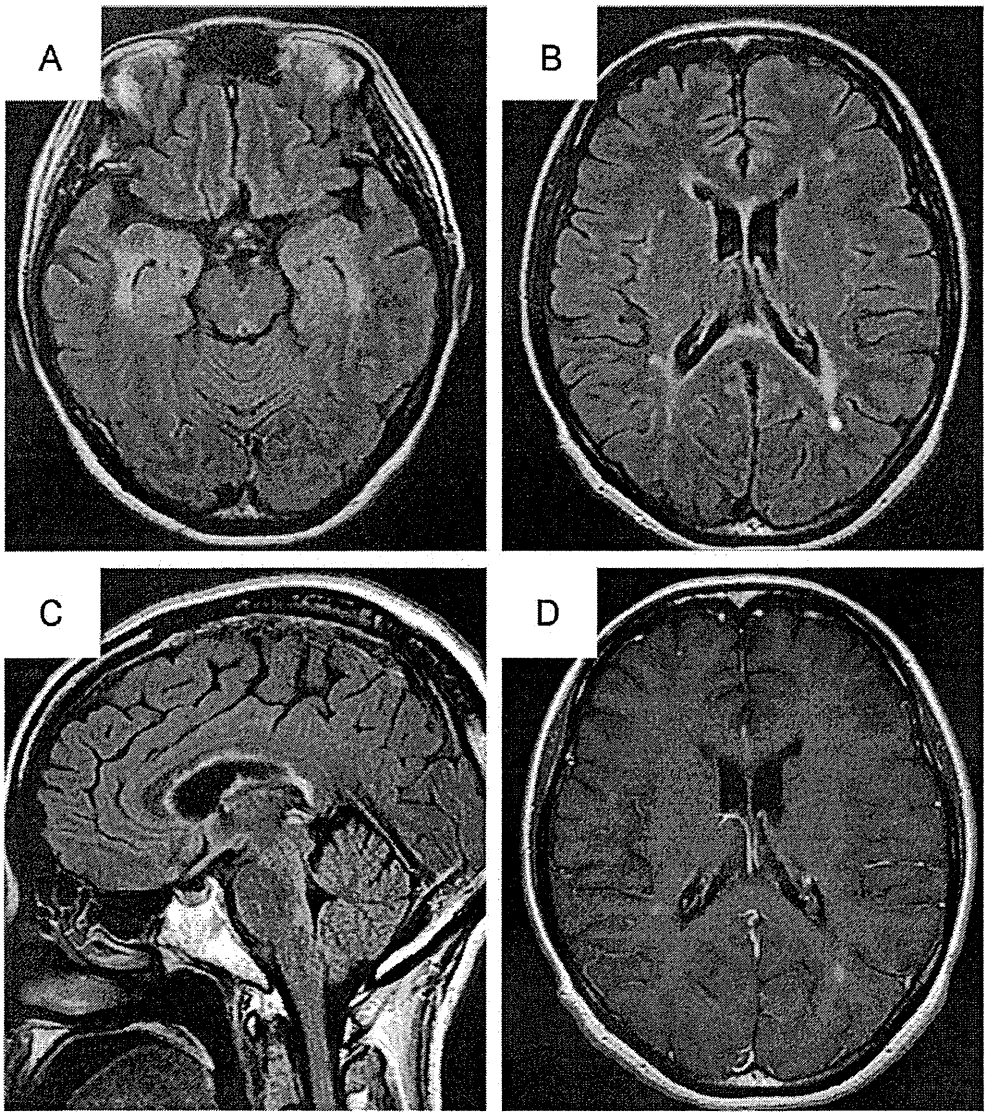

Fig. 1. Brain MRI performed on admission showing a high-intensity lesion on FLAIR images of the bilateral medial temporal lobes (A); some MS plaques (ovoid lesions) are noted around the lateral ventricle (B and C); an image obtained with gadolinium enhancement (D) is also shown.

3. Literature review

The coexistence of ON or neuromyelitis optica (NMO) with anti-NMDAR or anti-GluR antibody positive encephalitis has attracted attention recently. Four previously reported cases [2–5] and the present case are summarized in Table 1. Among these 5 patients, 2 were diagnosed with NMO, 2 with ON and 1 with MS. All 5 patients were female, and this predominance fits the clinical characteristics of their respective diseases. In all cases, anti-NMDAR or anti-GluR antibodies were detected in the CSF. The symptoms of the 3 patients with ON or MS preceded the development of anti-NMDAR encephalitis. Interestingly, no patient developed tumours and showed poor clinical prognoses.

