decline of PB concentration and this therapy was discontinued in only one.

PB is one of the barbiturate drugs that depress neuronal excitability by enhancing the γ-aminobutyric acid receptor mediated chloride current [8]. PB is commonly used to treat epilepsy orally and status epilepticus intravenously [9,10]. Since Crawford et al. reported that intravenous high-dose PB was found to be effective for refractory status epilepticus in children, high-dose PB therapy has been performed as an optional therapy for refractory status epilepticus [1,2]. Furthermore, non-intravenous high-dose PB therapy was used and found to be effective for intractable epilepsy [5–7]. According to our knowledge, however, most of the previous reports were anecdotal. That was the reason why we evaluated the effectiveness and safety of non-intravenous high-dose PB therapy for intractable epilepsy during childhood.

Of various types of seizure, those with motor components are supposed to be the best candidate for this therapy. Even among the patients for whom this therapy was not effective, seizure frequencies of Cases 7, 8, 9, and 11 in Table 1 decreased during the course of this therapy. When PB serum levels declined, their seizure frequencies had gradually increased. Since PB is an effective AED in the treatment of generalized tonic–clonic and simple partial seizures [11], high-dose PB therapy might be effective for intractable partial seizures with motor components. Meanwhile, present study showed that high-dose PB therapy might be an adjunctive therapy in order to discontinue continuous MDL infusion. Sudoh et al. also reported in three cases that non-intravenous high-dose PB therapy discontinued continuous MDL therapy in children [4]. These results indicate that high-dose PB therapy may be considered as an additional therapy for intractable partial epilepsy.

Some previous studies suggested that maintenance PB serum level remained from 40 to 80 μg/ml in high-dose PB therapy [1,2,5]. In our study, the maintenance PB serum level was more than 60 μg/ml among the patients for whom high-dose PB therapy was effective. This result was consistent with previous studies. However, a variation of the PB serum level was found in our study. One possible reason for this variation might be the difference in the methods of administration: intravenous and non-intravenous. We evaluated the effectiveness of this therapy at the steady-state of PB, and during this period patients received PB orally. Rossi et al. reported that the PB serum level of oral administration depended on the age and body weight of children with epilepsy or febrile seizures [12]. Fukuoka et al. reported that the PB serum level of oral administration depended on total body weight, total body water volume, body surface area and extracellular water volume in epilepsy patients including children [13]. We suggested that the variation of PB serum level in our study would depend on age and body weight rather than administration method.

Adverse effects were found in seven of 13 patients (54%). Severe adverse effects which required medical treatment or discontinuation of this therapy were found in two of 13 patients (15%). One of them required respiratory support, and the other showed Stevens–Johnson syndrome. Crawford et al. reported that all but one of the 40 patients required respiratory support and their maximum median PB serum level was 114 μg/ml [1]. Although there were two patients whose PB serum level was over 100 μg/ml during this therapy in our study, they did not have respiratory depressions. One patient who needed respiratory support showed airway obstruction due to hypersecretion of saliva. He was bedridden and tube-fed. His PB serum level at that time was about 50 μg/ml. We supposed that respiratory impairments might depend on the individual condition more than the PB serum level. Concerning respiratory support, non-intravenous high-dose PB therapy might have an advantage, compared to intravenous high-dose PB therapy. Meanwhile, PB is one of the potential causes of Stevens–Johnson syndrome [14]. We discontinued high-dose PB therapy on one patient due to Stevens–Johnson syndrome. This result indicates as follows: (1) we should take this syndrome into account, (2) we should discontinue the therapy immediately, and (3) we should perform the appropriate treatments such as steroid therapy for this syndrome. In the other five patients, their adverse effects were reversible without any medical treatments as their PB serum level decreased. These results indicated that high-dose PB therapy is relatively safe during childhood. To reduce the risk of the adverse effects during high-dose PB therapy, we suggest that it is preferred to maintain PB serum level less than 100 μg/ml and to monitor PB serum level frequently. Further investigations may be necessary to decide the dosage of PB.

The limitation of this study was the retrospective study in a small number of the patients. In addition, we could not compare the effectiveness and advantages of non-intravenous high-dose PB therapy with those of intravenous high-dose PB therapy. However, non-intravenous high-dose PB therapy was thought to be low risk of respiratory depression, and adverse effects of this therapy were reversible and treatable. Furthermore, it is easy to shift sequentially to the oral PB therapy. This is another advantage of this therapy.

We conclude that non-intravenously high-dose PB therapy is effective and may be considered as an additional treatment for intractable partial epilepsy in childhood.

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. None of the authors has any conflict of interest to disclose.

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Original article

Early infantile manifestations of incontinentia pigmenti mimicking acute encephalopathy

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Abstract

Objective: We retrospectively reviewed six patients with incontinentia pigmenti (IP) who had encephalopathic manifestations during early infancy.

Methods: We enrolled six patients who met the following criteria from the mailing list of the Annual Zao Conference: (1) diagnosis of IP; (2) encephalopathic manifestations with reduced consciousness and clusters of seizures by 6 months of age; and (3) no evidence of central nervous system infection or metabolic derangement.

Results: The onset of the encephalopathic events was within the first 2 months of life in all but one patient. All had clusters of focal clonic seizures. The duration of seizures was typically 5 min. The seizures ceased within 5 days in all patients. Various degrees of reduced consciousness were observed in association with the frequent seizures. Diffusion-weighted imaging during the acute phase showed reduced water diffusion in the subcortical white matter, corpus callosum, basal ganglia, thalami, and internal capsule in two patients. Scattered subcortical white matter lesions were observed on fluid-attenuated inversion-recovery images in two patients.

Conclusions: The encephalopathic manifestations in patients with incontinentia pigmenti were characterized by seizure clusters and reduced consciousness, albeit of relatively short duration. Magnetic resonance imaging abnormalities were predominant in the subcortical areas in most patients.

Keywords: Incontinentia pigmenti; Encephalopathic manifestation; MRI; Diffusion-weighted image; Early infancy

1. Introduction

Incontinentia pigmenti (IP) is a rare neurocutaneous syndrome characterized by skin lesions and disorders of various organs, including the central nervous system (CNS), eyes, teeth, and hair. The skin lesions specific to IP are present at birth or develop soon after birth. The skin lesions are classified into four stages: the vesicular, verrucous, pigmented, and atrophic scarring stages. Mutations of the NEMO (NF-κB essential modulator) gene located at Xq28 are responsible for IP [1]. NEMO is required for the activation of NF-κB, which protects against apoptosis and controls immune and

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inflammatory responses and cell adhesion [2]. IP cells with NEMO mutations lack NF-κB activation completely and are exquisitely sensitive to tumor necrosis factor alpha (TNF-α)-induced apoptosis [3]. The pathology of IP is characterized by extensive X-inactivation skewing [3], which reflects an efficient mechanism of counter-selection affecting cells expressing the mutated X chromosome. This extensive skewing is not seen in the antenatal epidermis, but in the epidermis after IP dermatosis.

One third of the patients with IP have CNS disorders, which manifest as seizures, microcephaly, mental retardation, hemiparesis, and spasticity. Several reports have described the neuroradiological findings and pathogenesis of IP, whereas the CNS manifestations of patients with IP are not fully understood.

We treated a patient with IP who had a cluster of severe seizures accompanied by reduced consciousness at 1 month of age. Although acute encephalopathy of unknown origin was first suspected in this patient, we later attributed the event to the CNS involvement of IP itself. We presented this patient at the Annual Zao Conference on Pediatric Neurology, where the clinical and neuroimaging features attracted the attention of the participants. Consequently, we attempted to clarify the features of the early infantile manifestations in children with IP mimicking acute encephalopathy. We present the results of a retrospective review of six patients with IP who had encephalopathic manifestations during early infancy.

2. Patients and methods

We collected patients who met the following criteria through the mailing list of the Annual Zao Conference on Pediatric Neurology: (1) diagnosis of IP based on the characteristic skin lesions; (2) encephalopathic manifestations with reduced consciousness, and seizure clusters or status epilepticus before 6 months of age; and (3) no evidence of CNS infection or metabolic derangement. The mailing list of the Annual Zao Conference includes more than 400 pediatric neurologists from all over Japan. This study was approved by the institutional review board of Juntendo University School of Medicine.

The patients were collected after we presented our patient (Patient 1) at the Annual Zao Conference in February 2007. Six patients who met the entry criteria were recruited, including our patient. We sent a structured questionnaire to each patient's attending pediatric neurologist. Magnetic resonance imaging (MRI) data were also collected. We reviewed the MRI and clinical features of the patients. At present, the mutation of the NEMO gene has not been examined in any of the patients.

