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高梨潤一	けいれん、意識障害－その時どうする　急性壊死性脳症、出血性ショック脳症症候群	小児内科	43	501-505	2011
高梨潤一	可逆性脳梁膨大部病変を有する脳炎・脳症	小児科診療	74(6)	973-980	2011
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IV. 研究成果の刊行物・別刷

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Original article

Carnitine palmitoyl transferase II polymorphism is associated with multiple syndromes of acute encephalopathy with various infectious diseases

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Abstract

The high incidence of acute encephalopathy in East Asia suggests the role of genetic factors in its pathogenesis. It has recently been reported that variations of the *CPT II* (carnitine palmitoyl transferase II) gene may be associated with fatal or severe cases of influenza-associated encephalopathy. In the present study, we examined the genotype of *CPT II* in cases of acute encephalopathy associated with various preceding infections. Twenty-nine Japanese patients with acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) or acute necrotizing encephalopathy (ANE) were studied. The frequency of F352C of *CPT II* exon 4 was significantly higher in patients than in controls. All patients who had allele C in F352C had allele I in V368I and allele M in M647V (CIM haplotype), which reportedly decreases CPT II activity to one third of that with FIM or FVM haplotype. The frequency of CIM haplotype was significantly different between patients and controls, but not between AESD and ANE. Our results revealed that having at least one CIM allele is a risk factor for the onset of acute encephalopathy, regardless of its antecedent infections.

Keywords: Acute necrotizing encephalopathy; Acute encephalopathy with biphasic seizures and late reduced diffusion; Carnitine palmitoyltransferase II; Single nucleotide polymorphism

1. Introduction

Acute encephalopathy is an acute brain dysfunction which usually occurs at the early stage of infectious diseases with high fever. Its main symptoms are impaired consciousness and signs of increased intracranial pressure, often accompanied by convulsions or seizures. Its incidence is highest in infancy and early childhood [1]. Acute encephalopathy consists of several different syndromes. In some syndromes, such as acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) and acute necrotizing encephalopathy (ANE) [2,3], the diagnosis is made easily for most

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patients, whereas in other syndromes, the diagnostic criteria are less clear cut.

The antecedent infection of acute encephalopathy is viral, such as influenza [4] and exanthema subitum [5], in the majority of cases. There is no specific relationship between the viruses and the types of encephalopathy, suggesting that the pathogenesis of acute encephalopathy is mediated primarily by host factors. Moreover, the incidence of acute encephalopathy is higher in East Asians than in Caucasians, implying a role of genetic factors [1].

It has recently been reported that genotypic variants of the carnitine palmitoyl transferase II *(CPT II)* gene are associated with fatal or severe cases of influenza-associated encephalopathy [6]. CPT II is an enzyme localized on the mitochondrial inner membrane, and removes fatty acids from carnitine [7]. Mutations of the *CPT II* gene cause CPT II deficiency, an inborn metabolic error affecting mitochondrial fatty acid β oxidation. When patients with CPT II deficiency are infected with viruses, some develop energy failure, and show a clinical course resembling that of acute encephalopathy [8].

It has recently been reported that thermolabile phenotype variations, formed by single nucleotide polymorphisms (SNPs) of the *CPT II* exon 4 [1055T > G/F352C and 1102G > A/V368I] and exon 5 [1939A > G/M647V] produce thermolabile phenotypes [6]. These variations cause a severe reduction of CPT II activity at high body temperature, although this reduction is minimal or mild at normal body temperature.

In the previous study [6], the subjects were limited to severe cases of acute encephalopathy associated with influenza. In the present study, we conducted a single nucleotide polymorphisms (SNPs) analysis of *CPT II* in patients with acute encephalopathy, including those following antecedent infections other than influenza, and those with a better prognosis. We focused on cases of AESD and ANE in which a diagnosis was definitely made.

2. Materials

2.1. Patients

We recruited patients with AESD and ANE from hospitals in the Kanto District, Japan. In this study, we regarded AESD as synonymous with acute encephalopathy with febrile convulsive status epilepticus (AEFCSE) [1]. Of the various syndromes of acute encephalopathy, we selected AESD (or AEFCSE) and ANE because concrete diagnostic criteria are available [3,9]. In total, twenty-nine patients, nineteen with AESD and ten with ANE, participated in this study. All the patients were Japanese, aged from 8 months to 8 years and 5 months. About 80% of patients were under 2 years of age. Nineteen patients were female, and ten were male. Pathogens of antecedent infections included human herpes virus 6 (HHV6), influenza virus, respiratory syncytial (RS) virus, rotavirus, adenovirus and mycoplasma. The most frequent virus was HHV6 (8 cases), followed by influenza virus (4 cases). The preceding pathogens were not identified in 11 cases (Table 1). We obtained written informed consent from the parents of the patients. This study was approved by the Ethics Committee of the University of Tokyo.

2.2. Controls

We also analyzed the *CPT II* genotype of control subjects, consisting of 100 healthy Japanese adults, 50 male and 50 female, at 20–69 years of age. Purified DNA from controls was extracted from PSC (Pharma SNP Consortium) B cell lines, and supplied by the Human Science Research Resources Bank.

3. Methods

3.1. CPT II genotyping

Peripheral blood samples were collected from the patients. Genomic DNA was extracted from the blood using standard protocols. PCR amplification of *CPT II* exons 4 and 5 were performed using AmpliTaq PCR kits (Applied Biosystems). The reaction mixture contained 2 μl buffer, 2 μl of 2 mM dNTP, 1 μl forward and reverse primers (10 pmol), 0.12 μl AmpliTaq and 1 μl genomic DNA (30 ng). Primer sequences for exons 4 and 5 were constructed based on the GenBank database in the National Center for Biotechnology Information (NCBI). For exon 4, forward and reverse primers were 5′-GGAAATCCAGGCACATCTGA-3′ and 5′–TAGCTGCTGTGATGCCTGTC-3′, respectively, and for exon 5, 5′-TCCTGAGACTCTGGTTTTCCA-3′ and 5′-TGATGGTAGCTTTTCATCTGC-3′. The PCR amplification protocol was as follows: denaturation at 95 °C for 9 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min. The final extension was performed at 72 °C for 7 min. The sequences of the PCR products of *CPT II* exons 4 and 5 were analyzed with an ABI PRISM BigDye Terminator Cycle Sequencing FS Ready Reaction Kit using a sequencer (310 Genetic Analyzer; Applied Biosystems). When two heterozygous genotypes ([1055T > G/F352C] and [1102G > A/V368I]) in *CPT II* exon 4 were recognized in the patients, TA cloning was performed (Invitrogen). After purification of *CPT II* exon 4 PCR products by the QIAquick purification kit (Qiagen), they were subcloned into pCR2.1 vector (Invitrogen). The cloned *CPT II* gene was sequenced,

Table 1
Patients, clinical information and *CPT II* genotype.