4. Discussion

The early clinical course of this patient was typical of MS and the later course was compatible with anti-NMDAR encephalitis. The patient's fulminant neuropsychiatric manifestations and seizures, including the lesions that were present in the bilateral medial temporal lobes on MRI and positivity for the anti-GluRε2 antibody in the CSF, are atypical of MS, which encouraged the diagnosis of anti-NMDAR encephalitis with overlapping MS. GluRε2 (NR2B) is a subunit of NMDAR that is predominantly expressed in the hippocampus and forebrain and is involved in memory function. The mild cognitive impairment of the present case appears to have been caused by lesions in these areas.

Antibodies against NR1/NR2B heteromers are specific to NMDAR-associated encephalitis, with or without ovarian teratoma, whereas GluRε2 antibodies are also found in some other disorders, such as Rasmussen's encephalitis and progressive epilepsia partialis continua, and may lack syndrome specificity. Hence, the detection of anti-GluRε2 antibodies in the present patient's CSF may be related to her epilepsy or the destruction of her central nervous system. However, an activated autoimmune system in such patients may be related to the production of anti-NMDA antibodies; previous cases [4,5] and present case have manifested ON or

Table 1
Characteristics of patients with presenting with NMO, ON or MS with anti-NMDA or anti-GluR receptor antibody positive encephalitis.

Authors	Age/sex	Disease	Onset	Anti-NMDAR Ab in CSF	Epilepsy	NMO-IgG/ anti-AQP4 Ab	Spinal lesion	Optic nerve lesion	Tumour	Prognosis
Honda [2]	39 years/F	NMOSD	NA	Anti-GluRε2	Absent	Anti-AQP4 Ab (+)	+ (>3VL)	–	–	Good
Kruer [3]	15 years/F	NMO	NMDA → NMO	Anti-NMDAR	Present	NMO-IgG (–)	+ (>3VL)	+	–	Good
Motoyama [4]	10 years/F	ON	ON → NMDA	Anti-NMDAR	Present	Anti-AQP4 Ab (–)	–	+	NA	Good
Ishikawa [5]	12 years/F	ON	ON → NMDA	Anti-GluRε2	Present	NA	–	+	NA	Good
Our case	34 years/F	MS	MS → NMDA	Anti-GluRε2	Present	Anti-AQP4 Ab (–)	+ (<3VL)	+	–	Good

NMO: neuromyelitis optica; NMOSD: neuromyelitis optica spectrum disorder; MS: multiple sclerosis; ON: optic neuritis; NMDAR: *N*-methyl D-aspartate receptor; GluR: glutamate receptor; Ab: antibody; AQP4: aquaporin 4; NA: not available; VL: vertebral segments in length.

MS before the development of anti-NMDAR encephalitis. A previous review reported that 59% of anti-NMDAR encephalitis patients have tumours and 36% of patients without tumours showed severe deficits or died [1]. Most noteworthy were the facts that all reported cases [2–5] and present case who presented with ON, NMO or MS with anti-NMDAR encephalitis did not present with tumours, and that those cases demonstrated good recoveries. Positive outcomes may be possible following the use of intensive immune-modulating therapies.

5. Conclusion

We reported the first case of a patient who developed anti-NMDA glutamate receptor antibody-positive encephalitis with good recovery during the course of MS. There may be a possible linkage between these diseases, and concurrent autoimmune responses may be important for the development of autoimmune encephalitis. Anti-NMDAR encephalitis should be recognized as a rare manifestation that can occur in MS patients who develop psychiatric symptoms and seizures. However, further investigation of patients with related disorders and analysis is needed.

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Case report

Anti-glutamate receptor δ2 antibody-positive migrating focal encephalitis

Takuya Fukuoka[a,*], Hidetaka Takeda[a], Yasuko Ohe[a], Ichiro Deguchi[a], Yukitoshi Takahashi[b], Norio Tanahashi[a]

[a] Department of Neurology, Saitama Medical University International Medical Center, Japan
[b] National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Japan

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1. Introduction

In recent years, increasing attention has been given to acute and subacute encephalitis related to glutamate receptors (GluR). GluRs are involved in excitatory neurotransmission on cell membrane surfaces of the spinous processes in dendrites of mammalian central nervous systems. Abnormal activity of GluR channels is thought to contribute to the neuronal death observed in acute and chronic encephalitis. GluRs have two types: ionotropic and metabotropic (Table 1). Ionotropic GluRs are classified pharmacologically as N-methyl-D-aspartate (NMDA)-type, non-NMDA-type (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid {AMPA}-type, kainate-type), and δ-type with unknown ligands. NMDA-type GluR ε2 antibody is frequently found in acute limbic encephalitis or widespread encephalitis [1]. Moreover, NMDA receptor-related encephalitis is well known to be associated with ovarian teratoma. Much remains unknown regarding the pharmacological properties of the GluR δ2 subunit that is localized in the forebrain after birth, but it is known to be selectively expressed in cerebellar Purkinje cells.

