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Case report

Immunomodulatory therapy in recurrent acute necrotizing encephalopathy ANE1: Is it useful?

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Abstract

Acute Necrotizing Encephalopathy (ANE) is a rare disorder characterized by fever, seizures and rapid progression to coma after the onset of a viral infection. Most cases are sporadic, however the observation of multiple cases in the same family with recurrent episodes of ANE led to the identification of a genetic form of the disorder, called ANE1, and to the discover of the causative mutation in *RANBP2* gene. We report the first Italian child with ANE1 carrying the common c.1880C > T mutation in the *RANBP2* gene, who presented three episodes of acute encephalopathy in the first two years of life. The child showed a less severe clinical and neuroradiological course with respect to the previously reported patients. During the acute encephalopathy episodes he was treated with steroids and immunoglobulin. A very low steroid maintenance therapy was administered after the second episode until the onset of the third. Thirty days after the last episode he started monthly intravenous immunoglobulin that might be used for prevention of viral infections. At the moment he is still continuing a low steroid maintenance therapy and monthly IVIG. We could hypothesize that the less severe clinical presentation of the third episode might be correlated to the steroid treatment or that the patient grew older. Despite there is no evidence to support that ANE1 is an immune-mediated disease, immunomodulatory therapy might be considered in the management of ANE1 cases especially in early childhood, in which a fatal course has been frequently

Abbreviations: ANE, acute necrotizing encephalopathy; *RANBP2*, RAN-binding protein 2; ICU, intensive care unit; GCS, Glasgow coma scale; MRI, magnetic resonance imaging; IVIG, intravenous immunoglobulin; CSF, cerebrospinal fluid

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[3] These authors critically evaluated the neurological and neuroradiological differential diagnosis and they selected the MRI images.
[4] These authors contributed to data acquisition and analysis.
[5] This author contributed to differential diagnosis and conception, design and critical revision of the article.
[6] This author performed genetic analysis.
[7] All the authors approved the final version of the article.

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reported. Further studies will be necessary to define the clinical, immunological and genetic aspects, as well as the outcome of immunomodulatory therapy in patients with ANE1.

Keywords: Acute; Recurrent; Necrotizing; Encephalopathy; *RANBP2*; Steroid

1. Introduction

Acute Necrotizing Encephalopathy (ANE) is a rare disorder characterized by fever, seizures and a rapid progression to coma within days after the onset of a viral infection. More commonly influenza A, but also influenza B, parainfluenza, and HHV6 affect children with apparent normal growth and development. The first cases have initially been reported in Asia by Mizuguchi [1–3] and then described worldwide [4].

The brain Magnetic Resonance Imaging (MRI) hallmark is represented by symmetrical lesions in the thalami, brainstem tegmentum, cerebellum, and periventricular white matter [1–3]. Most cases are sporadic and the disease is typically monophasic. However the observation of multiple cases in the same family with recurrent episodes of ANE led to the identification of a genetic locus on chromosome 2q [5,6], with subsequent identification of causative mutations in the gene *RANBP2 (OMIM 601181)*. Thus, in addition to the sporadic ANE, a genetic form of the disorder, ANE1, has been recognized [7].

Here we report the first Italian child with ANE1 carrying the common c.1880C > T mutation in the *RANBP2* gene, who presented three episodes of acute neurological involvement in the first two years of life.

2. Case report

Male, 3 years and 9 months old. The first episode of encephalopathy occurred when he was 5 month-old during an episode of gastroenteritis. He presented with hypotonia and focal seizures and was admitted to Intensive Care Unit (ICU), intubated and ventilated. Brain MRI showed bilateral involvement of the thalami with moderate enlargement of lateral ventricles (Fig. 1). He was started empiric, symptomatic and immunomodulatory therapy. The infectious and metabolic investigations

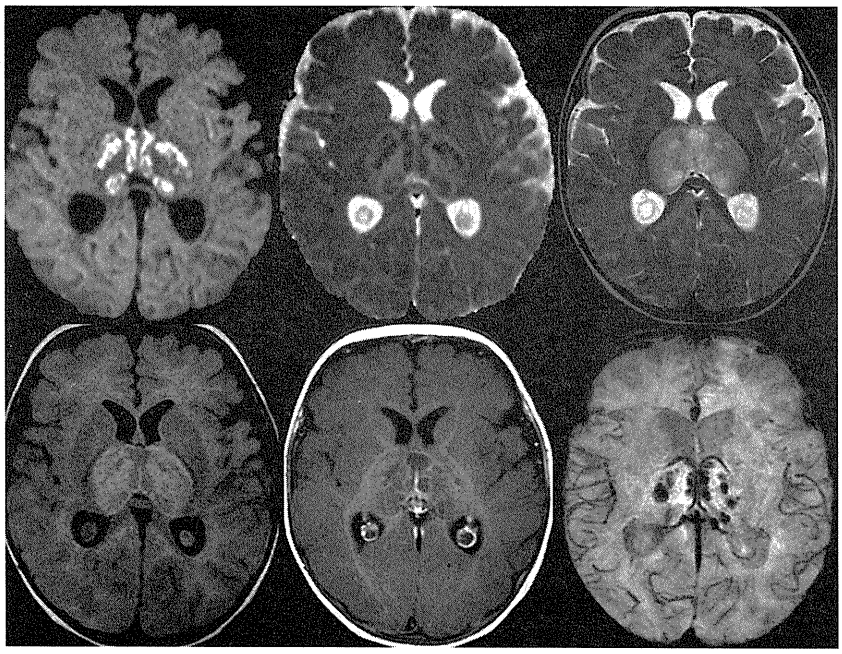

Fig. 1. MRI at presentation. (A) Axial diffusion-weighted image; (B) Axial ADC map; (C) Aaxial T2-weighted image; (D) Axial FLAIR image; (E) Contrast-enhanced axial T1-weighted image; (F) Axial susceptibility-weighted image. Diffusion-weighted images (A) and corresponding ADC map (B) clearly show multiple areas of restricted diffusion against a background of increased diffusion involving both thalami, which are swollen. On T2-weighted (C) and FLAIR (D) images, the thalami are markedly swollen and hyperintense. On T1-weighted images obtained after intravenous gadolinium chelate injection (E), multiple necrotic portions are well delineated by peripheral faint, linear enhancement. Incidental choroid plexus cysts are detected. Susceptibility-weighted image (F) shows multiple hypointense spots, consistent with petechial hemorrhage.

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Table 1
Clinical characteristics of recurrent episodes of ANE in our case.

	1st Episode	2nd Episode	3rd Episode
Age	5 mo	18 mo	26 mo
Concomitant infection	Rotavirus	RSV	Not identified
Duration of fever before encephalopathy	2 days	4 days	4 days
AST/ALT (U/L)	Normal	Normal	Normal
CSF	16 cells/mmc glucose 94 mg/dL protein 172 mg/dL	16 cells/mmc Glucose 60 mg/dL Protein 495 mg/dL	6 cells/mmc Glucose 48 mg/dL Protein 72 mg/dL
Other infectious investigations	Negative	Negative	Negative
Metabolic investigations	Normal	–	–
Lymphocytes subset, NK function, perforin expression	–	Normal	–
Start of therapy after onset	48 h	33 h	12 h
Therapy of the acute episode	Antibiotic Acyclovir Phenobarbital Mannitol Methylprednisolone (30 mg/kg/day iv for 5 days), followed by oral dexamethazone (0,5 mg/kg/day) with tapering IVIG (2 g/kg in 5 days)	Antibiotic Acyclovir Phenobarbital Dexamethazone (0.4 mg/kg/day iv for 10 days), followed by oral tapering IVIG (2 g/kg in 5 days)	Antibiotic Acyclovir Phenobarbital Methylprednisolone (30 mg/kg/day iv for 3 days), followed by oral dexamethazone (0,3 mg/kg/day) with tapering IVIG (2 g/kg in 2 days)

–, Not performed; RSV, respiratory syncytial virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

were negative (Table 1). The general and neurological conditions improved and he was discharged after 22 days. MRI at 9 months showed almost complete resolution of the previous picture (Fig. 2).