Patient number	Pathogen	Sex	Age, y:m	Diagnosis	Outcome		*CPT II* genotype
					Motor	Intellectual	
1	Influenza A	Female	3:01	AESD	Mild	Moderate	6
2	NI	Female	1:01	AESD	Severe	Severe	8
3	HHV6	Female	0:09	ANE	Mild	Mild	8
4	NI	Male	1:00	ANE	Normal	NA	6
5	HHV6	Male	0:08	AESD	Moderate	Normal	6
6	HHV6	Female	1:00	AESD	Normal	Normal	6
7	HHV6	Female	0:10	AESD	NA	NA	5
8	HHV6	Female	0:11	AESD	Mild	Mild	6
9	NI	Female	0:09	ANE	NA	NA	8
10	Influenza	Female	1:05	ANE	Severe	Severe	5
11	RS virus	Male	0:11	ANE	Profound	Profound	9
12	NI	Female	0:11	AESD	NA	NA	8
13	NI	Female	1:11	AESD	NA	NA	6
14	RS virus	Female	1:09	AESD	NA	NA	6
15	HHV6	Male	1:00	AESD	Profound	Profound	9
16	HHV6	Male	0:10	AESD	Normal	Normal	7
17	NI	Female	3:04	AESD	NA	NA	9
18	Rotavirus	Male	1:03	ANE	Normal	Normal	6
19	HHV6	Female	1:11	AESD	NA	NA	8
20	Influenza B	Female	3:02	AESD	Severe	Severe	9
21	Adenovirus	Male	1:02	AESD	Normal	Normal	1
22	NI	Male	0:09	ANE	Normal	Normal	8
23	RSvirus	Female	0:07	AESD	Normal	Normal	5
24	NI	Female	1:01	ANE	NA	NA	6
25	NI	Female	3:00	AESD	NA	NA	6
26	Mycoplasma	Male	0:09	AESD	Normal	Normal	1
27	NI	Male	1:07	ANE	Normal	Normal	9
28	Influenza	Female	8:05	ANE	Normal	Normal	9
29	HHV6	Female	1:05	AESD	Normal	Normal	7

NI, not identified.
NA, not available.
CPT II genotype numbers are the same as those defined by Chen et al. [5].

and the haplotype of *CPT II* was determined. Instead of direct sequencing, real-time PCR was conducted using a Taq-Man probe in control subjects. Two genotypes in *CPT II* exon 4 were discriminated after PCR amplification using Faststart Universal Probe Master ROX (Roche). The reaction mixture contained 12.5 μl Faststart Universal Probe Master ROX, 2.2 μl of each primer, 6.4 μl distilled water, 1.2 μl of each probe, and 1 μl genomic DNA (30 ng). Sequences of the primers and real-time PCR probes were constructed based on the GenBank database in the NCBI. For real-time PCR, forward and reverse primers were 5′-ATTAAGGACCTTGTCCACT-3′ and 5′-TGAGCACTGCCACACCATCA-3′, respectively. Real-time PCR probes for F352C were 5′-FAM-ACA-AACCGCTGGTTTGATAAA-3′ and 5′-VIC-ACAAA CCGCTGGTGTGATAAA-3′, and those for V368I were 5′-FAM-TGGCTCTACTGCCGTCCACTTT-3′ and 5′-VIC-TGGCTCTACTGCCATCCACTTT-3′. Real-time PCR was performed under the following conditions: the first denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, and annealing at 60 °C for 1 min using the ABI PRISM 7000 sequence detection system (Applied Biosystems).

3.2. Statistical methods

Differences in the demographic characteristics of the genotypes between patients and controls were assessed by χ^2-test, or when the χ^2-test was not valid, Fisher's exact test was used for categorical data. Significant differences were defined as $p < 0.05$ in conditional analysis. Odds ratios and associated 95% confidence intervals were calculated using Microsoft Office Excel 2007.

4. Results

Direct sequencing of exons 4 and 5 showed heterozygosity for [1055T > G/F352C], [1102G > A/V368I], and [1939A > G/M368I] in a subpopulation of both patients and controls. When a patient showed heterozygosity in two SNPs of exon 4, TA cloning was performed. We screened 100 controls by allelic discrimination using Taq-Man probes before direct sequencing. TA cloning was also performed in the controls classified into the heterozygous SNP group. According to the genotype definition of *CPT II* by Chen et al. [6], twenty-nine patients and one hundred controls were assorted into

six and eight genotype groups, respectively. In regard to the three polymorphic variations of *CPT II*, F352C, V368I and M647V, nine of the thrity-six expected genotypes were observed in the controls. The [1055T > G/F352C] substitution has been reported only in East Asians and not in Caucasians (rs2229291 on NCBI, http://www.sanger.ac.uk), whereas [1102G > A/V368I] and [1939A > G/M647V] substitutions have been reported in both races [9,10]. The [1939A > G/M647V] polymorphism has rarely been reported in humans. In this study, allele C in F352C was linked to both allele I in V368I and allele M in M647V in patients. Polymorphism [1939A > G/M647V] of *CPT II* was not found in patients; therefore, only six groups were recognized in the patients. The most frequent genotype was FVM–FIM (type 6) in both patients and controls. Each genotypic distribution revealed no significant difference between patients and controls. ($p = 0.176$, Table 2).

Next, we studied the allelic frequency of F352C between patients and controls, since this SNP was present only in Asian populations (rs2229291 on NCBI, http://www.sanger.ac.uk). The frequency of F352C was significantly higher in patients than in controls ($p = 0.011$, OR = 2.44, 95%CI = 1.21–4.94) (Table 3). The frequency of C allele was higher in patients (27.6%) than in controls (13.5%). All patients who had C allele in F352C had C-I-M combination. CPT II enzymatic activity with CIM haplotype is reported to be about one third of that with the FVM or FIM haplotype [6]. Therefore, we compared the frequency of having 0, 1 and 2 CIM alleles in patients and controls (Table 4). The frequency of having the CIM allele was significantly higher in patients than in controls ($p = 0.029$). To investigate whether the genotypes relate to different syndromes, we compared genotype distribution between patients with AESD and ANE, and found no difference in genotype distribution between them ($p = 0.773$) (data not shown).

Finally, we compared the allelic frequency of F352C between patients with a good and poor prognosis.

Table 4
The number of CIM haplotype in patients and controls.

CIM	Patients		Control	
	N	Frequency (%)	N	Frequency (%)
2	2	0.07	1	0.01
1	12	0.41	25	0.25
0	15	0.52	74	0.74
Total	29		100	

The frequency of having CIM haplotype was significantly different between patients and controls ($p = 0.029$).

Table 2
Genetic distribution of *CPT II*.

	Genotypes			Alleles	Patients		Controls	
	F352C	V368I	M647V		*N*	Frequency (%)	*N*	Frequency (%)
Type 1	FF	VV	MM	FVM–FVM	2	0.069	8	0.080
Type 2	FF	II	VV	FIV–FIV	0	0.000	0	0.000
Type 3	FF	VI	MV	FVM–FIV*	0	0.000	4	0.040
Type 4	FF	II	MV	FIM–FIV	0	0.000	10	0.100
Type 5	FF	II	MM	FIM–FIM	3	0.103	18	0.180
Type 6	FF	VI	MM	FVM–FIM	10	0.345	34	0.340
Type 7	CC	II	MM	CIM–CIM	2	0.069	1	0.010
Type 8	FC	II	MM	FIM–CIM	6	0.207	13	0.130
Type 9	FC	VI	MM	FVM–CIM	6	0.207	12	0.120
Total					29		100	

The nine genotypes were classified according to the diffinition by Chen et al. [6].
The difference in distribution between patients and controls was not statistically significant ($p = 0.176$).
* Haplotypes not determined.

Table 3
Allelic frequency of F352C and V368I.

Polymorphism	Allele	Patients($N = 29$)		Controls($N = 100$)		Test for allele frequency	
		N	Frequency (%)	*N*	Frequency (%)	*p* Value	Odds ratio (95%CI)
F352C	F	42	72.41	173	86.5	0.011	2.44
	C	16	27.59	27	13.5		(1.21–4.91)
V368I	V	20	34.48	66	33	0.832	1.07
	I	38	65.52	134	67		(0.58–1.98)

p value was calculated by chi-square test.
Odds ratio was shown for minor allele (C in F352C and V in V368I) versus major allele (F in F352C and I in V368I).

Table 5
Comparison of outcomes among genotypes in F352C of patients.

