TABLE I. Features of Epilepsy in Patients With ID and MICPCH Associated With *CASK* Mutations.

Pt	Age (Mo)	M/F	Epilepsy/seizure type	Onset (Mo)	EEG	Therapy	Response	*CASK* mutation
1	190	F	GE/atonic	65	bil. F. and diffuse slow SWC	VPA, CZP, ESM	PR	Pt. No. in Hayashi et al. [2012]
2	42	F						c.2302 + delT
3	33	F	ES, CPS	17	bil. F. spikes			c.173_173 + 1gelGG
4	24	F				VPA, ACTH, TPM, LTG	Intractable	c.316C > G p.Arg106Stop
5	95	F	LGS/atonic, myoclonic, spasms	43	diffuse slow SWC			c.1910G > A p.Gly637Asp
6	147	F	CPS	17	left O. SWC	VPA, CLB, TPM, LTG	CR	del(X)(p11.3p11.4)
7	63	F			Normal	VPA, GBP	PR	9
8	111	F	FLE	60	bil. F. spikes			3
9	61	F				VPA, CBZ	CR	10
10	36	F	ES	34	Normal			1
11	49	F				VPA	CR	4
12	124	F	EMA	108	bil. F. SWC, bil. P.O. spikes			8
13	194	F	GE/tonic	130	left C.P., right C.P. spikes	VPA, CZP, ESM	Intractable	5
14	184	F			Normal	VPA	CR	7
15	27	F						6
16	63	M	WS, GE/tonic, myoclonic	4	4 Mo hyps, 5 years left F.C. spikes			2
						ACTH, VPA, PHT	Intractable	c.1061T > C p.Leu348Pro

Abbreviations: Pt, patient; M, male; F, female; Mo, month; GE, generalize epilepsy; ES, epileptic spasms; CPS, complex partial seizure; LGS, Lennox–Gastaut syndrome; FLE, frontal lobe epilepsy; EMA, epilepsy with myoclonic absences; WS, West syndrome; bil, bilateral; F, frontal; SWC, spike and wave complex; O, occipital; P, parietal; C, central; hyps, hypsarrhythmia; VPA valproate; CLB clobazam; TPM, topiramate; LTG, lamotrigine; GBP, gabapentin; CBZ, carbamazepine; ESM, ethosuximide; ACTH, adrenocorticotropic hormone; PHT, phenytoin; PR, partial remission, CR, complete remission.

could control their head at ages 3–6 (4.6 ± 1.2) months. Sitting independently was possible in 13/15 patients between 7 and 25 (14.2 ± 5.7) months, and walking independently in five between 29 and 72 months. All 15 female patients could smile, only four could babble, and one could utter a word.

The male patient presented with West syndrome (epileptic spasms and hypsarrhythmia) at 4 months, followed by generalized epilepsy, which was intractable to antiepileptic drugs. Eight of the 15 female patients (53%) had epilepsy, with onset between 17 and 130 (mean, 60) months, including two with epileptic spasms (no hypsarrhythmia), one with Lennox–Gastaut syndrome. EEG showed variable abnormalities, the most common finding being bilateral frontal spikes or spike and wave complexes in four patients. The response to antiepileptic drugs was also variable.

MRI Findings

The representative MRI (Fig. 4) and MRI measurements (areas of cerebrum, cerebellar hemispheres, pons, and corpus callosum), and the cerebrum/corpus callosum ratio of the controls and patients are shown in Figure 5. The areas of all measured regions increased with increasing age in the controls, whereas the cerebrum/corpus callosum ratio decreased with age, reaching the adult value at around age 5 years. In the CASK patients, the area of the cerebrum,

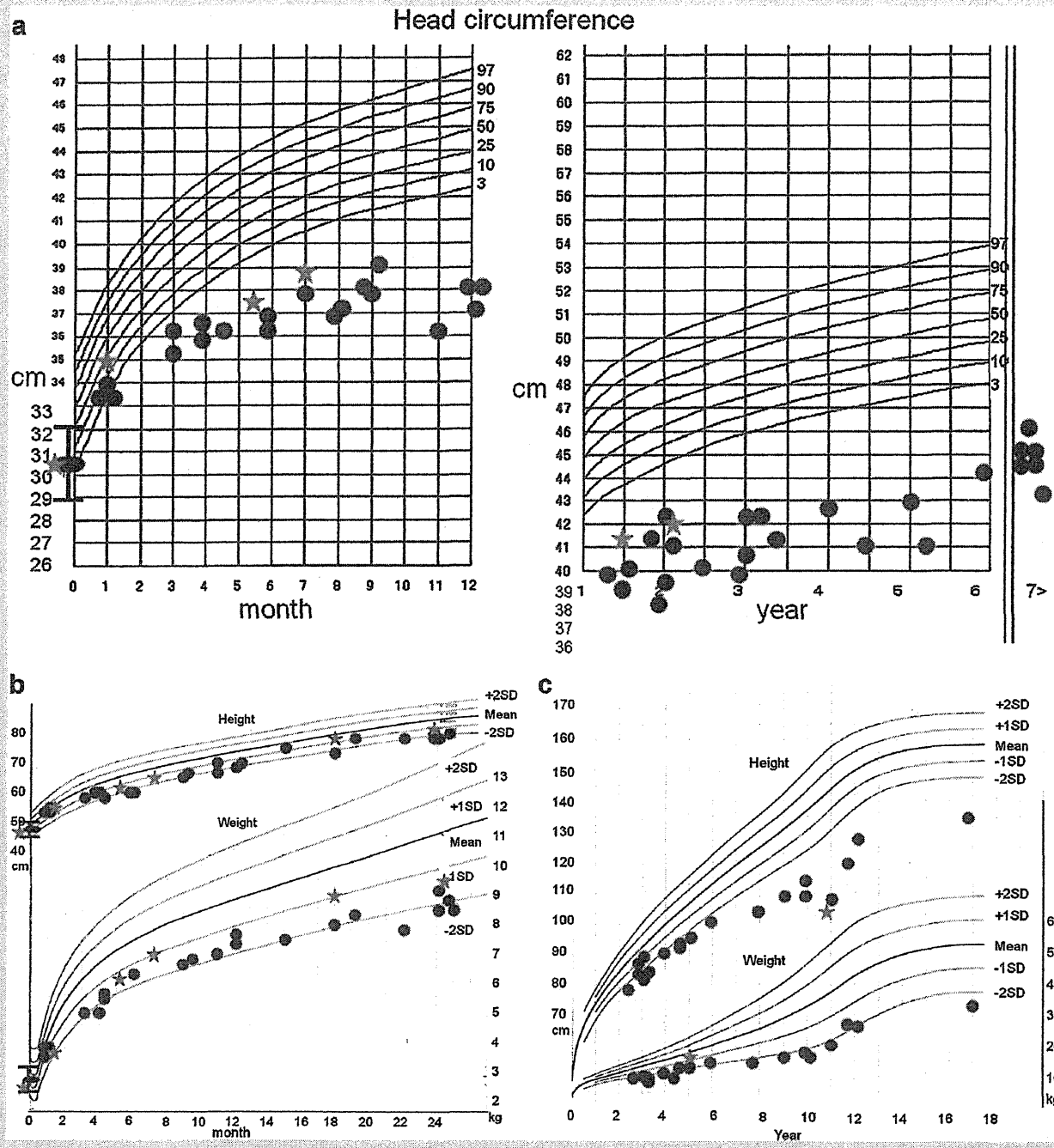

FIG. 1. Growth curves for head circumference (a), height, and weight (b,c). Black circles represent female patients, and stars a male patient. Large circle with bars represent mean ± standard deviation at birth.

cerebellar hemispheres, and pons (Fig. 5a–c) were much reduced in size when compared to controls, even in early infancy (after 4 months), and showed little size increase with age. The midline corpus callosum area was normal or in the low-normal range in all patients (Fig. 5d), appearing abnormally thick compared to the small cerebrum. The cerebellum/corpus callosum ratio was low normal or low in all patients with ID and MICPCH associated with *CASK* mutations (Fig. 5e). No obvious malformations were seen in the cerebral hemispheres in the 16 patients. In all five patients with non-*CASK*-related pontine hypoplasia, the corpus callosum was reduced in size and the cerebrum/corpus callosum ratio was high.

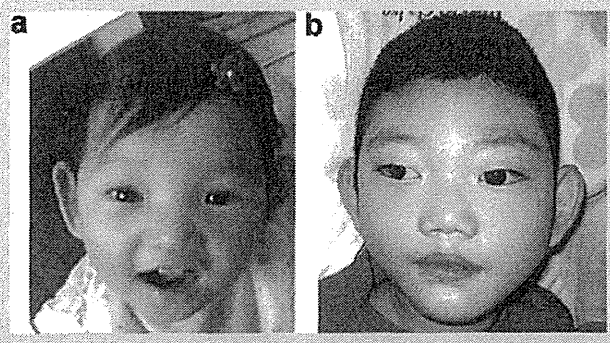

FIG. 2. Facial photograph of female Patient 11 (a) and male Patient 16 (b) showing an oval face, large eyes or irises, large ears, a broad nasal bridge, a broad nasal tip, and epicanthal folds.

DISCUSSION

This study revealed or confirmed several important clinical and radiological findings in Japanese patients with ID and MICPCH associated with *CASK* mutations. First, their head circumference at birth is within the normal range in about half, and birth height and weight are frequently normal; followed by postnatal growth retardation. Severe microcephaly develops usually within the first 4 months of life. Second, female patients acquire head control almost normally (between 3 and 6 months), but this is followed by motor delay. Third, more than half the female patients have epilepsy. In addition, MRI analyses showed that microcephaly and pontine and cerebellar hypoplasia develop in early infancy, and the corpus callosum is normal in size but appears large because the cerebrum is small. Finally, a male patient had a more severe clinical phenotype.