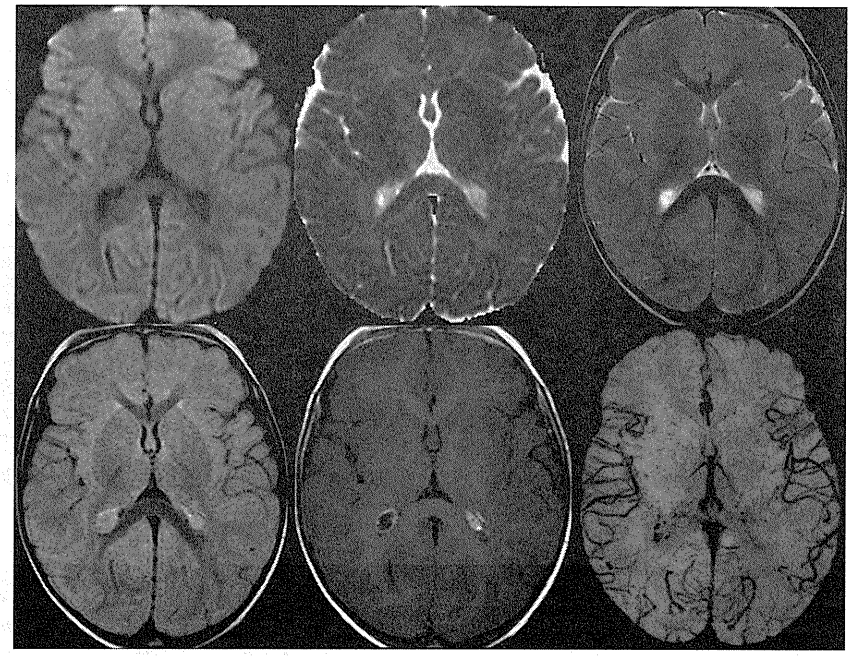

Fig. 2. MRI at 9 months. (A) Axial diffusion-weighted image; (B) Axial ADC map; (C) Axial T2-weighted image; (D) Axial FLAIR image; (E) Contrast-enhanced axial T1-weighted image; (F) Axial susceptibility-weighted image. There is almost complete resolution of the previous picture with only a tiny residual gliotic area in the right thalamus.

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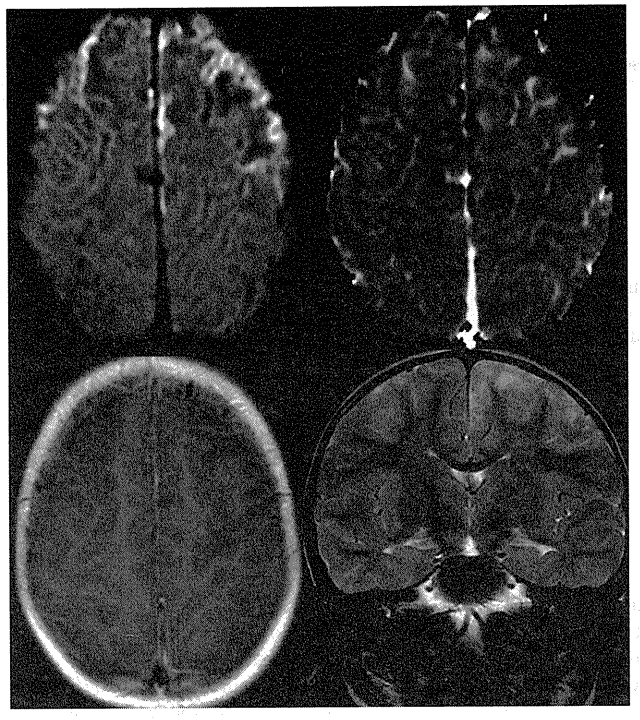

Fig. 3. MRI at 18 months. (A) Axial diffusion-weighted image; (B) Axial ADC map; (C) Contrast-enhanced axial T1-weighted image; (D) Coronal T2-weighted image. There is restricted diffusion at level of the frontal cortex bilaterally (A, B) consistent with cytotoxic edema. The blood-brain barrier is intact (C) although the cortex is swollen (C, D).

He was well with normal psychomotor development up to the age of 18 months when he had a 2nd episode of encephalopathy with fever and irritability and was admitted to ICU with Glasgow Coma Scale (GCS) 10/15 for focal seizures. MRI showed both supra and infratentorial cortical and subcortical lesions with diffuse swelling (Fig. 3). He showed a rapid improvement and was discharged after 18 days with oral dexamethazone tapering (Table 1). He continued oral dexamethazone at maintenance dose of 0.02 mg/kg/day.

At the age of 26 months he showed an episode of pharyngitis with fever, rapidly followed by irritability, sopor, balancing and swallowing disturbances. MRI showed less extensive lesions with respect to the first exam (Fig. 4). He then underwent to an empiric, symptomatic and immunomodulatory therapy (Table 1).

The child rapidly improved and was discharged after 17 days. Thirty days after the last episode he started monthly Intravenous Immunoglobulin (IVIG) (400 mg/kg). IVIG infusion was started to try to obtain a passive prevention of viral infections; indeed the child at the onset of the 3rd encephalopathic episode had serum IgG level at the inferior normal values for age (457 mg/dL). He continued therapy with dexamethazone (0.075 mg/kg/day) and the vaccination regime was stopped, waiting to gradually reduce the steroid therapy.

At the last follow up, at 3 years and 9 months of age, the child presented with progressive cognitive and developmental improvement. At the moment he is still

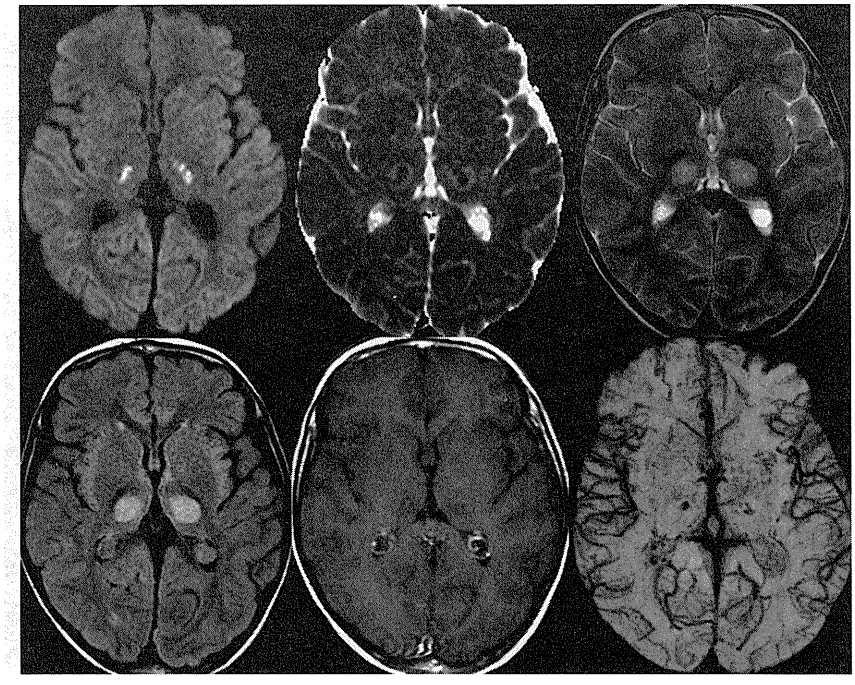

Fig. 4. MRI at 26 months. (A) Axial diffusion-weighted image; (B) Axial ADC map; C. axial T2-weighted image; (D) Axial FLAIR image; (E) Contrast-enhanced axial T1-weighted image; (F) Axial susceptibility-weighted image. MRI shows relapse of the disease, with findings analogous to those at presentation (compare with Fig. 1), albeit less severe.

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Table 2
Clinical data of published patients with recurrent or familial ANE with genetic studies.

