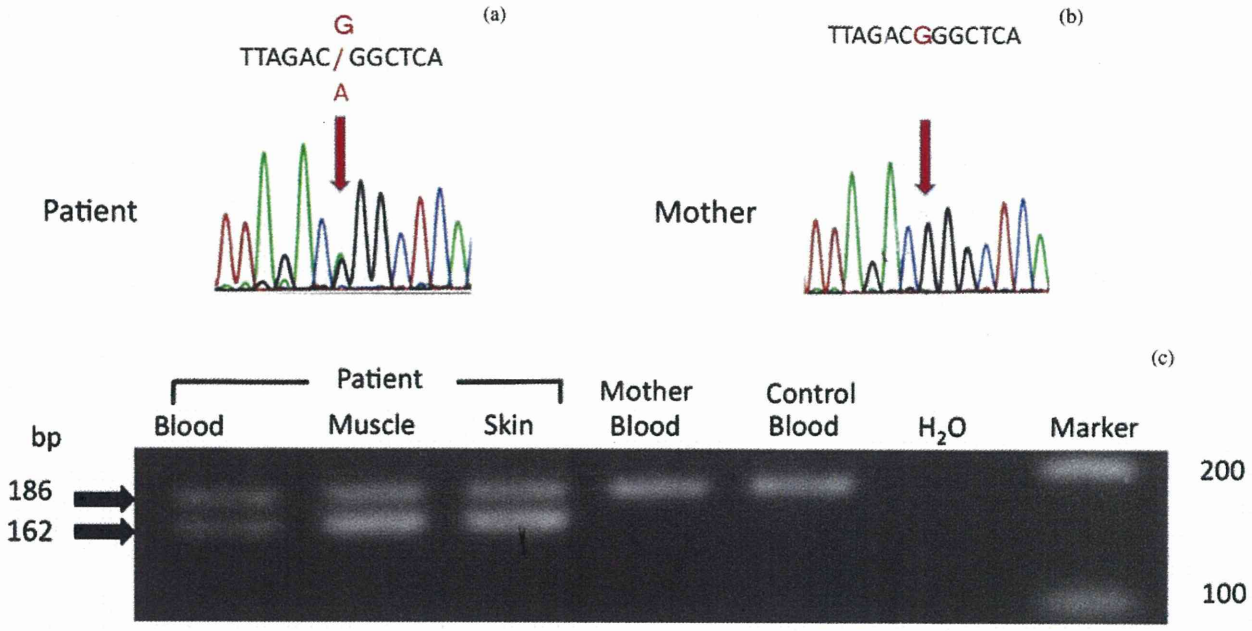

FIG. 2

Detection of the heteroplasmic m.625G>A transition. Diagrams represent screening of the patient's peripheral blood (a) and her mother's peripheral blood (b) for whole mitochondrial DNA genomes, and show the heteroplasmic m.625G>A transition in the patient's blood but not the mother's blood. (c) Electrophoretic strip showing that, in the presence of the m.625G>A mutation, the 186 base pair (bp) fragment was cleaved into 162 and 24 bp fragments (the latter not shown) by (BstOI is manufactured by Promega, Madison, WI, USA). This mutation was present in a heteroplasmic state in the patient's blood, muscle and skin, but was not detected in the mother's blood. Wild-type clones contained only the m.625G sequence.

Histological analysis

Unfortunately, many artifactual opaque fibers were observed in the temporalis muscle biopsy. Nevertheless, a few cytochrome c oxidase (COX) negative fibres were identified, although there were no ragged red fibres or strongly succinate dehydrogenase (SDH) reactive blood vessels (data not shown).

Genetic analysis

Whole mitochondrial DNA genome analysis, using peripheral blood DNA, detected two heteroplasmic base transitions: m.625G>A (Figure 2a) and m.5231G>A (data not shown).

The m.625G>A transition was present in a heteroplasmic state in the patient's blood, muscle and skin, but was not detected in her mother's blood (Figures 2b). The proportion of m.625G>A in muscle and skin was higher than that in blood (the approximate mutation load was 80 per cent in muscle and skin, and 70 per cent in blood) (Figure 2c). This transition was not present in 50 healthy controls.

The heteroplasmic m.5231G>A transition was present in both the patient's and her mother's blood.

Biochemical analysis

Respiratory chain enzyme assay showed that complex III activity was markedly decreased (30 per cent relative to citrate synthase, 17 per cent relative to complex II) while complex IV activity was slightly decreased (55 per cent relative to citrate synthase, 31 per cent relative to complex II).

Discussion

Mitochondrial sensorineural hearing loss is divided into the nonsyndromic type associated with m.1555A>G and the syndromic type associated with m.3243A>G. The complex of mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (known as MELAS) is representative of the latter.[1]

We considered our case to be the syndromic type, because the patient had short stature and suffered from hypertrichosis and epilepsy. Moreover, she showed high lactate levels in her blood and cerebrospinal fluid, and mild brain atrophy on MRI.