Microcephaly (−4 SD) and diminished body weight (−2 SD) became obvious after 4 months, while diminished stature (−2 to −3 SD) noted after 4 years. These findings are similar to those in a previous report of patients with *CASK* mutations in European countries [Moog et al., 2011]. MRI revealed that the area of the cerebrum is reduced in size after age 4 months, which was compatible with the OFC measurement. If MRI was performed in the neonatal period, it would confirm that the microcephaly observed in ID and MICPCH associated with *CASK* mutations predominantly develops postnatally. Therefore, the clinical diagnosis at birth may be difficult, and assessment of postnatal growth, especially OFC, is important in the diagnostic process.

The 15 females with *CASK* mutations developed head control normally or were mildly delayed, but had marked motor delay afterwards. A large majority (13/15) could sit alone between 7 and 25 months, but only 4/15 could walk without support between 2 and 6 years. This may be related to the fact that the cerebrum in neonates was often normal in size with no cerebral malformation on MRI. As 7 of 11 patients who could not walk at their most recent

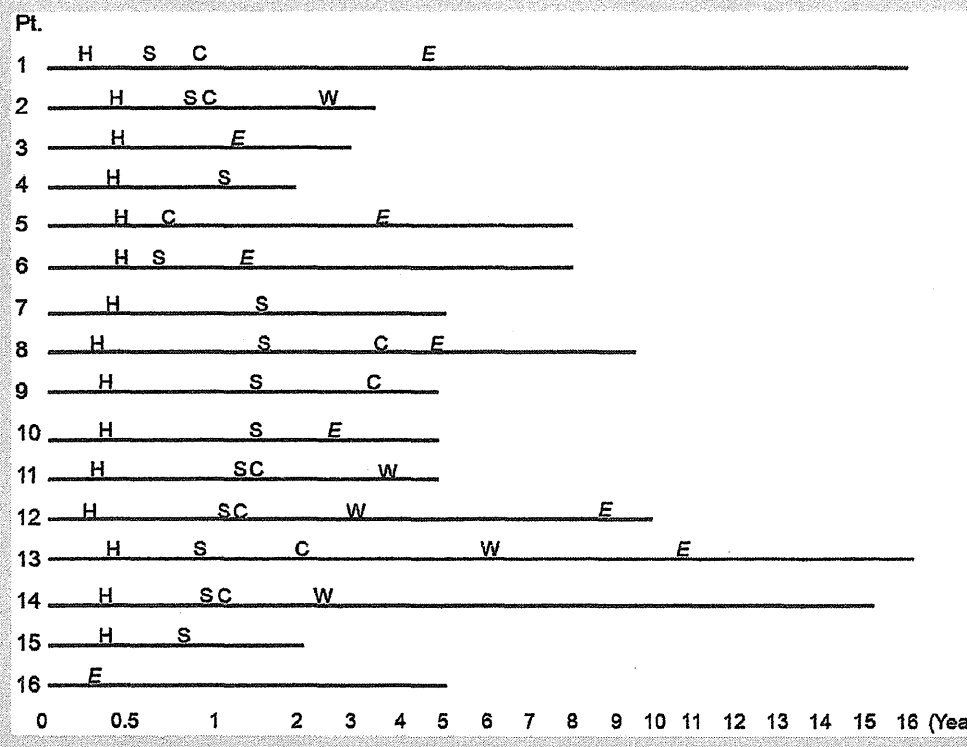

FIG. 3. Chart showing motor development and onset of epilepsy in patients with ID and MICPCH associated with *CASK* mutations. H, head control; C, crawl; S, sitting independently; W, walking independently; E, epilepsy.

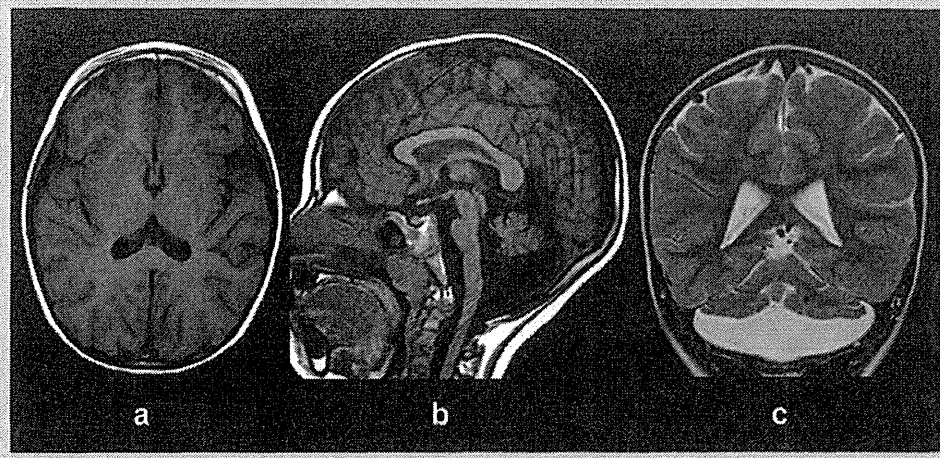

FIG. 4. Axial (a), mid-sagittal (b), and coronal MRI (c) of a 50-month-old female with *CASK* mutation (Patient 11). Note the microcephaly (a), hypoplastic pons (b), cerebellar hemispheres (c) and vermis (b) with normal appearance of the corpus callosum (a,b).

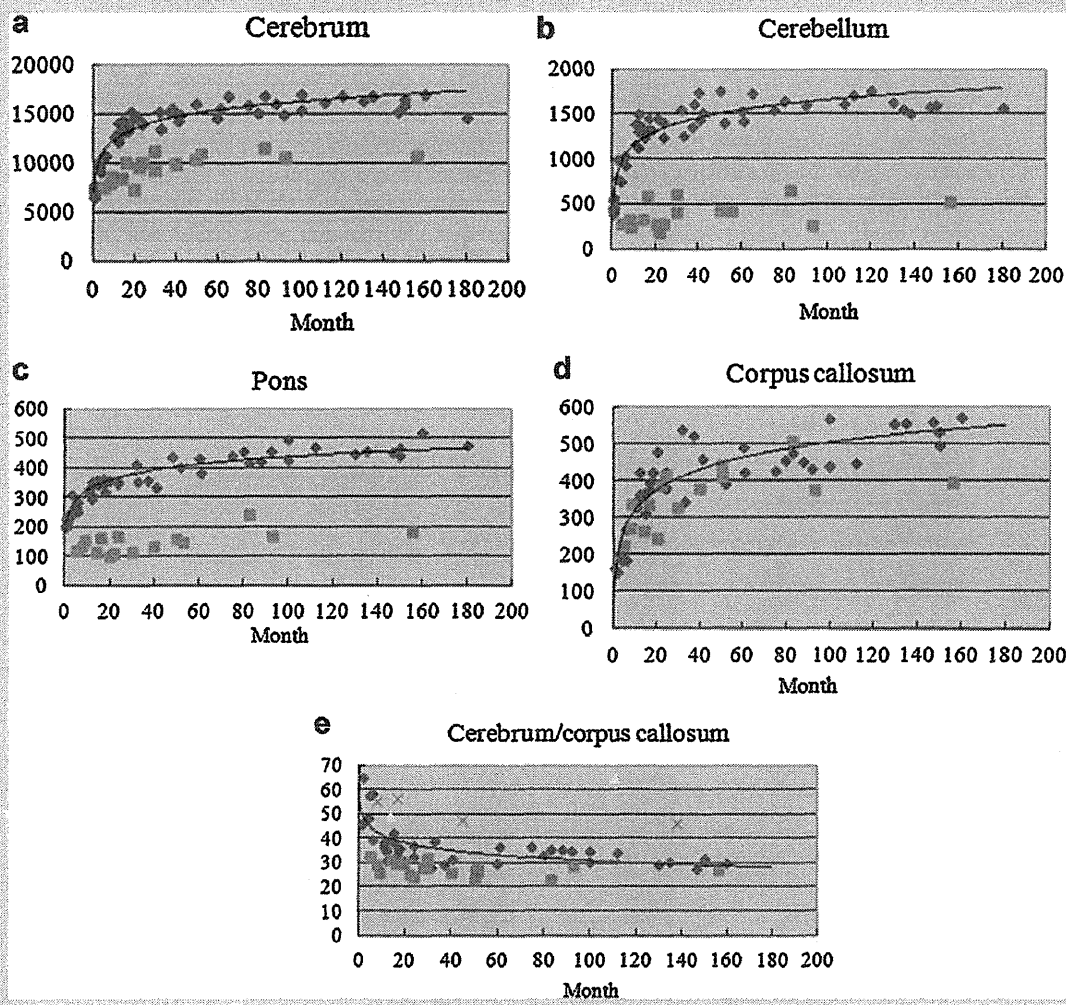

FIG. 5. Longitudinal changes in the cerebrum (a), cerebellar hemisphere (b), pons (c), and corpus callosum (d) areas, along with the cerebrum/corpus callosum area ratio (e). Diamonds represent control patients, squares patients with *CASK* mutations, triangles (e) the patient with PEHO syndrome, and Xs (e) the patients with other pontocerebellar malformations. The relatively normal callosal size combined with reduced size of other structures, resulting in the small cerebrum/corpus callosum ratio, appears to be a fairly unique characteristic of patients with *CASK* mutations.

evaluation were younger than 6 years, some might be expected to acquire this ability in the future.

Epilepsy was present in more than half of the females (53%) in this series, which is more frequent than the previously reported frequency (32%; 8/25) in European countries [Moog et al., 2011]. Seizure onset was between 17 and 130 months (mean, 60 months), and the epilepsy syndrome or seizure type was variable, similar to the previous report of onset at 1–8 years with various types of seizures [Moog et al., 2011]. Because most females without epilepsy were less than 6 years old, the frequency of epilepsy may be higher at subsequent re-evaluations. Child neurologists should be aware that epilepsy associated with ID and MICPCH due to *CASK* mutations has a relatively late onset. The neurological symptoms or facial features in the females were similar to those reported in European patients [Moog et al., 2011], however, hypohidrosis and hyposensitivity to pain were previously unrecognized. Although further clinical study is necessary to evaluate the frequency or severity of hypohidrosis and hyposensitivity to pain, ID and MICPCH associated with *CASK* mutations should be considered in the differential diagnosis of these symptoms.