3. Results

3.1. Patient report

The clinical course of Patient 1 was as follows. The patient was born after 38 weeks of gestation with a birth weight of 3354 g. Her mother had been diagnosed with IP, although the patient's older sister was not affected. Her perinatal history was unremarkable, although she was diagnosed with IP based on the histopathological findings of the characteristic skin lesions, which had appeared immediately after birth. She had a cluster of generalized convulsions lasting for a few minutes at 44 days of age. On admission, she was semi-comatose and had verrucous skin lesions. Her body temperature was 36.3 °C. The physical and neurological examination did not reveal any other abnormalities. Mild increases in white blood cells and eosinophils were observed (white blood cell count 15,800/μl with 12% eosinophils); no other abnormalities were found in the hematological, blood chemistry, or cerebrospinal fluid examinations. MRI the day after admission revealed patchy reduced diffusion in the subcortical and deep white matter, predominantly in the right frontal area, right thalamus, and basal ganglia (Fig. 1). On the same day, the electroencephalogram (EEG) showed right frontal dominant slowing of the background activity. Initially, she was diagnosed with acute encephalopathy of unknown origin and treated with glycerol, midazolam, dexamethasone, and acyclovir. Her convulsions were controlled after the dose of midazolam was increased to 0.3 mg/kg/h. She regained consciousness 10 days after the onset.

At 32 months of age, she presented with moderate mental retardation and mild left hemiplegia. Focal epilepsy developed at 9 months of age. Her seizures were characterized by clonic convulsions of the right upper and lower extremities with preserved consciousness. Phenobarbital was ineffective, and her seizures were controlled after gabapentin was added at 23 months of age. MRI at 10 months of age showed cystic encephalomalacia in the right frontal area predominantly (Fig. 1).

3.2. Patient characteristics (Table 1)

The patients were all female. Their pregnancy and delivery were unremarkable. Three patients had family histories of IP. All patients had vesicular eruptions appearing immediately after birth and were diagnosed with IP clinically or pathologically. Four patients had disorders in organs other than the skin and CNS: three had ocular disorders, one had a dental disorder, and one had superior vena cava syndrome. The average follow-up period was 47 months (range 7–123 months).

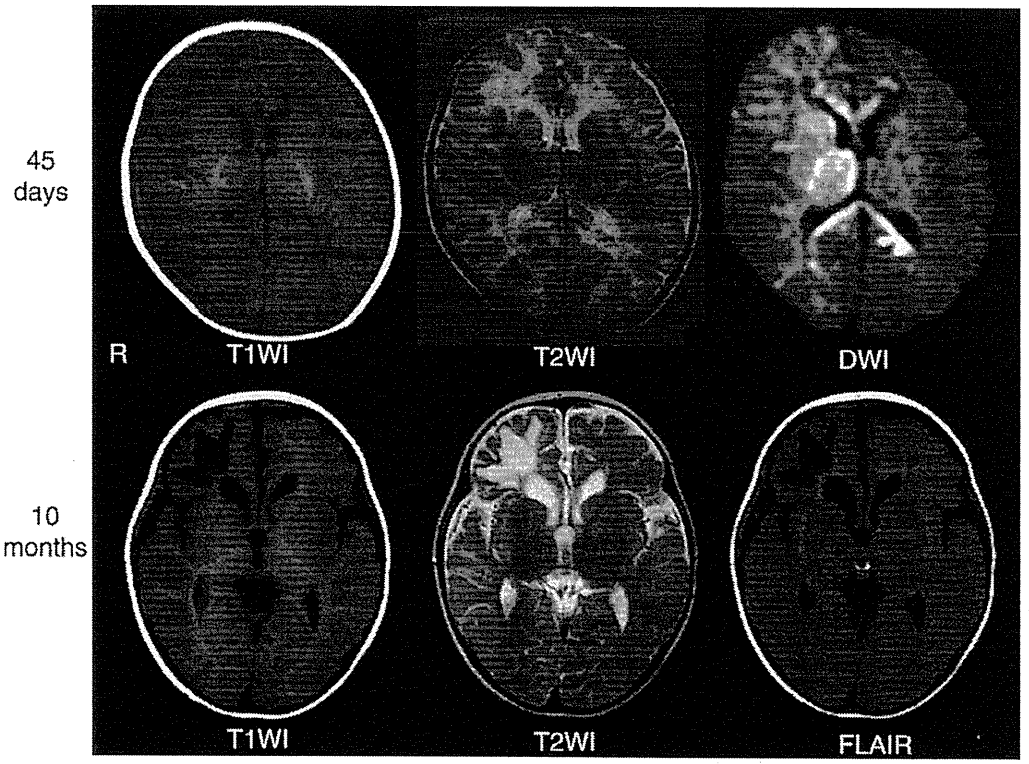

Fig. 1. MRI findings of Patient 1. Top: MRI at 45 days of age. Diffusion-weighted images revealed reduced diffusion predominantly in the subcortical white matter in the right frontal area. Reduced diffusion was also observed in the basal ganglia, thalamus, corpus callosum, and posterior limb of the internal capsule. In T2-weighted images, mildly increased signal intensities were seen in the subcortical white matter in the right frontal area. Bottom: MRI at 10 months of age. Marked encephalomalacia was seen in the right frontal lobe. The frontal horn of the right lateral ventricle was mildly dilated. T1WI, T1-weighted images; T2WI, T2-weighted images; FLAIR, fluid-attenuated inversion-recovery images; DWI, diffusion-weighted images; R, right.

Table 1
Patient characteristics.

Patient	Sex	Gestational age (weeks)	Birth weight (g)	Family history of IP	Complications other than skin and CNS	Age at the last follow-up (months)
1	F	38	3354	Mother	None	14
2	F	41	3200	Mother	Retinopathy	48
3	F	40	2472	None	Microphthalmia, retinal bleeding and detachment	76
4	F	38	2432	Mother, maternal aunt, and grandmother	None	14
5	F	39	2782	None	Retinopathy, absence of teeth	123
6	F	40	2316	None	Superior vena cava syndrome	7

IP, incontinentia pigmenti; CNS, central nervous system.

3.3. Encephalopathic events and outcome (Table 2)

The encephalopathic events began within the first 2 months of life in all but one patient. All had clusters of focal clonic seizures, and secondary generalized seizures were seen occasionally. Each seizure typically lasted for no more than 5 min. Two patients (Patients 1 and 2) had prolonged seizures lasting for 30 min or longer. The seizures ceased within 5 days in all patients. Various degrees of reduced consciousness were observed in all patients in association with the frequent seizures. The duration of reduced consciousness ranged from 4 to 10 days. Several antiepileptic drugs were administered. The seizures were suppressed by phenobarbital in three of the six patients. The patients recovered consciousness in parallel with the cessation of seizures.

At the last follow-up, four patients had delayed development, three had motor impairment, and three had epilepsy. Patient 4 had a non-accidental head injury after discharge, and her outcome has likely worsened as

Table 2
Encephalopathic events and outcome.

Patient	Age at onset	Seizure types	Duration of seizures (minutes)	Persistence of seizures (days)	Treatment	Motor impairment	Delayed development	Epilepsy
1	44 days	Focal CS	3–60	5	MDZ	Yes	Yes	Yes
2	5 days	Focal CS	2–30	5	MDZ, LID	No	No	Yes
3	6 months	Focal CS	2–5	3	PB	Yes	Yes	No
4	58 days	Focal CS Secondarily GS	2–5	2	MDZ, PB, PHT	No	Yes[a]	No
5	44 days	Focal CS	2–5	3	PB	Yes	Yes	Yes
6	1 day	Focal CS	3–5	5	PB, MDZ, thiopental	No	No	No

CS, clonic seizure; GS, generalized seizure; MDZ, midazolam; LID, lidocaine; PB, phenobarbital; PHT, phenytoin.
[a] This patient had non-accidental head injury after discharge.

a result. No patient has experienced a recurrence of encephalopathic manifestations with seizures clusters or reduced consciousness.

3.4. Neuroimaging

The MRI findings are summarized Table 3. MRI was performed during the acute stage in four patients (Fig. 2). Diffusion-weighted imaging (DWI) was performed in two patients during the acute phase of the encephalopathic event. Patchy areas of reduced diffusion were common in the subcortical white matter in both of these patients. Abnormal signal intensities were also common in the corpus callosum, basal ganglia, and thalami. Internal capsule involvement was observed in two patients. The other two patients underwent conventional MRI only during the acute phase. Scattered subcortical white matter lesions were observed on fluid-attenuated inversion-recovery images in both patients. One patient had a brainstem lesion.

Magnetic resonance imaging was obtained during the remote stage in five patients. Four patients had atrophic changes of varying degrees in areas corresponding to the regions with diffusion abnormalities in the acute stage. The remaining patient was complicated by a non-accidental head injury with a subdural hemorrhage, and no MRI was obtained.

3.5. EEG findings

The EEG findings are summarized in Table 3. EEG was performed during the acute stage in all but one patient. Three patients had slowing of the background activity to varying degrees. One patient had low-voltage background activity, and the remaining patient had widespread spikes. Ictal EEG changes were observed in two patients. An EEG during the remote stage was obtained in three patients: two had focal or multifocal spikes, whereas the EEG was normal in the other.