In the syndromic type of mitochondrial hearing loss, the retrocochlear auditory pathways require investigation, specifically to establish whether the auditory peripheral nerve and central nervous system (CNS) are intact or not. However, successful cochlear implantation has been reported in patients with the mitochondrial encephalopathy, lactic acidosis and stroke-like episode complex.[4–6] Sue *et al.* have reported successful cochlear implantation in such a patient, who had profound, bilateral hearing loss.[6]

Our case, too, underwent successful cochlear implantation, despite possible CNS disorders. In patients with many types of mitochondrial, profound, sensorineural hearing loss, we speculate that cochlear implantation may represent a promising treatment, because hearing loss associated with mitochondrial disorders is more likely to be caused by cochlear dysfunction than retrocochlear abnormalities.[1,6,7] Results from a guinea pig cochlear model also suggest that chronic mitochondrial dysfunction may most predominantly affect the stria vascularis and supporting cells.[8] Therefore, we believe that cochlear implantation should be considered in patients with progressive sensorineural hearing loss associated with a mitochondrial disease, regardless of whether their hearing loss is syndromic or nonsyndromic.

Of course, this treatment option should be reviewed for the potential complications; it may develop contraindications on the MRI scan (unless the magnet in the receiver-stimulator has been moved), or adverse events such as post-implant meningitis due to bacterial cellulitis.[9]

In our patient, whole mitochondrial DNA genome analysis detected two different heteroplasmic, one-base substitutions: m.625G>A and m.5231G>A.

Although heteroplasmic single nucleotide polymorphisms are rare, the m.5231G>A transition is unlikely to be pathogenic, because it has been listed as a single nucleotide polymorphism in the Mitomap database,[10] and because it was carried by our patient's healthy mother.

On the other hand, the m.625G>A transition (which involves the transfer RNA gene for phenylalanine) has not previously been reported in association with disease. This transition lies in close vicinity to the site of the m.622G>A mutation, which has been reported to be present in mild mitochondrial disease with hearing impairment.[11] Moreover, other mutations in the same transfer RNA gene for phenylalanine (e.g. m.582T>C, m.583G>A, m.606A>G, m.608A>G, m.611G>A, m.618T>C, m.636A>G and m.642T>C) have been recognised and listed in Mitomap, with deafness frequently mentioned as a clinical symptom.[12–14] In our patient, respiratory enzyme studies revealed a significant defect in complex III and a possible slight defect in complex IV, relative to citrate synthase and complex II. These results resembled those for other mutations of the same mitochondrial transfer RNA gene for phenylalanine, such as m.622G>A and m.618T>C.[11,15] Moreover, m.625G>A was not identified in our patient's mother's peripheral blood DNA, implying a *de novo* origin of the mutation, although this is not conclusive because only blood DNA was available from the mother. Such sporadic mutations have been reported in other patients with the same mitochondrial transfer RNA phenylalanine gene mutation.[12,16]

- **This report describes the case of a girl with mitochondrial sensorineural hearing loss**
- **Cochlear implantation was effective, and improved the patient's quality of life**
- **The mitochondrial DNA 625G>A mutation may be pathogenic for syndromic hearing loss**

Accordingly, we conclude that the m.625G>A transition may cause mitochondrial respiratory dysfunction and syndromic hearing loss. Another standard muscle biopsy and cybrid study would clarify the pathogenicity of the m.625G>A transition.

Conclusion

We report a sporadic case of progressive sensorineural hearing loss and epilepsy due to a mitochondrial disorder, successfully treated with cochlear implantation. The novel, heteroplasmic m.625G>A transition in the mitochondrial transfer RNA gene for phenylalanine may have been the pathogenic mutation in this case.

Cochlear implantation should be considered for patients with progressive, profound, bilateral, sensorineural hearing loss due to mitochondrial disease other than that due to the m.3243A>G mutation of the transfer RNA (tRNA(leu)) gene, or the m.1555A>G mutation of the 12s rivosomal RNA.

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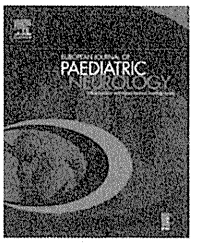

Case study

Iomazenil hyperfixation in single photon emission computed tomography study of malformations of cortical development during infancy

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ABSTRACT

We present 2 cases of malformations of cortical development and early onset epilepsy. The first case is of a patient with left hemimegalencephaly who developed focal epilepsy at the age of 2 days and cluster spasms at 1.5 months. After left functional hemispherectomy, seizures originated from the contralateral hemisphere, which had shown normal signals in the preoperative magnetic resonance imaging study. The second case is of a patient with lissencephaly, caused by a missense mutation in the doublecortin gene, who developed West syndrome at the age of 5 months. In both the cases, ^{123}I-iomazenil single photon emission computed tomography performed during infancy showed significant hyperfixation in the dysplastic lesions. This finding indicates the immaturity of the affected neurons and a gamma-aminobutyric acidergic involvement in epileptogenesis associated with malformations of cortical development during infancy.