The MRI findings in this case series confirmed the previous report of a normal to low-normal size of the corpus callosum and a low cerebrum/corpus callosum ratio with reduced areas of the cerebrum, pons, and cerebellar hemispheres. As five disease controls with pontine hypoplasia showed thinning of the corpus callosum and a high cerebrum/corpus callosum ratio, the normal size of the corpus callosum relative to a small cerebrum, which gives an impression of callosal thickening, is an important imaging clue for *CASK* mutations.

The growth pattern, neurologic development, neurological symptoms, and facial features were similar in the 15 females with loss-of-function mutations, which corresponds to previous reports showing that mutations resulting in a null allele are associated with a characteristic pattern of ID and MICPCH in females [Moog et al., 2011; Hayashi et al., 2012]. The one male in this study showed similar growth pattern, facial appearance, and MRI findings to the female patients; however, his neurologic manifestations were more severe, with no motor development and early onset, intractable epilepsy. Though it has been reported that *CASK* missense mutations in males can cause milder phenotypes, such as mild to severe ID with or without nystagmus, microcephaly, and/or dysmorphic features [Hackett et al., 2010] or FG syndrome [Piluso et al., 2009], their clinical features are distinct from ID and MICPCH. FG syndrome entails relative macrocephaly, agenesis of the corpus callosum, and mild ID with congenital nystagmus; microcephaly or cerebellar hypoplasia is rare. The striking difference in clinical severity between the two groups of *CASK* mutations might be explained by the different nature of the mutations; hypomorphic missense mutations in males are likely to have a relatively mild impact on protein structure and function, thus leading to less severe phenotype than the null mutations in females. Null mutations of *CASK* in males would be expected to cause a more severe phenotype than in female patients, usually resulting in prenatal or neonatal lethality. A partly penetrant *CASK* splice site mutation was reported in a severely affected male with MICPCH who died at 2 weeks [Najm et al., 2008]. In silico analysis in the present male patient with ID and MICPCH (Patient 16) showed the de novo missense mutation likely damaged and affected protein function with no splice site disruption, however, the functional CASK studies will be necessary to confirm the pathogenicity of this mutation. To clarify the details of clinical and radiologic features, it would be important to evaluate *CASK* in males with severe psychomotor delay and the characteristic facial appearance or MRI findings.

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Case report

Focal encephalopathy with recurrent episodes of epileptic status and cluster mimicking hemiconvulsion–hemiplegia–epilepsy syndrome

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Abstract

Hemiconvulsion–hemiplegia–epilepsy syndrome is characterized by unilateral convulsions during fever, transient hemiplegia, and subsequent partial epilepsy with atrophy in the cerebrum. A 9-year-old boy with a history of West syndrome and hypoglycemic attacks had three episodes of epileptic status and clusters mimicking HHE syndrome over a 2-year period. Magnetic resonance imaging revealed the involvement of the right and left cerebrums. Because no abnormalities were detected in an endocrine examination, screening tests for metabolic errors, or magnetic resonance spectroscopy, a diagnosis of metabolic errors was not supported. Immunohistochemistry using the patient's sera showed binding of the serum immunoglobulin with neurons in the temporal and occipital cerebral cortices, indicating the possible involvement of autoimmune mechanisms in this case. Focal encephalopathy should be considered in children showing convulsions, psychiatric disorders, and/or involuntary movements for several months in a row. In such cases, immunohistochemistry using the patient's sera may be useful for the investigation of the pathogenesis of the illness.

Keywords: Status epilepticus; Cluster; Hemiconvulsion–hemiplegia–epilepsy syndrome; Immunohistochemistry

1. Introduction

Autoimmune encephalitis includes various entities, such as Rasmussen encephalitis [1], non-herpetic acute limbic encephalitis [2], and anti-*N*-methyl-D-aspartate (NMDA) receptor antibody encephalitis [3]. Some authors have proposed a syndrome called subacute encephalopathy, which is characterized by delayed worsening, mild cortical atrophy, and poor neurologic outcome [4]. Hemiconvulsion–hemiplegia–epilepsy (HHE) syndrome is characterized by unilateral convulsions during fever, transient hemiplegia, and subsequent partial epilepsy with atrophy in the involved hemisphere [5]. Herein, we present the case of a boy who had 3 episodes mimicking HHE syndrome over a 2-year period. Immunohistochemistry using the patient's sera showed binding of the serum immunoglobulin with the neurons in the involved cerebral cortex.

2. Case report

A 9-year-old boy was the first child born to nonconsanguineous healthy parents. There was no family history of congenital metabolic errors or neuromuscular diseases. He was born by a prolonged delivery 38 weeks after an uneventful pregnancy. He had no perinatal distress, and

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his birth weight was 3146 g. At the age of 3 months, he was diagnosed with West syndrome, and adrenocorticotropin (ACTH) therapy was administered. He developed severe developmental delay and intractable epilepsy and had undergone a corpus callosotomy at the age of 2 years. Treatment with sodium valproate and zonisamide decreased the frequency of his epileptic seizures. At the age of 4, he had repetitive hypoglycemic attacks. However, no abnormalities were detected in endocrine tests, blood amino acid analyses, or on urine organic acid profiles. The hypoglycemic attacks stopped when the patient was started on a supplementary diet.

At the age of 6, he had a status of clonic convulsions of the left hand 10 days after a varicella infection. Brain magnetic resonance imaging (MRI) revealed high-intensity signals on T2-weighted images in the subcortical white matter in the right parietal and temporal cortices (Fig. 1A and B). On electroencephalogram (EEG), spikes were scattered in the right frontal and central area. A cerebrospinal fluid (CSF) examination did not indicate an increase in cells or cytokine levels, and a polymerase chain reaction (PCR) analysis failed to detect genomes of varicella zoster virus. However, the levels of 8-hydroxy-2′deoxyguanosine and total tau protein, which are the markers of oxidative DNA stress and axonal damage, respectively, were elevated (Table 1). Treatment with gabapentin gradually relieved the convulsions, although an MRI scan showed exacerbation of the brain atrophy in the right cerebrum. At 1 year and 3 months after the first status episode, he developed clusters of tonic and clonic convulsions in the left face and arm that were accompanied with left paresis. The right parietal white matter appeared as high-intensity areas in T2-weighted MRI images. There was no change in the levels of CSF markers. The convulsions were finally ameliorated with lamotrigine. After 3 months, he developed a cluster of tonic seizures in the right face in addition to paresis in the right arm, indicating the occurrence of a third episode. On MRI, white matter of the left temporal and occipital lobe showed high-intensity areas in T2-weighted images (Fig. 1C and D), in addition to edema in diffusion-weighted images (Fig. 1E and F). On EEG, slow waves were increased in the left frontal area. The levels of total tau protein and interleukin (IL)-6 were increased in the CSF (Table 1). The seizure in the face was controlled gradually by increasing the dose of lamotrigine, although an MRI scan showed brain atrophy in the left cerebrum.

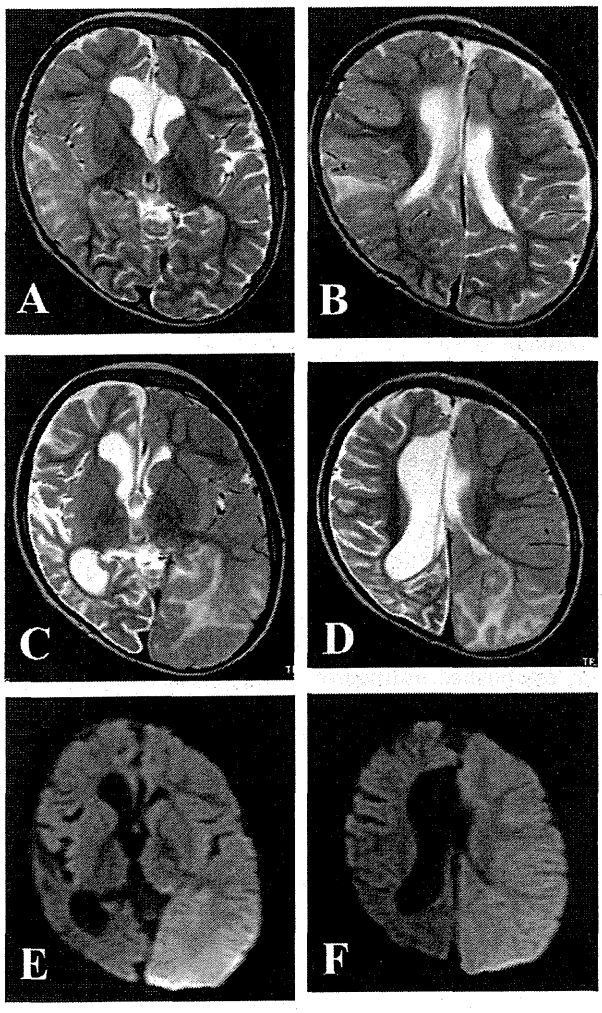

Fig. 1. Magnetic resonance imaging (MRI) changes. At the time of the first epileptic episode (A, B), high-intensity signals of T2-weighted images on brain MRI were seen in the subcortical white matter in the right parietal and temporal cortices. At the time of the third episode, the white matter of the left temporal and occipital lobes showed high intensity in T2-weighted images (C, D) and edema in diffusion-weighted images (E, F), in addition to the right cerebral atrophy.