4. Discussion

The CNS is often involved in patients with IP, although the CNS disorders in patients with IP are not fully understood. We report a unique early infantile CNS manifestation in patients with IP. The CNS

Table 3
MRI findings.

Patient	Acute stage								Remote stage	
	Age at MRI[a]	Subcortical WM	Deep WM	Basal ganglia	Thalamus	Corpus callosum	Internal capsule	Brainstem	Age at MRI	MRI findings
1	45 days (1)	++	+	+	+	++	++	–	10 months	Cystic encephalomalacia with atrophic changes in the right frontal area
2	14 days (9)	++	+	+	–	–	–	–	21 months	Patchy gliotic changes in the right subcortical WM
3	6 months (3)	++	–	–	+	–	–	+	36 months	Marked atrophic changes in the left hemisphere
4	60 days (2)	++	+	+	+	++	–	–	ND	ND
5	ND	ND	ND	ND	ND	ND	ND	ND	72 months	Patchy gliotic changes, mild left ventricular dilation
6	ND	ND	ND	ND	ND	ND	ND	ND	34 days	Marked atrophic changes in the left hemisphere

ND, not done. WM, white matter.
[a] The number in parentheses indicates days after the onset of encephalopathic manifestations.

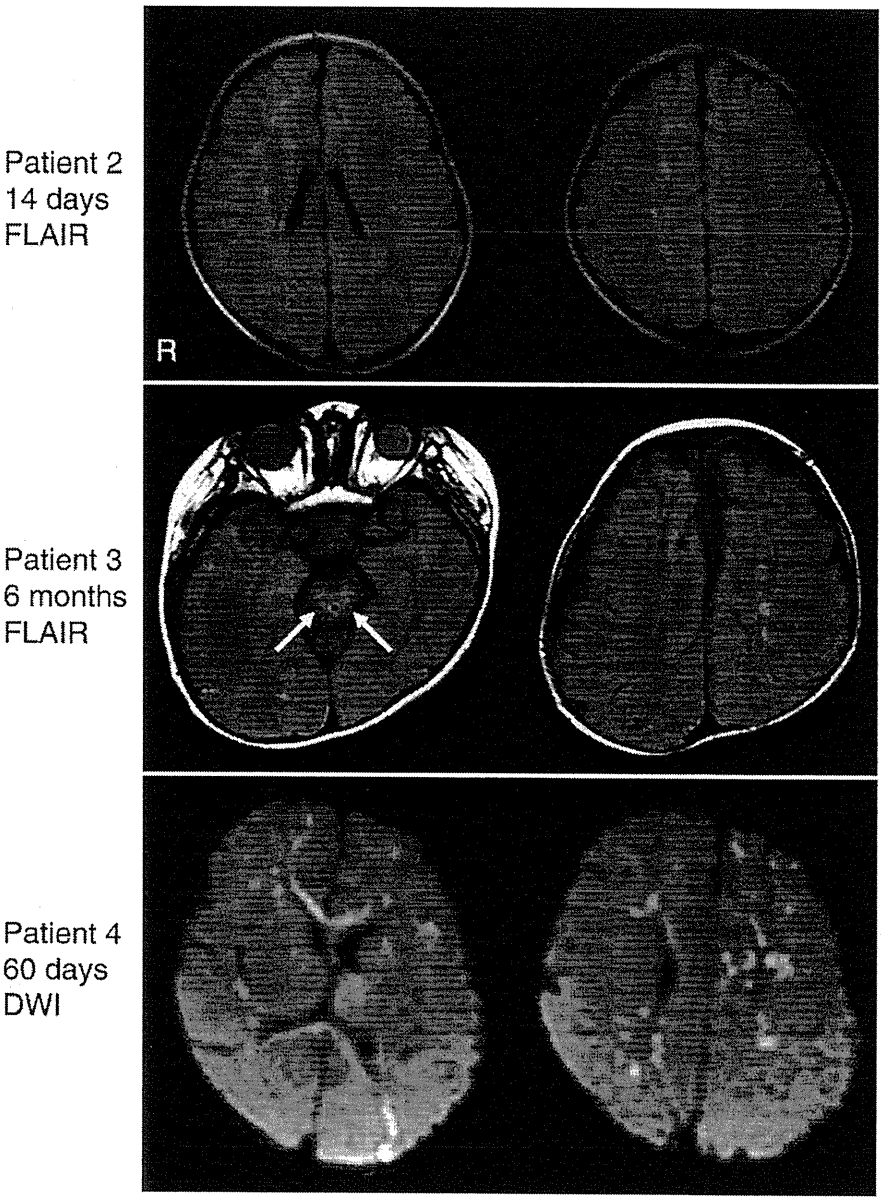

Fig. 2. MRI findings during the acute stage of encephalopathic manifestation. Top: FLAIR of Patient 2 at 14 days of age. Patchy high-intensity areas were seen in the subcortical areas predominantly in the right hemisphere. Middle: FLAIR of Patient 3 at 6 months of age. Linear high intensities were observed in the dorsal area of the brainstem (arrows). Patchy high-intensity areas were also present in the subcortical areas of the left hemisphere. Bottom: DWI of Patient 4 at 60 days of age. Patchy restricted diffusion was recognized in the subcortical areas and corpus callosum. FLAIR, fluid-attenuated inversion-recovery images; DWI, diffusion-weighted images; R, right.

symptoms of our patients were characterized by clusters of seizures and reduced consciousness, resembling acute encephalopathy. Several authors have reported similar patients [4–11]. A majority of these patients share points in common with our patients: onset during early infancy, seizures in clusters, and similar neuroimaging findings. These facts suggest that early infantile encephalopathic manifestations are a characteristic of the CNS disorders in patients with IP (Table 4).

The pathomechanism of CNS lesions in patients with IP is not clear. Several mechanisms have been considered, including destructive [12,13], vascular [4–8,14–16], and inflammatory [17–19] mechanisms. From an analysis of the mouse models, a sequence of events was postulated to occur during IP dermatosis [3,20,21]. At birth, the epidermis of IP patients is a mosaic of cells, including keratinocytes, either expressing wild-type or mutated NEMO protein. At this stage, cells expressing the mutated NEMO with a defect in NF-κB activation start to produce large quantities of interleukin 1β (IL-1β). The IL-1β likely acts on neighboring cells, possibly with other molecules. In response, TNF-α is synthesized

Table 4
EEG findings.

Patient	Acute stage		Remote stage	
	Age at EEG	EEG findings	Age at EEG	EEG findings
1	45 days (1)	Right frontal dominant slowing	12 months	Focal spikes on the right frontal area
2	7 days (2)	Right hemisphere dominant mild slowing Ictal discharges on the right fronto-centro-parietal area	48 months	Normal
3	ND	ND	ND	ND
4	59 days (1)	Mildly low voltage	ND	ND
5	45 days (1)	Widespread spikes	76 months	Multifocal spikes
6	2 days (1)	Left hemisphere dominant mild slowing Ictal discharges on the left frontal area	ND	ND

ND, not done.
The number in parentheses indicates days after the onset of encephalopathic manifestations.

and acts on the NEMO-mutated cells, inducing their apoptosis. Because the brain, like the skin, is of ectodermal origin, brain injury can result from the same pathogenesis. During the first weeks of life, several stimuli can induce an inflammatory response, including bacterial colonization of the skin and gastrointestinal tract, oxidative stress due to the transition from intrauterine to extrauterine life, and exposure to various environmental antigens. The occurrence of brain injury from the neonatal through the early infantile period lends some support to this hypothesis.

It is noteworthy that the seizures resolved within 5 days, although they were severe and mimicked acute encephalopathy with clusters or prolonged seizures that were relatively refractory to antiepileptic drugs, accompanied by reduced consciousness. Although additional seizures occurred in some patients as remote symptomatic epilepsy, none had a recurrence of the encephalopathic events. A recurrent encephalopathic event is likely uncommon, although recurrence of the CNS injury has rarely been reported [22,23]. These facts suggest that the encephalopathic events in infants with IP are self-limiting. This may also be explained by the hypothesis that the CNS injury is related to the increased sensitivity to the apoptosis of NEMO-mutated cells. After the NEMO-mutated cells are eliminated, a large majority of the surviving cells lack the mutation. The reduction in the number of cells with the mutation may be related to the paucity of the recurrence of the encephalopathic events.