1. Introduction

Malformation of cortical development (MCD) is one of the major causes of intractable childhood epilepsy.[1] Defects in some processes of corticogenesis, including migration, proliferation, or differentiation of neurons, can cause diverse types of MCDs. Here, we report 2 cases of patients with severe MCD and early onset epilepsy, who underwent ^{123}I-iomazenil single photon emission computed tomography (SPECT) during infancy. Neither of the patients had received benzodiazepines around the time of the SPECT scans.

2. Case study

2.1. *Case 1*

The patient was a boy with left hemimegalencephaly. His detailed clinical history, including seizure development

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associated with functional hemispherectomy, has been previously reported.[2] He experienced seizures consisting of motion arrest, apnoea, and facial cyanosis at the age of 2 days. Magnetic resonance imaging (MRI) revealed enlargement of and cortical dysplasia in the left cerebral hemisphere (Fig. 1A). The right hemisphere appeared to be normal, except for the slightly blurred grey-white matter demarcation in the medial orbito-frontal cortex and some frontocentral regions. Ictal electroencephalography (EEG) revealed that seizure activities originated in the left centro-parietal region. Valproate and phenobarbital were ineffective in ameliorating the seizures. At 1.5 months of age, the patient developed epileptic spasms in clusters. The EEG performed at the age of 2 months showed a suppression-burst pattern on the left side. Because of early onset intractable epilepsy, a left functional hemispherectomy was performed in another hospital, at the age of 3 months. However, intractable seizures originating from the right hemisphere developed shortly after the operation. Follow-up MRIs performed at the age of 7, 20, and 29 months showed frontocentral cortical lesions in the right hemisphere, with signs of progression of myelination, and an increase in the thickness of the cortex (Fig. 1D).

We performed ^{123}I-iomazenil SPECT twice before surgery. The first scan performed at age of 19 days showed significant hyperfixation in a large cortical area of the left hemisphere, particularly in the central region, and in the right frontocentral cortex (Fig. 1B), corresponding to the dysplastic lesions. Hyperfixation was also observed in the medial frontal and medial temporal regions. These findings were very distinct from the fixation patterns observed in the 3 control infants. All axial images of the control subjects are presented as Supplementary Figure, and some representative images of a 5-month-old boy with benign infantile convulsions are shown as Fig. 1H. The binding levels of iomazenil of the control subjects were high in the occipito-parietal region, especially in the primary visual cortex, and central region, but low in the fronto-temporal region. Although all the control infants suffered mild seizure episodes, they never had any other neurological abnormalities or organic lesions identifiable by MRI. The second SPECT performed at the age of 2 months showed reduced fixation in the frontal and the left parieto-temporal regions and enhanced fixation in the occipital cortex, which was considered a normal developmental change (Fig. 1C).

2.2. *Case 2*

The patient was a boy with type I lissencephaly caused by a missense mutation in doublecortin gene (c.2T > A). His postnatal psychomotor development was retarded. Cluster spasms appeared at the age of 5 months. His interictal EEG revealed a pattern of hypsarrhythmia, and hence, he was diagnosed with West syndrome. MRI performed upon admission revealed lissencephaly with an anterior severity in the anterior-posterior direction (Fig. 1E). The spasms were temporarily relieved by adrenocorticotropic hormone treatment. When he was 12-months-old, we added valproate to the treatment regimen. His seizures changed into focal seizures consisting of upward rolling of his eyeballs for a few seconds, and the seizure frequency decreased to a few times a day. The interictal EEG performed at the age of 17 months showed occasional spikes in the right occipital derivation.

^{123}I-iomazenil SPECT performed at the age of 6 months before the adrenocorticotropic hormone treatment, revealed pronounced hyperfixation across the entire cortex (Fig. 1F). This finding persisted in the follow-up scan at the age of 17