Genotypes	Outcome		
	Good	Poor	Total
F/F	9	1	10
F/C	4	4	8
C/C	2	0	2
Total	15	5	20

There was no statistical difference of genotype distribution between good and poor prognosis ($p = 0.154$).

Descriptions of the prognoses were available in twenty out of twenty-nine patients (Table 1). Of the ten patients who had F/F genotype, nine recovered completely or survived with mild neurological dysfunction, whereas one patient (Case 10) had severe sequelae. Of the eight patients who had the F/C genotype, four had a good prognosis, and the remaining four were left with severe neurological dysfunction. Both patients with the C/C genotype recovered completely. In regard to genotype distribution, there was no statistical difference between the good and poor prognosis ($p = 0.154$) (Table 5).

We paid attention to the clinical findings of two patients with the C/C genotype (Cases 16 and 29). In both cases, the antecedent infection was exanthema subitum. Clinical symptoms and brain MRI findings were typical of AESD. Case 16 had rhabdomyolysis, with a transient elevation of serum creatine kinase up to 30,040 U/ml, whereas Case 29 showed no particular laboratory data, except for transient thrombocytopenia.

5. Discussion

The results of the present study demonstrated that several SNPs in the *CPT II* gene are a risk factor for the onset of AESD and ANE, following various antecedent infections. The frequency of acute encephalopathy is higher in East Asians than in Caucasians [1]. It is noteworthy that the frequency of this substitution was significantly higher in our patients than in controls, since the [1055T > G/F352C] SNP in *CPT II* exon 4 has been reported only in East Asians, and not in Caucasians (rs2229291 on NCBI, http://www.sanger.ac.uk). Moreover, it has already been reported that this substitution induces an alteration of enzyme activity [11]. Chen et al. measured CPT II activity at 37 and 41 °C in COS-7 cells over-expressing *CPT II* variants, and found that the CIM haplotype shows the lowest activity in both conditions. They also reported a difference in the frequency of the FVM–CIM genotype between patients with influenza-associated encephalopathy and control subjects [6]. In the present study, we demonstrated that the frequency of the CIM haplotype, including not only FVM-CIM but also CIM–CIM and FIM–CIM, was significantly higher in patients than in controls. Considering the lowest enzyme activity of CPT II with the CIM genotype, our finding is in good agreement with the previous study [6], and extends the association to acute encephalopathy following various infectious diseases caused by HHV6, RS virus, rotavirus, adenovirus and mycoplasma. With regard to the F/F, F/C and C/C genotypes and the clinical outcome, the present study failed to show a significant correlation, possibly due to the limited number of patients.

We paid special attention to the clinical findings in the two patients with the homozygous C/C genotype, since this genotype is assumed to show the most prominent thermolability, thereby producing the most severe clinical presentation. Contrary to our expectations, the two patients recovered without any sequelae; however, the clinical features of Case 16 were noteworthy because this patient presented with transient rhabdomyolysis during the clinical course of acute encephalopathy. In this context, there is a report of a patient with CPT II deficiency due to homozygous S113L mutation, who showed recurrent rhabdomyolysis and myoglobinemia [12]. In some patients with CPT II deficiency, virus infection or long fasting triggers an episode resembling acute encephalopathy [8]. These similarities in clinical picture implicate the alteration of CPT II activity in the cerebral and muscular disorders of Case 16 with the C/C genotype.

We also compared *CPT II* genotype distribution between AESD and ANE patients, and found no significant difference. Our results show that the same polymorphism of *CPT II* is associated with both syndromes. This finding is interesting because their clinical phenotypes are quite different from each other, suggesting distinct pathomechanism. One possible explanation is that AESD and ANE share a common route of pathogenesis, which is affected by the alteration of CPT II activity at high body temperature. Another possibility is that changes of CPT II are linked to the pathogenesis via a pathway specific to each syndrome.

In conclusion, the present study extended the findings of the previous study of Chen et al. that revealed the association of a single CPT II genotype with influenza-associated encephalopathy with a poor prognosis [6]. We found that the frequency of the CIM allele was associated with the onset of acute encephalopathy with various preceding infections and variable prognosis. The thermolabile phenotype of CPT II variation predisposes infants and children to two distinct syndromes, AESD and ANE.

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Case Report

Pandemic Influenza A-Associated Acute Necrotizing Encephalopathy Without Neurologic Sequelae

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ABSTRACT

We describe an 8-year-old girl with the mildest form of acute necrotizing encephalopathy, associated with pandemic influenza A. She manifested a convulsion engendering deterioration of consciousness, although cranial computed tomography and magnetic resonance imaging within 4 hours after the convulsion revealed no abnormalities. Cranial magnetic resonance imaging 20 hours after the convulsion revealed lesions of the thalamus bilaterally, brainstem tegmentum, internal capsule, and white matter. She was diagnosed with acute necrotizing encephalopathy. Typically, the prognosis of acute necrotizing encephalopathy with a brainstem lesion is poor. Nevertheless, she recovered almost completely, after early intervention with pulsed methylprednisolone and high-dose γ-globulin therapy. She manifested a thermolabile phenotype of carnitine palmitoyltransferase II variants such as cystine-isoleucine-methionine phenotype type 9 (FVM-CIM; Phe352Cys-Val388Ile-Met647Met alleles), resulting in a predisposition to encephalopathy during influenza infection. This case is the first, to the best of our knowledge, of pandemic influenza A-associated acute necrotizing encephalopathy with a good outcome despite severe magnetic resonance imaging findings.

Introduction

Acute necrotizing encephalopathy is a rare but well-defined clinical syndrome first described by Mizuguchi [1]. It is rare, and predominantly affects infants and young children in eastern Asia, but the literature includes some case reports from outside eastern Asia [2-4]. Its clinical features include high-grade fever, seizures, and alterations in mental status that rapidly progress to coma and multiorgan dysfunction. The neuroimaging features of acute necrotizing encephalopathy are characterized by multiple symmetric lesions in the thalami bilaterally, frequently with accompanying lesions in the upper brainstem tegmentum, periventricular white matter, internal capsule, putamen, and cerebellar medulla. Affected patients exhibit high mortality and severe irreversible neurologic sequelae. Recently, mild cases were reported [5,6]. The pathogenesis of acute necrotizing encephalopathy remains poorly understood, but it can be triggered by infectious agents. Its prognosis is poor. The associated mortality rate is about 30-50%, and about 60-70% of patients, especially those with hemorrhagic lesions and brainstem involvement, manifest neurologic sequelae. Early diagnosis and prompt treatment are necessary for a good prognosis, but almost all severe cases lead to death or severe neurologic sequelae.

In patients with the thermolabile variant of carnitine palmitoyltransferase II, mitochondrial β-oxidation and the generation of adenosine triphosphate are hampered during high-grade fever, resulting in a predisposition to encephalopathy during infection with influenza [7].

We present a patient with a good outcome after acute necrotizing encephalopathy accompanying pandemic influenza A, in whom magnetic resonance imaging had demonstrated a severe pattern involving a hemorrhagic lesion in the bilateral thalami and reduced diffusion in the bilateral brainstem tegmentum. She also manifested a thermolabile phenotype of carnitine palmitoyltransferase II variants, e.g., cystine-isoleucine-methionine phenotype type 9 (FVM-CIM; Phe352Cys-Val388Ile-Met647Met alleles [F352C-V368I-M647M]).