The MRI findings in patients with IP include atrophic changes [13,15], hypoplasia of the corpus callosum [8,13,17,24], subcortical or deep white matter lesions [4–8,13–15], and hemorrhagic necrosis [15,17]. Pascual-Castroviejo et al. reported that the most severe lesions were located in the subcortical white matter [13]. Several authors have also reported CNS lesions in the subcortical or periventricular white matter in patients with IP [4–8,13–15]. MRI abnormalities were observed in the subcortical white matter in all of our patients. This indicates that the subcortical white matter is the most common site of CNS lesions in patients with IP. Diffusion-weighted image abnormalities during the acute phase were also impressive in our patients. Restricted water diffusion was seen in the corpus callosum, internal capsule, and basal ganglia in addition to the subcortical white matter. Some authors have reported that DWI showed reduced diffusion in the corpus callosum and subcortical white matter [5,11]. This is very similar to the images of our patients. These facts suggest that cytotoxic edema characterizes the CNS lesions of patients with IP and that DWI is useful for detecting the extent of the affected regions during encephalopathic events in patients with IP.

In conclusion, we report the clinical and neuroimaging features of encephalopathic manifestations in patients with IP during early infancy. The encephalopathic manifestations were characterized by clusters of seizures and reduced consciousness, although the duration of the episode was relatively short. MRI abnormalities were predominant in the subcortical areas in most patients. Further studies are necessary to determine the pathogenesis of the encephalopathic manifestations in patients with IP.

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Original article

Thermolabile CPT II variants and low blood ATP levels are closely related to severity of acute encephalopathy in Japanese children

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Abstract

Despite the decrease in Reye syndrome after the discontinuation of aspirin, acute encephalopathy (non-Reye syndrome type) has been continually reported in Japan. Recent studies suggested that the thermolabile phenotype of carnitine palmitoyltransferase II (CPT II) variation [F352C] was closely related to the pathomechanism of influenza-associated encephalopathy (IAE) in Japanese, causing mitochondrial ATP utilization failure during periods of high fever, resulting in brain edema. So, we analyzed CPT II polymorphism and peripheral blood ATP levels as a signal of "energy crisis" in 12 and 10 patients with acute encephalopathy, respectively. Out of the 12 patients with acute encephalopathy, six showed thermolabile CPT II variants [F352C], and of these six, two patients died in spite of intensive care. In contrast, the remaining six patients with no thermolabile CPT II variant [F352C] showed a relatively mild clinical course. Blood ATP levels of the 10 patients in the acute phase of encephalopathy were significantly lower than those during the convalescent phase and also those of patients with febrile seizure status. Our data suggest that the thermolabile F352C CPT II variant, found only in Japanese, might be one of the predisposing factors to trigger the pathomechanism of acute encephalopathy in the Japanese population, and that it is causally related to the severity of disease. The decreased blood ATP level seems to reflect systemic mitochondrial dysfunction including the blood brain barrier during the acute phase of encephalopathy.

Keywords: Acute encephalopathy, Carnitine palmitoyltransferase II; Thermolabile variants; ATP; Mitochondrial dysfunction

1. Introduction

Acute encephalopathy in children is clinically characterized by high fever, prolonged consciousness disturbance associated with brain edema, and prolonged or multiple generalized seizures. Acute encephalopathy distinct from Reye syndrome is not rare in Japan. The precise pathogenesis of acute encephalopathy including influenza-associated encephalopathy (IAE) remains unclear. An epidemiological study revealed that aspirin use was closely related to the pathogenesis of Reye syndrome [1]. However, despite the decrease in Reye syndrome after the discontinuation of aspirin, acute encephalopathy (non-Reye syndrome type) has been continually reported in Japan [2].

Recently, acute encephalopathy was classified into several types according to magnetic resonance imaging (MRI) findings together with the clinical course, such

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as acute necrotizing encephalopathy [3], acute encephalopathy with biphasic seizures and late reduced diffusion [4], and hemorrhagic and shock encephalopathy [5,6]. Although the influenza virus and HHV-6 (human herpes virus-6) are the main causative agents of these acute encephalopathies, many other viruses are also considered to be responsible for the disease [3,4,7].

It is estimated that more than 100 children die of IAE every year in Japan [8,9]. According to the first nationwide clinical survey of IAE in Japan, in many patients with IAE, multiple-organ failure developed, and rates of mortality (31.8%) and disability (27.7%) were high [2]. Although clinical and neuropathological studies suggested that blood–brain barrier destruction and hypercytokinemia in cerebrospinal fluid were closely related to the pathogenesis of IAE, the pathophysiology and mechanisms of disease onset are still unclear [3,7,10,11].

Recently, Chen et al. [12] reported that the thermolabile phenotypes of carnitine palmitoyltransferase II (CPT II) variations, [1055T > G/F352C] alone, and [1055T > G/F352C] + [1102G > A/V368I] were closely related to the pathomechanisms of IAE. The CPT system is a pivotal component of ATP generation through mitochondrial fatty acid oxidation in mammals [13]. Yao et al. [14] further characterized the enzyme properties of the CPT II variants as follows: (1) dominant-negative effect, (2) reduced activities, (3) thermal instability, and (4) short half-lives compared with the wild-type. They demonstrated that the thermolabile CPT II variants might cause mitochondrial fuel utilization failure in various organs and endothelial cells during periods of high fever, and, thus, might play an important role in the pathogenesis of brain edema in IAE. In the present study, we analyzed the CPT II polymorphism and peripheral blood ATP levels as a signal of "energy crisis" in patients with acute encephalopathy with and without influenza virus infection, septic encephalopathy, and febrile delirium during influenza virus infection, and analyzed the relationships among these data, age, and clinical manifestations.

2. Patients and methods

2.1. Patient profile for the study of CPT II polymorphism

This investigation was approved by the Ethics Review Committee for human genome analysis of our institution. All participants' caregivers gave written informed consent. Fifteen patients were included in the study. The clinical details are summarized in Table 1. The diagnoses of the 15 patients were as follows: 12 patients with acute encephalopathy (7 IAE, one human herpes virus type 6 (HHV-6) associated, one varicella-associated, one septic encephalopathy associated with *Hemophilus influenzae* type b, two acute encephalopathy with an unknown pathogenesis, highly suspected of being of viral origin), and three febrile delirium associated with influenza virus infection. Two patients (Case 1, IAE, and Case 2, septic encephalopathy) died 30 and 3 days after admission, respectively, despite intensive care. All 12 patients with acute encephalopathy were diagnosed based on prolonged seizures with high fever and/or consciousness disturbance lasting longer than 12 h associated with brain CT or MRI abnormalities.

3. Representative case presentations

3.1. Case 1

This 4-year-old girl was admitted to our hospital because of feeding difficulty, a lethargic state, and high fever lasting longer than 12 h. A rapid test for influenza A virus antigen in the nasal discharge was positive. She has been followed at our outpatient clinic with a diagnosis of severe psychomotor delay and epilepsy due to chromosome abnormality (46, XX, dup(2)(q21.1q24.2)) since the age of 3 years. Her seizure disorder was well-controlled with phenobarbital. On admission, except for a lethargic tendency, she showed no neck stiffness, involuntary movement, or convulsion, and her respiratory and circulatory conditions were stable. She was also able to follow an object. Neurological examination revealed normal light and corneal reflexes and normal deep tendon reflexes. Pathological reflexes were not induced. Her consciousness level, however, deteriorated 12 h after admission. On laboratory tests, blood glucose, ammonia, the white blood cell count (WBC), hemoglobin (Hb), and platelet count (Plt) were within normal ranges, and cereblospinal fluid (CSF) findings were unremarkable. Blood and CSF cultures were negative. Because she also showed sudden respiratory insufficiency and reduced blood pressure, she was immediately resuscitated and intubated. After that, she could not move and all brainstem reflexes disappeared. On brain CT the next day, as shown in Fig. 1a, cisterns surrounding the brainstem and cerebellum were not identified and auditory brainstem responses (ABR) showed only bilateral wave I. Rapid consciousness deterioration as well as brain CT and ABR findings suggested cerebral herniation due to influenza-associated brainstem encephalopathy. On the second CT 3 weeks later, severe brain edema and subarachnoid hemorrhage were observed. Despite intensive care, she died on the 31st day of hospitalization. She had a thermolabile F352C CPT II variant.

3.2. Case 2

This previously healthy 2-year-old boy was admitted to our hospital because of consciousness disturbance, a brief seizure cluster, and high fever lasting 24 h. On admission, neurological examination revealed coma, the absence of light and corneal reflexes, dilated and anisocoric pupils, and flaccid extremities. Neck stiffness was

Table 1
Clinical summary of patients and CPT II polymorphism.