	Patients and episodes of ANE and age at onset	Trigger infection	Therapy	Clinical course	Familiarity for encephalopathy	RANBP2 mutation (found: Y; not found: N)
Neilson DE, 2003	(1) F at 2 y (seizures, coma)	Not reported	Not reported	Death after 12 h	Another 11 cases in the family (clinical data not reported)	
	(2) M (brother of pt 1) at 9 m (status epilepticus)	Not reported	Not reported	Death after 12 h		
	(3) M 1st episode at 18 m (hallucination, unresponsive)	Not reported	Not reported	Residual abducens palsy		Heterozygous c.1880C > T; p.Thr585Met mutation (Genetic testing in 2009)
	2nd episode at 4 y (coma)	Influenza A	Not reported	Severe mental retardation and spastic quadriplegia		
	(4) M 1st episode at 3 y (coma, decorticated posture, focal seizures)	Not found	Not reported	Full recovery after 1 m		Heterozygous c.1880C > T; p.Thr585Met mutation (Genetic testing in 2009)
	2nd episode at 4 y 8 m (coma)	Parainfluenza type II	Not reported	Residual ataxia, aphasia, tremor and disconjugate gaze		
	(5) M (twin of pt 4) at 3 y (coma, decorticated posture, focal seizures)	Not found	Not reported	Full recovery after 3 m		Heterozygous c.1880C > T; p.Thr585Met mutation (Genetic testing in 2009)
Lopez-Laso E, 2009	(1) M 1st episode at 3 m (generalized seizures, coma)	Not found	Vitamins, carnitine	Full recovery after 1 week	Another 1 case in the family at 15 mo with fatal course (clinical data not reported)	Genetic testing in cases 1 and 2 and in another 4 family members: N
	2nd episode at 7 m (focal seizures, coma, decerebration)	Not found	Not reported	Death after 2 days		
	(2) M at 3 y (obnubilation, ataxia, myoclonic seizures)	Not found	Not reported	Full recovery after 17 days		
	(3) F at 10 m (status epilepticus, coma)	Not found	Not reported	Death		
Gika AD, 2010	F 1st episode at 9 m (encephalopathy with seizures; ventilated for 10 days)	Not found	No	Slow but complete recovery	Her mother presented an episode of encephalitis/ polyneuritis after a viral infection at 19 y	Heterozygous c.1880C > T; p.Thr585Met mutation in the patient and her mother
	2nd episode at 2 y (VI nerve palsy)	Not found	No	Recovery		
	3rd episode at 9 y (decreased consciousness: coma with intubation and ventilation)	Influenza A	Antiviral, antibiotic, immunomodulatory (high-dose of steroids after 24 h of disease onset and IVIG)	Neurological and cognitive sequelae		

(*continued on next page*)

Table 2
(*continued*)

	Patients and episodes of ANE and age at onset	Trigger infection	Therapy	Clinical course	Familiarity for encephalopathy	RANBP2 mutation (found: Y; not found: N)
Marco EJ, 2010	3 brothers (parents first cousins)					Genetic testing in case 3: N
	(1) M at 11 m (intermittently responsive, focal seizures with secondary generalization)	Serum IgM for Enterovirus positive	Case 1 and 2: antiviral (acyclovir), antibiotic, antipyretic, anticonvulsant, intracranial pressure management, hemodynamic support	Fatal after 3 days		
	(2) M at 23 m (focal seizures; after 16 h unresponsive)	Influenza A		Fatal after 16 h		
	(3) M (twin of pt 2) 1st episode at 18 m (ataxia, myoclonic jerks)	Not found	No	Apparent full recovery		
	2nd episode at 23 m(decreased consciousness with decorticated posturing)	Not found	Antiviral (oseltamivir), antibiotic, antipyretic, anticonvulsant, vitamins, coenzyme Q10, zinc, intracranial pressure management, hemodynamic support, immunomodulatory (dexamethasone, plasmapheresis)	Severe neurological sequelae Death at 8 y for status epilepticus		
Loh NR, 2010	M 1st episode at 18 m (reduction of consciousness, admission to ICU)	Adenovirus in stool positive	Antibiotic, antiviral (acyclovir), anticonvulsant	Discharged at day 19 with full recovery		Heterozygous 3238 C > T mutation The same mutation was found in his apparently healthy brother and mother
	2nd episode 3 y 11 m (confusion, lethargy)	Influenza A (H3N2)	Antibiotic and antiviral	Discharged at day 12 with full recovery		
Gilson C, 2010	F at 5 y (GCS 7/15: intubation and ventilation, decerebrated posture)	Not found	Anticonvulsant and antibiotic	Dysarthria and severe motor deficit; after one year she presented bilateral hemiparesis but speech and language improvement		Heterozygous c1754C > T:p.Thr585Met mutation. The same mutation was found in her asymptomatic mother, brother and maternal grandmother. In the patient was found c.833T > C; p.1278T mutation in CBS gene associated to homocysteinuria.

continuing a monthly IVIG infusion and oral dexamethazone (0.033 mg/kg/day) without any negative effects on his stature and ponderal growth, while he is starting again the vaccination regime.

Due to the recurrent encephalopathic episodes, ANE1 was suspected and molecular analysis of the *RANBP2* gene was performed after recruiting the informed consent. So we were able to identify a heterozygous missense mutation (c.1880C > T; p.Thr585Met). The genetic testing of his mother resulted negative for the *RANBP2* mutation, while his apparently healthy father and uncle (brother of his father) were carrying the same c.1880C > T; p.Thr585Met mutation on *RANBP2* gene. No other cases of encephalitis/encephalopathy were reported in the family.

3. Discussion

We report a new combined steroid and immunoglobulin therapy in the first Italian ANE1 case. The steroid therapy associated with IVIG was administered during the acute encephalopathy events, while the maintenance therapy was applied, using a very low steroid dosage, and a further monthly IVIG therapy for prevention of viral infections. ANE1 onset appears mainly in the earlier childhood, but rare cases have been reported in the adolescence or even in adult age [7]. In particular, this child presented the first ANE episode at 5 months of age, the youngest onset ever reported in ANE1 patients (Table 2). ANE1 must be always considered as differential diagnosis in any patient presenting with ANE and one of the following characteristics: (1) family history of neurological symptoms, occurring during an infection; (2) recurrent encephalopathy following fever, with or without findings of ANE; (3) MRI changes of ANE with additional lesions in any of the following areas: medial temporal lobe, insular cortices, claustrum, external capsule, amygdale, hippocampi, mammilary bodies, spinal cord [3,7,8].

In this reported case, due to the manifestation of recurrent ANE events associated to the presence of MRI lesions in the subcortical cerebral white matter, in the cerebellum and in the hippocampi, suggested us to hypothesize ANE1 diagnosis, so genetic analysis of *RANBP2* gene was performed [8].

This child was carrying the already reported most frequent c.1880C > T mutation in the *RANBP2* gene [7], that was transmitted by his healthy father, who he still never presented episodes of encephalitis/encephalopathy. ANE1 is an autosomal dominant disease with an incomplete penetrance [7]. The *RANBP2* mutation predisposes to ANE1, but additional factors are required to make the phenotype fully penetrant like other still unknown genes, type of virus, dose of inoculum, route of infection, nutritional status, priory infection and inflammatory state [8]. Since some familiar cases resulted being negative to *RANBP2* mutational screening, it is likely that other genetic loci could be involved in the pathogenesis of ANE [7–10].

Some authors previously proposed an empiric treatment with high dose of steroids in ANE patients [4,11]. Okumura et al. retrospectively evaluated the clinical course and the outcome of 34 children with isolated ANE, treated with steroids and gammaglobulin. They observed that steroid therapy within 24 h from the symptoms' onset was associated with a better outcome in children without brainstem lesions. They further reported that the outcome was not correlated with gammaglobulin treatment at the dose of 1–2 g/kg [12].

In 88% of ANE1 cases brainstem lesions are present and they are less predictive as they produce a full range of outcomes, independently from the steroid therapy [8]. In a recent paper Loh et al. [13], reported a patient with ANE1 who presented two recurrent encephalopathy episodes and a good clinical outcome without immunomodulatory therapy after one year follow-up.

Differently from the already published cases [14,15], the presently reported child showed a less severe clinical and neuroradiological course. We could then hypothesize that this mild clinical course might be correlated to the very low steroid maintenance therapy that was administered to the child after the 2nd episode or that the patient grew older. We could then suggest that a monthly IVIG therapy might be used for prevention of viral infections. Despite there is no evidence to support that ANE1 is an immune-mediated disease, immunomodulatory therapy might be considered in the management of ANE1 cases especially in early childhood in which fatal course has been frequently reported.

Further studies will be necessary to define the clinical presentation, the immunological and genetic aspects, the outcome of immunomodulatory therapy in the acute episodes of ANE1.

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References

1 Mizuguchi M, Abe J, Mikkaichi K, Noma S, Yoshida K, Yamanaka T, et al. Acute necrotising encephalopathy of childhood: a new syndrome presenting with multifocal, symmetric brain lesions. J Neurol Neurosurg Psychiatry 1995;58:555–61.

