute necrotizing encephalopathy; AERRPS, acute encephalitis with refractory, repetitive partial seizures; DS, Dravet syndrome; GEFS+, genetic (generalized) epilepsy with febrile seizure plus; BFNIS, benign familial neonatal-infantile seizures.

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inflammation-mediated status epilepticus (AEIMSE) (Nabbout et al., 2011). AESD is prevalent in Japan, affecting hundreds of children every year (Hoshino et al., 2012), whereas IHHS is encountered worldwide. When IHHS occurs during an infectious disease, it is regarded as a subgroup of AESD (Takanashi et al., 2006).

The main pathogenetic mechanism of AESD is considered to be excitotoxicity, based on the magnetic resonance spectroscopy findings showing an increase in glutamine/glutamate in the cerebral lesions (Mizuguchi et al., 2007; Takanashi et al., 2009). The genetic background of AESD remains to be elucidated. Polymorphisms in genes controlling neuronal excitability are candidates for risk factors of AESD. Recently, polymorphism of genes encoding adenosine receptor 2A (*ADORA2A*) and carnitine palmitoyltransferase II (*CPT2*) has been identified as genetic predisposition for AESD (Shinohara et al., 2013, 2011). However, some AESD patients have no such polymorphism, suggesting the involvement of other genes. Voltage-gated sodium channels are essential for neuronal excitability. We hypothesized that intrinsic susceptibility to seizures may predispose children to AESD and focused on the *SCN1A/SCN2A* genes, whose mutations are known to cause genetic epilepsy characterized by hyperthermia-induced seizures. Mutations in genes encoding a voltage-gated sodium channel subunit protein, *SCN1A*, cause a variety of genetic epileptic syndromes including Dravet syndrome (DS, severe myoclonic epilepsy of infancy) and genetic (generalized) epilepsy with febrile seizures plus (GEFS+) (Escayg et al., 2000; Claes et al., 2001; Escayg and Goldin, 2010). Recently, we and other researchers have reported that some patients with various types of acute encephalopathy have truncation or missense mutations of *SCN1A* (Ohmori et al., 2008; Sakakibara et al., 2009; Takayanagi et al., 2010; Kobayashi et al., 2010; Saitoh et al., 2012). *SCN2A* mutations cause benign familial neonatal-infantile seizures (BFNIS) (Heron et al., 2002; Berkovic et al., 2004), which are usually inherited from an affected parent. Several de novo *SCN2A* mutations have been reported in severer phenotypes such as DS (Shi et al., 2009) and early-onset epileptic encephalopathy including Ohtahara syndrome (Nakamura et al., 2013). On the other hand, missense *SCN2A* mutations have recently been identified in a patient with acute encephalitis with refractory, repetitive partial seizures (AERRPS), a typical syndrome of AEIMSE (Kobayashi et al., 2012), and in a patient with recurrent acute encephalopathy (Fukasawa et al., 2015).

To elucidate the genetic basis of AESD, we conducted an analysis of the *SCN1A* and *SCN2A* genes. This is the first report that evaluated the frequency of sodium channel mutations in a large number of patients with AESD and clarified them as a genetic risk factor.

Table 1
Clinical characteristics of patients with AESD ($n = 92$).

	Patients (%)
Female	50 (54%)
Age	
0–6 months	1 (1%)
7–12 months	28 (30%)
13–24 months	32 (35%)
>24 months	31 (34%)
Pathogen of preceding infection	
Human herpesvirus-6	30 (33%)
Influenza virus	8 (9%)
Adenovirus	3 (3%)
Respiratory syncythial virus	2 (2%)
Mumps virus	1 (1%)
Varicella zoster virus	1 (1%)
Mycoplasma pneumoniae	1 (1%)
Others (not identified)	46 (50%)
Duration of first convulsion	
<15 min	19 (21%)
15–30 min	55 (60%)
>30 min	14 (15%)
Not recorded	4 (4%)
Biphasic clinical course	76 (82%)
MRI findings distribution of lesions	
Frontal	21 (23%)
Hemispheric	16 (17%)
Diffuse	34 (37%)
Others	18 (20%)
Not particular	2 (2%)
Not available	1 (1%)
Prognosis (Intellectual/motor)	
Full recovery	16 (17%)/29 (31%)
Mild disability	16 (17%)/21 (23%)
Moderate disability	10 (11%)/9 (10%)
Severe disability	30 (33%)/21 (23%)
Death	0 (0%)
Not determined	15 (16%)/7 (7%)
Not available	5 (6%)

Methods

Subjects

We recruited patients with AESD from hospitals in Japan during 2008–2011 based on the diagnostic criteria (Hoshino et al., 2012). 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, 'probable' when either clinical or CT/MRI features were present (Saitoh et al., 2015). Patients diagnosed with definite or probable AESD were included in this study. Ninety-two Japanese patients, 42 male and 50 female aged from five months to six years and eleven months (median, two year and one month) participated in this study. The summary and details of clinical data are shown in Table 1 and Supplementary table, respectively. All patients had their first convulsion, mostly status epilepticus, within 24 h from the onset of fever, followed by impairment of consciousness that improved on the second day in most cases. On the fourth to sixth day of illness, there was a recurrence of convulsions or a cluster of partial seizures, followed again by impairment of consciousness in most cases (82%, Table 1). Such a biphasic clinical course is one of the characteristics of AESD. Typically, cranial MRI was normal on the first to second day of illness, but showed lesions in the cerebral subcortical white matter on the third to ninth day. Pathogens of antecedent infections included human herpesvirus 6 (30 cases), influenza virus (8 cases), respiratory syncytial virus, rotavirus, adenovirus, mumps virus and *Mycoplasma pneumoniae*.

Controls

To search the variants frequency in normal control population, we used Human Genetic Variation Browser (http://www.genome.med.kyoto-u.ac.jp/SnpDB (Japanese Genetic Variation Consortium, A reference database of genetic variations in Japanese population. in preparation) (Narahara et al., 2014) and Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.or) accessed on June 29, 2015.

Procedures

Peripheral blood samples were collected from the patients. Genomic DNA was extracted from the blood using standard protocols. All exons of *SCN1A* and *SCN2A* were polymerase chain reaction (PCR) amplified with flanking intronic primers and standard PCR conditions (Ohmori et al., 2002; Saitoh et al., 2012). PCR products

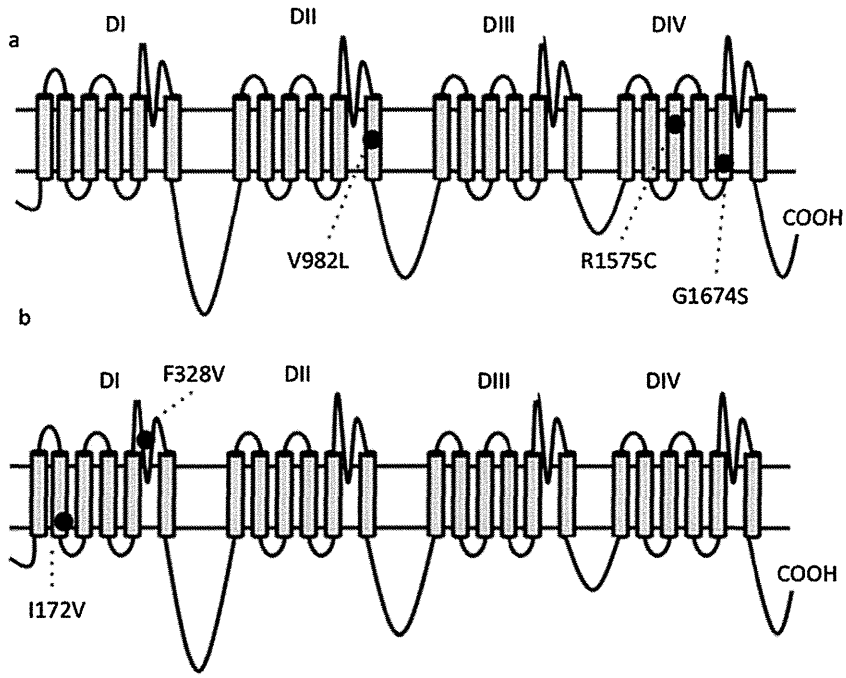

Fig. 1. Structure of human Nav1.1 (a) and Nav1.2 (b) channels with localization of *SCN1A* and *SCN2A* mutations. The locations of missense mutations, three of *SCN1A* and two of *SCN2A*, are shown.

of *SCN1A* and *SCN2A* were sequenced on 310 Genetic Analyzer, 3100 Genetic Analyzer or 3130xl Genetic Analyzer (Life Technologies, Carlsbad, CA, USA). Sequence changes were identified as mutations for missense ones if they were not reported as normal variant in database (http://asia.ensembl.org/Homo_sapiens/Transcript/Variationß), and resulted in non-conservative amino acid substitution. Reference sequence of mRNA was based on information available from GenBank (accession number: Human *SCN1A*. NM_001165963.1 and *SCN2A*. NM_001040143). The effect of a mutation change was predicted in silico using Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html).