Case no.	Age at onset	Pathogen	Diagnosis	CPT II polymorphism	Duration of high fever	Duration of seizure (min)	Therapy	Outcome
1[c]	4 years 10 months	Flu A	IAE	F352C	24 h	(−)	Gly, IVIG, m-PSL, Venti	Died
2	2 years 2 months	*H. influenza*	Hib septic AE	F352C, V368I	2 days	3	Venti, Epin, DOA, CTX	Died
3[c]	1 year	Unknown	AEU	F352C, V368I	2 days	30	Mann, MDZ	Severe MR, MD, Epi
4[c]	1 year 7 months	Flu A	IAE	(−)	30 h	40	Gly, m-PSL, Venti	Moderate MR, MD, Epi
5[a]	4 years 5 months	Flu A	IAE	F352C, V368I	5 days	90	Gly, MDZ, Pen, IVIG, m-PSL	Moderate MR
6[a]	2 years 1 months	Varicella	Varicella AE	F352C, V368I	24 h	90	Mann, MDZ, m-PSL	Mild MR
7[c]	6 years	Unknown	AEU	F352C, V368I	2 days	30	Mann, MDZ, m-PSL, HT	Mild MR
8[a]	1 years 4 months	Flu A	IAE	V368I	5 days	60	Gly, MDZ, Pen, PB, m-PSL	Mild MR
9[a]	2 years	Flu A	IAE	V368I	5 days	60	Gly, MDZ, Pen, IVIG, m-PSL	Mild MR
10[a]	11 months	HHV-6	HHV-6 AE	V368I	36 h	100	Gly, MDZ, Pen, m-PSL	Good
11[b]	2 years 5 months	Flu A	IAE	V368I, M647 V	24 h	40	MDZ, PB, m-PSL, HT	Good
12[a]	3 years 11 months	Flu A	IAE	V368I	2 days	40	MDZ, PB, m-PSL, HT, Venti	Good
13	4 years 9 months	Flu A	FD	F352C, V368I	4 days	2	(−)	Good
14	9 years 5 months	Flu A	FD	(−)	3 days	(−)	(−)	Good
15	11 years	Flu A	FD	V368I, M647V	3 days	(−)	(−)	Good

IAE: Influenza-associated encephalopathy, AEU: acute encephalopathy of unknown pathogen, FD: febrile delirium, Flu A: influenza A, HHV-6: human herpes virus-6, MR; mental retardation, MD: motor delay, Epi: epilepsy, Mann: mannitol, MDZ: midazolam, m-PSL: methylpredonisolone, HT: hypothermia, Venti: artificial ventilator, Epin: epinephrine, DOA: dopamine, CTX: cefotaxim, PB: Phenobarbital, Pen: pentobarbital, IVIG: intravenous infusion of gamma-globlin, Gly: glycerole.

[a] AESD (acute encephalopathy with biphasic seizures and late reduced diffusion).

[b] This case partially resembles ANE (acute necrotizing encephalopathy).

[c] Unclassified acute encephalopathy.

not observed. A rapid test for influenza virus antigen in the nasal discharge was negative. His head CT demonstrated diffuse brain edema, as shown in Fig. 1b. On laboratory investigation, blood glucose and ammonia, as well as liver and renal functions were within normal limits. WBC was 18,000/μL, Hb 11.6 g/dL, Plt 3,60,000/μL, and prothrombin time 68.7 s. Blood culture identified *H. influenzae* type b. Spinal tap was not performed because of the risk of cerebral herniation. The blood ATP level was 0.58 mM on admission. The acylcarnitine ratio ((C16 + C18:1)/C2) was high, at 0.203, on admission, compared with the upper cutoff value of 0.048 [12]. We diagnosed him with septic encephalopathy. Despite intensive care including antibiotics, ventilator support, and catecholamine infusion, he died 2 days later. He had compound thermolabile CPT II variants [F352C + V368I].

3.3. Case 12

This previously healthy 3-year-old boy was admitted to our hospital because of a febrile seizure status and high fever lasting longer than 24 h. His generalized tonic clonic seizure was suppressed with pentobarbital infusion 40 min after the onset. A rapid test for influenza virus antigen in the nasal discharge was positive for flu A. Brain CT revealed mild brain edema. So, he was sedated and intubated. Methylpredonisolone (m-PSL) pulse and hypothermia therapies were immediately started based on the diagnosis of IAE. The blood ATP value was 0.77 mM on admission, and it increased to 1.35 mM 2 weeks later. On the 6th day of hospitalization, he developed brief right-sided clonic seizure. Brain MRI (diffusion-weighted images) showed an abnormal high intensity in the left hemisphere (Fig. 1e). The clinical course and MRI findings were compatible with acute encephalopathy with biphasic seizures and late reduced diffusion [4]. Additional m-PSL therapy was given and the hypothermia therapy gradually discontinued. His neurological condition subsequently showed a full recovery. No apparent mental, motor, and social skill impairment was noted during follow-up 1 year later. He had a V368I CPT II variant.

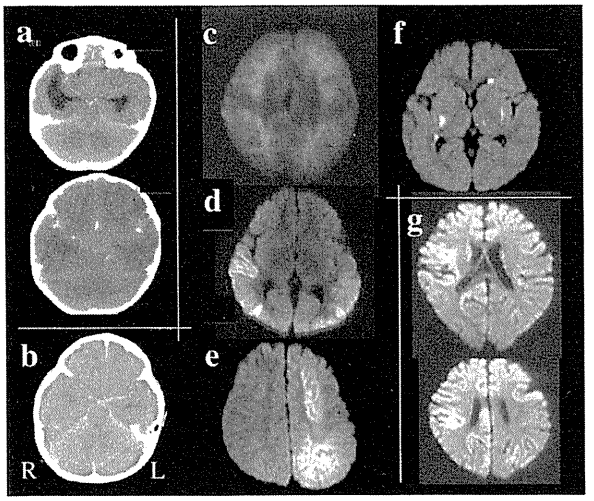

Fig. 1. (a) Brain CTs on 2nd and 21st day of hospitalization in Case 1 showing disappearance of cistern between brainstem and cerebellum (upper image) and severe brain edema (lower image). (b) Brain CT on admission showing severe brain edema in Case 2. (c–g) Brain MRIs showing abnormal high intensities in diffusion-weighted images in Cases 6,7,12,11 and 10, respectively.

3.4. Patient profile for the study of the blood ATP level

Twenty-five patients were included in this study. The diagnoses of the 25 patients were as follows: 10 patients with acute encephalopathy (mean age: 3 years and 11 months, age range: 7 months–10 years and 8 months, one IAE, one *Salmonella*-associated, one HHV-6-associated, three unknown virus-associated, one methylmalonic aciduria, one hepatic encephalopathy, one hemolytic uremic syndrome, and one septic encephalopathy (Case 2 in Table 1)), nine febrile seizure status (mean age: 1 year and 5 months, age range: 4 months–4 years 9 months), and six mitochondrial disease (mean age: 9 years and 8 months, age range: 2–25 years, two partial cytochrome c oxidase deficiency, three Leigh syndrome, and one chronic progressive external ophthalmoplegia). All 10 patients with acute encephalopathy were analyzed regarding the blood ATP levels in the acute phase (within 24 h of disease onset), and five of the 10 patients were also analyzed in the convalescent phase. Among the 15 patients who were analyzed for CPT II polymorphism, only Cases 2 and 12 were included in this study.

4. Methods

4.1. Analysis of CPT II polymorphism

Genomic DNA from whole blood was purified as previously described [15]. PCR of five exons of the CPT II gene was carried out with intron-based primers in genomic DNA. For haplotype analysis, the CPT II exon four region was cloned into the pCR® 2.1 vector (Invitrogen). The sequences of the PCR products and cloned CPT II gene were analyzed employing the ABI DyeDeoxy Terminator Cycle Sequencing Kit with an ABI-PRISM 3100 Genetic Analyzer (PE-Applied Biosystems). Each PCR product was sequenced at least twice independently.

4.2. Preparation of patients' lymphoblasts and culture

Blood samples (2 mL) were obtained from patients by venipuncture into a sterile EDTA blood collection tube. Lymphocytes were separated from peripheral blood, diluted (1:1, v/v) with sterile saline, by centrifugation ($800\times g$, 20 min) over 2 mL of Lymphoprep (Nycomed). The lymphocyte layer was recovered and washed twice with PBS by centrifugation at $250\times g$ for 10 min each, and then maintained in PRMI-1640 (GIBCO) supplemented with 12.5% FCS. Cells were incubated with 5% CO_2 at 37 °C for 7 days. Lymphoblastic cell lines were established by infecting peripheral blood lymphocytes with the Epstein Barr virus. Cells were grown in suspension in an SC flask (Greiner 658190) in an upright position, in 10 ml of PRMI-1640 medium that contained 12.5% FCS, maintained at 37 °C. Fluid was routinely changed every 2 days by removing the medium above the settled cells and replacing it with an equal volume of fresh medium.

4.3. Analysis of CPT II activity

CPT II activities of patients' lymphoblasts were analyzed as previously described [14]. To prepare whole cell extracts, cells were harvested and washed twice with PBS (−) at $250\times g$ for 10 min and then lysed with 0.5 mL of ice-cold lysis buffer (5 mM Tris–HCl buffer, pH 7.4, containing 1% Tween-20 and 0.5 M KCl), then centrifuged at $147{,}600\times g$ for 1 h at 4 °C. To analyze the heat stability of CPT II, cell lysates were pre-incubated at 30, 37 and 41 °C for 0–120 min. Protein concentrations in the cell lysates were measured using the BCATM Protein Assay Kit (Thermo SCIENTIFIC).