Case Report

The patient was a previously healthy 8-year-old girl, born after an uneventful pregnancy to nonconsanguineous healthy parents. She was born at term, with a normal birth weight. Her family had no history of neuromuscular disease,

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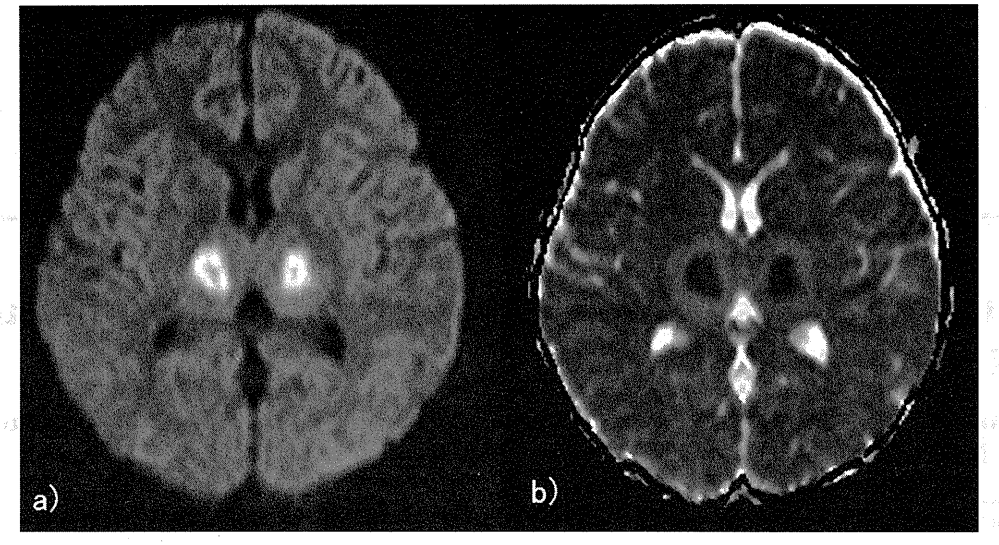

Figure 1. Cranial magnetic resonance imaging at 20 hours after the patient's convulsion. (a) Axial diffusion image indicates reduced diffusion (manifested as hyperintensity) in the symmetric thalamic lesions. (b) Axial apparent diffusion coefficient mapping indicates small, high lesions at the center of the thalami, with low lesions and high lesions around them.

metabolic disorders, or related illnesses. One day before admission, she developed coughing and fever. On the day of admission, she manifested a high-grade fever of 40°C and a generalized tonic-clonic seizure of about 30 seconds' duration. She was transferred to an emergency center. Nasal-wash rapid antigen testing produced positive results for influenza A virus. The deterioration of her consciousness progressed rapidly, and she was transferred to our hospital for additional examination and treatment. On arrival, she demonstrated normal blood pressure and no signs indicative of shock. Laboratory examination revealed normal blood cell counts, liver function, and renal function. Serum levels of electrolytes, blood glucose, and ammonia were also within normal range. Cerebrospinal fluid analysis demonstrated 1 cell/μL and protein at 17.3 mg/dL. The level of interleukin-6 in her cerebrospinal fluid was 143 pg/mL (normal range, <3.2 pg/mL), and the immunoglobulin-G index of her cerebrospinal fluid was 0.63 (normal range, <0.70). Levels of organic acids in the urine, amino acids in the blood and urine, and acylcarnitine in the blood indicated no abnormality. On the day of admission, an examination of the patient's head, using computed tomography and magnetic resonance imaging, yielded no remarkable findings. She manifested delirium, stupor, and abnormal neurologic findings such as anisocoria and unstable blood pressure. Our diagnosis was of acute encephalopathy. About 4 hours after her convulsion, early medical intervention comprising oseltamivir, pulsed methylprednisolone (30 mg/kg/day × 3 days), and high-dose γ-globulin therapy (1 g/kg/day × 2 days) was initiated. Twelve hours after the convulsion, computed tomography of the patient's brain revealed a low-density area in the bilateral thalami and upper brainstem tegmentum. Twenty hours after the convulsion, conventional cranial magnetic resonance imaging revealed increased T_2-weighted imaging and a fluid attenuation inversion recovery signal in the bilateral thalami. Apparent diffusion coefficient mapping revealed small high lesions at the center of the thalami, which were low at the periphery of the central thalamic lesions, and high outside them, and an area of low intensity in the upper brainstem tegmentum (Fig 1). We finally rendered a diagnosis of acute necrotizing encephalopathy-associated influenza A. Later, polymerase chain reaction confirmed the infection with pandemic influenza A. On hospital day 2, an electroencephalogram indicated high-voltage slow waves on the left central and occipital area, without apparent epileptic discharge. She gradually recovered consciousness. On hospital day 2, she responded to verbal instructions. On hospital day 6, she was able to read written characters. Physically, she developed transient left hemiparesis. On hospital day 16, she was able to walk without assistance. Her recovery was almost complete. On hospital day 26, we examined her cognitive function, using the Wechsler Intelligence Scale for Children-Third Edition. She manifested mild disturbances in cognitive function (i.e., difficulty in completing a maze and a show of intense effort at thought), although she fully recovered within 1-2 months. Cranial magnetic resonance imaging performed 6 months later revealed a deposition of hemosiderin at the center of the bilateral thalamus, with no atrophy (Fig 2). Chen et al. reported that the thermolabile phenotype of variations in carnitine palmitoyltransferase II, such as the cystine-isoleucine-methionine haplotype, was evident in patients with influenza-associated encephalopathy [7]. Analysis of the thermolabile phenotype of our patient's carnitine palmitoyltransferase II variation revealed her cystine-isoleucine-methionine haplotype to be type 9 (F352C-V368I-M647M).

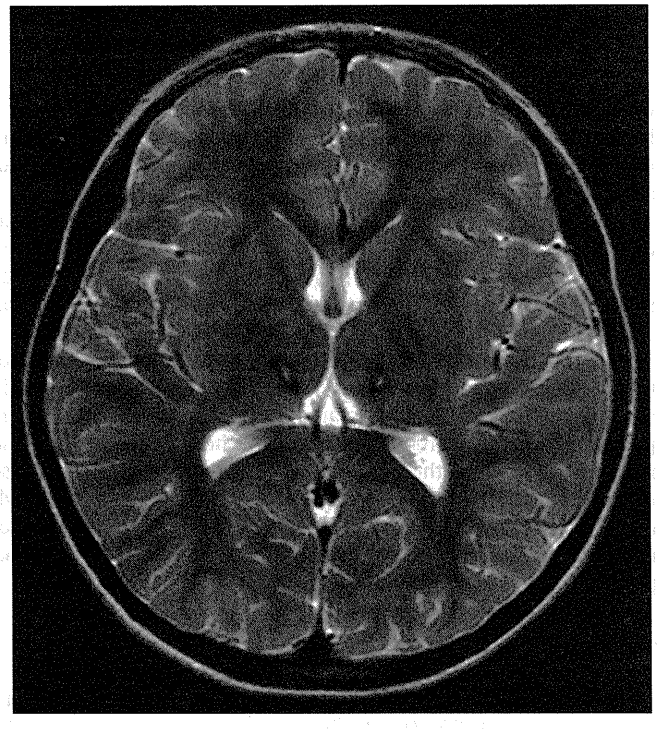

Figure 2. Cranial magnetic resonance imaging at about 6 months after the onset of acute necrotizing encephalopathy. Axial T_2-weighted magnetic resonance imaging reveals a small, high-intensity area indicating the deposition of hemosiderin at the center of the thalamus bilaterally, with no atrophy.

Discussion

This report describes a patient with acute necrotizing encephalopathy associated with pandemic influenza A infection. She exhibited severely destructive findings on cranial magnetic resonance imaging. Nevertheless, she recovered fully, with no neurologic sequelae.