Standard protocol approvals, registrations, and patient consents

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.

Results

Of the 92 AESD patients studied, three had missense mutations of *SCN1A* and two had those of *SCN2A* (5.4%). The localization of these mutations is shown in Fig. 1. Clinical features of the six patients and the predicted effects of mutations are shown in Table 2.

Identification of SCN1A *mutations*

The V982L mutation was found in Case 1 with partial epilepsy and AESD. The valine 982 residue is located on the transmembrane segment 6, domain II of *SCN1A* protein (Fig. 1a). This mutation had previously been reported in a patient with atypical DS without myoclonic seizures and ataxia who had an additional *SCN9A* mutation (Singh et al., 2009). The G1674S mutation in Case 2 was a novel mutation, which had not been reported previously. Another amino acid substitution at the same position (G1674R) had previously been reported in a patient with DS (Ohmori et al., 2002). The glycine 1674 residue is located on the transmenbrane segment 5, domain IV of *SCN1A* protein (Fig. 1a). The R1575C mutation, found in Case 3, had previously been reported in Rasmussen encephalitis, AERRPS and mimicking acute necrotizing encephalopathy (Ohmori et al., 2008; Kobayashi et al., 2010; Saitoh et al., 2012). Located on the transmemebrane segment 2, domain IV (Fig. 1a), this mutation markedly alters the electrophysiologic properties of the sodium channel (Ohmori et al., 2008). In normal 1208 Japanese population (Human Genetic Variation Browser), none had the identified *SCN1A* mutations in the present study. All the three mutations were predicted as damaging by both the Polyphen-2 and SIFT scoring (Table 2).

The clinical course of Case 1, who had taken theophylline before the onset of AESD, was described previously (Saitoh et al., 2012; Saitoh et al., 2015). Case 2, a 2-year-1-month-old boy, was born uneventfully to non-consanguineous parents. Before the onset of AESD, he had recurrent febrile seizures. At the onset of AESD, he had a right-side dominant clonic seizure, followed by a generalized tonic–clonic seizure lasting 2 h and persistant post-ictal consciousness disturbance. After he became alert, he could not speak for a month. He showed transient right hemiparesis 10 days after the onset. His mother had also suffered from acute encephalopathy at the age of two, and had left hemiparesis as the sequela. Neither the patient nor mother had epileptic attacks. The mother had the same *SCN1A* mutation with her son. Case 3 was born at 25 gestational week weighing 695 g. He had no febrile or afebrile seizures and could walk before the onset of AESD. At the age of 2 years and 11 months, during the course of a febrile respiratory infection, he had status epilepticus lasting more than 15 min, followed by a cluster of focal seizures three days later. Cranial CT revealed normal findings on day 1, and diffuse cerebral cortical edema on day 4. MRI on day 20 showed diffuse cerebral atrophy. He was eventually left with spastic quadriplegia, severe mental deficit and epilepsy.

Identification of SCN2A *mutations*

The I172V mutation was found in Case 4 with febrile seizures and AESD. The isoleucine 172 residue is located on the transmembrane segment 2, domain I of *SCN2A* protein (Fig. 1b), and is well

Table 2
Characteristics of acute encephalopathy and preceding epilepsy in *SCN1A*/*SCN2A* mutation positive cases.

Case	Age	Sex	Mutation/inheritance	Preceding epilepsy/seizure, family history	Initial seizure duration (min)/late seizure	Neuroimaging	Prediction of functional effect			
							Prognosis	Polyphen-2	SIFT	Reference
			SCN1A							
1	2y3m	F	V982L NA	Focal epilepsy, unremarkable	GS (>15)/+	CT/day 2, unremarkable; CT/day 5, diffuse brain edema peri-Rolandic sparing	Spastic quadriplegia, severe MR	1.000 Probably damaging	0.00 Damaging	Singh et al. (2009)
2	2y1m	M	G1674S Inherited (M)	FS, HH	Right hemiconvulsion, secondary generalized (>120 min)/– (intravenous barbiturate, day1–9)	CT/day 2, unremarkable; brain perfusion scintigram/day 15, left hypoperfusion	Complete recovery	1.000 Probably damaging	0.03 Damaging	Novel*, This study
3	2y11m	M	R1575C NA	None, unremarkable	GS (>15)/+	CT/day 1, unremarkable; CT/day 4, diffuse brain edema; MRI/day 20, diffuse cortical atrophy	Spastic quadriplegia, severe MR	0.994 Probably damaging	0.03 Damaging	Kobayashi et al. (2010), Ohmori et al. (2008), Saitoh et al. (2012)
			SCN2A							
4	1y2m	F	I172V Inherited (F)	None, unremarkable	Right-side myoclonic jerk (>15)/+	MRI/day 4, left hemispheric high signal intensity in cortex and subcortical white matter	Right spastic heniplegia, mild MR	0.018 Benign	0.98 Tolerated	Novel, This study
5	0y11m	F	F328V NA	FS, FS	GS (>15)/+	MRI/day 3, right hemispheric high signal intensity in cortex and subcortical white matter	Complex focal epilepsy, mild MR	0.000 Benign	0.45 Tolerated	Shi et al. (2009),

NA, not available; HH, hemiconvulsion–hemiplegia syndrome; GS, generalized seizure.
* The mutation at the same position had been reported by Ohmori et al. (2002).

conserved among multiple sodium channels, such as *SCN1A* and *SCN3A*, and animal species including mouse and zebrafish. This mutation had not been reported previously. I172V was inherited from the asymptomatic father. The F328V mutation was found in Case 5. This mutation is located in a linker between segment 5 and 6, domain I of SCN2A protein (Fig. 1b), and had previously been reported in a patient with DS (Shi et al., 2009) who had a co-existing *SCN1A* mutation. In normal 1208 Japanese population (Human Genetic Variation Browser), none had these two mutations. These two mutations were predicted tolerant by both the Polyphen-2 and SIFT scoring (Table 2).

Case 4, a 1-year-2-month old female, was born uneventfully. Her familial history was not remarkable. She had biliary atresia and left subdural hemorrhage due to vitamin K deficiency at two months of age and underwent surgery. During the course of pandemic 2009 H1N1 influenza infection, she developed status epilepticus lasting more than 15 min. The seizures were focal and clonic, affecting the right angle of mouth and right hand. The serum levels of aminotransferases were elevated. On day 5, she had clusters of partial seizures. On day 6, she showed right hemiparesis. Brain MRI revealed cortical/subcortial edema of the left cerebral hemisphere. She was eventually left with mild motor disability and moderate intellectual deficit. She had episodes of status epilepticus with febrile diseases, seizures during taking a bath and afebrile tonic seizures after the onset of AESD and took antiepileptic drugs. Case 5, an 11-month-old female was previously healthy with unremarkable family history. She developed status epilepticus lasting more than 15 min during a course of human herpesvirus-6 infection. Several days later, a cluster of focal seizures occurred. Brain MRI showed lesions in the right parietal, temporal and occipital lobes. Her intellectual development was delayed (IQ, 63). She had focal seizures with motor arrest.

Discussion

We identified here five *SCN1A*/*SCN2A* mutations in five out of 92 patients with AESD. There was no truncated mutation of the two genes in the present study. A missense mutations in *SCN1A*, V982L (Singh et al., 2009) and one in *SCN2A*, F328V (Shi et al., 2009), had previously been reported in the patients with DS. G1674S in *SCN1A* located at the same position of a reported case with DS (Ohmori et al., 2002). Previous studies have shown that truncated mutations of *SCN1A* cause DS, the severest phenotype in genetic epilepsy due to sodium channel defects. On the other hand, many missense mutations cause only a modest alteration in channel function (Escayg and Goldin, 2010). The same missense mutation of *SCN1A* sometimes displays a wide range of severity from DS to GEFS+ in inherited cases (Catterall et al., 2010; Escayg and Goldin, 2010). In this study, none of our five patients revealed clinical findings of typical DS during the follow-up period ranging from 4 months to 21 years. The diversity of clinical phenotypes may be explained by the characteristics of *SCN1A* mutations. For example, the R1575C mutation found in Case 3 had previously been reported in various types of encephalitis/encephalopathy, but not in epileptic syndromes. Another possibility is that the phenotype is influenced by additional genetic and/or environmental factors, which may be different between epileptic syndromes and acute encephalopathy. In patients with AESD, additional genetic and/or environmental factors may have been sufficient to produce AESD, but not DS.