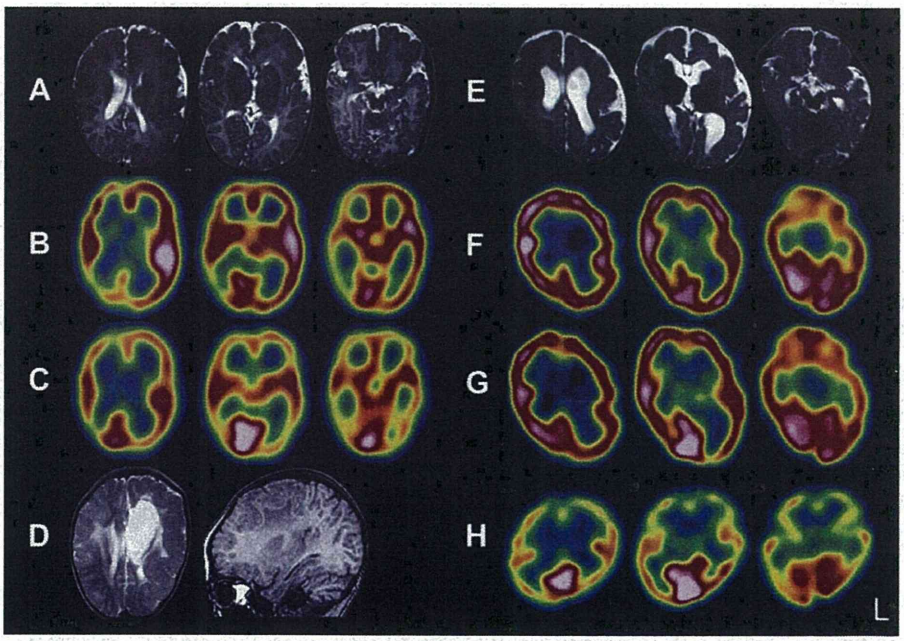

Fig. 1 – MRI and ^{123}I-iomazenil SPECT images of the 2 patients and a control subject. (A, E) T2-weighted images and (D) T2-weighted axial image (left) and T1-weighted sagittal image (right). (A–D) case 1: (A, C) 2 months, (B) 19 days, and (D) 29 months. (E–G) case 2: (E, F) 6 months, and (G) 17 months. (H) Control SPECT images of a 5-month-old infant with benign infantile convulsions. MRI, magnetic resonance imaging; SPECT, single photon emission computed tomography.

months (Fig. 1G). Compared to the control images (Supplementary Figure and Fig. 1H), the frontal hyperfixation was obviously abnormal; however, the hyperfixation in the occipital region, where the MRI showed a normal-shaped cortex, may be a physiological change.

3. Discussion

In both the patients with severe MCD, ^{123}I-iomazenil SPECT demonstrated abnormal hyperfixation in the lesions during early infancy. During normal development of the brain, postnatal neuronal maturation occurs early in the occipital and central regions and later in the frontal region. This development is corroborated by a synchronous progression of myelination and an increase in regional cerebral blood flow, which can be detected by MRI and SPECT. The images of the control infants in this study indicate that the iomazenil binding is symmetrically high initially in these regions, particularly in the occipital area, and is usually low in the fronto-temporal region during infancy (Supplementary Figure and Fig. 1H). In both patients, however, the hyperfixation areas extended beyond these physiological regions and included the frontal region. In case 1, the iomazenil binding in the left occipito-temporal region with a dysplastic cortex was asymmetrically higher than that in the right region with a normal-shaped cortex. Because ^{123}I-iomazenil SPECT is usually evaluated by visualizing the regional differences in the tracer binding level, we could not determine whether the absolute binding amount had increased in the dysplastic lesions. The relative fixation level, as compared to the physiological hyperfixation in the occipital region, however, indicates that the iomazenil binding in the lesions may have actually increased beyond the physiological level.

There are several potential mechanisms for the iomazenil hyperfixation in lesions. One of them is the association of abundant $GABA_A$ receptor expression with immaturity of the affected neurons and increased number of neurons and synapses. During brain development, GABA is the first active neurotransmitter, and it induces excitatory effects through $GABA_A$ receptors.[3] This GABAergic excitation has been found to be crucial to the progress in corticogenesis. A positron emission tomography study using flumazenil, a ligand of the $GABA_A$ receptor, suggested that $GABA_A$ receptor expression is higher in younger children.[4] These studies indicate that the immature neurons may express the abundant $GABA_A$ receptors.

In MCD lesions in paediatric patients, the presence of immature neurons and predominance of GABAergic synaptic activity have been demonstrated.[5] In addition, increased cortical thickness, delayed neurogenesis, and excessive neuro- and synaptogenesis have been suggested in hemimegalencephaly studies.[6,7] In case 1, the iomazenil hyperfixation in the cortical lesions might represent these actively ongoing abnormal corticogenesis. This notion is supported by the postnatal increase in the thickness of the dysplastic cortex in the right frontal lobe seen in this case. These results indicate that abundant expression of $GABA_A$ receptors in MCD lesions may play a critical role.

The excitatory GABAergic effects and abundant $GABA_A$ receptors in affected neurons may induce or enhance the epileptogenesis in MCDs during early infancy. Recent investigations indicate that the GABAergic excitation of immature neurons that are connected to the normal pyramidal cells might be crucial in inducing clinical seizures.[5,8,9] In the epileptogenic lesions of cortical dysplasia and hippocampal sclerosis, low expression of the potassium chloride co-transporter KCC2 has been identified.[10,11] The upregulation of KCC2 is crucial to the developmental and functional transition from excitatory to inhibitory GABAergic neurotransmission, which may start shortly after birth.[12] The failure of the upregulation of KCC2 would lead to prolonged excitation of the abundant $GABA_A$ receptors in the dysplastic lesions.

Another possible mechanism for iomazenil hyperfixation is the upregulation of postsynaptic $GABA_A$ receptors by a reduction in- or an impaired function of GABAergic neurons, as suggested in previous reports.[13,14] Interestingly, the involvement of *DCX* in the maturation of GABAergic neurons was recently reported.[8,15] The *DCX* mutation reported in case 2 supports this hypothesis.