4.4. Measurement of blood ATP levels

ATP concentrations in whole blood lysate were measured by an ENLITEN® ATP assay system bioluminescence detection kit (Promega) according to the instructions provided by the manufacturer and the values were expressed as ATP levels in whole blood.

5. Results

5.1. CPT II polymorphism in the patients

As shown in Table 1, among the 15 patients studied, seven had a thermolabile F352C CPT II variant (1 F352C only and six [F352C + V368I]), four V368I only,

two [V368I + M647 V], and two no polymorphisms. In 12 patients with acute encephalopathy (Cases 1–12), six (Cases 1–3 and 5–7) had a thermolabile F352C CPT II variant (1 F352C only and five [F352C + V368I]), and five (Cases 8–12) had the V368I CPT II variant (4 V368I only and one [V368I + M647 V]) and one (Case 4) showed no CPT II variant. Two patients with acute encephalopathy who died (Cases 1 and 2) had a thermolabile F352C CPT II variant (1 F352C only and the other [F352C + V368I]). In three patients with febrile delirium associated with influenza infection (cases 13–15), only case 13 (brief febrile seizure and unusually long febrile delirium) had the [F352C + V368I] CPT II variant. No other reported CPT II mutations or polymorphisms were detected.

There was no significant difference in the age at onset (41.0 ± 23.3 vs. 24.3 ± 12.7 months of age, $p = 0.18$), duration of high fever (52.0 ± 35.3 vs. 63.0 ± 44.9 h, $p = 0.28$), and duration of seizures (40.5 ± 40.1 vs. 56.7 ± 23.4 h, $p = 0.12$) between the six patients with acute encephalopathy with a thermolabile F352C CPT II variant (Cases 1–3,5–7) and six patients with acute encephalopathy without this thermolabile variant (Cases 4,8–12) (Mann–Whitney U-test).

5.2. Lymphocyte CPT II activity in the patients

As shown in Fig. 2(b), CPT II activity using peripheral lymphocytes of a patient with a thermolabile F352C CPT II variant was significantly reduced to about 50% during incubation for 120 min at 41 °C as compared to those at 30 and 37 °C. All patients with a thermolabile F352C CPT II variant showed a significant reduction of CPT II activity at 41 °C.

Fig. 2(a) shows CPT II activity in a patient with the V368I CPT II variant without reduction even at 41 °C.

5.3. Blood ATP levels in patients with acute encephalopathy

As shown in Fig. 3, ATP levels in the extracts of whole blood in the acute phase of encephalopathy during high fever were significantly low (0.58 ± 0.16 mM, $n = 10$) compared with those in the convalescent phase (1.08 ± 0.27 mM, $n = 5$) and with those of patients with febrile seizure status (1.01 ± 0.36 mM, $n = 9$). The blood ATP levels in the acute phase of encephalopathy revealed no significant difference when compared to those of patients with mitochondrial disease exhibiting several symptoms (0.79 ± 0.39 mM, $n = 6$).

6. Discussion

Although the precise pathomechanisms of acute encephalopathy have yet to be clarified, it is postulated that some genetically-determined factors might be involved, because some types of acute encephalopathy are more frequent in Japanese than in Caucasians. Chen et al. [12] demonstrated that the thermolabile phenotype of CPT II variations such as the F352C CPT II variant or complex [F352C + V368I] CPT II variant might be a principal genetic background of IAE in Japanese. On the basis of the analysis of fatty acid oxidation and cellular ATP production in COS-7 cells transfected with wild-type and variant *CPT2* cDNAs at 37 and 41 °C, Yao et al. [14] suggested that the compound *CPT2* variants with thermolabile phenotypes are the main cause of multiple-organ failure, particularly in high ATP-consuming organs as well as endothelial cells and play a major role in the etiology of IAE.

In the 12 patients with acute encephalopathy studied, six patients (Cases 1–3 and 5–7) had thermolabile F352C CPT II variants (F352C CPT II variant alone in one case and complex [F352C + V368I] CPT II variants in five cases), which were reported to be frequently noted in severe IAE patients [12,14]. Of the six patients, two patients (Case 1, IAE and Case 2, *Hemophilus influenzae*-associated septic encephalopathy) died despite intensive care. Case 2, who died of fatal septic encephalopathy [16], showed a high acylcarnitine ratio ((C16 + C18:1)/C2:0.203) on admission. This value corresponded to the ratio (>0.09) of the high-risk group of patients with IAE showing a fatal outcome, thus reflecting the disorder of mitochondrial •-oxidation. [12]. The remaining six patients (Cases 4 and 8–12) with acute encephalopathy without a thermolabile F352C CPT II variant followed a relatively mild clinical course (Table 1). Out of the six patients, five had a V368I CPT II variant.

As shown in Fig. 2, the CPT II activities of lymphocyte in patients with the F352C CPT II variant showed thermal instability, that is, a marked activity reduction at 41 °C, while those in patients with the V368I CPT II variant did not. There was no significant difference in the age at onset, duration of high fever, and duration of seizures between the six patients with the F352C CPT II variant (Cases 1–3 and 5–7) and six patients without this variant (Cases 4 and 8–12). Therefore, taken together, it seems likely that a thermolabile F352C CPT II variant might be related to the severity of disease, that is, the rapidity of progression of brain edema. In Caucasians, two polymorphisms of CPT II, p.V368I and p.M647 V, occur with a frequency of 0.5 and 0.25, respectively, exhibiting a Hardy–Weinberg equilibrium. A third polymorphism, p.F352C, occurs with a frequency of 0.21 exclusively in the Japanese population [17]. Therefore, this thermolabile F352C CPT II variant might be one of the predisposing factors to trigger the pathomechanism of acute encephalopathy in Japanese.

The CPT system regulates the entry of long-chain fatty acids into the mitochondrial matrix for •-oxidation. Fatty acid oxidation is an important source of acetyl-CoA for maintaining the tricarboxylic acid cycle.

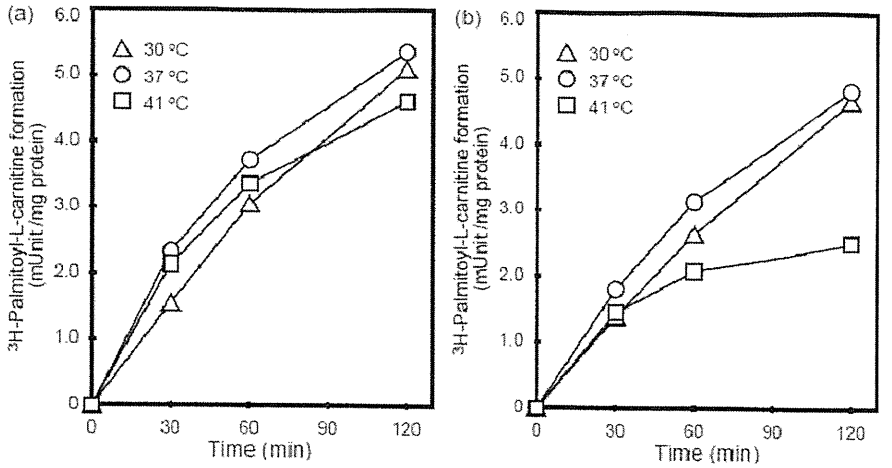

Fig. 2. (a) Lymphocyte CPT II activity in case 12 (influenza-associated encephalopathy) with V368I CPT II variant at 30, 37 and 41 °C. No definite reduction of CPT II activity was observed at 41 °C. (b) Lymphocyte CPT II activity in Case 1 (influenza-associated encephalopathy) with a thermolabile F352C CPT II variant at 30, 37 and 41 °C. At 41 °C, the CPT II activity decreased to about 50% of that at 37 °C after 2-h-incubation.

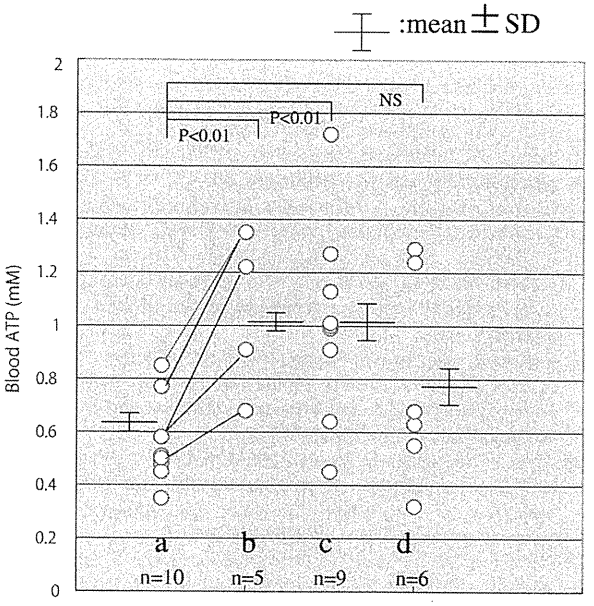

Fig. 3. ATP levels in whole blood in patients with acute encephalopathy (acute (a) and convalescent phase (b)), febrile seizure status (c) and mitochondrial disease (d). In five patients with acute encephalopathy, blood ATP level recovered at convalescent phase.