Most of the *SCN2A* mutations cause benign epileptic syndromes such as BFNIS (Heron et al., 2002). Several de novo missense mutations of *SCN2A* associated with severe phenotypes, such as DS, locate outside transmembrane domains (Heron et al., 2002; Berkovic et al., 2004; Kamiya et al., 2004; Herlenius et al., 2007; Ogiwara et al., 2009). The F328V mutation found in Case 5 also locates in the same domain. However, the patient had no clinical features of DS. Additional genetic and/or environmental factors might be necessary to cause DS. We suggest that the *SCN1A/SCN2A* mutations found in this study are not the single causative factor, but one of the multiple predisposing factors of AESD. Such mutations may be different from those in more severe epileptic syndrome in the severity of functional effect. Although these variants, V982L in *SCN1A* and F328V in *SCN2A*, were previously reported in severe epilepsy syndromes, they co-existed with *SCN9A* and *SCN1A* mutations, respectively (Singh et al., 2009; Shi et al., 2009). The clinical characteristics of 5 cases, such as seizure types at onset and MRI findings, were also different from each other, suggesting the involvement of other factors associated with AESD.

We found a novel missense mutation, I172V, in Case 4. This patient had some clinical features reminiscent of DS, including fever-induced seizure susceptibility. The alteration in channel function in this patient remains to be elucidated. I172V found in Case 4, was inherited from the asymptomatic father, and was predicted as tolerant variant in silico analysis. Despite a very rare variant, I172V may probably be a benign polymorphism.

We examined the frequency of Japanese control population with *SCN1A/2A* rare variants (We put alternative allele frequency <0.005) using the Japanese exome database, HGVB. For *SCN1A* and *SCN2A*, the number of variants meeting the criteria was 28 and 26, respectively, and the frequency of such rare variants in normal Japanese was 2.32% and 2.15%. In this study, the mutation-positive frequency in AESD cases was 3.26% for *SCN1A*, and 2.17% for *SCN2A*. These data may suggest the pathogenicity of these variants for *SCN1A*, but not for *SCN2A*.

We further searched the database of Exome Aggregation Consortium. Two (R1575C in *SCN1A* and F328V in *SCN2A*) out of the 5 mutations were found in normal East Asian with allele frequency of 0.000816 and 0.00106, respectively. Considering that AESD (except IHHE) has been reported only from Japan, it is interesting that F328V was found only in East Asia. R1575 was also very rare in other population (allele frequency less than 8.814×10^{-5}). The existence of these rare variants in East Asia may account for the high incidence of AESD in Japan.

In conclusion, we demonstrated that mutations in *SCN1A* and *SCN2A* are one of the predisposing factors of AESD. Altered channel activity caused by these mutations may promote seizures and excitotoxic brain damage in patients.

Conflict of interest statement

None.

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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.eplepsyres.2015.08.001

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PAEDIATRIC NEURORADIOLOGY

Disrupted glutamate-glutamine cycle in acute encephalopathy with biphasic seizures and late reduced diffusion

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Abstract
Introduction Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is the most common subtype of infectious pediatric encephalopathy in Japan. It is sometimes difficult to make an early diagnosis of AESD; excitotoxicity is postulated to be the pathogenesis based on elevated glutamine (Gln) and glutamate (Glu) complex (Glx = Glu + Gln) observed on MR spectroscopy. It is uncertain whether Gln or Glu contributes to the elevated Glx, or whether MR spectroscopy is useful for an early diagnosis.
Methods Five Japanese patients with AESD (three boys and two girls, 1 year of age) were enrolled in this study. MR spectroscopy was acquired from the frontal white matter (repetition time (TR) of 5000 ms, echo time (TE) of 30 ms) with a 1.5- or 3.0-T scanner. MR spectroscopy was performed four times for two patients, three times for one patient, and two times for two patients. Quantification of Glu and Gln was performed using LCModel.
Results Glu was elevated in three of four studies on days 1–4 and became normal or low afterward. Gln was normal in three studies on days 1–2, elevated in all seven studies on days 4–12, and became normal or low afterward.
Conclusion These findings suggest that MR spectroscopy may be useful for an early diagnosis. Acute Glu elevation changes to subacute Gln elevation, suggesting that a disrupted Glu-Gln cycle may play an important role.

Keywords Encephalopathy · Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) · MR spectroscopy · Glutamine · Glutamate

Introduction

Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is the most common subtype of infectious pediatric encephalopathy in Japan [1], occurring in about 200 Japanese children (most often around 1 year of age) per year. AESD is clinically characterized by a prolonged febrile seizure (early seizure) on day 1, followed by late seizures associated with deterioration of the consciousness level on days 4 to 6, and radiologically by delayed reduced diffusion in the frontal or fronto-parietal subcortical white matter, the so-called bright tree appearance, on days 3 to 9 [1–3]. Between the biphasic seizures, some patients (around 30 %) exhibit almost normal consciousness levels with normal magnetic resonance imaging (MRI) findings, which leads to an initial misdiagnosis of febrile seizure status. The exact pathogenesis of AESD is uncertain; excitotoxic injury with delayed (or apoptotic) neuronal death is hypothesized to be a possible mechanism [4]. We have reported elevated glutamine (Gln)/glutamate (Glu) complex (Glx = Gln + Glu) observed on MR spectroscopy with a 1.5-T magnet in patients with AESD on days 3 to 7, when diffusion-weighted image (DWI) has already shown the bright tree appearance [5]. To determine whether or not MR spectroscopy findings allow the diagnosis of AESD and febrile seizure status before the bright tree

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appearance, and are thereby useful for an early diagnosis, and to determine whether Glu or Gln contributes to the elevation of Glx on MR spectroscopy during the course of AESD, we separately examined Glu and Gln on MR spectroscopy in additional five patients with AESD.

Materials and methods

Five Japanese patients with AESD (three boys and two girls, 1 year of age), diagnosed based on the clinical and radiological criteria previously reported [1, 3], were included in this study. Patients 1, 2, 3, and 5 developed normally until the onset of AESD; patient 4 exhibited developmental delay, epilepsy, and pontocerebellar hypoplasia before the onset of AESD. All five patients presented with a seizure of longer than 30 min as the initial neurological symptom within a day of the onset of fever (day 1), followed by late seizures (most often complex partial seizures) on days 4 to 7. Their clinical information is summarized in Table 1.

Diffusion-weighted images (DWIs) of the five patients showed restricted diffusion in the subcortical white matter (bright tree appearance) on days 5 to 10 (Fig. 1). MR spectroscopy was acquired from the frontal white matter with a 1.5-T scanner (Magnetom Avanto; Siemens, Enlargen, Germany) for patients 1 to 3, or with a 3.0-T scanner (Achieva 3.0T TX; Philips Healthcare, Best, Netherlands) for patients 4 and 5. Point-resolved spectra were obtained using TR of 5000 ms, TE of 30 ms, and NEX of 32, with a voxel size of 4.5 cm^3. MR spectroscopy was performed four times for patients 1 (days 1, 4, 8, and 28) and 3 (days 2, 5, 11, and 22), three times for patient 4 (days 2, 7, and 13), and two times for patients 2 (days 10 and 27) and 5 (days 7 and 18), respectively. Quantification of Glu and Gln was performed using the modified water scaling method of LCModel (35.88 mol/l for default value of water proton density) [6]. MR spectroscopy data were compared with those for aged-matched controls (1 year of age, $n=7$ for 3 T, $n=8$ for 1.5 T), who were studied to evaluate disorders such as mild psychomotor retardation or an enlarged head circumference and had

Table 1 Clinical information, and Glx, Glu, and Gln concentrations on MR spectroscopy

Pt	Age/sex	Magnet	Day	BTA	Glx (mM)	Glu (mM)	Gln (mM)
	Past history						
	Pathogen						
	Prognosis						
1	1/M	1.5 T	1	−	9.7 ↑	7.9 ↑	1.8
	Healthy		4	−	15.0 ↑	9.1 ↑	5.9 ↑
	Unknown		8	+	10.1 ↑	3.8 ↓	6.3 ↑
	ID, Epi		28	−	5.5 ↓	4.4 ↓	1.1 ↓
2	1/M	1.5 T	10	+	8.8	4.5	4.3 ↑
	Healthy		27	−	5.4 ↓	4.3 ↓	1.1 ↓
	Unknown						
	Tetraplegia, severe ID, Epi						
3	1/F	1.5 T	2	−	9.3 ↑	6.7 ↑	2.6
	Healthy		5	+	8.0	4.6	3.4 ↑
	Unknown		11	−	8.8	5.6	3.2 ↑
	Mild ID, Epi		22	−	7.2	5.3	1.9
Normal range of 1.5 T (mM, mean±2 SD)					7.8±0.6	5.5±0.5	2.3±0.4
4	1/M	3 T	2	−	8.5	5.8	2.7
	PCH, ID		7	+	11.0 ↑	5.8	5.2 ↑
	HHV-6		13	−	8.1	5.4 ↓	2.7
	Mild progression						
5	1/F	3 T	7	+	13.9 ↑	5.7	8.2 ↑
	Healthy		18	−	5.7 ↓	3.0 ↓	2.7
	HHV-6						
	Tetraplegia ID						
Normal range of 3 T (mM, mean±2 SD)					8.8±0.4	6.7±0.5	2.1±0.4

↑, over mean+2 SD; ↓, under mean−2 SD

Pt patient, *BTA* bright tree appearance, *Glx* glutamate + glutamine, *Glu* glutamate, *Gln* glutamine, *M* male, *F* female, *T* tesla, *ID* intellectual disability, *Epi* epilepsy, *PCH* pontocerebellar hypoplasia, *HHV* human herpes virus

no identified MRI abnormality. Because normal data depends on field strength and is different between 1.5- and 3-T magnets, metabolite concentrations were considered abnormally high or low if they differed from the controls by more than 2 SD. Glx, Glu, and Gln were also evaluated as percentages relative to the means for controls.