Please cite this article in press as: Bergamino L et al. Immunomodulatory therapy in recurrent acute necrotizing encephalopathy ANE1: Is it useful? Brain Dev (2011), doi:10.1016/j.braindev.2011.08.001

2 Mizuguchi M. Acute necrotizing encephalopathy of childhood: a novel form of acute encephalopathy prevalent in Japan and Taiwan. Brain Dev 1997;19:81–92.
3 Mizuguchi M, Yamanouchi H, Ichiyama T, Shiomi M. Acute encephalopathy associated with influenza and other viral infections. Acta Neurol Scand 2007;115:45–56.
4 Mastroyanni SD, Gionnis D, Voudris K, Skardoutsou A, Mizuguchi M. Acute necrotizing encephalopathy of childhood in non-Asian patients: report of three cases and literature review. J Child Neurol 2006;21:872–9.
5 Neilson DE, Eiben RM, Waniewski S, Hoppel CL, Varnes ME, Bangert BA, et al. Autosomal dominant acute necrotizing encephalopathy. Neurology 2003;61:226–30.
6 Neilson DE, Feiler HS, Wilhelmsen KC, Lynn A, Eiben RM, Kerr DS, et al. Autosomal dominant acute necrotizing encephalopathy maps to 2q12.1-2q13. Ann Neurol 2004;55:291–4.
7 Neilson DE, Adams MD, Orr CM, Schelling DK, Elben RM, Kerr DS, et al. Infection-triggered familial or recurrent cases of acute necrotizing encephalopathy caused by mutations in a component of the nuclear pore, *RANBP2*. Am J Hum Genet 2009;84:44–51.
8 Neilson DE. The interplay of infection and genetics in acute necrotizing encephalopathy. Curr Opin Pediatr 2010;22:751–7.
9 López-Laso E, Mateos-González ME, Pérez-Navero JL, Camino-León R, Briones P, Neilson DE. Infection-triggered familial or recurrent acute necrotizing encephalopathy. An Pediatr (Barc) 2009;71:235–9.
10 Marco EJ, Anderson JE, Neilson DE, Strober JB. Acute necrotizing encephalopathy in 3 brothers. Pediatrics 2010;125:e693–8.
11 Manara R, Franzoi M, Cogo P, Battistella PA. Acute necrotizing encephalopathy: combined therapy and favorable outcome in a new case. Childs Nerv Syst 2006;22:1231–6.
12 Okumura A, Mizuguchi M, Kidokoro H, Tanaka M, Abe S, Hosoya M, et al. Outcome of acute necrotizing encephalopathy in relation to treatment with corticosteroids and gammaglobulin. Brain Dev 2009;31(3):221–7.
13 Loh NR, Appleton DB. Untreated recurrent acute necrotising encephalopathy associated with *RANBP2* mutation, and normal outcome in a Caucasian boy. Eur J Pediatr 2010;169:1299–302.
14 Gika AD, Rich P, Gupta S, Neilson DE, Clarke A. Recurrent acute necrotizing encephalopathy following influenza A in a genetically predisposed family. Dev Med Child Neurol 2010;52:99–102.
15 Gilson C, McFarland R, Forsyth R. Autosomal dominant acute necrotising encephalopathy: a case report with possible disease-expression modification by coincidental homocysteinuria. Eur J Paediatr Neurol 2011;15:174–6.

Please cite this article in press as: Bergamino L et al. Immunomodulatory therapy in recurrent acute necrotizing encephalopathy ANE1: Is it useful? Brain Dev (2011), doi:10.1016/j.braindev.2011.08.001

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FULL-LENGTH ORIGINAL RESEARCH

Mutations of the *SCN1A* gene in acute encephalopathy

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Summary

Purpose: Acute encephalopathy is the most serious complication of pediatric viral infections, such as influenza and exanthema subitum. It occurs worldwide, but is most prevalent in East Asia. Recently, there have been sporadic case reports of epilepsy/febrile seizure and acute encephalopathy with a neuronal sodium channel alpha 1 subunit (*SCN1A*) mutation. To determine whether *SCN1A* mutations are a predisposing factor of acute encephalopathy, we sought to identify *SCN1A* mutations in a large case series of acute encephalopathy including various syndromes.

Methods: We analyzed the *SCN1A* gene in 87 patients with acute encephalopathy, consisting of 20 with acute necrotizing encephalopathy (ANE), 61 with acute encephalopathy with biphasic seizures and late reduced diffusion (AESD), and six with nonspecific (unclassified) acute encephalopathy.

Key Findings: Three patients had distinct point mutations. Two of them had epileptic seizures prior to acute encephalopathy. Clinical and neuroradiologic findings of acute encephalopathy were diverse among the three patients, although all had a prolonged and generalized seizure at its onset. The first patient with V982L had partial epilepsy and AESD. The second patient with M1977L had febrile seizures and nonspecific acute encephalopathy. The third patient with R1575C had no seizures until the onset of ANE. M1977L was a novel mutation, whereas the remaining two, V982L and R1575C, have previously been reported in cases of Dravet syndrome and acute encephalopathy, respectively.

Significance: These findings provide further evidence that *SCN1A* mutations are a predisposing factor for the onset of various types of acute encephalopathy.

KEY WORDS: *SCN1A*, Ion channel gene defect, Acute encephalopathy status epilepticus, Seizure susceptibility.

Acute encephalopathy (AE) refers to brain dysfunction of acute onset that usually follows an infectious disease with fever. Pathologic substrate of AE is diffuse, noninflammatory brain edema. AE is most common in infants and young children, and is manifested clinically with stupor/coma and a febrile seizure, which is often severe and prolonged.

Based on clinical and neurologic findings, AE is classified into multiple syndromes, such as Reye's syndrome, acute necrotizing encephalopathy (ANE) (Mizuguchi et al., 1995), and acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) (Takanashi et al., 2006). These syndromes show distinct computed tomography/magnetic resonance imaging (CT/MRI) features: diffuse brain edema in Reye's syndrome; bilateral, symmetric thalamic lesions in ANE; and cerebral cortical edema of subacute onset (usually appearing on the third to ninth day of illness, and sparing the perirolandic region) in AESD (Mizuguchi et al., 2007); however, about one-third of patients with AE show no such features and are unable to be classified into these syndromes (nonspecific AE). Pathogenesis of AE is complex, and much remains to be elucidated. The main pathomechanism differs among syndromes: metabolic disorder in Reye's syndrome, cytokine storm in ANE, and excitotoxicity in AESD (Mizuguchi et al., 2007). Delayed neuronal death after a severe/prolonged febrile seizure may play a major role in the pathophysiology of AESD (Mizuguchi et al., 2007; Takanashi et al., 2009).

Mutation of the neuronal sodium channel alpha 1 subunit (*SCN1A*) is the most common cause of hyperthermia-induced seizure susceptibility in patients with generalized epilepsy with febrile seizure plus (GEFS+) and Dravet syndrome (Escayg et al., 2000; Claes et al., 2001; Escayg et al., 2001; Wallace et al., 2001, 2003; Ohmori et al., 2002; Sugawara et al., 2002; Nabbout et al., 2003; Fukuma et al., 2004; Mantegazza et al., 2005; Escayg & Goldin, 2010). By contrast, *SCN1A* mutations are rare in febrile seizures other than GEFS+ and Dravet syndrome (Malacarne et al., 2002).

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Recently, there have been sporadic case reports of epilepsy/febrile seizure and AE with an *SCN1A* mutation (Sakakibara et al., 2009; Kobayashi et al., 2010; Takayanagi et al., 2010). In addition, children with Dravet syndrome occasionally have AE, which often causes death (Sakauchi et al., 2011). These cases suggest the role of *SCN1A* mutations in the pathogenesis of AE. To validate the hypothesis that *SCN1A* mutations are a predisposing factor of AE, we conducted *SCN1A* gene analysis in a large case series of AE, including various syndromes.

Methods

Patients

The Ethics Committees of the University of Tokyo approved this study. The parents or legal guardians of participants signed an informed consent form as approved by the ethics committees. Eighty-seven patients, 48 female and 39 male, who were diagnosed with AE and treated by 29 hospitals in Japan between September 2008 and August 2010, participated in this study. All the patients were of Japanese ethnicity. Diagnosis of AE was based on the following criteria: (1) impairment of consciousness showing acute onset, rapid progression and duration for more than 24 h, with or without seizures; (2) onset during the course of a febrile and/or infectious disease; and (3) exclusion of similar conditions, such as postictal stupor/coma, effect of anticonvulsants, meningitis, encephalitis, toxic encephalopathy, and metabolic errors. In addition, the patients were divided to three subgroups. Diagnosis of ANE and AESD was based on the criteria described previously (Hoshino et al., 2011). According to syndromic classification, 20 patients had ANE, 61 had AESD, and 6 had nonspecific AE (Table 1). There was no case of classical Reye's syndrome. Seven patients had preexisting febrile seizures and one patient had partial epilepsy before the onset of acute encephalopathy (Table 1). One hundred healthy adult Japanese volunteers without a history of AE served as control subjects.