The etiology and pathogenesis of acute necrotizing encephalopathy remain unknown, although influenza virus, human herpes virus-6, rotavirus, herpes simplex virus, and mycoplasma were reported as causative agents. Hypercytokinemia, as caused by these

infections, engenders the proteolytic destruction of the blood-brain barrier through the action of trypsin and the activation of matrix metalloprotease-9, which increases vascular permeability and causes brain edema, petechial hemorrhage, and necrosis. The increasing permeability of the vascular wall is caused not only by the pathophysiologic factors of hypercytokinemia, but also by genetic factors of susceptibility to energy failure. One genetic factor in the susceptibility to energy failure is the thermolabile carnitine palmitoyltransferase II polymorphism.

Our patient manifested a cytogenic edematous lesion in the bilateral thalami with central hemorrhagic necrosis, and also cytogenic edematous lesions in the upper brainstem tegmentum and internal capsule. Albayram et al. [8] reported on the diffusion-weighted magnetic resonance imaging findings of acute necrotizing encephalopathy. Reportedly, a simple alteration of vessel wall permeability without disruption (mild cases) or vessel wall necrosis (severe cases) engenders vascular occlusion, neural tissue necrosis, and a transudation of serum, resulting in perivascular and parenchymatous edema [8]. In our patient, apparent diffusion coefficient maps indicated high apparent diffusion coefficient values at the center of the thalamic lesions, low apparent diffusion coefficient values at the periphery of the central thalamic lesions, and high apparent diffusion coefficient values outside of them, suggesting hemorrhagic necrosis, cytotoxic edema, and vasogenic edema. Wong et al. reported on the positive correlation between clinical outcomes and magnetic resonance imaging scores, which involve a composite of characteristic features including the presence of hemorrhage, cavitation, and the location of lesions in the brainstem and white matter [9]. Kim et al., in their study of acute necrotizing encephalopathy in Korean infants and children, reported that the presence of hemorrhage and localized tissue loss on magnetic resonance imaging might be correlated with a poor prognosis [10]. Our patient manifested a severe magnetic resonance imaging pattern. Nevertheless, she recovered almost completely, with no neurologic sequelae. The timing of therapeutic intervention is probably important for a good clinical prognosis, regardless of the magnetic resonance imaging findings.

Analysis of the thermolabile phenotype of the patient's carnitine palmitoyltransferase II variation revealed her cystine-isoleucine-methionine haplotype as type 9 (F352C-V368I-M647M). Chen et al. reported that the thermolabile phenotype of the carnitine palmitoyltransferase II variation such as the cystine-isoleucine-methionine haplotype, including type 9 (F352C-V368I-M647M) is apparently associated with influenza associated encephalopathy [7]. Although this idea is merely speculative, the thermolabile carnitine palmitoyltransferase II phenotype with decreased enzymatic activities may reduce the utilization of mitochondrial fuel below the phenotypic threshold during high-grade fever, and the impaired mitochondrial β-oxidation and the generation of adenosine triphosphate in the cerebral microvascular endothelial cells may engender the increasing permeability of the vascular wall and the development of brain edema, resulting in neurologic sequelae with influenza-associated encephalopathy. Early intervention (e.g., with pulsed methylprednisolone) may prove effective in suppressing the inflammatory response. In addition, our patient became normothermic without hypothermia therapy. The prompt recovery of carnitine palmitoyltransferase II activity may have engendered the good prognosis of our patient. Considering the transient energy metabolism disorder with the thermolabile phenotype of carnitine palmitoyltransferase II polymorphic variants, we think that hypothermic therapy, the administration of L-carnitine for the activation of long-chain fatty acid β-oxidation, and the administration of glucose to increase the rate of the citric acid cycle may prove to be highly effective therapeutic strategies.

In conclusion, when we encounter patients with influenza infection and a rapidly progressive deterioration of consciousness and multiorgan dysfunction, neuroimaging modalities including diffusion weighted imaging are not enough to detect early pathological changes in brain structure. However, early effective therapeutic strategies may contribute to good clinical prognosis.

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Original article

Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes

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Abstract

A research committee supported by the Japanese government conducted a nationwide survey on the epidemiology of acute encephalopathy in Japan using a questionnaire. A total of 983 cases reportedly had acute encephalopathy during the past 3 years, 2007–2010. Among the pathogens of the preceding infection, influenza virus was the most common, followed by human herpesvirus-6 (HHV-6) and rotavirus. Among syndromes of acute encephalopathy, acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) was the most frequent, followed by clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS), acute necrotizing encephalopathy (ANE) and hemorrhagic shock and encephalopathy syndrome (HSES). Influenza virus was strongly associated with ANE and MERS, HHV-6 with AESD, and rotavirus with MERS. Mortality was high in ANE and HSES, but was low in AESD, MERS and HHV-6-associated encephalopathy. Neurologic sequelae were common in AESD and ANE, but were absent in MERS.

Keywords: Acute encephalopathy; Epidemiology; Acute necrotizing encephalopathy; Acute encephalopathy with biphasic seizures and late reduced diffusion; Clinically mild encephalitis/encephalopathy with a reversible splenial lesion

1. Introduction

Acute encephalopathy is a severe complication of common infections of childhood, such as influenza, exanthem subitum and acute viral gastroenteritis. It usually affects children who have previously been healthy, and often causes death or severe neurological handicaps. There are two classifications of acute encephalopathy [1]. One is based on the pathogen of the preceding infection, such as influenza encephalopathy, human herpesvirus-6 (HHV-6) encephalopathy and rotavirus encephalopathy, whereas the other is based on clinical, laboratory, imaging and pathological findings of encephalopathy. With recent advances in this syndrome classification,

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many novel syndromes, such as acute necrotizing encephalopathy (ANE) [2], acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) [3] and clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS) [4], have been established.

At present, epidemiologic data on acute encephalopathy are limited. In the United States, the California Encephalitis Project has collected a large number of cases of central nervous system infection since 1988; however, this study focused primarily on encephalitis, not on encephalopathy [5]. In Japan, several attempts have previously been made to estimate the morbidity and mortality of acute encephalitis/ encephalopathy [6–10]; however, none has used syndrome classification of acute encephalopathy.

In 2010, supported by a grant from the Ministry of Health, Labour and Welfare of Japan, we started the Committee for the Research on the Etiology, Diagnosis and Treatment of Severe and Intractable Acute Encephalopathy, and conducted a nationwide survey of acute encephalopathy in Japan. This study used for the first time both classifications, pathogenic (virological) and syndrome (clinico-pathological) [1], and elucidated the relationship between viruses and syndromes.

2. Material and methods

In this study, we defined acute encephalopathy based on the following criteria: (1) acute onset of impaired consciousness after a preceding infection, and (2) exclusion of well-defined intracerebral inflammation. According to the second criterion, we excluded meningitis/ encephalitis, such as herpes simplex virus (HSV) encephalitis and acute disseminated encephalomyelitis, in which inflammatory pathology is clearly established. On the other hand, we included several conditions in which the distinction between encephalitis and encephalopathy is unclear, such as MERS [4] and acute encephalitis with refractory, repetitive partial seizures (AERRPS) [11]. We also included cases even if the respondent inadvertently failed to answer a single item.

In June 2010, we mailed a questionnaire to the heads of the Department of Pediatrics of 520 hospitals that had been qualified as institutions for training pediatric specialists by the Japanese Pediatric Society. The hospitals included all the pediatric referral centers in Japan, and were distributed all over the country.

The questionnaire items were (1) the number of cases of acute encephalopathy treated by each hospital during the last 3 years (from April 2007 to June 2010), (2) date

Table 1
Diagnostic criteria for three major syndromes.