Results

The concentrations of Glx, Glu, and Gln are shown in Table 1. MR spectroscopy showed elevated Glx in three of four studies on days 1 to 4 before the bright tree appearance on DWI, elevated Glx in three of four studies on days 5 to 8, and normal (four studies) or low (three studies) levels afterward (Figs. 1 and 2a). Glu was elevated in three of four MR spectroscopy studies on days 1 to 4 and became normal (six studies) or low (five studies) afterward (Figs. 1 and 2b). On the other hand, Gln was normal in three MR spectroscopy studies on days 1 and 2, elevated in all seven studies on days 4 to 12, and became normal (three studies) or low (two studies) afterward (Figs. 1 and 2c). Longitudinal changes of Glx, Glu, and Gln (relative to the means for controls) are shown in Fig. 2.

Discussion

The most important finding in this MR spectroscopy study is that Glx elevation in AESD is observed from the acute stage before the bright tree appearance on DWI, and the elevation of Glx consists of two phases, that is, acute Glu elevation (days 1–4) changing to subacute Gln elevation (days 4–12). The acute Glu elevation corresponds to the clinical stage of the early seizure and consciousness disturbance between biphasic seizures, and the subacute Gln elevation almost corresponds to the clinical stage of late seizures and secondary

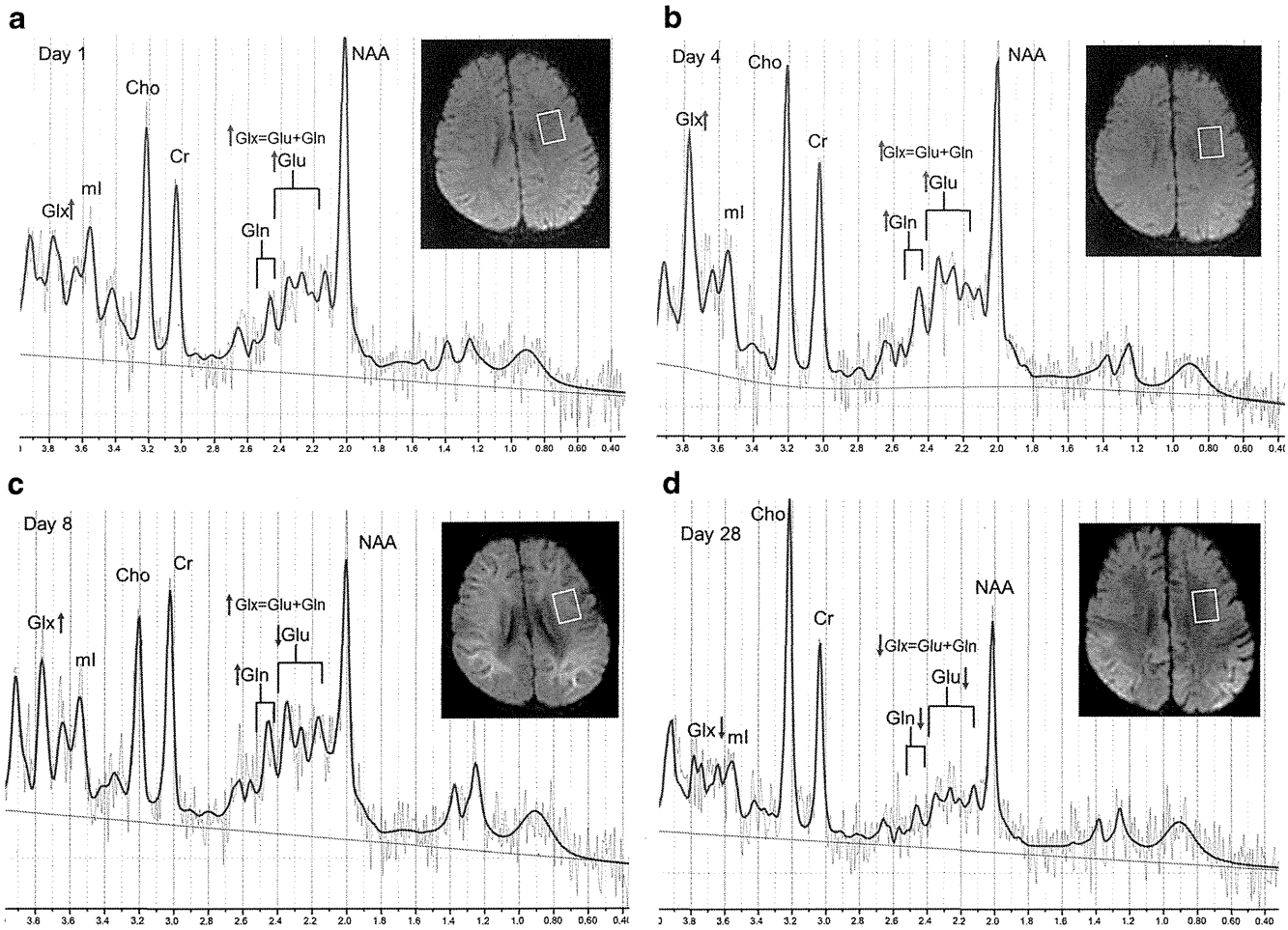

Fig. 1 DWI and MR spectroscopy of patient 1 on days 1, 4, 8, and 28. DWI shows no abnormality on days 1 (**a**) and 4 (**b**) but reveals subcortical dominant high intensity (bright tree appearance) on day 8 (**c**), which had subsequently disappeared on day 28 (**d**) with a high signal in the parietal cortex. MR spectroscopy of the frontal white matter (*white square*, 15× 20 mm) shows increased Glx on day 1 (**a**, increased Glu and normal Gln), day 4 (**b**, both increased Glu and Gln), and day 8 (**c**, decreased Glu and increased Gln), and decreased Glx (both decreased Glu and Gln) on day 28 (**d**). MR spectroscopy also shows *N*-acetylaspartate (NAA), creatine (Cr), choline (Cho), and myo-inositol (mI)

deterioration of the consciousness level with the bright tree appearance on DWI.

It is sometimes difficult to make an early diagnosis, and thus to start early treatment for AESD; because the consciousness disturbance between biphasic seizures is relatively mild (around 30 % of AESD patients being clear during that period), anticonvulsants for early seizures (most often status epilepticus), such as phenobarbital or benzodiazepine, may cause the patients to become drowsy, and MRI is unremarkable during the first few days [2, 3], which may lead to a misdiagnosis of febrile seizures. Some laboratory examinations of blood or cerebrospinal fluid (CSF), such as of total tau, S100B, and neuron-specific enolase, are proposed for an early diagnosis of AESD [7, 8]; however, these are not possible in most practices, and they do not exhibit high sensitivity or specificity. MR spectroscopy performed between days 1 and 4, before the bright tree appearance, revealed elevated Glx in three of four studies, while MR spectroscopy for patients with febrile seizure status (eight patients, 1 to 2 years) between days 2 and 5 showed no elevation of Glx, compared with that in age-matched controls [5]. This suggests that Glx observed on MR spectroscopy could be a marker for predicting AESD among pediatric patients with prolonged febrile seizures.

The MR spectroscopy finding that acute Glu elevation changes to subacute Gln elevation may strengthen the hypothesis of hyperexcitotoxicity as a pathogenesis of AESD. Glutamatergic neurotransmission plays a variety of roles in normal development and brain function [9]; however, too much Glu is harmful. Glutamatergic neurons in the human cerebral cortex release Glu into the synaptic cleft, where it is taken up by surrounding astrocytes through glutamate transporters to maintain a proper Glu concentration [10, 11]. Glu taken up by nearby astrocytes is amidated to a harmless compound, Gln, by glutamine synthetase and returned to the neurons for re-use as Glu, completing the Glu (in neuron)-Gln (in astrocyte) cycle [12]. Under excitotoxic conditions, astrocytes are thought to be neuroprotective due to their ability to clear and metabolize extracellular Glu into Gln by glutamine synthetase, which is present only in astrocytes [12–14]. If Glu is released in quantities that cannot be processed by astrocytes or if the astrocytes are not functioning properly, the excessive Glu results in excessive activation of *N*-methyl-D-aspartate receptors, which allows entry of Ca^{2+} into the postsynaptic neurons, triggering various processes resulting in necrotic cell death or apoptosis [10, 11, 15]. Because the normal concentration of Glu in the synaptic cleft (2–4 μM) is much lower (<1/1000) than that in glutamatergic neurons (around 10 mM) [16], MR spectroscopy (normal Glu, 4–8 mM) cannot detect intrasynaptic Glu itself. The Glu elevation in acute AESD observed on MR spectroscopy thus reflects its accumulation within neurons or astrocytes participating in the Glu-Gln cycle.