The CPT II is ubiquitously expressed in all tissues that require fatty acid oxidation as an energy-producing pathway [18]. CPT II deficiency is a disorder of long-chain fatty acid oxidation. It is classified into three clinical types based on the age at onset and disease severity: lethal neonatal form, severe infantile hepatocardiomuscular form, and myopathic form. It is clear that our patients' clinical manifestations did not correspond to any of these three types. The thermolabile instability of the F352C CPT II variant in our cases explains the situation whereby impaired energy metabolism could occur during high fever due to a secondary CPT II deficiency in spite of the absence of symptomatic manifestations of CPT II disorder in daily life at a normal temperature [12,14].

Olpin et al. [19] reported based on mutation analysis that when CPT II activities are above 20% of controls, fatty acid oxidation in fibroblasts is usually within the normal range (>70% of controls). However, under heat stress, fasting, acidosis, and seizures, moderately lowered CPT II activity due to the thermolabile F352C CPT II variant may accelerate the disease process of acute encephalopathy.

Blood ATP levels in the acute phase of encephalopathy during high fever were significantly lower than those in the convalescent phase and also with those of patients with febrile seizure status. This suggests that mitochondrial energetic failure may be more severe in patients with acute encephalopathy, and the pathological process of acute encephalopathy should differ from the febrile seizure status. The low levels of ATP in the acute phase of encephalopathy were normalized in the convalescent phase in line with clinical recovery. Interestingly, blood ATP levels in the acute phase of encephalopathy corresponded to those of mitochondrial disease with several symptoms. Yao et al. [14] showed that COS-7 cells transfected with thermolabile [F352C + V368I] CPT II variants exhibited significantly decreased fatty acid oxidation and subsequent intracellular ATP reduction at 41 °C. The decreased ATP levels seemed to reflect systemic mitochondrial dysfunction including the blood brain barrier (BBB) at the acute phase of encephalopathy in our cases. The ATP demand per body weight is so high in infants that a thermolabile CPT II variant induced-ATP reduction might lead to a greater susceptibility to the pathophysiology of encephalopathy in children than in adults.

The brain capillary endothelium is characterized by a greater density of mitochondria than that of peripheral capillaries [20]. This greater mitochondrial density is required to maintain the significant active transport mechanisms, electrochemical gradients, autoregulatory adjustments, and regulation of tight junctional complexes. As such, the requirement of a constant ATP supply may make the BBB particularly susceptible to acute hypoxic insult [21]. From a similar perspective, BBB breakdown may occur at an initial stage of encephalopathy under the condition of ATP reduction, thus leading to subsequent brain edema due to complex cascade of hypercytokinemia, excitotoxicity, and oxidative stress. Although there is one hypothesis that cytokine storm due to virus–glial cell interaction might cause endothelial cell damage (BBB breakdown) leading to brain edema and neuronal injury [11], we consider that endothelial cell damage might induce in turn cytokine production resulting in neuronal damage in patients with thermolabile F352C CPT II variant irrespective of encephalopathy type .

In three patients with febrile delirium associated with influenza virus infection (Cases 13–15), Case 13 with a thermolabile F352C CPT II variant developed a short seizure and an intermittent confused state with visual hallucinations and agitation lasting 6 h. Cases 14 and 15 without F352C CPT II variant showed short-term consciousness alteration and abnormal behavior without seizures. All patients' brain MRIs were normal, and they fully recovered. Although more extensive study is needed, the grade of febrile delirium associated with influenza virus was more severe in a case with a thermolabile F352C CPT II variant when compared with that in cases without F352C CPT II variant.

Given that a thermolabile CPT II variant might be one of the predisposing factors for acute encephalopathy, we should revise the therapeutic strategy from the acute phase. Considering the rapid progression of encephalopathy and associated low CPT II activity during high fever, immediate hypothermia, sufficient glucose infusion, and L-carnitine supplementation should be adopted as treatment options. We speculate that the immediate hypothermia led to the recovery of the lowered CPT II activity and, thus, mitochondrial energy failure became minimal in many tissues including the brain capillary endothelium, leading to less severe damage to the central nervous system.

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CLINICAL REPORT

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West Syndrome Associated With Mosaic Duplication of *FOXG1* in a Patient With Maternal Uniparental Disomy of Chromosome 14

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FOXG1 on chromosome 14 has recently been suggested as a dosage-sensitive gene. Duplication of this gene could cause severe epilepsy and developmental delay, including infantile spasms. Here, we report on a female patient diagnosed with maternal uniparental disomy of chromosome 14 and West syndrome who carried a small supernumerary marker chromosome. A chromosomal analysis revealed mosaicism of 47,XX, + mar[8]/46,XX[18]. Spectral karyotyping multicolor fluorescence in situ hybridization analysis confirmed that the marker chromosome was derived from chromosome 14. A DNA methylation test at *MEG3* in 14q32.2 and microsatellite analysis using polymorphic markers on chromosome 14 confirmed that the patient had maternal uniparental disomy 14 as well as a mosaic small marker chromosome of paternal origin containing the proximal long arm of chromosome 14. Microarray-based comparative genomic hybridization analysis conclusively defined the region of the gain of genomic copy numbers at 14q11.2-q12, encompassing *FOXG1*. The results of the analyses of our patient provide further evidence that not only duplication but also a small increase in the dosage of *FOXG1* could cause infantile spasms. © 2011 Wiley-Liss, Inc.

Key words: West syndrome; maternal uniparental disomy; chromosome 14; supernumerary marker chromosome; *FOXG1*; mosaic duplication

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INTRODUCTION

Mutations in *FOXG1* on chromosome 14 are associated with the congenital variant of Rett syndrome [Shoichet et al., 2005; Jacob et al., 2009]. Recently, *FOXG1* was described as a dosage-sensitive gene. The duplication of this gene could cause severe epilepsy and developmental delay, including infantile spasms [Yeung et al., 2009; Brunetti-Pierri et al., 2011]. Maternal uniparental disomy 14 (upd(14)mat) is characterized by pre- and postnatal growth retardation, neonatal hypotonia, small hands and feet, feeding difficulty, precocious puberty, and truncal obesity [Kotzot and Utermann, 2005; Mitter et al., 2006]. Upd(14)mat syndrome demonstrates a Prader-Willi-like phenotype during infancy [Mitter et al., 2006; Hosoki et al., 2009] but complications of seizures are rarely observed. Upd(14)mat is reported in carriers of Robertsonian translocations involving chromosome 14 and is also found in patients with normal karyotypes and supernumerary marker chromosomes (SMCs) [Mitter et al., 2006]. The presence of SMCs has often increased chromosome dosage, which results in the increased expression of dosage-sensitive genes.

To add new insight regarding the genetic cause of West syndrome phenotype, we report on a female patient diagnosed with upd(14)mat and West syndrome who carried a small SMC derived from the chromosome 14q11.2 to 14q12 region encompassing *FOXG1*.

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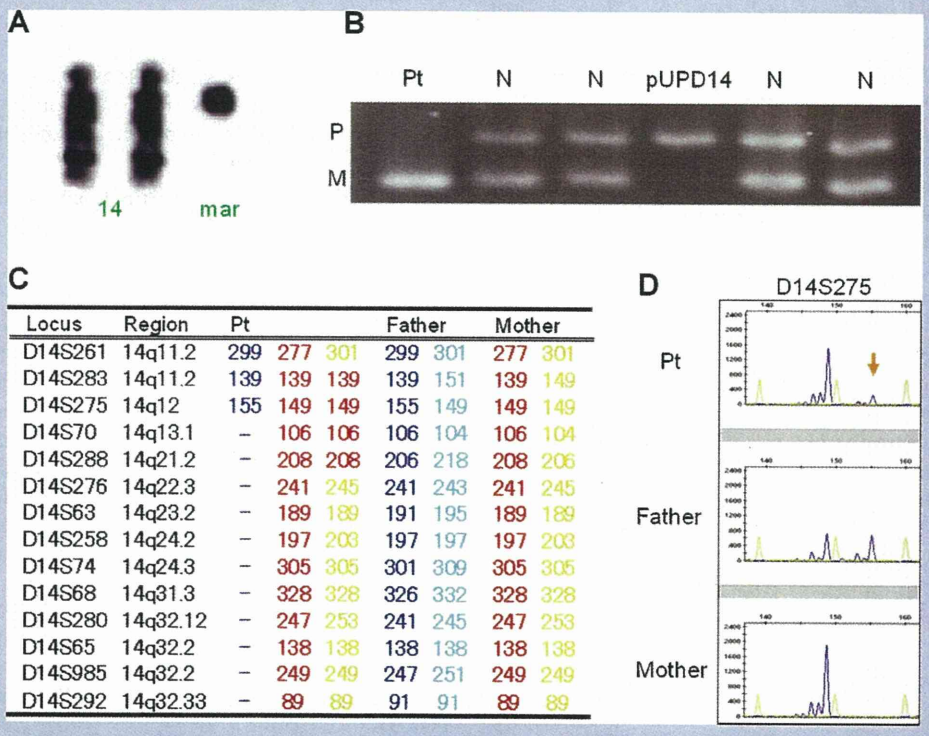
CLINICAL REPORT