In both the MCD cases reported, ^{123}I-iomazenil SPECT showed lesional hyperfixation during early infancy. This finding may be associated with the immaturity of the affected neurons and ongoing corticogenesis and may help in explaining epileptogenesis. Further investigation is required to elucidate the exact mechanisms and pathogenic significance of this finding.

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None of the authors has any conflict of interest to disclose.

Appendix. Supplementary material

The supplementary data associated with this article can be found in the on-line version at doi:10.1016/j.ejpn.2011.03.007.

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Original article

Effectiveness and safety of non-intravenous high-dose phenobarbital therapy for intractable epilepsy during childhood

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Abstract

High-dose phenobarbital (PB) therapy is effective for refractory status epilepticus. We reviewed medical records of patients with intractable partial epilepsies on whom performed non-intravenous high-dose PB therapy. Thirteen patients received PB rectally or orally at a dosage of 20–30 mg/kg/day initially, and the PB dosage was gradually reduced to a maintenance dosage of 5–10 mg/kg/day orally. We evaluated the effectiveness and safety of this procedure after 14 days at the maintenance dosage level. Twelve patients had partial seizures and one had secondary generalized seizures. In six of 13 patients (46%), seizure frequencies decreased more than 50%, and two of 13 patients (15%) became seizure free. In five of seven patients who were treated by continuous midazolam infusion therapy, we were able to discontinue the midazolam therapy. Adverse effects were found in seven of 13 patients. We were able to continue high-dose PB therapy in six patients because their adverse effects were transient and improved after a decrease in PB concentration, but we discontinued this therapy in the patient who developed Stevens–Johnson syndrome. Respiratory depression and hypotension were not found in our study. We conclude that high-dose PB therapy is effective and may be considered as an additional treatment for intractable partial epilepsy in childhood.

Keywords: High-dose phenobarbital therapy; Intractable partial epilepsy; Status epilepticus; Non-intravenous; Stevens–Johnson syndrome

1. Introduction

High-dose phenobarbital (PB) therapy, usually given intravenously, is effective for refractory status epilepticus [1,2]. In Japan, where intravenous PB therapy was not available until October 2008, there were some reports that non-intravenous high-dose PB therapy was effective for refractory status epilepticus [3–5]. In these reports, PB was given intramuscularly, rectally, or orally during high-dose PB therapy.

Only a few anecdotal studies reported that non-intravenous high-dose PB therapy was effective for intractable epilepsy in childhood as an additional therapy [6,7]. We performed non-intravenous high-dose PB therapy for intractable partial epilepsies during childhood, and evaluated the effectiveness and safety of this therapy.

2. Methods

We reviewed medical records of patients on whom non-intravenous high-dose PB therapy performed between January 1994 and July 2008 at Saitama Children's Medical Center, Saitama, Japan. Intractable partial epilepsy in this study was defined that epileptic partial seizures occurred daily even though we treated

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Table 1
Patients' characteristics, outcome, and adverse effects in high-dose PB therapy.

Case	Sex	Age at high-dose PB therapy	Past history and/or complications	Seizure type (manifestations)	Concomitant AEDs	Duration of high-dose PB therapy (days)	PB serum levels at evaluation (μg/ml)	Seizure outcome	Adverse effect
1	F	2 mo	Neonatal asphyxia, mutlicystic encephalomalacia, West syndrome, MR	Tonic posturing of the legs, myoclonus of the arms	VPA	29	53	Effective	No
2	M	10 mo	MR	Rolling up of the eyes, apnea, clonic movements of the arms	CLB, ZNS, MDL infusion	21	69	Effective (seizure free)	Drowsiness
3	F	11 mo	Subcortical band heterotopia, MR	Head rotation to the right, tonic posturing of the arms	PHT, VPA	21	66	Effective (seizure free)	Drowsiness
4	M	4 yr 7 mo	MR	Rolling up of the eyes, tonic posturing of the arms	AZA, CZP, PRM, MDL infusion	24	38	Effective	No
5	F	5 yr 10 mo	Huntington disease, MR	Clonic movements of the arms	CBZ, VPA, MDL infusion	18	71	Effective	No
6	F	6 yr 9 mo	Acute encephalopathy, MR	Oral automatism, deviations of the eyes to the right	AZA, PB[b], PHT, MDL infusion	50	82	Effective	Emotional instability
7[a]	M	1 mo	Neonatal asphyxia, MR	Rolling up of the eyes, tonic posturing of the arms	MDL infusion	30	44	Not effective	No
8	M	3 mo	West syndrome, MR	Tonic posturing of the legs	MDL infusion	31	54	Not effective	No
9	M	5 mo	MR	Rolling up of the eyes, tonic posturing of the arms	PHT, VPA	41	27	Not effective	Drowsiness
10	F	8 mo	West syndrome, MR	Rolling up of the eyes	PHT	22	50	Not effective	Drowsiness
11	M	1 yr 7 mo Hypersecretion of saliva	West syndrome, MR	Clonic movements of the arms, oral automatism	CLB, VPA, ZNS	21	40	Not effective	
12	F	9 yr 7 mo	MR	Loss of consciousness,	CLB, ZNS, PB[b]	18	32	Not effective	No
13	M	11 yr 10 mo	MR	Tonic posturing of the arms, secondary generalization	CLB, PHT, TPM, VPA, MDL infusion	8	ND	Aborted	Stevens–Johnson syndrome, liver dysfunction