Genetic analysis

Genomic DNA of patients with AE was prepared from ethylene diamine tetraacetic acid (EDTA)–treated whole blood samples using the QuickGene DNA whole blood kit (Fujifilm Corporation, Tokyo, Japan). *SCN1A* was screened by a direct sequencing method with an automatic sequencer, as described previously (Kobayashi et al., 2010). Reference sequence of mRNA was based on information available from GenBank (accession number: Human SCN1A. AF117907.1).

Results

Of the 87 AE cases studied, three had missense mutations—V982L, M1977L, and R1575C—none of which were found in the 100 controls. The *V982L* mutation was found in case 1 with partial epilepsy and AESD. The valine 982 residue is located on the transmembrane segment 6, domain II of SCN1A protein, is highly conserved among vertebrates, and shares homology with other types of sodium channels (Fig. 1). This mutation was previously reported in a patient with Dravet syndrome without myoclonic seizures and ataxia (Singh et al., 2009). The M1977L mutation was found in case 2 with GEFS+ and nonspecific AE. The methionine 1977 residue is located on near the C-terminus, and is conserved through mammalians (Fig. 1).

Table 1. Clinical data of 87 patients with acute encephalopathy (AE)

Diagnosis	Sex	Age at onset of AE (mean)	Family history of FS/epilepsy	Preexisting seizures	Preceding infection	Status epilepticus or cluster of seizures within 2 days after the onset of AE	Prognosis: Neurologic sequelae
ANE	Male 7	8 months to 9 years	FS 2	FS 1	HHV-6 3	Yes 3	Severe 9
(n = 20)	Female 13	7 months	Epilepsy 0	Epilepsy 0	Flu 4	No 2	Mild or none 9
		(2 years 4 months)	None 18	None 19	RSV 2	NA 15	NA 2
					Rota 1		
					NI 10		
AESD	Male 28	5 months to 6 years	FS 6	FS 3	HHV-6 18	Yes 33	Severe 14
(n = 61)	Female 33	(1 year 9 months)	Epilepsy 1	Epilepsy 1	Flu 8	No 23	Mild or none 41
			None 50	None 53	RSV 2	NA 5	NA 6
			NA 4	NA 4	Others 4		
					NI 29		
Nonspecific AE	Male 4	1 year 9 months to	FS 1	FS 3	Flu 2	Yes 5	Severe 0
(n = 6)	Female 2	6 years 1 month	None 5	None 3	NI 4	No 1	Mild or none 5
		(3 years 2 months)					NA 1

Patients were classified into three syndromes: acute necrotizing encephalopathy (ANE), acute encephalopathy with biphasic seizures and late reduced diffusion (AESD), and nonspecific AE. FS, febrile seizures; NA, not available; HHV-6, human herpesvirus 6; Flu, influenza virus; RSV, respiratory syncytial virus; Rota, rotavirus; NI, not identified.

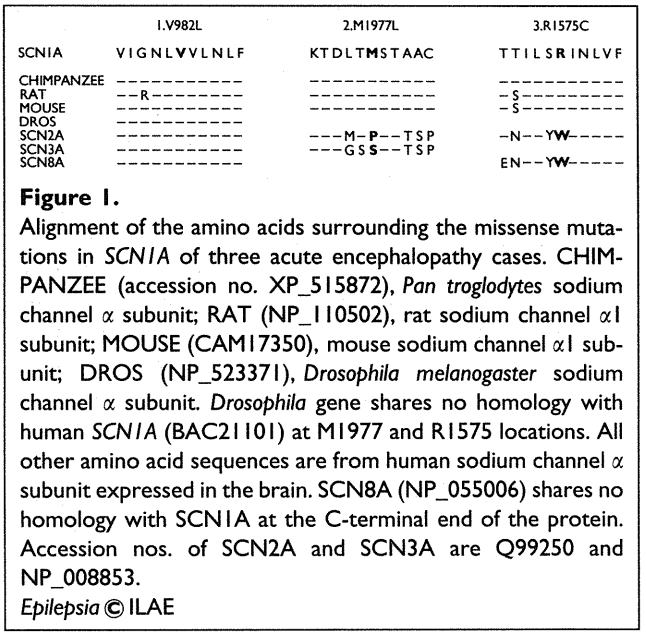

Figure 1.
Alignment of the amino acids surrounding the missense mutations in *SCN1A* of three acute encephalopathy cases. CHIMPANZEE (accession no. XP_515872), *Pan troglodytes* sodium channel α subunit; RAT (NP_110502), rat sodium channel α1 subunit; MOUSE (CAM17350), mouse sodium channel α1 subunit; DROS (NP_523371), *Drosophila melanogaster* sodium channel α subunit. *Drosophila* gene shares no homology with human *SCN1A* (BAC21101) at M1977 and R1575 locations. All other amino acid sequences are from human sodium channel α subunit expressed in the brain. SCN8A (NP_055006) shares no homology with SCN1A at the C-terminal end of the protein. Accession nos. of SCN2A and SCN3A are Q99250 and NP_008853.
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The R1575C mutation was found in case 3 with ANE. This mutation has been reported previously in a patient with acute encephalitis with refractory, repetitive partial seizures (AERRPS) (Kobayashi et al., 2010), as well as in a patient with Rasmussen encephalitis with an autoantibody against the glutamate receptor GluR3 (Ohmori et al., 2008), and markedly alters the electrophysiologic properties of the sodium channel. The arginine 1575 residue is highly conserved among vertebrates (Fig. 1). The clinical course of these three patients is described briefly below.

Case 1: AESD with V982L mutation

This patient, a 2-year-old girl, was born small-for-date weighing 2,008 g, after gestation of 39 weeks. There was no family history of epilepsy or seizure disorders. From the age of 3 months, she had recurrent, afebrile complex partial seizures. There was no febrile seizure, myoclonic seizure, mental delay, or ataxia. The diagnosis of partial epilepsy was made, and prophylaxis with carbamazepine was begun. From 1 year and 11 months, she also had bronchial asthma. At 2 years and 3 months of age, she underwent treatment with intravenous theophylline for an asthma attack. She then had a respiratory infection with fever and vomiting, and developed myoclonic seizures, which evolved into prolonged generalized tonic convulsion. Status epilepticus was refractory to anticonvulsants and lasted 2 hours, requiring general anesthesia and mechanical ventilation. Although cranial CT was normal on day 2, coma persisted. Serum levels of aminotransferases and lactic dehydrogenase were very high. On day 5, she had clusters of partial seizures. CT revealed diffuse cerebral cortical edema. Based on the biphasic clinical course and serial CT findings, the diagnosis of AESD was made. She was treated with continuous infusion of thiopental (until day 21) and methylprednisolone pulse therapy. CT and MRI thereafter showed severe atrophy of the cerebral cortex with sparing of bilateral perirolandic regions (Fig. 2). She was eventually left with spastic quadriplegia and severe mental deficit.

Case 2: Nonspecific AE with M1977L mutation

This patient, a 3-year-old boy, was born uneventfully to nonconsanguineous parents. His uncle had had febrile convulsions during infancy. From the age of 1 year 6 months, he had recurrent febrile seizures. At 3 years of age, he had a respiratory infection with fever, and then had his sixth attack consisting of repeated generalized convulsions, six times during 24 h. Each convulsion lasted from several seconds to 5 min. Phenytoin (15 mg/kg) was given intravenously to prevent their recurrence. Consciousness disturbance (Glasgow Coma Scale score, E4V4M5) persisted for 24 h, and was explained neither by postictal stupor nor by sedative effects of phenytoin. Cranial CT revealed mild diffuse edema. Electroencephalography (EEG) on day 1 showed high-voltage slow activity in the right parietal area. He was diagnosed with nonspecific AE and recovered completely; however, he developed a cluster of afebrile seizures 1 month later. EEG showed bilateral frontal spike-waves. Valproate treatment successfully prevented seizures. He had neither mental delay nor ataxia.