I. *Acute necrotizing encephalopathy of childhood (ANE)*
1. Acute encephalopathy following a viral febrile disease. Rapid deterioration in the level of consciousness. Convulsions
2. No CSF pleocytosis. Increase in CSF protein commonly observed
3. CT or MRI evidence of symmetric, multifocal brain lesions. Involvement of the bilateral thalami. Lesions also common in the cerebral periventricular white matter, internal capsule, putamen, upper brain stem tegmentum and cerebellar medulla. No involvement of other CNS regions
4. Elevation of serum aminotransferases of variable degrees. No increase in blood ammonia
5. Exclusion of resembling diseases.
   A. Differential diagnosis from clinical viewpoints.
   Overwhelming bacterial and viral infections, and fulminant hepatits; toxic shock, hemolytic uremic syndrome and other toxin-induced diseases; Reye syndrome, hemorrhagic shock and encephalopathy syndrome, and heat stroke.
   B. Differential diagnosis from radiological viewpoints.
   Leigh encephalopathy and related mitochondrial cytopathies; glutaric acidemia, methylmalonic acidemia, and infantile bilateral striatal necrosis; Wernicke encephalopathy, and carbon monoxide poisoning; acute disseminated encephalomyelitis, acute hemorrhagic leucoencephalitis, other types of encephalitis and vasculitis; arterial or venous infection, and the effects of severe hypoxia or head trauma

II. *Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD)*
1. Onset with convulsion (status epilepticus convulsivus in most cases) within 24 hours from the onset of fever
2. Subsequent, transient improvement in consciousness
3. Recurrence of convulsions (clustering partial seizures in most cases) on the fourth to sixth day of illness, followed by impairment of consciousness
4. Pathogens of precedent infection: influenza virus and HHV-6, 7 in many cases
5. Variable prognosis: mild to severe psychomotor retardation. Typical cases show impaired speech and voluntariness
6. Normal MRI on the first to second day of illness
7. High signal intensity lesions in the cerebral subcortical white matter on diffusion-weighted images on the third to ninth day of illness. T2-weighted and FLAIR images may show high signal intensities along U-fibers

III. *Clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS)*
1. Onset with neuropsychiatric symptoms, such as abnormal speech and/or behavior, and impaired consciousness and convulsion, within one week after the onset of fever
2. Complete recovery without sequelae, mostly within ten days after the onset of neuropsychiatric symptoms
3. High signal intensity lesion in the splenium of corpus callosum, in the acute stage. T1 and T2 signal changes are mild
4. Lesion may involve the entire corpus callosum and the cerebral white matter in a symmetric fashion
5. Lesion disappears within a week, with neither residual signal changes nor atrophy

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(year/month) and age at onset of each case, (3) sex, (4) syndrome of acute encephalopathy (e.g. ANE, AESD, MERS and others), (5) pathogen of preceding infection (e.g. influenza virus, HHV-6, unknown and others), and (6) prognosis. With regard to syndrome diagnosis (item #4), we also sent the diagnostic criteria of three major syndromes, ANE [12], AESD [13,14] and MERS [13] (Table 1), together with their typical neuroimaging findings. Diagnosis of hemorrhagic shock and encephalopathy syndrome (HSES) and other syndromes was based on previously published criteria [1,11,15]. As for prognosis (item #6), sequelae were judged as severe if the patient was unable either to walk independently or to utter meaningful words. Responses were sent back either by mail or by fax.

Statistical data were compared among the three syndromes, ANE, AESD and MERS. For numerical data (age), statistical significance was evaluated with one-way ANOVA. The homogeneity of the variances was analyzed by the Levene test; in case of P less than 0.05, pairwise comparisons were made and corrected by Bonferroni method. For categorical data (outcome), we used chi square tests with residual analysis.

This study was based on the Ethical Guideline for Epidemiological Researches published by Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare, Japan.

3. Results

3.1. Acute encephalopathy as a whole

Of the 520 hospitals, 265 (51.0%) responded. The total number of cases of acute encephalopathy was 983. The calculated annual incidence was 302 cases per year.

There were 497 males (51.0%) and 477 females (49.0%); no gender difference was noted.

Age at onset ranged from infancy to puberty. The incidence was most high in infancy and early childhood (Fig. 1). The average/standard deviation was 4.0 ± 3.7 years, and the median was 3 years.

Syndrome classification revealed that AESD was the most common (282 cases, 28.7%), followed by MERS (153 cases, 15.6%), ANE (39 cases, 4.0%), HSES (20 cases, 2.0%), limbic encephalitis (15 cases, 1.5%), Reye-like syndrome (7 cases, 0.7%), AERRPS (6 cases, 0.6%), Reye syndrome (4 cases, 0.4%) and posterior reversible encephalopathy syndrome (PRES) (4 cases, 0.4%). Thirteen cases (1.3%) had other syndromes, and 431 cases (43.8%) remained unclassified.

Among pathogenic viruses of preceding infection, influenza virus was the most common (263 cases, 26.6%), followed by HHV-6 (168 cases, 17.0%), rotavirus (40 cases, 4.0%), respiratory syncytial virus (RSV) (17 cases, 1.7%), mumps virus (9 cases, 0.9%), adenovirus (7 cases, 0.7%), HHV-7 (6 cases, 0.6%), HSV (6 cases, 0.6%), norovirus (5 cases, 0.5%), Epstein Barr virus (3 cases, 0.3%), varicella-zoster virus (3 cases, 0.3%), human parechovirus (2 cases, 0.2%) and measles virus (1 case, 0.1%). Bacterial pathogens, such as enterohemorrhagic *Escherichia coli* and *Salmonella*, were detected in 16 cases (1.6%), and *Mycoplasma pneumoniae* in 9 cases (0.9%). Concomitant infections, such as HHV-6/RSV and rotavirus/*Campylobacter jejuni* or *coli*, were found in 5 cases (0.5%). Pathogens remained unidentified in 401 cases (40.8%).

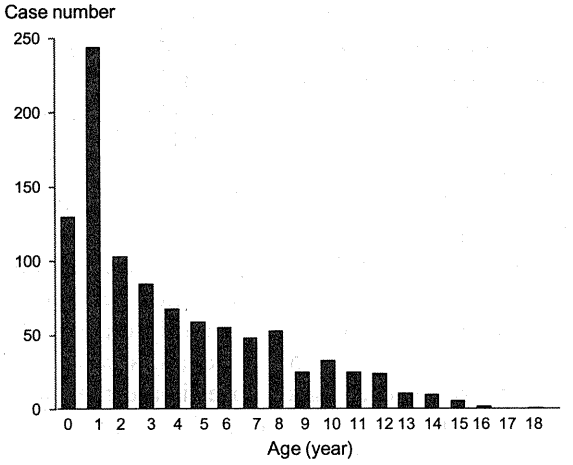

Fig. 1. Age distribution of acute encephalopathy.

The outcome of acute encephalopathy varied. Full recovery was noted in 552 cases (56.2%), mild to moderate sequelae in 218 (22.1%), severe sequelae in 133 (13.5%), and death in 55 (5.6%).

3.2. Major syndromes of acute encephalopathy

3.2.1. AESD

AESD was the most frequent syndrome (282 cases), with 114 male (40.4%) and 167 female (59.6%) patients. Age distribution showed a high incidence in infancy (average/standard deviation 1.7 ± 2.2 years, median 1 year) (Fig. 2).

Pathogens of the preceding infection were HHV-6 in 108 cases (38.2%), influenza virus in 27 (9.5%), HHV-7 in 5 (1.8%), rotavirus in 4 (1.4%) and RSV in 4 (1.4%). There were no cases of bacterial infection.