Several recent reports [2–5] have revealed anti-GluR δ2 antibodies in the serum and cerebrospinal fluid of patients with autoimmune encephalitis.

We report herein a rare case involving a 42-year-old woman with anti-glutamate receptor δ2 antibody-positive encephalitis and migrating focal lesions mainly in the cerebral cortex.

2. Case report

A 42-year-old woman presented with loss of consciousness and convulsions after catching a cold. She was examined by a family physician the following day. No obvious abnormalities were apparent on brain computed tomography, but magnetic resonance imaging (MRI) with fluid-attenuated inversion recovery (FLAIR) showed high-intensity areas in bilateral frontal cortices, the left parietal cortex, and the cortex and subcortex of bilateral occipital lobes, and she was referred to our hospital for emergency hospitalization. On admission, body temperature was 37.4 °C and she was in a stupor. No cranial nerve or motor system abnormalities were identified, and nuchal rigidity was identified.

Blood tests showed a leukocyte count of 11,810/μl (neutrophils, 90.0%) and C-reactive protein level was 1.28 mg/dL. Thyroid function was normal, free T_3, 2.23 pg/ml, free T_4, 1.34 ng/dl, TSH, 097 μIU/ml. Anti-thyroglobulin antibody-RIA was ≤0.3 U/ml, Anti-thyroid receptor antibody <1.0 IU/ml, Antinuclear antibody <5 I.C., Anti-cardiolipin antibody IgG ≤8.0 U/ml, NSE-RIA 6.9 ng/ml, Anti-aquaporin 4 antibody, anti-Hu antibody, anti-Yo antibody were negative. Tumor markers did not show any abnormalities (CEA; 2.6 ng/ml, AFP 4.5 ng/ml, CA19-9 17.9 U/ml). Immune system findings showed an activated immune status. Immunoglobulin (Ig)M was mildly elevated to 275 mg/dl and among peripheral blood cells, the T lymphocyte subset of CD4/interleukin-2 receptor antibody-positive cells comprised

* Corresponding author at: Department of Neurology, Saitama Medical University International Medical Center, 1397-1 Yamane, Hidaka City, Saitama 350-1298, Japan. Tel.: +81 42 984 4359; fax: +81 42 984 0664.
E-mail address: tfukuoka@saitama-med.ac.jp (T. Fukuoka).

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Table 1
Subunit of glutamate receptor channel.

Subtype	Subfamily	Subunit
AMPA type	GluR1-4(GluRα)	GluR1(GluRA, GluRα1) GluR2(GluRB, GluRα2) GluR3(GluRC) GluR4(GluRD)
Kainic acid type	GluR5-7(GluRβ)	GluR5 GluR6(GluRβ2) GluR7
	KA1, 2	KI 1 KI 2(GluRγ2)
δ type	GluRδ	GluRδ1 GluRδ2
NMDA type	GluRε	GluRε1 GluRε2 GluRε3 GluRε4
	NR1(GluRζ1)	NMDAR1(GluRζ1, NR1)
	GluRχ	GluRχ(NR3A) NR3B

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; KA, kainic acid, GluR, glutamate receptors, NMDA, N-methyl-D-aspartate.

23.1% (normal: 6.3–19.5%), CD4/CD29-positive cells comprised 27.3% (9.0–27.2%), and suppressor–effector cells comprised 1.02% (1.3–9.42%). Cerebrospinal fluid (CSF) examination showed elevated protein levels (61 mg/dl) and polynuclear cell-dominant cytosis (cell count, 54/μl; mononuclear cells, 12/μl; polynuclear cells, 42/μl), but glucose level was normal (72 mg/dl). Herpes simplex virus IgG 0.3 EIA, EB virus anti-EBNA IgG antibody <1 C.I, EB virus anti-VCA IgM antibody <1 C.I. Antibody titers against herpes simplex virus and Epstein–Barr virus in cerebrospinal fluid were negative. Oligoclonal bands was negative. All cultures of blood and cerebrospinal fluid likewise yielded negative results. Results for anti-GluR IgG-δ2 antibody were positive in serum, but negative results were obtained for IgG-ε2, IgM-ε2, and IgM-δ2 subtypes. Blood and CSF levels of lactate were normal. Whole body CT including enhanced did not show any abnormality.