Case 3: ANE with R1575C mutation

The patient is now a 12-year-old boy who was born uneventfully and showed normal development. At the age 9 months, he had acute gastroenteritis with fever (temperature 38°C) and diarrhea. Four days later, he was taken to

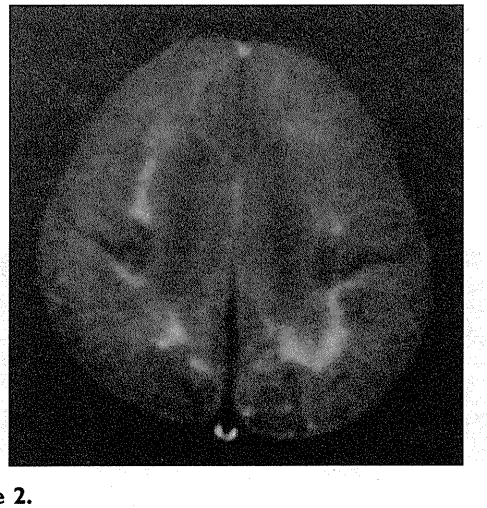

Figure 2.
Cranial MRI of a patient (case 1) with acute encephalopathy with biphasic seiaures and late reduced diffusion (AESD). Imaging at 4 years after AE (fluid-attenuated inversion recovery, FLAIR) showed cerebral atrophy, high signal intensity of the white matter, and sparing of the bilateral perirolandic regions.
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hospital because of generalized seizure after several bouts of vomiting. On admission, he was afebrile and mentally obtunded, and had recurrent generalized tonic–clonic seizures. Blood examination showed slight elevation of C-reactive protein, and cerebrospinal fluid examination showed an increased protein level (370 mg/dl). MRI revealed abnormal signals in the bilateral thalamus and subcortical white matter on T_2-weighted images (Fig. 3A). Contrast T_1-weighted imaging showed medullary streaks in the white matter (Fig. 3B). Based on the clinical and imaging findings, the diagnosis of ANE was made. He was treated with intravenous anticonvulsants and gammaglobulin, and recovered without any sequelae. Two years later, his younger sister was also affected by ANE at the age of 8 months, and was eventually left with severe motor and cognitive impairment. She did not have the R1575C mutation.

Discussion

AE is a complex disorder in which multiple factors, both genetic and environmental, are involved. Environmental factors include not only infectious agents, such as influenza virus and human herpesvirus 6 (HHV-6), but also drugs, such as aspirin and theophylline (Mizuguchi et al., 2007). With regard to genetic factors, single nucleotide polymorphisms (SNPs) of carnitine transpalmitoyl transeferase II (*CPTII*) and Toll-like receptor 3 (*TLR-3*) have been identified as predisposing factors of AE (Chen et al., 2005; Hidaka et al., 2006; Shinohara et al., 2011). In our previous study on the *CPT II* gene, we found in two of the present cases (cases 2 and 3) thermolabile SNPs associated with susceptibility to AE (Shinohara et al., 2011). The relationship of these SNPs and AE is complex. For example, *CPTII* SNPs occur in association with two syndromes: AESD and ANE (Shinohara et al., 2011). For each syndrome, unidentified genes other than *CPTII* are likely to be also involved. On the other hand, there is one syndrome of AE caused by mutations of a single gene: *ANE1* (familial recurrent variant of ANE) due to mutation of the Ran-binding protein 2 (*RANBP2*) gene (Neilson et al., 2009).

In this study, we tested the possibility that mutation of another gene, *SCN1A*, is a genetic predisposition for the onset of AE. We performed *SCN1A* gene analysis in 87 Japanese patients with AE, and found point mutations, V982L, M1977L, and R1575C, in three (3.4%) of them. These results are comparable to those of a previous study, which found an *SCN1A* mutation in one of 15 AE patients (Kobayashi et al., 2010). Our study dealt with a large case series of AE, and found multiple patients with *SCN1A* mutations, thereby establishing the association between *SCN1A* mutations and AE.

In addition to our three patients, three additional patients with AE and a *SCN1A* mutation have been reported previously. Clinical and neuroradiologic findings of these six patients are variable (Table 2). With regard to epilepsy, two patients had Dravet syndrome, one had GEFS+, one had partial epilepsy, one had febrile seizures, and the remaining one had none. On the other hand, *SCN1A* missense mutations have been identified not only in patients with Dravet syndrome and GEFS+, but also in unaffected subjects without a seizure disorder (Ohmori et al., 2008). Therefore, their contribution to epilepsy and AE requires critical evaluation and further investigation.

With regard to AE, three had AESD, one had AERRPS, one had ANE, and one had nonspecific AE. Clinical features varied among the cases, although all had either prolonged seizures (status epilepticus; four cases) or a cluster of brief seizures (three cases), mostly with fever (five cases), on day 1 or 2, in contrast to mutation-negative patients in whom such seizures were absent in about 40% (Table 1). Prognosis also varied largely from severe psychomotor deficit to complete recovery. Notably, two patients with the same *SCN1A* mutation, R1575C, showed clinical phenotypes quite different from each other. Case 3 in this study had no epilepsy and ANE, whereas case 6 reported previously had

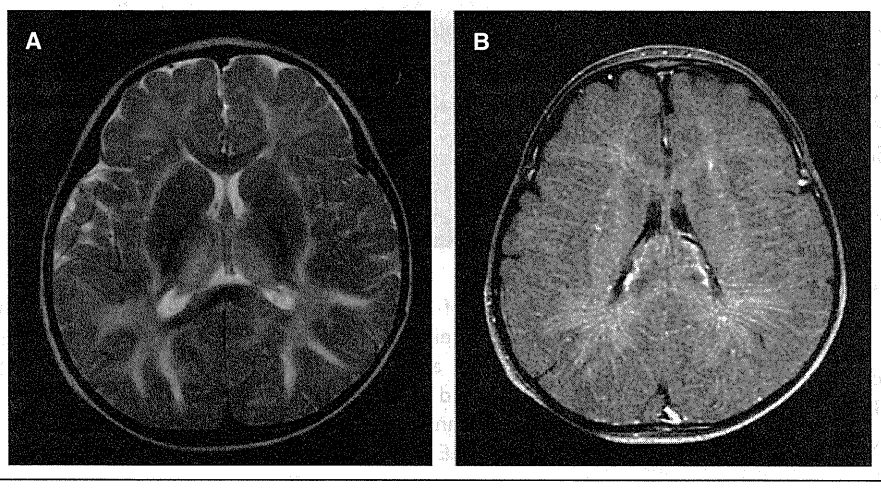

Figure 3.
Cranial MRI of a patient (case 3) with acute necrotizing encephalopathy (ANE). (**A**) T_2-weighted axial section shows high-intensity areas of bilateral thalami and the cerebral white matter. (**B**) Gadolinium-enhanced T_1-weighted axial image shows medullary streaks in the affected white matter lesions.
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Table 2. Characteristic of AE and preceding epilepsy in the [a]present (cases 1, 2 and 3) and previously reported cases

Case no.	Age	Gender	*SCN1A* mutation	Antecedent infection	Other etiologic factors	Precedimg eplilpsy or seizure dieorder	AE	Seizure at acute stage of AE	Neuroimaging findings	Prognosis	Reference
1[a]	2 years 3 months	F	V982L/ missense	Upper respiratory infection	Theophylline	Partial epilepsy	AESD	Status, febrile, on day1	CT/day 2, unremakable; CT/day 5, diffuse brain edema; follow-up MRI, central sparing	Spastic quadriplegia, severe MR	This study
2[a]	3 years	M	M1977L/ missense	Upper respiratory infection		GEFS+	Nonspecific AE	Cluster, febrile, on day1	CT/day 2, unremakable	Complete recovery	This study
3[a]	0 year 9 months	M	R1575C/ missense	Acute gastroenteritis	Family history of AE	None	Mimiking ANE	Cluster, afebrile, on day 1	MRI/day 2, bilateral thalamic lesions	Complete recovery	This study
4	1 year 4 months	F	R1892X/ nonsense	Rotavirus gastroenteritis		Dravet syndrome	AESD (HH)	Status, febrile, on day1	MR/day 6, left hemispheric edema	Mild MR (DQ = 71), right spastic heniplegia	Sakakibara et al., 2009
5	0 year 9 months	F	D43fs/ truncation	Fever of unknown etiology		Suspected Dravet syndrome	Atypical AESD	Status, febrile, on day1	MRI/day ×3, diffuse high signal intesity in cortex and subcortical white matter	Spastic quadriplegia, severe MR	Takayanagi et al., 2010
6	6 years 5 months	M	R1575C/ missense	Fever of unknown etiology		Febrile seizure	AERRPS	Cluster and status, febrile on day 2	CT and MRI, unremarkable	Mild MR	Kobayashi et al., 2010

Cases 4, 5, and 6 were reported in references.

GEFS+, generalized epilepsy with febrile seizure plus; AESD, acute encephalopathy with biphasic seizures and late reduced diffusion; AE, acute encephalopathy; ANE, acute necrotizing encephalopathy; HH, hemiconvulsion-hemiplegia syndrome; AERRPS, acute encephalitis with refractory, repetitive partial seizures.