The observation of acute Glu elevation in AESD may be explained by the same mechanism as in the human epileptic hippocampus, which also shows high Glu on MR spectroscopy [16]. It is hypothesized that slowing of the Glu-Gln cycle in astrocytes due to down regulation of glutamine synthetase results in increased astrocytic Glu, which could explain why the concentration of Glu has been found to increase several-fold even in the sclerotic hippocampus with 60–80 % neuronal loss and a doubled glial density [16, 17]. Glu has actually been found to increase fourfold in hippocampal astrocytes after inhibition of glutamine synthetase by the xenobiotic l-methionine-SR-sulfoximine [17, 18]. It is reasonable to suggest that the switching from Glu elevation to Gln elevation observed in the subacute stage of AESD may result from Gln accumulation in the astrocytes after recovery of the glutamine synthetase function, and the MR spectroscopy finding might be compatible with the previous report showing high Gln and

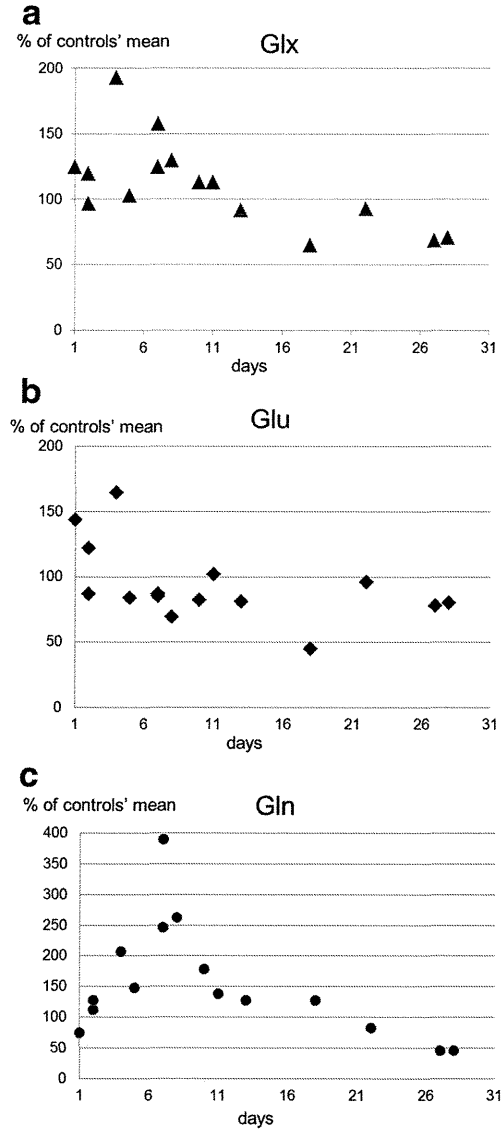

Fig. 2 Longitudinal changes of Glx (**a**), Glu (**b**), and Gln (**c**) in patients with AESD relative to the means of the controls

low Glu in CSF from patients with influenza-associated encephalopathy in Japan [19].

Perinatal hypoxic-ischemic encephalopathy (HIE) also shows biphasic evolution, a primary phase of injury (profound asphyxia) followed by a latent phase typically lasting approximately 6 h, during which EEG activity remains suppressed but high-energy phosphates are nearly normal [20]. The latent phase is frequently followed by secondary energy failure from 6 h to 3 days, characterized by stereotypic seizures, cytotoxic edema, and near-complete failure of cerebral mitochondrial activity [20]. The extracellular level of Glu in the cortex of newborn piglets with experimental HIE showed biphasic elevation; Glu increased after the hypoxic-ischemic insult, normalized shortly after resuscitation (latent phase), and began to increase again at 15 h after the insult (secondary energy failure) [21]. In contrast, the extracellular Gln concentration showed no such longitudinal change. Interestingly, the secondary increase in cortical Glu corresponds to the seizure activity, as observed in late seizures of AESD. Although the initial insult and time course differ between AESD and perinatal HIE, their clinical courses are similar; both involve a transient clinical recovery followed by secondary progression with seizures. If AESD has, in part, a similar pathomechanism to perinatal HIE, secondary elevation of extracellular Glu may result in accumulation of Gln in astrocytes during the subacute stage of AESD. In our patients, the Glu, Gln, and Glx levels became low in the chronic stage on days 27 and 28, possibly as a result of neuronal (Glu pool) and astrocytic (Gln pool) cell damage. In support of neuronal damage, MR spectroscopy was previously reported to show a progressive *N*-acetyl aspartate (neuronal marker) decrease in the subacute to chronic stages of AESD [5].

The limitations of our study are the small number of patients, because AESD is a rare disorder (only 200 patients per year), and quantitative MR spectroscopy cannot be performed in most Japanese hospitals. Further study of larger cohorts is necessary to confirm the observations on MR spectroscopy in this study.

In conclusion, elevated Glx observed on MR spectroscopy may be useful for an early diagnosis of AESD, and acute Glu elevation (days 1–4) changes to subacute Gln elevation (days 4–12), suggesting that a disrupted Glu-Gln cycle may play an important role in the pathogenesis of AESD.

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Ethical standards and patient consent We declare that all human and animal studies have been approved by Tokyo Women's Medical University Institutional Review Board and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. We declare that due to the retrospective nature of this study, informed consent was waived.

Conflict of interest We declare that we have no conflict of interest.

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Predictive score for early diagnosis of acute encephalopathy with biphasic seizures and late reduced diffusion (AESD)

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ABSTRACT

Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) at onset manifests an early seizure (ES) usually lasting more than 30 min. Following ES, some patients exhibit almost clear consciousness with no neurological symptoms, and no MRI abnormality for a few days, which may lead to an initial misdiagnosis of prolonged febrile seizures (PFS). To allow an early diagnosis of AESD, we retrospectively analyzed clinical manifestations, laboratory data, and radiologic and EEG findings in patients with AESD (n = 62) having ES of over 30 min, and ones with PFS (n = 54), using logistic regression analyses. Multivariate logistic regression analysis revealed that an age below 1.5 years and a Glasgow Coma Scale score of 14 or less than 14 (Japan Coma Scale score of 1 or higher) were high risk factors of developing AESD. We proposed an AESD prediction score system consisting of consciousness level, age, duration of convulsions, enforcement of mechanical intubation, and aspartate aminotransferase, blood glucose and creatinine levels (full score: 9), the mean scores in AESD and PFS being 5.9 and 1.8, which were significantly different ($p < 0.001$). We herein propose a scoring system for differentiating patients with AESD and PFS around the time of ES (score of 4 or more than 4 suggesting AESD), which may contribute to early therapeutic intervention and an improved neurologic outcome.

1. Introduction

Acute encephalopathy is a generic term for acute brain dysfunction caused by various agents, such as infection, metabolic disease, hepatic or renal dysfunction, and hypertension. The pathologic substrate of acute encephalopathy is diffuse or widespread, non-inflammatory brain edema. Thus, inflammatory cells are not usually found in the brain or cerebrospinal fluid (CSF), as included in the diagnostic criteria for acute necrotizing encephalopathy (ANE) [1]. In Japan, acute encephalopathy is usually associated with infection, most often by influenza virus or human herpes virus (HHV) 6 and 7, and its incidence is highest in infancy and early childhood [1–3]. The diagnosis is easy for patients with acute necrotizing encephalopathy (ANE), and hemorrhagic shock and encephalopathy syndrome (HSES), because they usually present monophasic, progressive consciousness disturbance. Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is the most common subtype of acute para-infectious encephalopathy in Japan, accounting for around 30% of patients (120–200 per year) [2]. AESD is clinically characterized by a febrile seizure usually lasting

Abbreviations: AESD, acute encephalopathy with biphasic seizures and late reduced diffusion; ALT, alanine aminotransferase; ANE, acute necrotizing encephalopathy; AST, aspartate aminotransferase; BS, blood sugar; BTA, bright tree appearance; Cr, creatinine; CSF, cerebrospinal fluid; DWI, diffusion weighted imaging, EEG, electroencephalography; ES, early seizure; GCS, Glasgow Coma Scale; HSES, hemorrhagic shock and encephalopathy syndrome; IL, interleukin; JCS, Japan Coma Scale; LDH, lactate dehydrogenase; LS, late seizures; MMP, matrix metalloproteinase; PFS, prolonged febrile seizures; TIMP, tissue inhibitor of metalloproteinase 1.