AEDs, antiepilptic drugs; AZA, acetazolamide; CBZ, carbamazepine; CLB, clobazam; CZP, clonazepam; F, female; M, male; MDL, midazolam; MR, mental retardation; MRI, magnetic resonance imaging; ND, not done; mo, month; PB, phenobarbital; PHT, phyntoin; PRM, primidone; TPM, topiramate; VPA, valpric acid; yr, year; ZNS, zonisamide.

[a] All cases, except Case 7, were received rectal high-dose PB therapy initially. Only Case 7 was received oral high-dose PB therapy initially.

[b] PB was administered orally at standard dose.

by oral antiepileptic drugs (AEDs) or continuous intravenous midazolam (MDL) therapy. All patients were hospitalized in our institution, and were treated. Medical records were reviewed as follows: age at high-dose PB therapy, past history and/or complications, seizure type, concomitant AEDs when high-dose PB therapy performed, dosage of PB, PB serum level, the duration of high-dose PB therapy, seizure outcome, interictal electroencephalogram (EEG) findings, and adverse effects.

Patients received PB orally or rectally at a dosage of 20–30 mg/kg/day initially for 2–4 days, after which the PB dosage was gradually reduced to the maintenance dosage of 5–10 mg/kg/day, as previous studies examined [6,7]. After that, all of the patients received oral PB medications at the same maintenance dosage. No AEDs were added during high-dose PB therapy.

We evaluated the effectiveness and safety of high-dose PB therapy after 14 days at the oral maintenance dosage level, because the duration of the PB serum level at steady-state was about 14 days. We evaluated the effectiveness of this therapy as follows: "Effective" represented a greater than 50% seizure reduction, "Not effective" represented a less than 50% seizure reduction, and "Exacerbation" represented an increase in seizure frequency.

3. Results

High-dose PB therapy was performed on 13 patients (seven males and six females). The age range of the patients who received high-dose PB therapy was 1 month to 11 years, and the age range of the patients at the onset of epilepsy was 1 month to seven years (Table 1). Only one patient (Case 7 in Table 1) was received oral high-dose PB therapy from the beginning of this therapy. A history of neurological disorders was found in all 13 patients: mental retardation in 13 patients, West syndrome in four, neonatal asphyxia in two, Huntington Disease in one, and acute encephalopathy in one. The four patients who previously had West syndrome underwent a combination therapy of vitamin B6, γ-globulin, adrenocorticotropic hormone (ACTH) therapy, and thyrotropin-releasing hormone (TRH) therapy. Abnormal findings of magnetic resonance imaging (MRI) were found in nine patients (69%): cerebral atrophy in six patients, multicystic leukoencephalomalacia in one, subcortical band heterotopia in one, and cerebellar atrophy in one. Partial seizures were found in 12 patients and secondary generalized seizures in one, but primary generalized seizures were not found. Seizures with motor component, such as tonic posturing and clonic movements, were common in 10 of 13 patients.

The concomitant AEDs were shown in Table 1 when non-intravenous high-dose PB therapy had performed. Phenytoin and valproic acid was used for five patients, clobazam for four, zonisamide for three, acetazolamide and PB for two, and carbamazepine, clonazepam, primidone, and topiramate for one. MDL continuous infusion therapy was performed on seven patients, who had no complications induced by changes in medication, intercurrent infections, acute neurological insults such as meningitis, trauma, and anoxia. Four of seven patients who received MDL therapy had status epilepticus (Cases 5, 6, 8, and 13 in Table 1). Other three patients had repetitive partial seizures more than 20 times per day (Cases 2, 4 and 7).

In six of the 13 patients (46%), non-intravenous high-dose PB therapy was effective, and two of them (Cases 2 and 3) became seizure free. We were able to discontinue MDL continuous infusion in five patients (71%). Concerning seizure type, this therapy was effective in five of nine patients who had seizures with motor components such as tonic posturing and clonic movements. There was no difference in the duration (average ± standard deviation days) of non-intravenous high-dose PB therapy between the patients for whom this therapy was effective and not effective, 27.2 ± 11.8 and 27.2 ± 8.5 days, respectively. The average PB serum level at evaluation was 63.2 μg/ml among the patients for whom high-dose PB therapy was effective, and 41.1 μg/ml among the patients for whom high-dose PB therapy was not effective. Concerning interictal EEG findings, all patients had frequently multifocal and/or generalized electric abnormalities, such as spikes, polyspikes, spike-wave complexes, and high-amplitude high voltage slow waves. In the patients for whom this therapy was effective, the frequencies of these abnormalities decreased and generalization disappeared, whereas there were no changes of electric abnormalities in the patients for whom this therapy was not effective.