Brain MRI findings on admission are shown in Fig. 1. High-intensity areas on T2-weighting image, FLAIR, and (apparent diffusion coefficient) ADC maps, iso- or hyperintense areas on diffusion-weighted imaging (DWI), and hypointense areas on T1-weighted imaging were seen in bilateral frontal and parietal lobes and the left occipital lobe. No gadolinium enhancement was seen. Meningoencephalitis was suspected at the time of admission, and

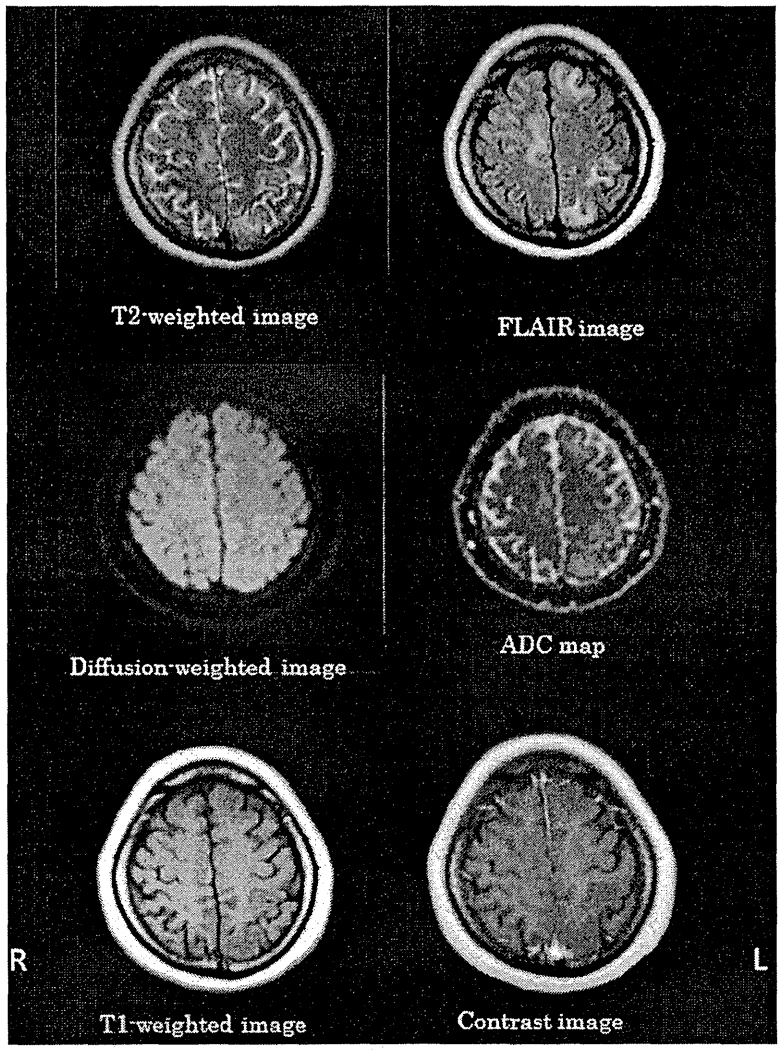

Fig. 1. Brain MRI on admission: bilateral cortex of the frontal, parietal and left occipital lobe showed high-intensity areas on T2-weighted, FLAIR and ADC images, iso- or hyperintense areas on DWI, but low-intensity areas o T1-weighted image. In contrast image, lesion was not enhanced.