There have been no further occurrences of epileptic status or clusters for 1 year after the third episode, and paresis has tended to improve. Magnetic resonance spectroscopy (MRS) did not reveal a lactate peak, and the concentrations of lactic and pyruvic acids were normal in the blood and CSF. Tests with an antibody against glutamate receptor δ2, but not ε2, were weakly positive in the CSF and moderately positive in the serum obtained after the second episode, whereas the test against glutamic acid decarboxylase was negative. In order to visualize the binding of the antineuronal autoantibody with the brain tissue, we performed immunohistochemistry using the patient's sera on 6-μm serial sections, including sections from the temporal and occipital cortices, basal ganglia, and cerebellar cortex from a 9-year-old control with no pathological changes in the brain and who was dying of acute leukemia. The sections were incubated with diluted sera obtained at the second episode and at the convalescent period 2 months after the third episode. Antibody binding was detected by adding rabbit polyclonal antibodies against human immunoglobulins (IgG, IgA, and IgM) (Dako, Glostrup, Denmark). The pyramidal neurons showed strong immunoreactivity, which was discriminated from

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Table 1
Summary of markers in the cerebrospinal fluid.

Episode	Protein (mg/dl)	8-Hydroxy-2′ deoxy-guanosine (ng/ml)	Hexanoyl lysine-adduct (nmol/L)	Total tau protein (pg/ml)	Neuron specific enolase (ng/ml)
1st	27	0.114▲	5.781	6140.473▲	12
2nd	19.1	<0.06	<2.6	413.295	7.1
3rd	25.1	<0.06	<2.6	1381.575▲	n/A
Cutoff	45	0.06	6	500	18

Episode	IL-4 (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	IL-10 (pg/ml)	TNF-α (pg/ml)
1st	<0.01	2.05	28.54	0.3	<0.06
2nd	<0.01	7.15	28.92	<0.11	<0.06
3rd	<0.01	12.62▲	23.2	0.31	<0.06
Cutoff	0.12	10.9	118.47	0.97	0.06

▲ and n/A denote an increase over the cutoff index and no assessment, respectively.

the weak immunoreactivity in the neuropil, in the temporal and occipital cortices (Fig. 2), but no immunoreactivity was seen in the hippocampus, basal ganglia, or cerebellar cortex with the serum obtained at the second episode. This reactivity was not seen using the serum obtained after convalescence.

3. Discussion

The cause of the brain disorder has not been identified in this case. The history of West syndrome and intractable hypoglycemic attacks suggest the possibility of congenital metabolic errors, and the repetition of MRI changes in the white matter mimics mitochondrial encephalopathy, myopathy, lactic acidosis, and stroke-like episodes (MELAS). However, the lack of abnormal data in the endocrine and metabolic tests using blood, urine, and CSF, and the absence of a lactate peak on MRS did not support the diagnosis of metabolic errors or MELAS. This patient had three episodes of status and clusters of partial seizures, paresis was found in two episodes, and MRI showed focal abnormalities with subsequent brain atrophy in the involved hemisphere. These findings were partly similar to those of the HHE syndrome [5]. However, the repetition of epileptic episodes is not observed in HHE syndrome. Although the IL-6 levels in the CSF are increased in epileptic status, the abnormalities of markers of DNA oxidative stress and axonal damage at the first episode (Table 1) indicated the possibility of encephalopathy [6]. Anticonvulsants, such as gabapentin and lamotrigine, seemed to be effective, although the improvement may reflect the natural course of disease. In addition, the corpus callosotomy at the age of 2 years is likely to lead to the repetition of hemiconvulsions.

In addition to Rasmussen encephalitis [1] and anti-NMDA receptor antibody encephalitis [3], focal encephalitis has been reported in Japan, and convulsions, psychiatric disorders, and/or involuntary movements persist for months in this disease. MRI, single photon emission computed tomography, or positron emission tomography (PET) occasionally revealed focal lesions in the CNS. A high dose of methylprednisolone and/or immunoglobulin ameliorated the symptoms in patients, and the involvement of autoimmune mechanisms was speculated. However, established antineuronal autoantibodies have not been detected in these cases. Immunohistochemistry using patient sera and brain sections verified the selective

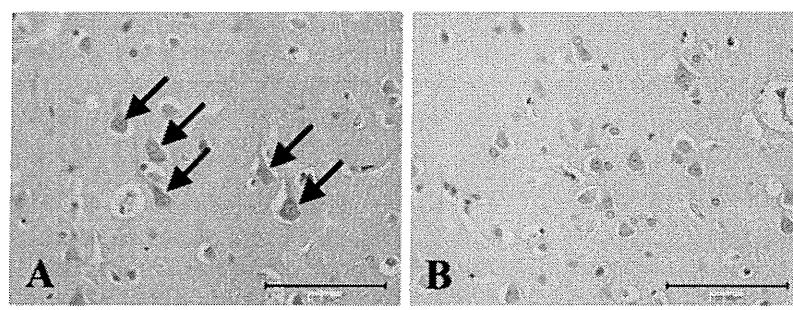

Fig. 2. Immunohistochemical findings The pyramidal neurons (arrows) showed strong immunoreactivity, which was discriminated from the weak immunoreactivity in the neuropil, in the middle temporal cortex in the control using serum obtained at the second episode (A). This reactivity was not seen with serum obtained after convalescence (B).

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binding of autoantibodies with the neurons in the hippocampus and striatum in patients with autoimmune limbic encephalitis [7] and autoimmune dystonia [8], respectively. We attempted such immunohistochemistry in a 1-year-old girl, who developed aphasia and dystonia. Neuronal immunoreactivity was detected in the cerebral cortex and basal ganglia, and these areas demonstrated abnormalities in fluorodeoxyglucose-PET study [9]. In this case, immunohistochemistry using serum obtained from the second episode but not from that obtained during convalescence revealed the binding of serum immunoglobulin with the neurons in the sections. The brain regions showing neuronal immunoreactivity were in accordance with the symptoms and MRI findings. It should be noted that focal encephalopathy that is characterized by convulsions, psychiatric disorders, and/or involuntary movements and that persists for months may occur in children, and immunohistochemistry of sera may be useful for clarifying the pathogenesis in such patients. The Purkinje cells in the cerebellar cortex express glutamate receptor δ2, and an autoantibody against this receptor is involved in cerebellitis [10]. Since this case showed neither cerebellar signs nor MRI changes in the cerebellum, the reason for the positive test results of the glutamate receptor δ2 antibody is not clear. On the basis of the immunohistochemical data and changes in the CSF markers, we speculate the possible involvement of an autoimmune process that was triggered by varicella infection in this case. However, the symptoms vanished in the absence of immunomodulatory treatment. Even in Rasmussen encephalitis, the mere presence of an autoantibody cannot verify the involvement of autoimmune process [1]. The exact pathomechanism in this case still remains to be investigated.

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Original article

Cutaneous adverse drug reaction in patients with epilepsy after acute encephalitis

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Abstract

Patients with epilepsy after encephalitis/encephalopathy (EAE) often have refractory seizures, resulting in polytherapy with the risk of adverse reactions due to anti-epileptic drugs (AEDs). We focused on the characteristics of cutaneous adverse reaction (CAR).

In this retrospective study, the medical records of 67 patients who were diagnosed as having EAE in our hospital were reviewed and the clinical characteristics were analyzed. Immunological biomarkers including cytokines, chemokines, granzyme B, soluble tumor necrosis factor receptor 1 (s-TNFR 1), matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) were measured in 22 patients. CARs attributed to AEDs were observed in 16 of 67 EAE patients (23.9%) (CAR group). High CAR rates were observed with phenytoin, lamotrigine, phenobarbital, and carbamazepine. Severe CARs were found in three of 67 patients (4.5%). The frequencies of CARs were significantly higher in patients with encephalitis onset older than five years of age. CAR occurred only in patients who had onset of EAE within 6 months after encephalitis. The durations from acute encephalitis to CARs were within one year for almost all AEDs, except lamotrigine. The proportion of patients with serumregulated on activation normal T cell expressed and secreted (RANTES) levels higher than the upper limit of normal range was significantly higher in CAR group than in non-CAR group. Patients in the early stage of EAE and patients with encephalitis onset older than five years of age may be at higher risk of CARs to AEDs, especially to phenytoin, lamotrigine, phenobarbital, and carbamazepine. RANTES may be a biomarker for susceptibility to CARs in EAE patients.

Keywords: Epilepsy; Encephalitis; Cutaneous adverse drug reaction; RANTES; Antiepileptic drug

1. Introduction

Epilepsy after encephalitis/encephalopathy (EAE) is reported to occur in 16.4% of patients with acute encephalitis [20,2]. Epileptic seizures in patients with EAE are often intractable. Among 383 pediatric patients admitted between 1993 and 1994 to our epilepsy center for the treatment of intractable epilepsy, 40 patients (10.4%) had EAE, 35 patients had epilepsy related to cerebral malformation, and 14 patients had epilepsy related to neuro-cutaneous syndrome [3]. Thus our data suggest that encephalitis/encephalopathy (encephalitis) is the most frequent etiology in pediatric patients with intractable epileptic seizures in Japan. Intractable epileptic seizures in patients with EAE tend to result in

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polytherapy with AEDs, with the risk of adverse reaction caused by AEDs.

Immunological mechanisms have been reported to be involved in the acute stage of some types of encephalitis [4–6]. Analysis of cytokine levels in influenza virus-associated encephalopathy revealed that cytokines are produced by peripheral blood mononuclear cells (PBMC), and that CSF IL-6 level is a useful indicator of the severity of disease [4]. The CSF concentrations of IL-6 in patients with nonherpetic acute limbic encephalitis (NHALE) were significantly higher than those in controls ($p < 0.001$) [5]. Antibodies to glutamate receptor (GluR) are known to contribute to the pathophysiological mechanisms in acute encephalitis including NHALE [6–8]. These immunological factors augmented by encephalitis may persist from the acute to chronic stage of acute encephalitis, and may affect EAE.