The female patient was the first daughter born to healthy, non-consanguineous Japanese parents with an unremarkable family history. Intrauterine growth retardation was noted during pregnancy. The child was delivered at 40 weeks and 5 days of gestation by cesarean because of cervical insufficiency. At birth, birth weight (BW) was 2,140 g (−2.4 SD), birth length (BL) was 48 cm (−0.4 SD), and occipitofrontal head circumference (OFC) was 28 cm (−3.9 SD). After delivery, the infant had episodic vomiting and was admitted to the neonatal intensive care unit. She received nasogastric tube feeding for 11 days due to feeding difficulty. During infancy, she had hypotonia. At 4 months, she developed epileptic seizures with upward eye deviation, and, at 5 months, infantile spasms. Her electroencephalogram (EEG) showed hypsarrhythmia. At 5 months, she was diagnosed as having West syndrome, and referred to our hospital. When she was admitted, her BW was 5.6 kg (−1.9 SD), BH was 62 cm (−1.1 SD), and OFC was 41 cm (+0.2 SD). She had mild dysmorphic features including a frontal bossing, small mouth, and small hands. A hemangioma on the left forehead was noted. A neurological examination revealed mild hypotonia without muscle weakness. A brain MRI and comprehensive metabolic screening were normal. Infantile spasms were not controlled despite an optimal dose of sodium valproate and zonisamide. Treatment with adrenocorticotropic hormone (ACTH) was started at age 6 months and successfully controlled her seizures. Subsequently, clobazam was added to improve her EEG, and she had no relapse of infantile spasms until she was 6 years old. Her EEG at 5 years 11 months was normal. At 3 years 11 months, her BH was 87.5 cm (−3.9 SD). Because of her short stature, growth hormone therapy was started at age four and was effective. The patient had mild psychomotor delay. At age six, she was able to speak a few words. Her intelligent quotient by a modified Binet method was 40 at age 5 years and 8 months.

Cytogenetic and Molecular Genetic Analysis

A chromosomal analysis was performed using the G-banding of cultured lymphocyte and spectral karyotyping (SKY) multicolor fluorescence in situ hybridization (FISH) method. Through the analysis of 26 metaphase cells, we found that the patient had a mosaic chromosome of 47,XX, + mar[8]/46,XX[18] (Fig. 1A). The origin of the SMC was not identified by conventional G banding.



Locus	Region	Pt			Father		Mother	
D14S261	14q11.2	299	277	301	299	301	277	301
D14S283	14q11.2	139	139	139	139	151	139	149
D14S275	14q12	155	149	149	155	149	149	149
D14S70	14q13.1	–	106	106	106	104	106	104
D14S288	14q21.2	–	208	208	206	218	208	206
D14S276	14q22.3	–	241	245	241	243	241	245
D14S63	14q23.2	–	189	189	191	195	189	189
D14S258	14q24.2	–	197	203	197	197	197	203
D14S74	14q24.3	–	305	305	301	309	305	305
D14S68	14q31.3	–	328	328	326	332	328	328
D14S280	14q32.12	–	247	253	241	245	247	253
D14S65	14q32.2	–	138	138	138	138	138	138
D14S985	14q32.2	–	249	249	247	251	249	249
D14S292	14q32.33	–	89	89	91	91	89	89

FIG. 1. Cytogenetic and molecular genetic examinations of chromosome 14. A: G-banding of chromosome 14 and the marker. B: *MEG3* methylation test. The *MEG3* methylation test demonstrated that the patient showed only a maternal unmethylated signal. P, paternal methylated signal; M, maternal unmethylated signal; Pt, patient; N, normal control; pUPD14, paternal uniparental disomy 14. C: Microsatellite analysis using polymorphic markers on chromosome 14. Putative haplotypes are indicated by color. The patient showed a combination of maternal uniparental heterodisomy and isodisomy of the entire chromosome 14, as well as additional paternal inheritance for only the proximal long arm of chromosome 14 (shown in blue). D: Fragment analysis at D14S275. Fragment analysis at D14S275 showed a small peak of paternal inheritance (indicated by arrow) showing the mosaic status of the marker of paternal origin. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833].

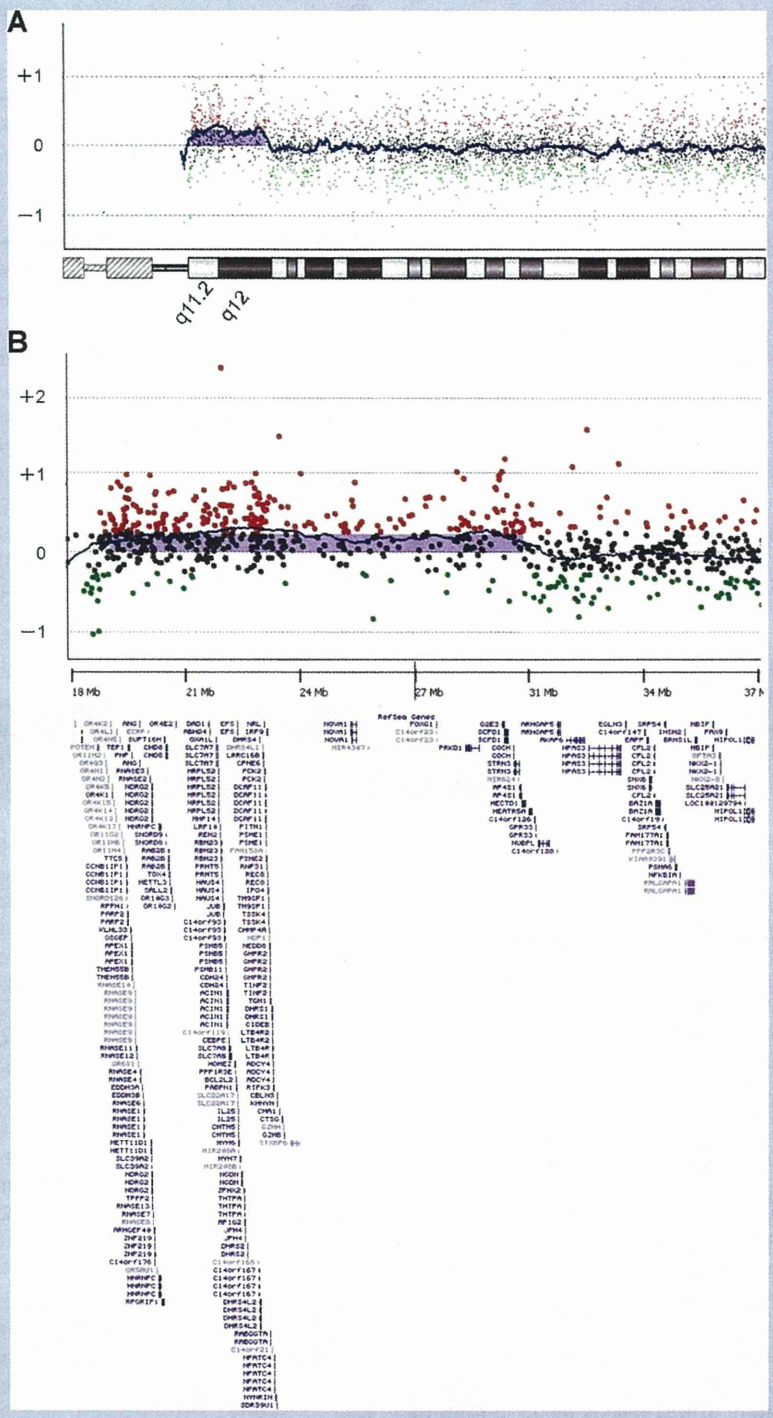


FIG. 2. Result of aCGH analysis for chromosome 14. A: Chromosome view indicating a genomic copy number gain of 14q11.2q12. The mean log2 ratio of this aberration region is 0.24, which indicates mosaicism of this marker chromosome. B: Aberration region expanded in gene view. The locations of the RefSeq Genes from the UCSC genome browser are shown under the gene view. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833].