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more than 30 min (early seizure [ES]) as the initial neurological symptom on day 1, followed by late seizures (LS), most often a cluster of complex partial seizures, associated with deterioration of the consciousness level on days 4 to 6, and mild to severe neurologic sequelae. MRI findings are typically normal on day 1 or 2, followed by a subcortical white matter lesion (bright tree appearance [BTA]), most obvious in diffusion weighted imaging (DWI), on days 3–9. Between the biphasic seizures, some patients exhibit continuous disturbance of the consciousness level, leading to an early diagnosis of encephalopathy and the initiation of therapy; but other patients (around 20–30%) exhibit normal to minimally disturbed consciousness with no neurological symptoms [3]. Clear consciousness and normal MRI after ES in AESD may lead to an initial misdiagnosis of prolonged febrile seizures (PFS), and a delay of therapeutic intervention for encephalopathy.

Therefore, we attempted to identify clinical, laboratory, neuroradiological and electroencephalography (EEG) findings useful for differentiating AESD from PFS in the acute stage, and also to develop a scoring system for predicting AESD.

2. Patients and methods

A questionnaire was sent to members of the Committee for Research on the Etiology, Diagnosis and Treatment of Severe and Intractable Acute Encephalopathy, Japan, and to some members of the Annual Zao Conference on Pediatric Neurology after institutional review board approval from the Kameda Medical Center. The diagnosis of AESD was based on the previously reported diagnostic criteria with modification [2] (Table 1). In this study, we evaluated AESD patients with ES lasting longer than 30 min. When MRI showed BTA, a diagnosis of AESD was made even if the patient lacked LS, because strong medication (such as pentobarbital) under intubation with a muscle relaxant may mask LS. PFS were defined as FS lasting longer than 30 min with a good prognosis, no LS, and no MRI lesions. The data for patients with PFS were collected from the Kameda Medical Center. The onset of fever was defined as day 1. Various clinical and laboratory data, and neuroradiological and EEG findings at the acute stage of the illness (prior to LS) were statistically analyzed (Table 2). The consciousness level was evaluated using the Glasgow Coma Scale (GCS) and Japan Coma Scale (JCS) at 12–24 h after ES. JCS is most often used to evaluate the consciousness level in Japan, with which consciousness is divided into 10 levels (JCS 0, 1, 2, 3, 10, 20, 30, 100, 200, and 300), ranging from clear consciousness (JCS 0) to deep coma (JCS 300) [4]. A close correlation has been reported between the GCS and JCS scores; GCS 15 is equivalent to JCS 0, GCS 14–13 to JCS 1–3, GCS 12–9 to JCS10–30, and GCS 8–3 to JCS 100–300 [5]. Univariate logistic regression analysis was performed in AESD and PFS patients, and the variables showing a significant difference were subjected to multivariate logistic regression analysis. The threshold was determined from the ROC curve for all variables showing a significant difference on univariate logistic regression analysis, and a scoring system for the early diagnosis of AESD was developed using these variables. Scores were based on the regression coefficients and point estimations of the variables. Fisher's test was used to confirm whether or not this scoring system effectively differentiates AESD and PFS. Statistical analysis was performed using SAS 9.3, and a p value < 0.05 was taken to indicate significance.

Table 1
Diagnostic criteria for AESD.

1. Onset with convulsions (status epilepticus convulsions in most cases) within 24 h from the onset of fever
2. Subsequent, transient improvement in consciousness
3. Recurrence of convulsions (clustered partial seizures in most cases) on fourth to sixth day of illness, followed by impairment of consciousness
4. Pathogens of infection: influenza virus and HHV-6, 7 in many cases
5. Normal MRI on first to second day of illness
6. High signal intensity lesions in cerebral subcortical white matter on diffusion-weighted imaging on third to ninth day of illness. T2-weighted and FLAIR images may show high signal intensity along U-fibers
7. Exclusion of resembling diseases, including ADEM, HHE, vasculitis and metabolism abnormality with white matter abnormality.

Table 2
Variables compared between AESD and PFS patients.

1. Patient characteristics: age, male/female, past history of febrile seizures, family history of febrile seizures, antipyretics before onset, pathogens
2. Clinical factors: onset day of early seizures, duration of seizures, laterality of seizures, maximum body temperature (°C), consciousness level 12–24 h after seizures, mechanical ventilation
3. Laboratory data: WBC, Hb, PLT, AST, ALT, LDH, AESD index: AST × LDH/ALT, CK, UN, Cr, blood glucose, sodium, K, Cl, PT, APTT, PH, PCO_2, BE and CSF (cell, protein and glucose), cytokines (IL-6 and TNF-α), lactic acid on admission
4. Brain CT around early seizures
5. Brain MRI around early seizures
6. EEG around early seizures

3. Results

3.1. Patient characteristics

62 AESD patients with ES lasting longer than 30 min (31 males and 31 females) and 54 PFS patients (27 males and 27 females) were enrolled in this study. The pathogen of infection was identified in 39 (62.9%) of the 62 patients with AESD; human herpes virus (HHV)-6 in 24 (38.7%), influenza virus A or B in 6 (9.7%), respiratory syncytial (RS) virus in 3 (4.8%), rota virus in 2 (3.2%), and other pathogens in 4 (6.5%).

3.2. Statistical analysis of clinical course, laboratory data, MRI, CT, and EEG

Univariate logistic regression analysis was performed for all variables shown in Table 2. Because data on cytokines, such as interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and lactate, were available only in a small number of patients (less than 10 patients with AESD), these variables were excluded from the statistical analysis. Univariate logistic regression analysis showed that age, duration of ES, consciousness level at 12–24 h after ES, presence or absence of intubation, abnormal EEG, and abnormal laboratory data, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), Na, creatinine (Cr), and blood sugar (BS) concentrations, were statistically significant variables indicating AESD relative to PFS ($p < 0.05$). The mean age of the 62 AESD patients was 1.7 years (range: 0.6–7.6 years), and that of the 54 PFS patients was 2.5 years (0.6–7.6 years). The median GCS was 9 (30 in JCS) in AESD, and 15 (0 in JCS) in PFS. The median and average ± SD of the duration of ES in AESD were 58 min and 65.5 ± 48.8 min, and those in PFS 35 min and 48.5 ± 24.3 min. Intubation was performed in 26/62 patients with AESD, and in 2/54 with PFS. The median and average ± SD of AST were 51 mEq/l and 81.7 ± 84.9 mEq/l in AESD, and 36 mEq/l and 37.7 ± 12.6 mEq/l in PFS. Those of Cr were 0.37 mg/dl and 0.37 ± 0.12 mg/dl in AESD, and 0.30 mg/dl and 0.31 ± 0.09 mg/dl in PFS. Those of BS were 230 mg/dl and 220 ± 91 mg/dl in AESD, and 129 mg/dl and 160 ± 57 mg/dl in PFS. EEG was abnormal in 26/34 patients with AESD and 7/26 patients with PFS, so examined. Multivariate logistic regression analysis revealed that an age below 1.5 years and a GCS score of 14 or less than 14 (JCS score of

Table 3
Regression coefficients and odds ratios of variables in which a significant difference was detected on univariate logistic regression analysis.

Prediction data	Odds ratio (95% CI)	*p* value	Regression coefficient
Age below 1.5 years	9.685 (1.970–47.610)	<0.005	2.271
GCS score of 14 or less than 14	233.26 (34.311–>999.999)	<0.001	5.452

1 or higher) (patients without clear consciousness) were high risk factors of AESD (Table 3).

3.3. AESD prediction score

Among the variables in which a significant difference was detected on univariate logistic regression analysis (age, duration of ES, consciousness level at 12–24 h after ES, presence or absence of intubation, and abnormal EEG, AST, ALT, Cr, and BS), ALT was excluded because it apparently correlated with AST. EEG was also excluded because it was only performed in approximately half of the patients with AESD or PFS. Thresholds were determined from the ROC curves for age, duration of ES, consciousness level at 12–24 h after ES, presence or absence of intubation, and AST, Cr, and BS concentrations. In practice, GCS 14–13 (JCS 1–3) and GCS 12–9 (JCS 10–30) were scored 2, GCS 8–3 (JCS 100–300) scored 3, age below 1.5 years scored 1, duration of ES above 40 min scored 1, mechanical intubation scored 1, AST on admission above 40 mEq/l scored 1, blood glucose on admission above 200 mg/dl scored 1, and Cr on admission above 0.35 scored 1 (Table 4). A scoring system (full score: 9) for early diagnosis of AESD was then developed from these thresholds. The cut-off value determined from the ROC curve of the AESD-predictive score (Fig. 1) was 4, that is, a score of 4 or more than 4 suggests AESD.

For patients for whom all variables were available (53 AESD and 49 PFS patients), the mean AESD-predictive scores were 5.9 and 1.8, respectively, which were significantly different ($p < 0.001$). The sensitivity of the AESD predicting system was 88.7% and its specificity was 90%. For patients with normal consciousness levels (GCS 15), that is, nine AESD and 49 PFS patients, the AESD-predictive scores were 3.22 and 1.65, respectively ($p < 0.001$). Among nine AESD patients with GCS 15, however, only four (44.4%) had AESD-predictive scores of 4 or over 4. All 49 patients with PFS scored 3 or less than 3 points.