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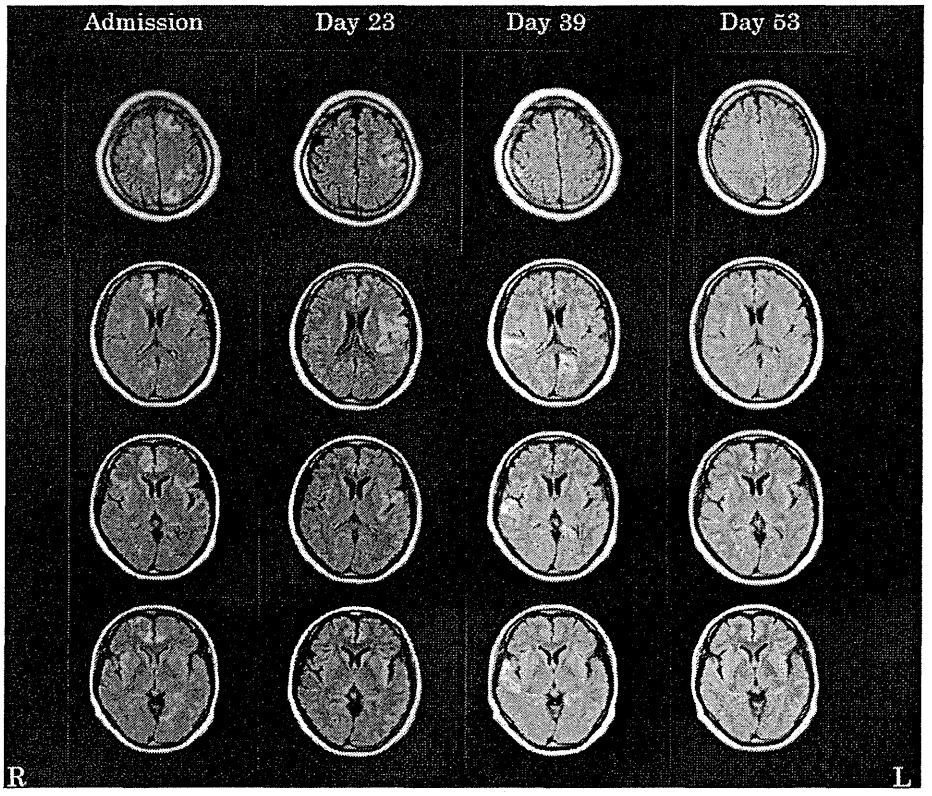

Fig. 2. Fluid-attenuated inversion recovery (FLAIR) MRI of the brain. High-intensity areas showed various changes with time after onset.

antibiotics (panipenem/betamiprom, 3 g/day), an antiviral agent (acyclovir, 1500 mg/day), and an antiedematous agent for cerebral edema (glycerol, 800 ml/day) were administered. Steroid pulse therapy (methylprednisolone 1000 mg/day) was performed for 5 days, followed by oral prednisolone at 40 mg/day. On day 2, the patient regained consciousnous. She was performed the neuropsychological test during day 7. Mini-mental state examination (MMSE) yielded a score of 27/30, digit span was 6 digits forward and 5 digits backward and Frontal Assessment Battery was 18/18. Cognitive behavior testing yielded a high score of 32 on the Frontal

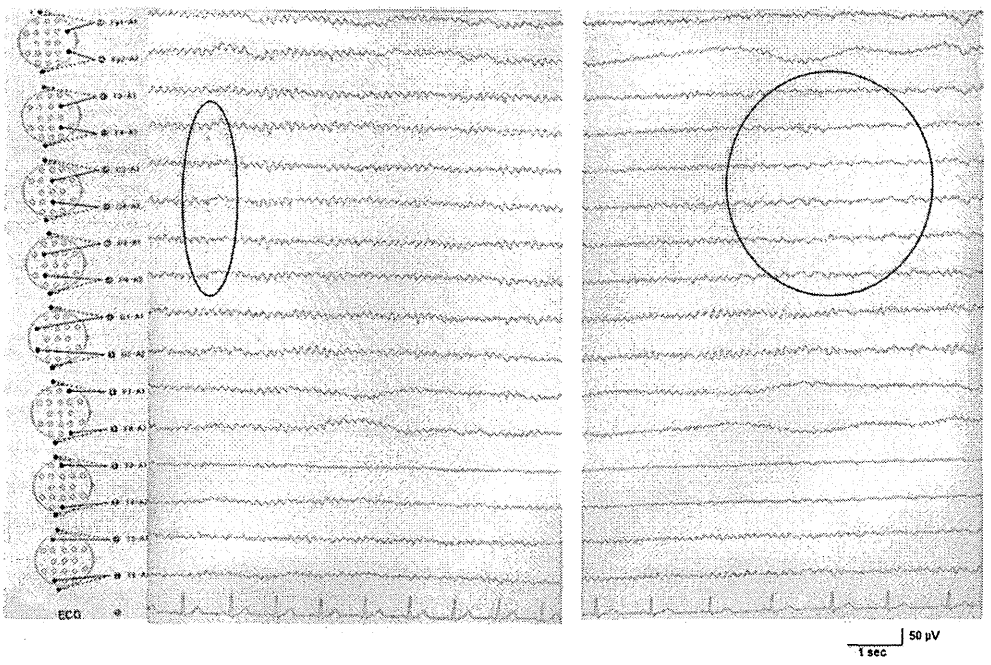

Fig. 3. Paroxysms and slow waves were seen with left dominance in the central and parietal regions.