Cutaneous adverse reactions (CARs) are divided into maculopapular exanthema, exanthema pustulosis, Steven Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug-induced hypersensitivity syndrome (DIHS). Maculopapular exanthema is the most common CAR [9]. Although the pathophysiological mechanisms of CAR have not been fully elucidated, maculopapular exanthema may be ascribed to delayed hypersensitivity reactions (type IV) with activation of eosinophils and cytotoxic T cells (CTL) [10]. On the other hands, recent studies of severe CARs including SJS and TEN suggest that HLA-linked T-cell mediated pathophysiology may be associated with SJS caused by carbamazepine (CBZ) [11–13]. Cytokines are thought to play a role in acute and/or immune-mediated adverse drug reactions due to their ability to regulate the innate and adaptive immune systems. They control both the intensity and type of immune response mounted by stimulating or suppressing different cells [9]. We investigated the clinical characteristics of CARs, and attempted to identify the immunological markers for CARs in patients with EAE.

2. Methods

A total of 67 patients (39 males, 28 females) with a diagnosis of EAE were treated in our epilepsy center between February 1996 and May 2009. We conducted a retrospective study by reviewing the medical records of these patients and sending questionnaire to their primary physicians. The 67 patients were divided into a group with (CAR group, $n = 16$) and a group without CAR (non-CAR group, $n = 51$). We examined the serum cytokine and chemokine profile at the remote stage after CAR. Serum concentrations of IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-γ, eotaxin, IL-8, IP-10, monocyte chemotactic protein (MCP-1), macrophage inflammatory protein (MIP)-1a, MIP-1b, tumor necrosis factor receptor (TNF)-α, and RANTES (regulated on activation normal T cell expressed and secreted, CCL5) were measured using the BioPlex suspension array system (BIO-RAD, San Francisco, CA). Soluble tumor necrosis factor receptor (sTNFR) 1 was determined by an enzyme-linked immunosorbent assay (ELISA) kit [Human sTNF-R (60 kDa) ELISA, Cosmo Bio BMS03], and granzyme B was also examined by an ELISA kit (Cat. No. KT-078, Kamiya Biomedical Company, Seattle, WA, USA). Serum concentrations of matrix metalloproteinase-9 (MMP-9) were determined using an activity assay kit and tissue inhibitor of metalloproteinase-1 (TIMP-1) with a sandwich-type ELISA kit (Amersham, Buckinghamshire, England) according to manufacturer's recommendation. The MMP-9 kit measures both the pro- and active forms of MMP-9. Statistical analyses were conducted by Mann–Whitney test and χ^2 test and singinicance was set at $p < 0.05$.

3. Results

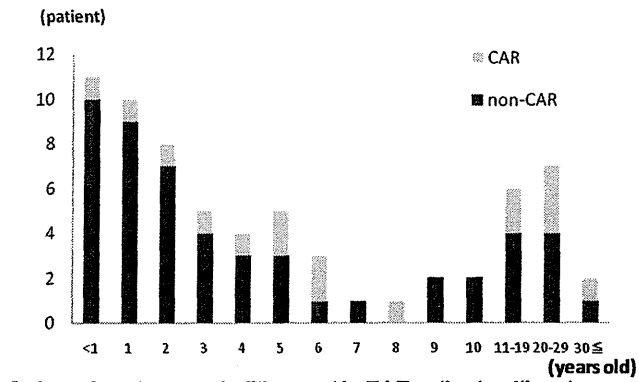
3.1. Background of 67 patients with EAE

The etiologies of acute encephalitis were known in 37 patients (55%), including influenza virus in 14 patients (21%), herpes simplex virus in 7 patients (10%), and human herpes virus (HHV)-6 in 4 patients (6%). Norovirus, rotavirus, adenovirus, Kawasaki disease, Coxsackie virus A, Coxsackie virus B, herpangina, *Escherichia coli* O-157, and pertussis were also recorded as causative disease or agent in single patient. The others' etiologies were unknown and we have no date of their antibodies to VGKC, GAD, GABA-B, NMDAR complex, or GluRs in acute stage of encephalitis. The mean age of acute encephalitis onset was 8 years and 4 months (2 months to 64 years, median age 3 years and 11 months ; $n = 67$) (Fig. 1A). The mean latency from onset of acute encephalitis to onset of epilepsy was 6 months (0 month to 7 years and 3 months, median 0 month; $n = 67$) (Fig. 1B).

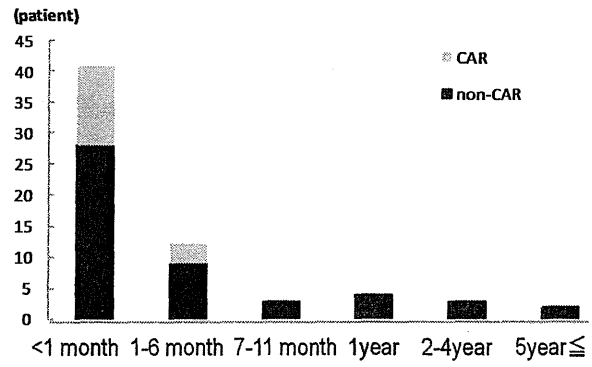
Adverse effects due to AEDs were recorded in 32 patients (47.8%), including sleepiness in 26 patients (38.8%) and CARs in 16 patients (23.9%) (Fig. 1C). Many patients were treated by polytherapy with AEDs, and the frequency of prescription was in the order of vaproic acid (VPA) > CBZ > zonisamide (ZNS) > clobazam (CLB) > phenobarbital (PB).

3.2. Clinical characteristics of CARs

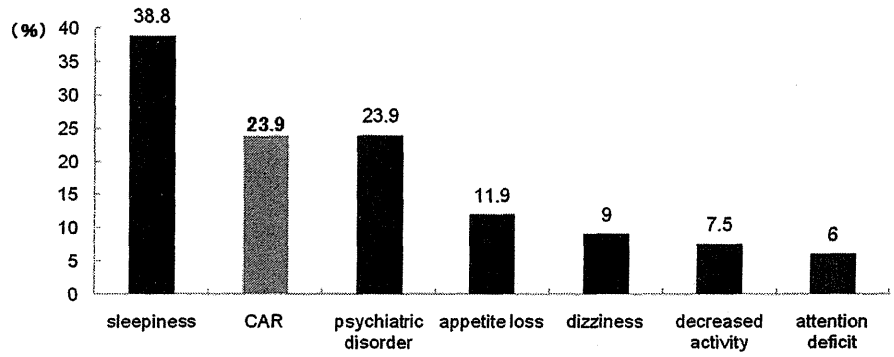
The overall frequency of CARs in EAE patients was 23.9% (16 of 67 patients). The sex ratio was not significantly ($p = 0.054$) different between CAR (6 males and 10 females) and non-CAR group (33 males and 18 females). The causes of encephalitis and the past histories were not significantly different between two groups.



A: Age of acute encephalitis onset in EAE patients with cutaneous adverse reactions (CAR) and those with non-cutaneous adverse reactions (non-CAR)



B: Latency from onset of acute encephalitis to onset of epilepsy in CAR and non-CAR patients



C. Frequencies of adverse reactions attributed to AEDs

Fig. 1. Clinical characteristics of adverse drug reactions to anti-epileptic drugs (AEDs) in patients with epilepsy after encephalitis/encephalopathy (EAE) ($n = 67$).

The CARs comprised of maculopapular exanthema (13 patients), SJS (two patients), and DIHS (one patient).

The mean onset age of acute encephalitis was 13 years and 3 months (8 months to 64 years and 6 months, median 6 years) in CAR group, and 7 years (0 month to 56 years and 1 month, median 3 years) in non-CAR group (Fig. 1A). The frequency of CAR was significantly ($p = 0.018$) lower in patients with encephalitis onset at 5 years or younger of age (13.2%) than in those with encephalitis onset over 5 years of age (37.9%).

The mean latency from onset of acute encephalitis to onset of epilepsy was not significantly ($p = 0.103$) different between CAR group (mean: 0.5 month; range: 0–5 months, median 0 month) and non-CAR group (mean: 8 months; range: 0 month to 7 years and 3 months, median 0 month). CARs occurred only in patients with latency less than 6 months (Fig. 1B).

CARs occurred almost exclusively within one year after onset of acute encephalitis for most AEDs, except lamotrigine (LTG) which was launched recently in Japan (Fig. 2). CAR occurred within 1 month from encephalitis onset in 61% of the patients, and within 6 months in 79%. The durations from the start of AEDs to appearance of CARs ranged from 1 to 3 weeks.

The frequencies of CARs caused by various AEDs are shown in Table 1. Phenytoin (PHT) and LTG had the highest frequency (25%), followed by PB and CBZ with frequencies higher than 10%. No CARs (0%) were attributed to ZNS, CLB, clonazepam (CZP) and gabapentin (GBP). The frequencies of CARs occurring within one year after encephalitis onset were 8/26 (30.7%) for PHT, 6/40 (15%) for CBZ, and 5/30 (16.6%) for PB. The mean numbers of AEDs used were 6.4 in CAR group and 5.4 in non-CAR group. The doses (mean ± standard deviation) were 136.6 ± 53.1 mg/day for PHT, 200 ± 100 mg/day for CBZ, 75 mg/day for PB, 62.5 ± 37.5 mg/day for LTG, and unknown for VPA. The mean serum levels were 3.1 μg/ml for PHT, 17.9 μg/ml for PB, and unknown for CBZ, VPA and LTG. The number of other co-administered AEDs to which CAR was observed ranged from 0 to 4 drugs (Table 2).