4. Discussion

We herein developed a scoring system for differentiating AESD from PFS in the acute stage, the former usually having a poor neurologic outcome and the latter a good outcome. This scoring system comprising age, duration of ES, consciousness level at 12–24 h after ES, presence or absence of intubation, and AST, Cr, and BS levels will be clinically useful, because these variables can be easily measured at any hospital. This scoring system enabled us to make a correct diagnosis in 47/53 AESD patients, and in 4/9 AESD patients with clear consciousness after ES, which may lead to early therapeutic intervention recommended by the guidelines for acute encephalopathy in Japan [1], including methyl-prednisolone pulse therapy. This scoring system will not miss patients with other types of encephalopathy, such as ANE and HSES, because they usually present with monophasic severe consciousness disturbance and intractable seizures at the onset, and usually have a predicting score of 4 or over 4, leading to the diagnosis of encephalopathy. In addition, patients with ANE usually exhibit bilateral thalamic lesions on CT or MRI, and those with HSES clinically present with hemorrhagic shock at the onset [6], which can be easily distinguished from AESD.

Table 4
AESD predictive-scores.

1. Consciousness level 12–24 h after seizures
GCS 15 or JCS 0, score 0
GCS 14–13 or JCS 1–3 & GCS 12–9 or JCS 10–30, score 2
GCS 8–3 or JCS 100–300, score 3
2. Age below 1.5 years, score 1
3. Duration of convulsions above 40 min, score 1
4. Mechanical ventilation, score 1
5. AST on admission above 40 mEq/l, score 1
6. Blood glucose on admission above 200 mg/dl, score 1
7. Cr on admission above 0.35 mg/dl, score 1

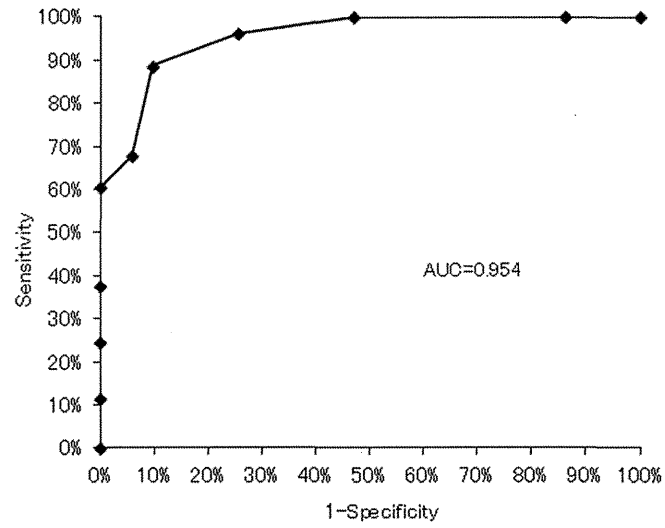

Fig. 1. The cut-off value of the AESD-predictive score. The cut-off value determined from an ROC curve was 4. The sensitivity was 88.7% and the specificity 90%.

Regarding the predictive variables, "disturbance of consciousness 12–24 h after ES" is one of the two factors detected on multivariate logistic regression analysis, which is compatible with the current concept that disturbance of consciousness is the basis for the diagnosis of acute encephalopathy [1], and this is consistent with a previous report that disturbance of consciousness (JCS ≧ 10) lasting 12 h after the onset indicated a high-risk of acute encephalopathy [7].

The age at onset in 62 AESD patients with ES lasting longer than 30 min was 1.7 years (SD 1.3, range 0.6–7.6 years, median 1.2 years), and that in 54 PFS patients was 2.5 years (SD 1.7, range 0.6–7.6 years, median 1.9 years). According to one report [2], the mean age of AESD was 1.7 years with SD of 2.1 years, which almost coincided with our data. Although the ages at onset overlap in patients with PFS and AESD, it is considered that an age over 1.5 years may suggest PFS rather than AESD. Because we could not determine the mean age for PFS in Japan, more data for PFS patients are necessary to confirm the difference in age between PFS and AESD.

The most significant difference was noted in AST among the hepatic enzymes, i.e., AST, ALT, and lactate dehydrogenase (LDH), thus, AST was adopted for this scoring system. Hepatic enzymes were reported to be highest approximately 2 weeks after the onset of AESD and became normalized within 1–2 months [8]. Although it has been reported that the 'AESD index = AST × LDH ÷ ALT' was significantly higher in AESD patients than in PFS patients until 3 days of illness [8], no significant difference was observed in our study. A cytokine storm is considered as the pathogenesis of ANE and HSES. An excess of inflammatory cytokines, such as IL-6 and TNF-α, can cause severe hepatic dysfunction due to vascular injury as a result of endothelial damage [9]. Excitotoxic neuronal damage has been postulated to play an important role in the pathogenesis of AESD [10], however, cytokine profiles in AESD also show mild elevation of IL-6 [11], which may explain the relatively mild hepatic dysfunction in AESD.

We identified an increase in Cr as a risk factor of AESD, which was consistent with a previous report that a high initial serum Cr level could be a useful marker in the differential diagnosis of AESD and PFS [12]. The increase in Cr could not directly result from renal dysfunction, because no renal failure was observed in any patient in the present and previous studies [12], or this one. One possible explanation may be that more Cr is produced from creatine stored in the brain under excitotoxic conditions to supply energy, and is released into the blood through the injured blood brain barrier [13–15].

Hyperglycemia is one of the risk factors of AESD, blood glucose levels of 150 mg/dl or higher were observed in 75% of AESD patients. A

significantly higher blood glucose level has been reported in patients with status epilepticus (>30 min) compared to that in patients with seizures lasting less than 30 min [16]. Hyperglycemia was previously considered to be a compensatory reaction to a relative lack of energy and to have no influence on the prognosis; however, a correlation between hyperglycemia and a poor clinical outcome in patients with head trauma [17,18], children with meningococcal septicemia [19], and children with septic meningitis [20] has recently been reported. Intercellular acidosis, extracellular glutamate accumulation, cerebral edema formation, and destruction of the blood brain barrier (BBB) have been postulated to account for hyperglycemia-induced brain damage [21,22]. Hyperglycemia induces progressive cerebrovascular damage and affects BBB permeability [23]. BBB dysfunctions in AESD have actually been proposed based on an increased ratio of matrix metalloproteinase-9 (MMP9), a major component of the cerebral epithelial cell basement membrane that is responsible for the integrity of BBB, and its inhibitor, tissue inhibitor of metalloproteinase 1 (TIMP-1) [24], which might partly result from hyperglycemia induced BBB dysfunction.

Although EEG abnormality was more frequent in AESD (27/34 having EEG on day 1 or 2) than in PFS (7/26), we had to exclude EEG abnormality from the AESD predicting scoring system because of the limited number of patients so examined in this study. In general, EEG in the acute stage of encephalopathy reveals high voltage slow waves. A previous study also showed EEG abnormalities in most patients with AESD (15/16) [3]. On the other hand, slow wave or paroxysmal discharges were recorded in only 16–46% of patients with PFS [25]. These findings suggested that EEG is a useful method for making an early diagnosis of AESD. It is necessary to perform EEG in a larger number of patients with AESD or PFS to develop a revised AESD predicting scoring system including EEG findings.

Imaging provided no clue for differentiating AESD from PFS in this study. This is reasonable because CT and MRI are usually normal in AESD on day 1 or 2, followed by BTA between days 3 and 9. These results suggested that imaging is not important for the early diagnosis of AESD, which is distinct from other types of encephalopathy, such as ANE and clinically mild encephalitis/encephalopathy with reversible splenial lesions (MERS). Lesions in the thalamus (ANE) or splenium of the corpus callosum (MERS) provide an important clue for an early diagnosis [2].

This study had several limitations. First, it was a retrospective one with no established protocol, which is why the timings of laboratory examinations, EEG, CT, and MRI were not uniform among the patients. Second, the number of patients was rather small. Because of these limitations, we had to use some variables exhibiting significant differences only on univariate logistic regression analysis to develop the scoring system. The present AESD predicting scoring system is not sufficient for AESD patients with clear consciousness levels after ES; four out of 9 AESD patients with clear consciousness had scores of 4 or over 4. Further analysis with uniform protocols including cytokines, MMP9, TIMP-1, and EEG will be necessary for more precise scoring system.

Conflict of interest

All authors declare no conflict of interest.