Adverse effects during the high-dose PB therapy were found in seven patients (54%): drowsiness in four patients, emotional instability in one, hypersecretion of saliva in one, and Stevens–Johnson syndrome in one. Drowsiness and emotional instability disappeared as the PB serum level decreased. Case 11 in Table 1 needed mandatory respirator support because hypersecretion of saliva obstructed his airway. Case 13 in Table 1 presented with Stevens–Johnson syndrome and liver dysfunction; we therefore discontinued high-dose PB therapy. He recovered after steroid therapy without any sequelae. In our study, respiratory depression and hypotension were not found.

4. Discussion

The present study showed that non-intravenous high-dose PB therapy was effective for intractable partial epilepsy in childhood. A reduction of more than 50% of seizure frequency was found in 46% of the patients, and discontinuation of MDL continuous infusion was in 71%. Adverse effects were found in seven patients; those in six patients were transient and improved after

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).

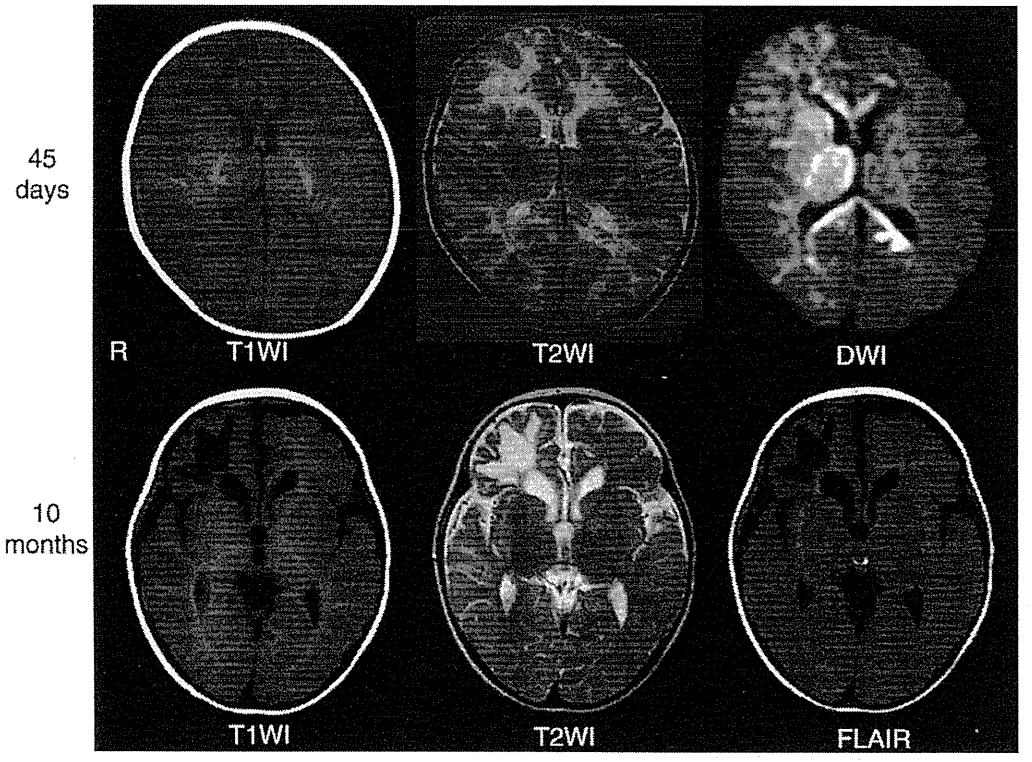

Fig. 1. MRI findings of Patient 1. Top: MRI at 45 days of age. Diffusion-weighted images revealed reduced diffusion predominantly in the subcortical white matter in the right frontal area. Reduced diffusion was also observed in the basal ganglia, thalamus, corpus callosum, and posterior limb of the internal 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.