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febrile seizure and AERRPS (Kobayashi et al., 2010). This difference suggests the involvement of factors other than *SCN1A* mutation in the pathogenesis of AE.

The family history of case 3 deserves attention. The younger sister of this patient also had the same type of AE, despite the absence of *R1575C* mutation, which strongly suggests the involvement of another, as yet unidentified factor in this familial ANE. Comparison between the siblings revealed a longer duration of status epilepticus in the brother (case 3), and a worse prognosis in the sister. Plausibly, the *SCN1A* mutation contributed more to the evolution of status epilepticus, and the unidentified factor more to the development of bithalamic lesions and the overall neurologic damage.

In summary, we found *SCN1A* mutations in 3 of 87 cases of AE, and identified them as a predisposing genetic factor of AE. As for both epilepsy and AE, clinical phenotypes were variable among patients with *SCN1A* mutations. This variability, together with the family history of one patient (case 3), suggested that factors other than *SCN1A* mutations are also involved in the pathogenesis of AE.

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Disclosures

None of author has any conflict of interest to disclosure.

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.

References

Chen Y, Mizuguchi H, Yao D, Ide M, Kuroda Y, Shigematsu Y, Yamaguchi S, Yamaguchi M, Kinoshita M, Kido H. (2005) Thermolabile phenotype of carnitine palmitoyltransferase II variations as a predisposing factor for influenza-associated encephalopathy. *FEBS Lett* 579:2040–2044.

Claes L, Del-Favero J, Ceulemans B, Lage L, Van Broeckhoven C, De Jonghe P. (2001) De novo mutations in sodium-channel gene *SCN1A* cause severe myoclonic epilepsy in infancy. *Am J Hum Genet* 68:1327–1332.

Escayg A, Goldin AL. (2010) Sodium channel *SCN1A* and epilepsy: mutations and mechanisms. *Epilepsia* 51:1650–1658.

Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A. (2000) Mutations of *SCN1A*, encoding a neuronal sodium channel, in two families with GEFS+. *Nat Genet* 24:343–345.

Escayg A, Helis A, MacDonald BT, Haug K, Sander T, Meisler MH. (2001) A novel *SCN1A* mutation associated with generalized epilepsy with febrile plus-and prevalence of variants in patients with epilepsy. *Am J Hum Genet* 68:866–873.

Fukuma G, Oguni H, Shirasaka Y, Watanabe K, Miyajima T, Yasumoto S, Ohfu M, Inoue T, Watanachai A, Kira R, Matsuo M, Muranaka H, Sofue F, Zhang B, Kaneko S, Mitsudome A, Hirose S. (2004) Mutations of neuronl voltage-gated Na+ channel alpha I subunit gene *SCN1A* in core severe myoclonic epilepsy in infancy (SMEI) and in bordrline SMEI (SMEB). *Epilepsia* 45:140–148.

Hidaka F, Matsuo S, Muta T, Takeshige K, Mizukami T, Nunoi H. (2006) A missense mutation of the Toll-like receptor 3 gene in a patient with influenza-associated encephalopathy. *Clin Immunol* 119:188–194.

Hoshino A, Saitoh M, Oka A, Okumura A, Kubota M, Saito Y, Tkanashi J, Horose S, Yamagata T, Yamanouchi H, Mizuguchi M. (2011) Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes. *Brain Dev* doi: 10.1016/j.braindev.2011.07.012 [In press].

Kobayashi K, Ouchida M, Okumura A, Maegaki Y, Nishiyama I, Matsui H, Ohtsuka Y, Ohmori I. (2010) Genetic seizure susceptibility underlying acute encephalopathies in childhood. *Epilepsy Res* 91:143–152.

Malacarne M, Madia F, Gennaro E, Vaccca D, Guney AI, Buono S, Bernardina BD, Gaggero R, Gobbi G, Lispi ML, Malamaci D, Melideo G, Roccella M, Sferro C, Tiberti A, Vanadia F, Vigevano F, Viri F, Vitali MR, Bricarelli FD, Bianchi A, Zara F. (2002) Lack of SCN1A mutations in familial febrile seizures. *Epilepsia* 43:559–562.

Mantegazza M, Cambardella A, Rusconi R, Schiavon E, Annesi F, Cassulini RR, Labate A, Carrideo S, Chifari R, Canevini MP, Canger R, Franceschetti S, Annesi G, Wanke E, Quattrone A. (2005) Identification of an Nav1.1 sodium channel (*SCN1A*) loss-of-function mutation associated with familial simple febrile seizures. *Proc Natl Acad Sci U S A* 102:18177–18182.

Mizuguchi M, Abe J, Mikkaichi K, Noma S, Yoshida K, Yamanaka T, Kamoshita S. (1995) Acute necrotising encephalopathy of childhood: a new syndrome presenting with multifocal, symmetric brain lesions. *J Neurol Neurosurg Psychiatry* 58:555–561.

Mizuguchi M, Yamanouchi H, Ichiyama T, Shiomi M. (2007) Acute encephalopathy associated with influenza and other viral infections. *Acta Neurol Scand* 115:S45–S56.

Nabbout R, Gennaro E, Bernardina BD, Dulac O, Madia F, Bertini E, Capovilla G, Chiron C, Cristofori G, Elia M, Fontana E, Gaggero R, Granata T, Guerrini R, Loi M, La Selva L, Lispi ML, Matricardi A, Romeo A, Tzolas V, Valseriati D, Veggiotti P, Vigevano F, Vallee L, Dagna Bricarelli F, Bianchi A, Zara F. (2003) Spectrum of *SCN1A* mutations in severe myoclonic epilepsy of infancy. *Neurology* 60:1961–1967.

Neilson DE, Adams MD, Orr CM, Schelling DK, Eiben RM, Kerr DS, Anderson J, Bassuk AG, Bye AM, Childs AM, Clarke A, Crow YJ, Di Rocco M, Dohna-Schwake C, Dueckers G, Fasano AE, Gika AD, Gionnis D, Gorman MP, Grattan-Smith PJ, Hackenberg A, Kuster A, Lentschig MG, Lopez-Laso E, Marco EJ, Mastroyianni S, Perrier J, Schmitt-Mechelke T, Servidei S, Skardoutsou A, Uldall P, van der Knaap MS, Goglin KC, Tefft DL, Aubin C, de Jager P, Hafler D, Warman ML. (2009) Infection-triggered familial or recurrent cases of acute necrotizing encephalopathy caused by mutations in a component of the nuclear pore, RANBP2. *Am J Hum Genet* 84:44–51.

Ohmori I, Ouchda M, Ohtsuka Y, Oka E, Shimizu K. (2002) Significant correlation of the *SCN1A* mutations and severe myoclonic epilepsy in infancy. *Biochem Biophys Res Commun* 295:17–23.

Ohmori I, Ouchida M, Kobayashi K, Jitsumori Y, Inoue T, Shimizu K, Matsui H, Ohtsuka Y, Maegaki Y. (2008) Rasmussen encephalitis associated with *SCN1A* mutation. *Epilepsia* 49:521–526.

Sakakibara T, Nakagawa E, Saito Y, Sakuma H, Komaki H, Sugai K, Sasaki M, Kurahashi H, Hirose S. (2009) Hemiconvulsion-hemiplegia syndrome in a patient with severe myoclonic epilepsy in infancy. *Epilepsia* 50:2158–2162.

Sakauchi M, Oguni H, Kato I, Osawa M, Horose S, Kaneko S, Takahashi Y, Takayama R, Fujiwara T. (2011) Retrospective multiinstitutional study of the prevalence of early death in Dravet syndrome. *Epilepsia* 52:1144–1149.

Shinohara M, Saitoh M, Takanashi J, Yamanouchi H, Kubota M, Goto T, Kikuchi M, Shiihara T, Yamanaka G, Mizuguchi M. (2011) Carnitine palmitoyl transferase II polymorphism is associated with multiple syndromes of acute encephalopathy with various viral infectious diseases. *Brain Dev* 33:512–517.

Singh NA, Pappas C, Dahle EJ, Claes LR, Pruess TH, De Jonghe P, Thompson J, Dixon M, Gurnett C, Peiffer A, White HS, Filloux F, Leppert MF. (2009) A role of *SCN9A* in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. *PLoS Genet* 5:e1000649.

Sugawara T, Mazaki-Miyazaki E, Fukushima K, Shimomura J, Fujiwara T, Hamano S, Inoue Y, Yamakawa K. (2002) Frequent mutations of *SCN1A* in severe myoclonic epilepsy in infancy. *Neurology* 58:1122–1124.