3.3. Severe CARs

Among 23 patients with CAR, two had SJS (caused by PHT in 1and by CBZ in 1) and one had DIHS (VPA + PB + PHT) (Table 2). The rate of severe CARs was three of 67 patients (4.5%), and SJS occurred

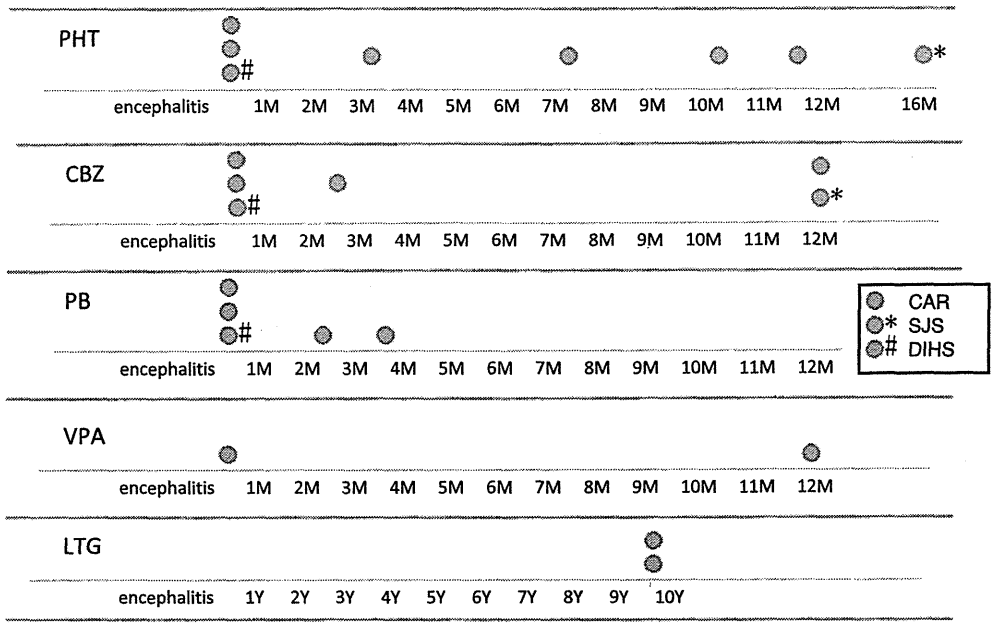


Fig. 2. The durations from acute encephalitis/encephalopathy to appearance of cutaneous adverse reactions (CARs) attributed to different AEDs.

Table 1
Frequencies of cutaneous adverse drug reactions attributed to various anti-epileptic drugs.

	Our data from 67 patients with epilepsy after encephalitis	Data of Arif et al. (2007) [15] from 1875 patients with epilepsy	Data of Hirsch et al. (2008) [16] from 1890 patients with epilepsy
Vaproic acid	3.6% (2 of 57 patients)	0.7%	1.7%
Carbamazepine	11.1% (6 of 54 patients)	3.7%	8.3%
Zonisamide	0% (0 of 48 patients)	Not shown	6.9%
Clobazam	0% (0 of 37 patients)	Not shown	2.1%
Phenobarbital	14.2% (5 of 35 patients)	Not shown	6.2%
Phenytoin	25% (8 of 32 patients)	5.9%	11.9%
Clonazepam	0% (0 of 23 patients)	Not shown	Not shown
Gabapentin	0% (0 of 13 patients)	0.3%	0.9%
Lamotrigine	25% (2 of 8 patients)	4.8%	8.9%

exclusively in patients with post-influenza EAE. One of two patients with SJS had encephalopathy caused by influenza B virus at 5 years and 5 months of age, and onset of epilepsy at 5 years and 9 months of age. She developed SJS one week after starting PHT at age 6 years and 9 months. The other had encephalitis caused by influenza virus at 11 years of age, and onset of epilepsy just after onset of encephalitis. She developed SJS a few days after starting CBZ. The patient with DIHS had encephalitis caused by unknown virus at 8 months of age, and onset of epilepsy just after onset of encephalitis. DIHS appeared 15 days after onset of encephalitis while midazolam, phenobarbital, ^{99m}Tc-ethyl cysteinate dimer, PB, VPA, and PHT were prescribed. Because PCR of HHV-6 was positive, the patient was diagnosed as DIHS and treated with steroid therapy. One of two patients with SJS has HLA-A*2402/2601, HLA-B*4002/5201, and the other has HLA-A*2402/0201 and HLA-B*1518/4002.

3.4. Successful re-start of AEDs that had caused CARs

Three patients were successfully re-started on the AEDs that had been discontinued because of CARs (Fig. 3). Patient 1 with allergy to eggs and milk had encephalitis caused by herpes simplex virus. She started LTG at 10 years after the onset of EAE. CAR appeared on her lip, face, thoracodorsal trunk and limbs on the day when the dose was increased to 100 mg/day from 50 mg/day. LTG therapy was ceased, in spite of complete control of seizures after a few days of LTG treatment. Since a drug-induced lymphocyte stimulation test (DLST) to LTG was negative, we attempted to re-initiate LTG treatment 14 months after the CAR, starting from an extremely low dose of 2 mg/day. Thereafter, the dose of LTG was titrated gradually in increments of 5 mg/day per week. Seizure decreased at the dose of 30 mg/day and disappeared at 35 mg/day. Patient 2 had no allergy history and encephalitis was caused by

Table 2
Clinical characteristics of cutaneous drug adverse reactions (CARs) attributed to various anti-epileptic drugs (AEDs).

	Phenytoin (PHT)	Carbamazepine (CBZ)	Phenobarbital (PB)	Vaproic acid (VPA)	Lamotrigine (LTG)
Numbers of patients	8	6	5	2	2
Severe CARs	SJS, 1 DIHS, 1 (PHT + PB + VPA)	SJS, 1	DIHS, 1 (PHT + PB + VPA)	DIHS, 1 (PHT + PB + VPA)	
Duration from acute encephalitis/encephalopathy to CARs	<1 M, <1 M, <1 M, 3 M, 7 M, 10 M, <1 Y, 1 Y	<1 M, <1 M, <1 M, 2 M, 1 Y, 1 Y	<1 M, <1 M, <1 M, 2 M, 3 M	<1 M, <1 Y	9 Y, 9 Y
Duration from AED initiation to CAR	7 d, 7 d, 10 d, 11 d, 15 d, unknown 2	6 d, unknown 5	12 d, 23 d, 24 d, unknown 2	Unknown 2	16 d, 17 d
Dose of AED	70 mg, 140 mg, 200 mg unknown 5	100 mg, 300 mg Unknown 4	75 mg unknown 4	Unknown 2	25 mg, 100 mg
Serum level of AED	3.1 μg/ml, Unknown 7	Unknown 6	17.9 μg/ml, unknown 4	Unknown 2	Unknown 2
Number of other co-administered AEDs to which CAR was observed	0 drug : 2 1 drug : 2 2 drugs : 3 3 drugs : 1	0 drug : 2 1 drug : 2 Unknown : 1	0 drug : 2 1 drug : 2 3 drugs : 1	2 drugs : 1 3 drugs : 1	2 drugs : 1 4 drugs : 1

SJS, Steven–Johnson syndrome; DIHS, drug-induced hypersensitivity syndrome. Three patients had severe AED-related eruption: 2 had SJS induced by PHT or CBZ, one had DIHS induced by PHT, PB and VPA. The durations from the start of AEDs to appearance of CARs ranged from 1 to 3 weeks.

unknown virus. His mother had a history of asthma. CBZ 300 mg/day was started 2 months after the onset of epilepsy, and discontinued on the 5th day because of CAR. At 3 months after the cessation of CBZ, we attempted to re-initiate from 100 mg/day, titrating in increments of 100 mg/day per week to 550 mg/day, but CAR did not recur. Patient 3 had no allergy history and encephalitis was caused by influenza virus. His grandfather had allergy to bee and rubber, and his mother had a history of asthma. PB 75 mg/day was started at 3 months after the onset of epilepsy, and discontinued after two weeks because of fever, pneumonia, CAR (face and limbs), and lip swelling. At 8 months after the cessation of PB, we re-initiated PB from 10 mg/day, titrating in increments of 5 mg/day per 2–4 weeks up to 75 mg/day. Partial seizure control was achieved (Fig. 3).

3.5. Cytokines and chemokines

We measured serum concentrations of cytokines and chemokines in 22 patients, and compared between CAR group (10 patients) and non-CAR group (12 patients). All samples were collected more than one year after CARs. Clinical background of the two groups was not different. IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, FGF basic, G-CSF, GM-CSF, IP10, MCP-1, MIP1a, MIP1b, IFNγ, eotaxin, MMP-9, TIMP-1, granzyme B were not significantly different between CAR group and non-CAR group. The proportion of patients with serum RANTES level higher than the upper limit of normal range was significantly ($p = 0.017$) higher in CAR group (90%) than in non-CAR group (50%) (Fig. 4).

4. Discussion

We studied the clinical characteristics and immunological markers of CARs in 67 patients with EAE. The frequency of CAR in EAE patients (23.9%) was very high compared with our data in consecutive intractable epileptic patients (0.64%) [14] and the data in general epileptic patients from the Columbia Comprehensive Epilepsy Center in USA (15.9%) [15]. The frequencies of CARs in patients with EAE treated with AEDs were 25% with PHT, 25% with LTG, 14.2% with PB, 11.2% with CBZ, and 3.6% with VPA. The frequencies of CARs to these AED in EAE were higher than the data for general epileptic patients provided by Japanese pharmaceutical companies and those published in the USA [15,16] (Table 1). PHT, LTG, PB and CBZ were associated with the highest rates of CARs both in Japanese EAE patients observed in the present study and in general epileptic patients in USA published by Arif et al. [15] and Hirsh et al. [16]. However, the CAR rates of PHT, LTG and PB in EAE patients more than double the rates in general epileptic patients.