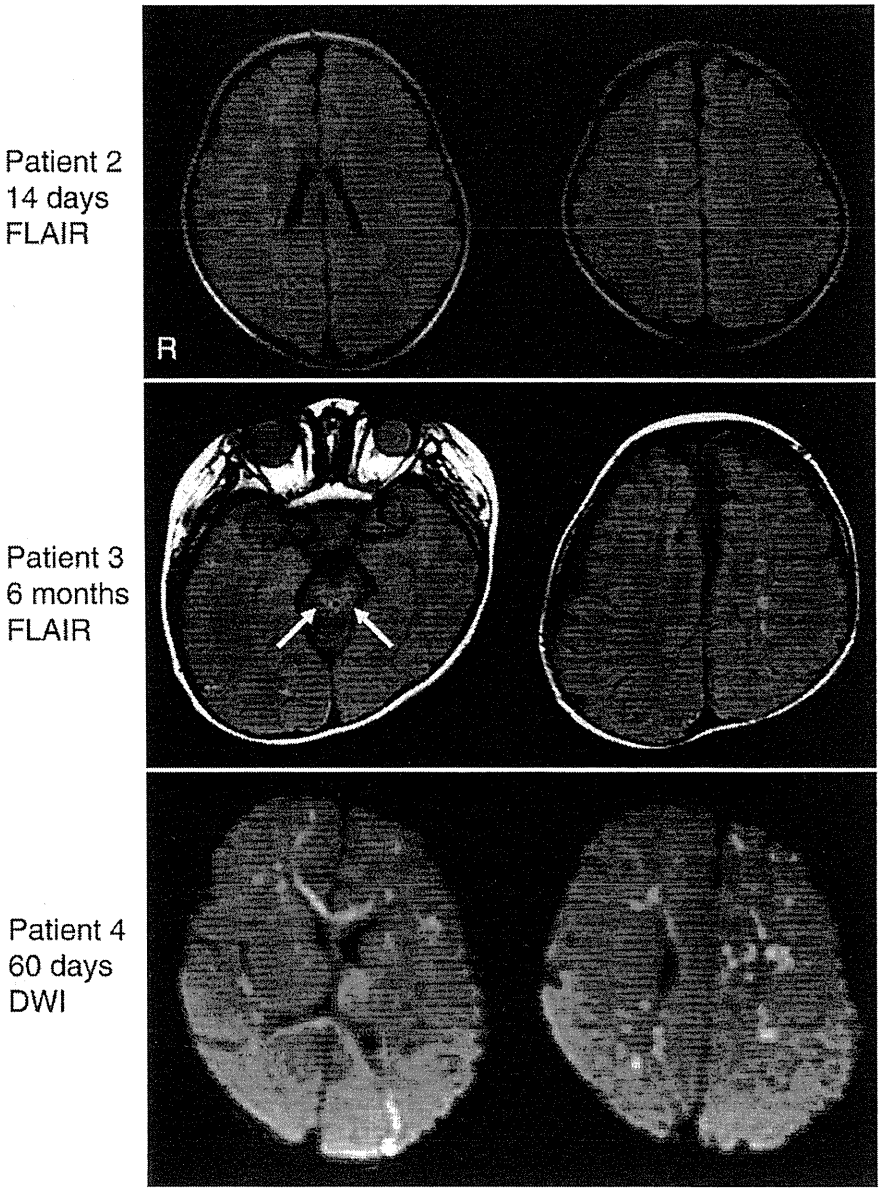

Fig. 2. MRI findings during the acute stage of encephalopathic manifestation. Top: FLAIR of Patient 2 at 14 days of age. Patchy high-intensity areas were seen in the subcortical areas predominantly in the right hemisphere. Middle: FLAIR of Patient 3 at 6 months of age. Linear high intensities were observed 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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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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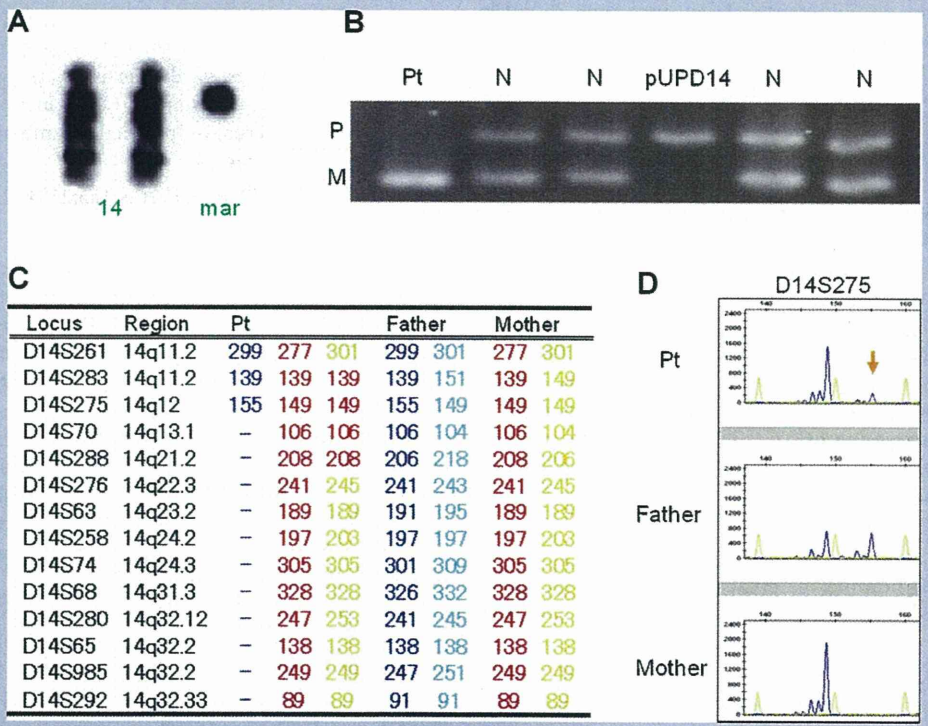
CLINICAL REPORT

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].