Takanashi J, Oba H, Barkovich AJ, Tada H, Tanabe Y, Yamanouchi H, Fujimoto S, Kato M, Kawatani M, Sudo A, Ozawa H, Okanishi T, Ishitobi M, Maegaki Y, Koyasu Y. (2006) Diffusion MRI abnormalities after prolonged febrile seizures with encephalopathy. *Neurology* 66:1304–1309.

Takanashi J, Tada H, Terada H, Barkovich AJ. (2009) Excitotoxicity in acute encephalopathy with biphasic seizures and late reduced diffusion. *Am J Neuroradiol* 30:132–135.

Takayanagi M, Haginoya K, Umehara N, Kitamura T, Numata Y, Wakusawa K, Hino-Fukuyo N, Mazaki E, Yamakawa K, Ohura T, Ohtake M. (2010) Acute encephalopathy with a truncation mutation in the *SCN1A* gene: a case report. *Epilepsia* 51:1886–1888.

Wallace RH, Scheffer IE, Barnet S. (2001) Neuronal sodium-channel α-subunit mutations in generalized epilepsy with febrile seizure plus. *Am J Hum Genet* 68:859–865.

Wallace RH, Hodgson BL, Grinton BE. (2003) Sodium channel α-subunit mutations in severe myoclonic epilepsy of infancy and infantile spasm. *Neurology* 61:765–769.

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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).

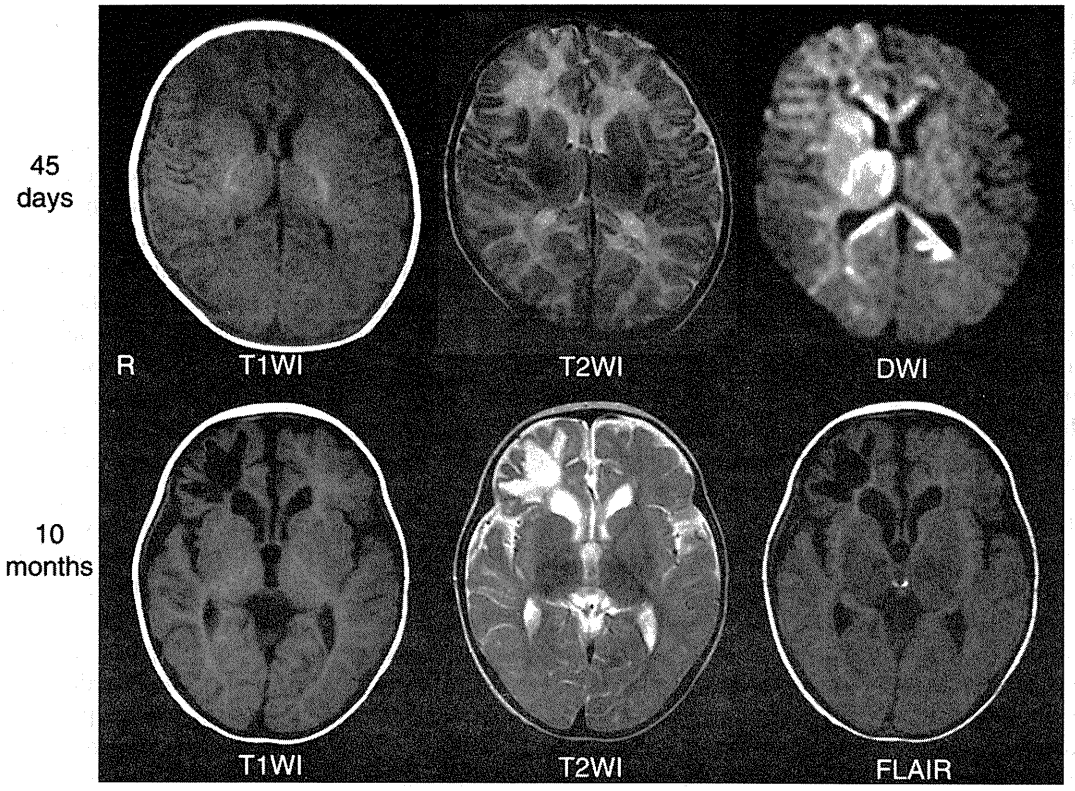

Fig. 1. MRI findings of Patient 1. Top: MRI at 45 days of age. Diffusion-weighted images revealed reduced diffusion predominantly in the subcortical white matter in the right frontal area. Reduced diffusion was also observed in the basal ganglia, thalamus, corpus callosum, and posterior limb of the internal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

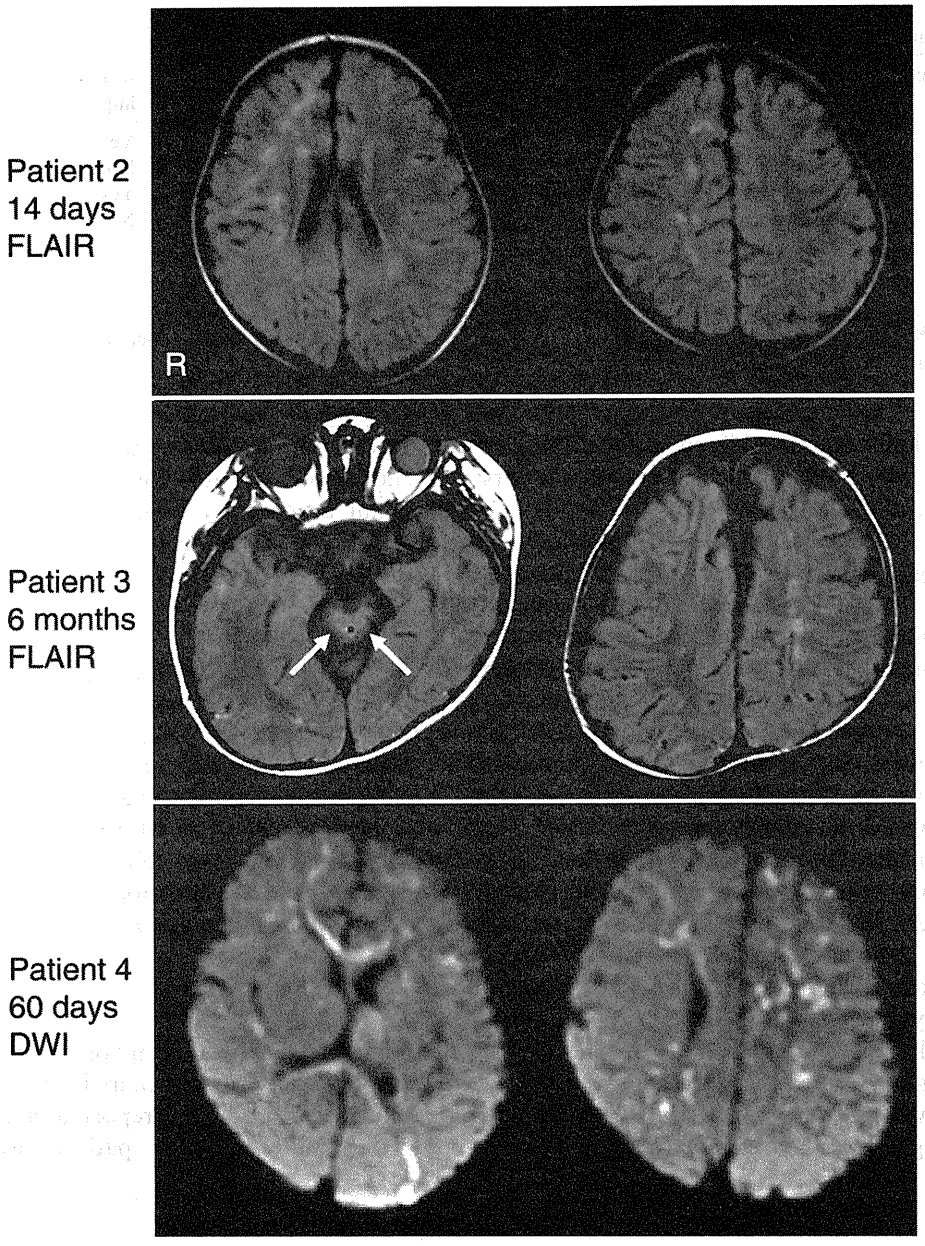

Fig. 2. MRI findings during the acute stage of encephalopathic manifestation. Top: FLAIR of Patient 2 at 14 days of age. Patchy high-intensity areas were seen in the subcortical areas predominantly in the right hemisphere. Middle: FLAIR of Patient 3 at 6 months of age. Linear high intensities were observed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