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Original article

Early predictors of status epilepticus-associated mortality and morbidity in children

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Abstract

Background: Early predictors of status epilepticus (SE)-associated mortality and morbidity have not been systematically studied in children, considerably impeding the identification of patients at risk. *Objectives:* To determine reliable early predictors of SE-associated mortality and morbidity and identify the etiology of SE-associated sequelae in Japanese children. *Methods:* We conducted a prospective multicenter study of clinical findings and initial laboratory data acquired at SE onset, and assessed

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[1] Appendix 1

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outcomes at the last follow-up examination. In-hospital death during the acute period and neurological sequelae were classified as poor outcomes. *Results:* Of the 201 children who experienced their first SE episode, 16 exhibited poor outcome that was most commonly associated with acute encephalopathy. Univariate analysis revealed that the following were associated with poor outcomes: young age (≤24 months); seizure duration >90 min; seizure intractability (failure of the second anticonvulsive drug); biphasic seizures; abnormal blood glucose levels (<61 or >250 mg/dL); serum aspartate aminotransferase (AST) ≥56 U/L; and C-reactive protein (CRP) levels >2.00 mg/dL. Multivariate analysis revealed that young age, seizure intractability, abnormal blood glucose levels, and elevated AST and CRP levels were statistically significant. *Conclusions:* Young age and seizure intractability were highly predictive of poor outcomes in pediatric SE. Moreover, abnormal blood glucose levels and elevated AST and CRP levels were predictors that might be closely associated with the etiology, especially acute encephalopathy and severe bacterial infection (sepsis and meningitis) in Japanese children.

Keywords: Status epilepticus; Prognosis; Predictors; Acute encephalopathy

1. Introduction

The incidence, causes, and prognosis of pediatric status epilepticus (SE) vary worldwide because of distinct environments, socioeconomic conditions, and genetic susceptibility to acute neurological disorders; i.e., the incidence of febrile seizures is much higher and that of bacterial meningitis is lower in Japanese children than in other children. SE predictors have been extensively studied in adults; however, these studies might not provide reliable information for the prediction of pediatric SE because the etiology in children is distinct from that in adults. Early SE predictors have not been systematically studied in children, considerably impeding the identification of patients at risk.

Influenza-associated encephalopathy cases, in which the initial presentation usually includes SE, have increased markedly in Japan since the 1994–1995 influenza epidemic; this prompted the Japanese Ministry of Health, Labour and Welfare to initiate a national survey of influenza-associated encephalopathy in 1998. During the 1998–1999 epidemic, 148 cases with a mortality of 31.8% and a morbidity of 27.7% were reported [1]. Such acute encephalopathies are caused by pathogens other than influenza, and although they are a common cause of acquired brain damage in Japanese children, the pathophysiology remains unknown. These encephalopathies have been collectively termed as acute encephalopathy with inflammation-mediated status epilepticus (AEIMSE) [2]. Early diagnosis of AEIMSE is often difficult because laboratory results, including data from cerebrospinal fluid (CSF) analysis and neuroimaging, are often unremarkable for several days after SE onset [3–5]. Therefore, we initiated a prospective multicenter status epilepticus study to determine the reliable early predictors of SE-associated mortality and morbidity and elucidate the etiology of SE-associated brain injury in Japanese children.

2. Methods

2.1. Study design

The Status Epilepticus Study group includes researchers belonging to 25 hospitals who have been studying the etiology and prognosis of pediatric SE since 2005. The 25 hospitals included 7 intensive care units and 18 local hospitals. Most pediatric SE patients in Tottori prefecture, eastern Shimane prefecture, and northern Okayama prefecture were referred to one of these participating hospitals. Tottori Prefecture Central Hospital serves severely ill pediatric patients living in the north-western part of Hyogo prefecture and Tottori prefecture. Kagoshima University Hospital, which serves severely ill pediatric patients in Kagoshima City, participated in this study. This group has been enrolling pediatric patients who suffered seizures ≥20 min. Between August 1, 2005 and March 31, 2010, over 250 patients aged 1 month–16 years were consecutively enrolled in this study.

SE was defined as any seizure >30 min or a series of recurrent seizures >30 min without complete recovery of consciousness in this study. Patients with SE of any etiology were the participants of this study. The episodes of first SE during the study period were used for statistical analysis.

We used the modified version of the classification of SE etiology proposed by Maytal et al. (Appendix 2) [6]. Clinical findings from each patient were used to identify outcome predictors: these included demographic data, previous neurological conditions, factors commonly associated with SE, SE characteristics, health status at SE onset, and laboratory data. Prior neurological conditions included past seizures, comparative mental and motor development, and past neurological insults. Factors associated with SE included pyrexia and exposure to proconvulsant drugs (theophylline

and antihistamines). SE characteristics included seizure duration, type (generalized or focal), mode (continuous or intermittent), intractability (failure of the second anticonvulsive drug), and biphasic seizures (seizure recurrence 2–6 days after SE onset). Initial laboratory data, which were acquired at SE onset, were used for analysis. They included white blood cell count, serum sodium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase, lactate dehydrogenase, C-reactive protein (CRP), and blood glucose levels. CSF analysis, including bacterial and viral cultures, was performed when encephalitis, meningitis, or AEIMSE was suspected. Virological tests included CSF gene analysis for herpes simplex virus type 1 and human herpesvirus 6, rapid influenza or rotavirus antigen detection test, and serum viral antibodies. Cranial computed tomography (CT) or magnetic resonance imaging (MRI), and electroencephalography (EEG) were performed to identify the cause of SE in patients with suspected acute symptomatic etiology and in those exhibiting neurological sequelae after SE. Additional metabolites, including plasma and urine amino acids, urine organic acids, and blood and CSF lactic acids, were analyzed in patients who exhibited neurological sequelae after SE as well. Clinical data using a uniform chart were collected every 6 months from each hospital. When necessary, additional information, including laboratory data, was obtained from medical records.

2.2. Assessment

Neurological status was initially assessed at discharge. When a patient had any developmental deterioration or neurological deficit at discharge, follow-up examinations were repeated by an experienced pediatrician or pediatric neurologist at each hospital. Neurological sequelae were assessed at the last follow-up examination. Enjoji Scale of Infant Analytical Development, Tanaka–Binet Intelligence Scale, or Wechsler Intelligence Scale for Children was used to determine the intellectual (IQ) or development quotient (DQ). IQ/DQ < 70 or new-onset motor deficit was regarded as a sequela when a patient had no prior neurological or developmental deficit. In addition, if a patient exhibited a predisposing developmental or neurological problem, IQ/DQ decline >10 or new-onset motor deficit after SE, it was regarded as a sequela. In-hospital death during the acute period and neurological sequelae were classified as poor outcomes.

AEIMSE was defined according to the following criteria [3,5,7]: (1) acute onset of impaired consciousness accompanied by seizures during a febrile infection; (2) exclusion of well-defined central nervous system (CNS) inflammation, head trauma, cerebrovascular disease, toxic encephalopathy, and systemic and metabolic diseases; (3) normal cell count in CSF and negative viral and bacterial culture of CSF samples; and (4) diffuse cerebral dysfunction and edema as indicated by symptoms, EEG findings, and neuroimaging findings. AEIMSE subtypes such as hemorrhagic shock and encephalopathy syndrome (HSES), acute necrotizing encephalopathy (ANE), acute encephalopathy with biphasic seizures and late reduced diffusion (AESD), and clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS) were classified according to previous reports [5,7]. Idiopathic hemiconvulsion–hemiplegia syndrome, characterized by long-lasting febrile seizures followed by hemiplegia, has clinical and imaging similarities to AESD [2,3,8]; therefore, it was included in the AESD category as well.

This study was approved by the ethics committees of Tottori University and each participating hospital.

2.3. Statistical analysis

Univariate analysis was performed for each candidate variable, with poor outcome as the dependent variable. Nonsignificant variables ($P > 0.05$) were excluded from further analysis. Multivariate logistic regression analysis was performed for those variables judged to be significant in univariate analysis to identify independent predictors for poor outcome. The SPSS 15.0J software (SPSS, Tokyo, Japan) was used for all the statistical analysis. All the tests were two-tailed, and P value <0.05 was considered to indicate statistical significance.

3. Results

3.1. Prognosis of status epilepticus

Of the 230 patients who met the inclusion criteria, 29 patients were excluded because of insufficient clinical or laboratory data (poor outcomes, none); therefore, in total, 201 patients were the subjects for the statistical analysis. Two patients died in the hospital during the acute period, and 1 of them had been diagnosed as having Prader–Willi syndrome. One infant who had had prior developmental delay (DQ, 67) due to bacterial meningitis exhibited developmental deterioration at discharge. She was judged as poor outcome (DQ < 20) at follow-up examination 1 month after SE and died unexpectedly thereafter. Fourteen patients without prior neurological or developmental problems who presented developmental deterioration or new-onset neurological deficits at discharge were followed-up for at least 7 months (median, 45 months; range, 7–60 months). At the last examination, 11 patients exhibited the following neurological sequelae: vegetative state ($n = 2$), profound mental retardation or developmental delay (IQ/DQ < 20) with motor palsy ($n = 1$), severe mental retardation or developmental delay (IQ/DQ < 35; $n = 2$), moderate mental retardation or developmental delay