Outcome of AESD was characterized by low fatality and a high incidence of neurologic sequelae. Full recovery was noted in 81 patients (28.7%), mild to moderate sequelae in 116 (41.1%), severe sequelae in 71 (25.1%) and death in only 4 (1.4%). The ratio of patients with mild to moderate sequelae was significantly higher than for ANE ($P < 0.01$) and MERS ($P < 0.01$).

3.2.2. MERS

MERS was the second most frequent syndrome (153 cases), with 80 male (52.3%) and 69 female (45.1%)

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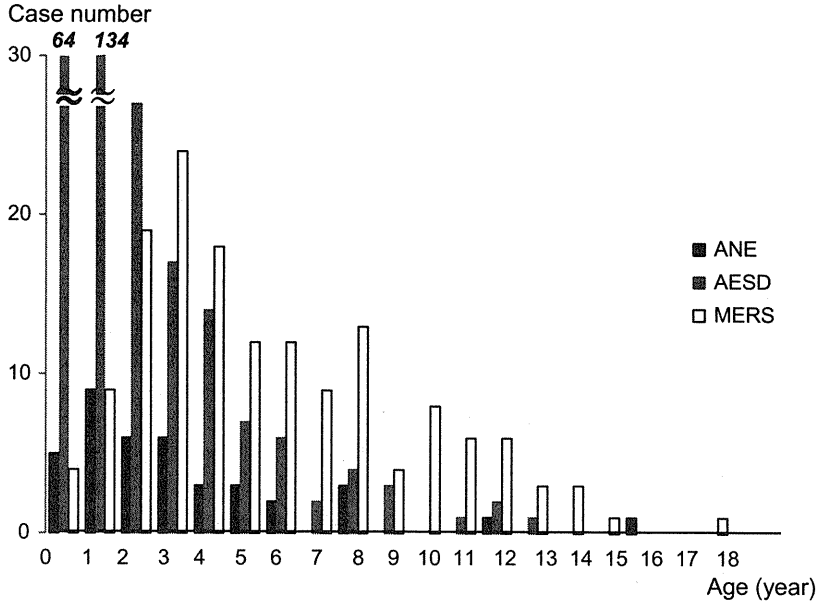

Fig. 2. Age distribution of major syndromes of acute encephalopathy. ANE, acute necrotizing encephalopathy; AESD, acute encephalopathy with biphasic seizures and late reduced diffusion; MERS, mild encephalitis/encephalopathy with a reversible splenial lesion.

patients. Age at onset varied (average/standard deviation 5.6 ± 3.7 years, median 5 years), and was significantly higher than for AESD ($P < 0.01$) (Fig. 2).

Pathogens of the precedent infection were influenza virus in 53 cases (34.4%), rotavirus in 18 (11.7%), mumps virus in 6 (3.9%), and HHV-6 in only 3 (2.0%). Notably, there were 5 cases (3.3%) following bacterial infections.

Outcome was good, with the vast majority of patients (138 cases, 90.2%) achieving a full recovery. The ratio of full recovery was significantly higher in MERS than in AESD ($P < 0.01$). In the remaining patients (11 cases, 7.1%), the sequelae were mild to moderate. There was no case resulting in severe handicap or death.

3.2.3. ANE

ANE ranked third with regard to incidence (39 cases); there were 23 male (59.0%) and 16 female (41.0%) patients. Age at onset of ANE showed the highest incidence in infancy (average/standard deviation 3.3 ± 3.4 years, median 2 years) (Fig. 2), and was significantly higher than for AESD ($P < 0.01$) and lower than for MERS ($P < 0.01$).

Pathogens of the preceding infection were influenza virus in 16 cases (41.0%) and HHV-6 in 8 (20.5%). There was no case of bacterial infection.

Outcome was poor in most patients. Full recovery was noted in only 5 patients (12.8%), mild to moderate sequelae in 9 (23.0%), severe sequelae in 13 (33.3%) and death in 11 (28.2%). Compared to AESD, the mortality of ANE was higher, whereas the probability of neurologic sequelae was comparable. The ratio of full recovery was significantly lower than for AESD ($P < 0.01$) and MERS ($P < 0.01$), and that of death significantly higher than for AESD ($P < 0.01$) and MERS ($P < 0.01$).

3.2.4. HSES

HSES was the fourth most common syndrome (20 cases), with 8 male (40.0%) and 12 female (60.0%) patients. Age at onset ranged from 0 to 8 years. The average and median age was 2.9 ± 2.9 years and 1 year, respectively.

Pathogens of the preceding infection were influenza virus in 3 cases, HHV-6 in 2, norovirus in 1, and RSV in 1.

Outcome was very poor. Eleven patients (55.0%) died, whereas only 2 (10.0%) showed full recovery. The remaining patients had neurologic sequelae, mild to moderate in 1 (5.0%) and severe sequelae in 5 (25.0%).

3.3. Major pathogens of acute encephalopathy

3.3.1. Influenza virus

Influenza virus was the most common pathogen (263 cases), with 153 male (58.2%) and 109 female (41.8%) patients. Age at onset of influenza-associated encephalopathy ranged widely from infancy to puberty (Fig. 2). The mean and median ages were 6.3 ± 3.4 and 6 years, respectively.

Syndrome classification revealed that MERS was the most common (53 cases, 20.2%), followed by AESD (27 cases, 10.3%), ANE (16 cases, 6.1%), HSES (3 cases, 1.1%), Reye, Reye-like and other syndrome (each 1 case, 0.4%). More than half of the patients (158 cases, 60.1%) were unclassified.

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The outcome varied. Although many patients achieved a full recovery (199 cases, 75.7%), fatal cases were not uncommon (18 cases, 6.8%). Neurologic sequelae were mild to moderate in 22 patients (8.4%), and severe in 22 (8.4%).

3.3.2. HHV-6

HHV-6 was the second most common pathogen (168 cases), with 73 male (43.5%) and 95 female (56.5%) patients. The vast majority of patients were infants under 2 years of age (Fig. 2). Age at onset of HHV-6-associated encephalopathy (average/standard deviation 0.8 ± 1.1 year, median 1 year) was significantly lower than with influenza-associated encephalopathy ($P < 0.001$).

Among encephalopathy syndromes, AESD was by far the most common (108 cases, 64.3%). Eight patients had ANE (4.8%). Other syndromes, such as MERS (3 cases, 1.8%), HSES (2 cases, 1.2%) and limbic encephalitis (1case, 0.6%), were rare. The number of unclassified cases was smaller (39 cases, 23.2%) than for influenza.

Half of the patients recovered (85 cases, 50.6%). Fatality was low (3 cases, 1.8%); however, many patients were left with neurologic sequelae, being mild to moderate (48 cases, 28.6%) or severe (28 cases, 16.7%).

3.3.3. Rotavirus

Rotavirus was the third most common pathogen (40 cases, 16 male and 23 female). The average and median ages were 2.8 ± 2.4 and 2 years, respectively. Eighteen patients had MERS (45.0%), four AESD (10.0%), and one ANE (2.5%). Full recovery was noted in 28 patients (70.0%), mild to moderate sequelae in 5 (12.5%), severe sequelae in 3 (7.5%), and death in 3 (7.5%).

3.3.4. RSV

RSV was the fourth most common pathogen (17 cases, 4 male and 13 female). The average and median ages were 1.4 ± 0.9 and 1 year, respectively. There were 4 cases of AESD, and 1 case each of MERS and HSES. Full recovery was noted in 12 patients (70.6%), mild to moderate sequelae in 3 (17.6%), severe sequelae in 2 (11.8%), and death in none.