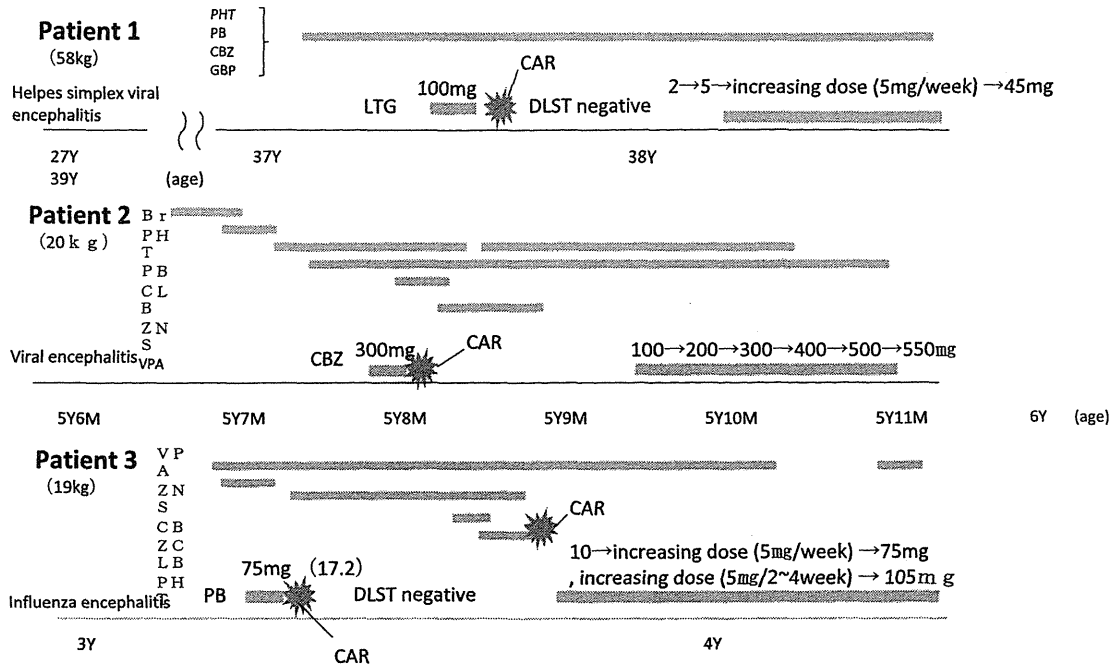


Fig. 3. Clinical courses of three cases of successful re-initiation of AEDs that had been discontinued due to CARs.

These data may suggest that patients with EAE have pathophysiological background that induces CARs to AEDs. On the other hand, we found no CARs attributed to ZNS, CLB, CZP, GBP in Japanese EAE patients. ZNS was associated with a high rate of CARs in general epileptic patients in USA, but did not cause CARs in EAE patients in Japan. Further evaluation of the contribution of genetic background to ZNS-associated CAR is required.

The study of epidemiological and clinical features of severe CARs suggested that they were predominantly female [17]. There may be female predominance in CAR group of EAE patients, although statistical significance between CAR and non-CAR group was not confirmed ($p = 0.054$).

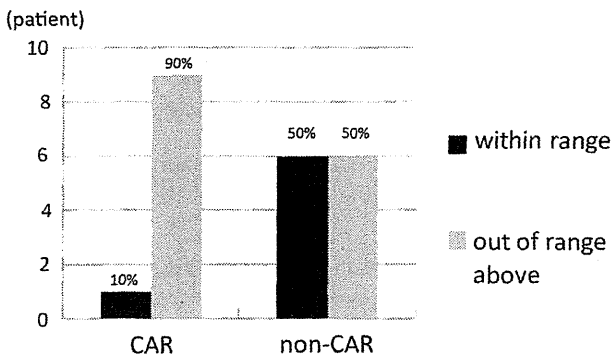


Fig. 4. Numbers of patients having serum RANTES levels within normal range or higher than upper limit of normal range in patients with cutaneous adverse reactions (CARs) and those with non-cutaneous adverse reactions (non-CAR). RANTES: regulated on activation normal T cell expressed and secreted.

The frequency of CAR was significantly higher in patients with encephalitis onset over five years of age. The frequency was extremely high (42.9%) in EAE patients who had onset of encephalitis between 20 and 29 years of age. NHALE is considered to have immunological pathophysiology including antibodies to GluR, and onsets of many patients with NHALE are between 20 to 29 years of age [18]. These may suggest that age-related distribution of immune-mediated encephalitis affects the incidence of AED-associated CARs.

CARs occurred only in patients who had onset of EAE within 6 months after encephalitis, and 61% of CARs occurred less than 1 month after the onset of encephalitis. These data suggest that CARs tend to occur in the early stage of EAE that develops early after encephalitis. Therefore, the pathophysiological condition of acute encephalitis may contribute to AEDs-related CARs, and the mechanisms of epileptogenesis in EAE may share common factors with the mechanisms of CARs.

CARs associated with traditional AEDs (CBZ, PB, PHT, VPA) appeared exclusively within one year from the onset of acute encephalitis, but CARs caused by LTG occurred a few years after encephalitis onset. These data may suggest that CARs caused by aromatic AEDs occur rarely later than one year after encephalitis while CARs caused by LTG (non-aromatic AED) can occur many years after encephalitis. However, the characteristic of LTG-related CARs is associated with a factor independent of encephalitis. LTG was launched in

Japan on 12th December 2008, and therefore we had few patients who were treated with LTG soon after encephalitis. Therefore, the rate of CAR related to LTG given soon after the onset of encephalitis should be further studied.

AEDs that caused non-serious CARs were tolerated in three patients by re-induction at lower initial doses and lower speed of titration. These data may suggest that non-serious CARs in EAE patients are not necessarily the idiosyncratic CAR defined by HLA [11,12], and that other evolutional factors; for example, immunological factors stimulated by encephalitis, may contribute to the CARs induced by AEDs.

Serious CARs were found in three patients, SJS in two and DIHS in one. The rate of severe CARs (4.5%) was also higher than that in general intractable epileptic patients in our epilepsy center (0.097%) [14] and in previous report (1/10,000) [10]. SJS occurred only in patients with EAE after influenza-associated encephalopathy and longer than one year after encephalopathy. Cytokine storm sometimes occurs in influenza-associated encephalitis [4]. In these cases, the strong immunological reactions in the acute stage of encephalopathy may continue until the recovery stage, and contribute to the development of SJS. Furthermore, HLA-B*1518 alleles have been shown to be associated with higher relative risk (above 10.0) of severe CARs [11], and one of two patients with SJS in our study has HLA-A*2402/0201 and HLA-B*1518/4002. Therefore, pharmacogenomic predisposition may contribute to the SJS also in patients with EAE.

We had three patients who could be successfully tolerated by the AEDs that had been discontinued because of CARs. In a patient, DLST of the AED was negative. Some reports described that DLST was useful to identify the causal relationship of drug allergies, and sensitivity and specificity of DLST were reported as 24.8% and 93.1%, respectively [19]. But there is no report to support that DLST is relied upon to determine as if a drug could be re-start. DLST may provide us with useful information, but further investigations are needed to argue whether DLST could be reliable biomarker for re-start.

We found elevated serum RANTES levels in patients who had completely recovered from CAR. RANTES is a chemokine secreted by NK cells, $CD4^+$T cells, $CD8^+$ T cells, and recruited T cells, eosinophils, and basophils [1,21,22]. CARs are causally related to four types (types IVa–d) of delayed hypersensitivity reactions mediated by T cell subpopulations, and type IVb reaction causes maculopapular exanthema with activated eosinophils [10]. Maculopapular exanthema is the most common type of delayed skin reaction showing infiltration of T cells and eosinophils in the skin, and disappears shortly after the discontinuation of drugs [9]. We hypothesize that increased chemotactic factors for eosinophils (RANTES) in EAE patients recruit eosinophils that contribute to the maculopapular exanthema. AEDs are known to cause CARs relatively frequently [9], and AED-specific reactive T cells have been demonstrated in the blood of patients [10]. T cells activated by encephalitis may cross-react with AEDs as a result of T cell receptor degeneracy. Further investigations are needed to examine the evolutional changes of immunological factors including RANTES, in order to confirm the mechanisms of how AEDs induce CARs in patients with EAE.

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Case report

An immunologic case study of acute encephalitis with refractory, repetitive partial seizures

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Abstract

Acute encephalitis with refractory, repetitive partial seizures (AERRPS) is a neurologic syndrome characterized by extraordinarily frequent and refractory partial seizures, which immediately evolve into refractory epilepsy. To elucidate the pathophysiology of AERRPS, we performed an immunologic study of an affected boy, revealing decreased natural killer (NK) cell activity in the peripheral blood mononuclear cells. IgG antibodies against the glutamate receptor (GluR)ε2, ζ1, and δ2 subunits were all positive in both the serum and cerebrospinal fluid (CSF). There were raised plasma concentrations of interleukin (IL)-2, IL-6, IL-10, tumor necrosis factor-α, and interferon-γ as well as an extremely elevated CSF level of IL-6. These findings suggest that AERRPS is immune-mediated encephalitis, in which both autoimmunity and exaggerated cytokine production are involved. NK cell dysfunction may be the underlying abnormality in this AERRPS case, which might have contributed to the production of GluR autoantibodies.

Keywords: Acute encephalitis with refractory, repetitive partial seizures; Natural killer cell; Glutamate receptor antibody; Autoimmunity; Hypercytokinemia

1. Introduction

Acute encephalitis with refractory, repetitive partial seizures (AERRPS) is a neurologic syndrome characterized by acute onset of extraordinarily frequent and refractory partial seizures. The seizures occur following antecedent febrile illness, and switch over to refractory epilepsy without a latent period [1]. Because the same entity appeared to be reported with different nomenclatures including AERRPS, a more comprehensive term "fever-induced refractory epileptic encephalopathy in school-aged children" has recently been proposed [2].