4. Discussion

In this study, the Research Committee on the Etiology, Diagnosis and Treatment of Severe and Intractable Acute Encephalopathy, supported by the Ministry of Health, Labour and Welfare of Japan, conducted a nationwide survey on the epidemiology of acute encephalopathy. In Japan, several studies have previously been performed on the epidemiology of acute encephalitis/encephalopathy [6–10]. All these studies classified encephalitis/encephalopathy pathogenically (virologically), but not syndromically (clinico-pathologically). They paid little attention to the distinction between encephalitis and encephalopathy. Some were performed prior to the advent of clinically useful virological methods, such as immunochromatography (rapid antigen detection) for influenza virus and rotavirus [6,7], resulting in inaccurate virological diagnosis in many cases. The present study is the first to focus on acute encephalopathy, and uses both pathogenic and syndrome classifications.

Our study, however, had several limitations. First, the rate of responding hospitals was not high (51.0%), excluding accurate estimation of the nationwide incidence. Second, this survey was a multi-center study in which many and varied hospitals participated. Among them, the medical activities, including various aspects of diagnosis and treatment, are diverse. Accordingly, the quality of the data obtained in this study are not well guaranteed. For instance, most cases of MERS, as well as many cases of AESD, cannot be properly diagnosed without magnetic resonance imaging (MRI) [13]. Poor access to MRI in some hospitals may cause underdiagnosis of these conditions. In addition, some institutions may have failed to perform proper virological examination for the diagnosis of exanthema subitum. It is thus plausible that several cases of HHV-7-associated encephalopathy were misdiagnosed into HHV-6-associated encephalopathy.

Despite these limitations, this study has several strengths. First, the study area covered all prefectures in Japan. Second, a large number of cases were collected. Third, recent advances in virological examination have facilitated rapid and accurate identification of pathogens. Fourth, diagnostic criteria have recently been established for multiple syndromes [12–14], enabling proper syndrome diagnosis in many cases. Taking advantage of this, this study successfully demonstrated many important features of each syndrome as to its age distribution, relation to pathogens, and prognosis.

Among the three major syndromes, ANE, AESD and MERS, there were striking differences. With regard to age distribution, the mean age was 1.7 years in AESD, 3.3 years in ANE, and 5.6 years in MERS. Most cases of AESD occurred in infancy (0–1 years), and those of ANE in infancy and early childhood (0–5 years). By contrast, MERS was often seen in schoolchildren (Fig. 2). These findings were comparable to those of previous studies on AESD [3,16], ANE [2,12] and MERS [4].

With regard to pathogens of the preceding infection, ANE and MERS were strongly associated with influenza. In AESD, by contrast, HHV-6 was the most common pathogen. The findings of ANE in this study are comparable to those reported in 1990's [2,12]. Comparison with previous data [3,4,16] suggests an increase of influenza-associated MERS and a decrease of influenza-associated AESD in this decade. In this study, it

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was noteworthy that five cases of MERS had a preceding bacterial infection. This finding is in agreement with previous data that 6 out of 54 MERS cases were infected with streptococcus and *E. coli* (3 cases each) [13]. In contrast, bacterial pathogens were identified in none of the ANE and AESD cases. Although there have previously been several reports of ANE following bacterial infections [17,18], such cases are exceptional.

The prognosis of ANE and HSES was poor. In many cases, ANE caused either death or neurologic sequelae. The findings were comparable to those in the 1980's and 1990's [2,12], indicating that the overall prognosis of ANE has not been improved substantially despite the efficacy of corticosteroids in some cases [19]. The prognosis of AESD was characterized by low mortality (1.4%) and the high possibility of neurologic sequelae (66.2%). These results are again comparable to those of previous studies [3,16], reflecting the failure of current therapies to protect patients from neurologic damage in AESD. By contrast, the prognosis of MERS was excellent, in agreement with the findings of previous reports [4,13].

A large population (43.1%) of patients remained unclassified into specific syndromes. This group may consist of (1) cases of mild encephalopathy showing no abnormal findings on cranial CT/MRI, (2) cases of unknown or uncommon types of encephalopathy, and (3) cases of MERS, AESD and other syndromes in which proper diagnosis could not be reached.

In this study, we also classified acute encephalopathy based on pathogens [1], and found differences between influenza virus and HHV-6 in age distribution, syndrome, and prognosis. With regard to age, HHV-6-associated encephalopathy was predominantly seen in infants, whereas influenza-associated encephalopathy was prevalent also in older children (Fig. 3). This difference is partially explained on the basis of age predilection of these viruses, namely the incidence of exanthem subitum and influenza in general. As to syndromes, HHV-6 was associated strongly with AESD, but not with MERS. By contrast, influenza was associated with all three major syndromes, AESD, ANE and MERS. Reasons for this discrepancy remain unclear. Multiple factors, such as neurovirulence of these viruses, the host response of inflammatory cytokines, and development of the human brain, may possibly be involved. With regard to prognosis, the number of deaths was higher with influenza-associated encephalopathy, whereas that of neurologic sequelae was higher with HHV-6-associated encephalopathy. These findings may merely reflect the difference in the proportion of syndromes.

In general, the data obtained in this study were comparable to those of previous studies for influenza-associated encephalopathy (1999–2002) [8] and HHV-6 encephalopathy (2003–2004) [10], with regard to the incidence, age distribution and sex ratio. As to the prognosis of influenza-associated encephalopathy, however, mortality has markedly decreased from 30% in 1999–2000 [8] to 7% in 2007–2010. This decline may have resulted from improved treatment and/or the altered incidence of each syndrome.

In conclusion, we conducted a national survey of acute encephalopathy in Japan during three years,

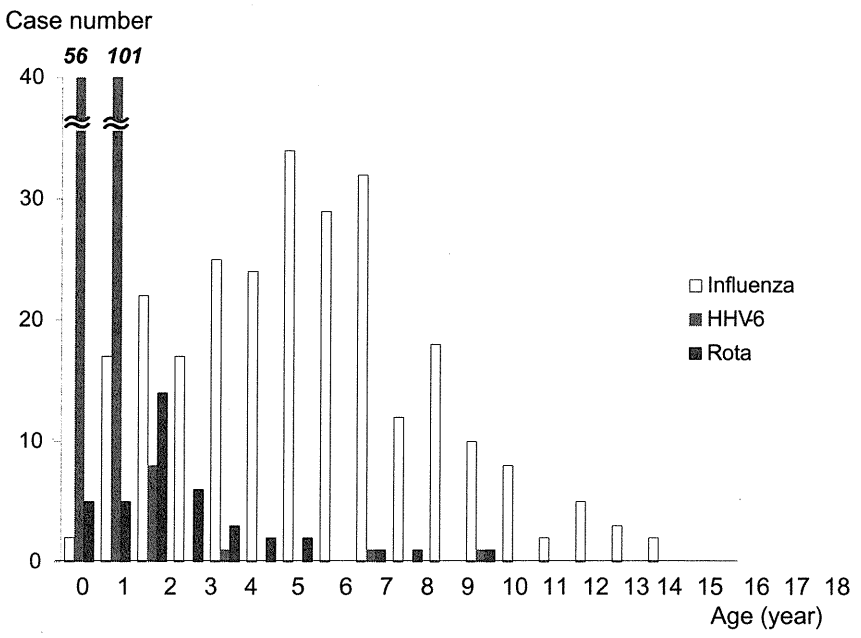

Fig. 3. Age distribution of influenza-, human herpesvirus-6- and Rotavirus-associated encephalopathy HHV-6, human herpesvirus-6; Rota, Rotavirus.

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2007–2010, and revealed the epidemiology of ANE, AESD, MERS and other syndromes. These syndromes showed marked differences in their age distribution, pathogens of preceding infection and prognosis, underscoring the necessity for therapies specific to each syndrome.

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