Although the exact pathophysiology of AERRPS remains unknown, it has been postulated that antecedent infection triggers early production of functional antibodies, which may gain access to the central nervous system (CNS) and cause neuronal hyperexcitability [1,3]. The autoimmune hypothesis is supported by the finding that some patients with AERRPS exhibit the presence of antibodies against the *N*-methyl-D-aspartate (NMDA)-type

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glutamate receptor (GluR)ε2 subunit [1]. However, extensive immunologic studies are required to elucidate the possible immune-mediated mechanism of AERRPS. In the present article, we performed the first thorough immunologic evaluation of a patient with AERRPS, which provided some insight into the pathophysiological mechanism underlying AERRPS.

2. Case report

A 7-year-6-month-old previously healthy boy was admitted to a local hospital because of a 5-day history of fever and cough. A physical examination was reported to have revealed a neurologically normal child. Laboratory examinations showed a white blood cell count of 4000/μl and a negative serum reactive C protein value. Serum antibodies against *Mycoplasma pneumoniae* and influenza virus were negative. Chest X-rays revealed consolidation in the right lung. A diagnosis of atypical pneumonia was made. Intravenous administration of clindamycin was initiated, and the fever subsided on the following day. On the 4th hospital day, he gradually lost consciousness and eventually suffered recurrent seizures. Because of this, the patient was admitted to our hospital. On admission, neurologic examination revealed a sluggish light reflex of the pupils and hyperactive tendon reflexes with a bilateral extensor plantar response. A physical examination was unremarkable. The family history was not contributory. Laboratory examinations revealed a white blood cell count of 15,200/μl and a negative serum reactive C protein value, with normal liver and kidney function, serum electrolyte, blood sugar, and plasma ammonia results. Serum antibodies against herpes simplex virus, cytomegalovirus, Epstein–Barr virus, *M. pneumoniae*, and *Coxiella burnetii* were negative. Chest X-rays were normal. Cranial magnetic resonance imaging (MRI) was unremarkable. The patient required artificial ventilation because of numerous recurrent partial seizures, which began with twitching of the left side of the face and subsequently propagated into jerking of the left side of the body, followed by convulsions of the right upper limb. The seizures recurred every 5–10 min, and were refractory to intravenous administration of midazolam, phenytoin, lidocaine, or thiopental. Ictal electroencephalogram (EEG) revealed that the seizure activity originated from the right occipital region, and then spread into the left occipital area. A lumbar puncture on the 2nd hospital day revealed a cell count of 203/μl (mono. 39, poly. 164), a protein level of 30 mg/dl, and a sugar level of 84 mg/dl. Oligoclonal band or myelin basic protein in the cerebrospinal fluid (CSF) was negative. Bacterial and viral cultures of the CSF were sterile. A diagnosis of AERRPS was made, and methylpredonisolone pulse therapy (30 mg/kg/day × 5 days i.v.) and intravenous administration of high-dose immunoglobulin (300 mg/kg/day × 3 days i.v.) were initiated on the 3rd hospital day, without effect. Brain single-photon emission computed tomography using ^{99m}Tc-ethyl-cysteinate dimer disclosed a circumscribed area of hyperperfusion in the medial region of the right occipital lobe (Fig. 1A). Second cranial MRI on the 4th hospital day revealed a high-intensity area in the same region in diffusion-weighted sequences (Fig. 1B). Fluid attenuated inversion recovery MRI of the brain was unremarkable. Although treatment with high doses of thiopental, midazolam, and lidocaine led to the disappearance of apparent clinical seizures, frequent electrographic seizures were still detectable on

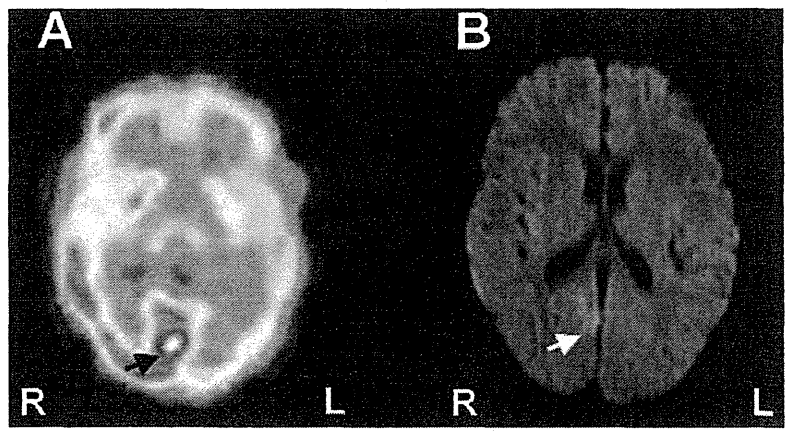

Fig. 1. Brain imaging of a 7-year-6-month-old boy with AERRPS. (A) Ictal ^{99m}Tc-ECD SPECT of the brain performed at the 3rd hospital day revealing a circumscribed area of hyperperfusion (arrow) in the medial region of the right occipital lobe. (B) Diffusion-weighted brain MRI (TR 3500, TE 90) performed at the 4th hospital day revealing a high-intensity area (arrow) located in the same region as that on ^{99m}Tc-ECD SPECT, suggestive of cytogenic edema closely related to the seizure focus.

long-term monitoring EEG. On the 9th hospital day, the patient died following sudden cardiac arrest. No autopsy was permitted.

3. Immunologic data (Table 1)

The immunologic examination was performed before the intravenous administration of methylpredonisolone and immunoglobulin. Natural killer (NK) cell activity in the peripheral blood mononuclear cells examined at effector/target cell ratios of 10:1 and 20:1 was 4.9% (normal, 8.9–29.5%) and 9.9% (normal, 17.1–48.7%), respectively. The CD 2 lymphocyte (T cells and most NK cells) and CD 20 lymphocyte (B cells) fractions in the peripheral blood were 51.2% (normal, 71.0–91.0%) and 46.9% (normal, 3.0–20.0%), respectively. The plasma concentrations of interleukin-2 (IL-2), IL-6, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were elevated, while only the IL-6 concentration was significantly elevated in the CSF. IgG antibodies against the GluRε2 subunit, the GluRζ1 subunit, and the GluRδ2 subunit were measured with the ELISA method, and were all above the mean value + 2 standard deviations in both the serum and CSF.

4. Discussion

The present case was consistent with the diagnostic criteria of AERRPS [1], although evolution into refractory epilepsy could not fully be documented because of the patient's death. To our knowledge, this is the first report describing a possible association between AERRPS and NK cell-related immune dysfunction. NK cells comprise a subset of peripheral circulating lymphocytes, and constitute a major component of the innate immune system [4]. They exert an initial prompt response to viral infection, and kill infected or transformed cells by releasing small cytoplasmic granules of proteins called perforin and granzyme or immunomodulatory chemokines and cytokines such as TNF-α and IFN-γ [4].

Recent reports have suggested a link between immunodeficiency and autoimmune encephalitis [5,6]. Akman et al. [5] described a patient in whom non-paraneoplastic limbic encephalitis developed as the first clinical manifestation of common variable immune deficiency. The patient had serum antoantibodies against glutamic acid decarboxylase, which is the rate-limiting enzyme for the synthesis of gamma-aminobutyric acid. A similar observation was made by Verhelst et al. [6], who described a

Table 1
Immunologic investigation of the patient with AERRPS.

1. Humoral immunity			4. Cytokines		
Serum protein fraction			*Plasma*		
Albumin	57.5%	(60.8–71.8)	IL-1β	0.89 pg/ml	(<3.4)
α1-Globulin	4.6%	(1.7–2.5)	IL-2	15.1 pg/ml	(<4.5)
α2-Globulin	12.2%	(5.7–9.5)	IL-4	3.0 pg/ml	(<15.0)
β-Globulin	10.9%	(7.2–11.1)	IL-6	46.6 pg/ml	(<19.9)
γ-Globulin	14.8%	(10.2–20.4)	IL-10	15.8 pg/ml	(<14.2)
Immunoglobulin			TNF-α	94.1 pg/ml	(<11.1)
IgG	911 mg/dl	(744–1719)	IFN-γ	204.2 pg/ml	(<42.9)
IgM	93 mg/dl	(27–215)	*CSF*		
IgA	152 mg/dl	(44–208)	IL-1β	0.80 pg/ml	(<1.4)
Complement system			IL-2	<2.6 pg/ml	(<4.6)
C3	130 mg/dl	(80–140)	IL-4	<2.6 pg/ml	(<11.6)
C4	51 mg/dl	(11–34)	IL-6	9035.8 pg/ml	(<9.7)
CH50	48 U/ml	(39–45)	IL-10	7.7 pg/ml	(<6.1)
Lymphocyte subset			TNF-α	4.0 pg/ml	(<6.2)
CD2	51.2%	(71–91)	IFN-γ	<7.1 pg/ml	(<46.6)
CD4	26.2%	(25–54)	5. Anti-glutamate receptor antibodies		
CD8	18.9%	(23–56)	*Serum*		
CD20	46.9%	(3–20)	IgG-GluRε2	Positive	
2. Cell-mediated immunity			IgG-GluRζ1	Positive	
Phytohemagglutinin	181,696 cpm	(59,481–179,402)	IgG-GluRδ2	Positive	
Stimulation index	449.7	(101.6–1643.8)	*CSF*		
Concavalin A	100,651 cpm	(51,307–131,043)	IgG-GluRε2	Positive	
Stimulation index	249.1	(74.3–1793.2)	IgG-GluRζ1	Positive	
3. Natural killer cell activity			IgG-GluRδ2	Positive	
10:1*	4.9%	(8.9–29.5)			
20:1*	9.9%	(17.1–48.7)			

AERRPS, acute encephalitis with refractory, repetitive partial seizures; CD, cluster of differentiation; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; CSF, cerebrospinal fluid; GluR, glutamate receptor. * denotes effector/target cell ratio. Normal ranges in parenthesis.