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Behavioral Inventory. Chronological changes on MRI FLAIR images are shown in Fig. 2. Paroxysms and slow waves were seen with left dominance in the central and parietal regions on Day 8 (Fig. 3). Ovarian teratoma was not found by pelvic MRI.

On Day 23, when the dose of prednisolone was tapered to 20 mg/day, motor aphasia and right-sided hemiplegia occurred for about 30 min. MRI revealed new lesions showing hyperintense areas in the left temporal and frontoparietal lobes on FLAIR imaging. Prednisolone was therefore increased again to 40 mg/day. No neurological symptoms appeared in the subsequent course. On Day 32, those lesions had disappeared, and new lesions had appeared in the left occipital and frontal lobes. On Day 39, when the dose was 20 mg/day, new lesions in the right temporal and left occipital areas were seen on MRI, and the dose of prednisolone was again increased to 40 mg/day and tapered gradually. On Day 53, MRI findings had nearly normalized, and normal results were also obtained on magnetic resonance angiography and magnetic resonance venography following this increase in prednisolone dose. At 34 months later, no neurological symptoms have appeared and there have been no abnormal MRI findings.

3. Discussion

Characteristics of the present case were as follows: (1) clinical features of meningoencephalitis and CSF pleocytosis; (2) localized cortical lesions were frequently seen in various sites over time on MRI; (3) both clinical symptoms and MRI lesions responded well to steroid therapy; and (4) positive results were obtained for anti-GluR δ2 antibody in the serum.

From the perspective of clinical feature and MRI findings, differential diagnoses included multiple sclerosis, acute disseminated encephalomyelitis (ADEM), posterior reversible leucoencephalopathy syndrome (PRES), Hashimoto's encephalopathy, and mitochondrial encephalopathies. In the present case, the lesions were mainly in the cortex, in contrast to the white matter lesions seen in those disorders. Hashimoto's encephalopathy was also thought to be unlikely due to negative findings for anti-thyroid antibody. Mitochondrial encephalopathies were ruled out based on lactate levels.

Anti-GluR δ2 antibody in the serum and CSF has occasionally been detected in patients with acute cerebellitis or acute limbic encephalitis [2,4]. However, Tomioka et al. [4] and Hirano et al. [5] presented two cases of anti-GluR δ2 antibody-positive autoimmune encephalitis with cerebral cortical lesions. The case described by Tomioka et al. [4] involved a 16-year-old boy who showed meningitis-like symptoms and seizures. Brain MRI showed only mild hyperintensity in the parietal cortex on FLAIR and clinical symptoms were monophasic. The case described by Hirano et al. [5] involved a 17-year-old boy who presented with right hemiplegia, aphasia, and seizures. Brain MRI showed a hyperintense lesion in the left parietal cortex on T2-weighted imaging. That case responded to steroids and showed repeated episodes of encephalitis without new lesions on MRI. Our case resembles these in terms of cerebral cortical lesions. However, the migration of cerebral cortical lesions over time as verified by MRI represents a highly unusual finding.

Cortical lesions in the present case were considered to represent extracellular edema based on MRI findings. Although the role of anti-GluR δ2 antibody in the pathogenesis of this autoimmune migrating cortical-dominant localized encephalitis is unclear, cellular immunity that had been activated as a result of an antecedent infection was thought to have caused a cross-reaction in the central nervous system, giving rise to autoimmune encephalitis as a result of anti-GluR antibody production and cytokine release. It is unclear whether anti-GluR antibodies themselves were involved in the encephalitis or whether antibodies were produced as a result of cell destruction from encephalitis.

Conflict of interest

The authors have no conflicts of interest to report.

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Short communication

Steroid-responsive focal epilepsy with focal dystonia accompanied by glutamate receptor delta2 antibody

Hideyuki Matsumoto [a,*], Shingo Okabe [a], Minako Hirakawa-Yamada [a], Yukitoshi Takahashi [b], Noboru Satoh [a], Yukifusa Igeta [a], Hideji Hashida [a]

[a] Department of Neurology, Japanese Red Cross Medical Center, 4-1-22 Hiroo, Shibuya-ku, Tokyo 150-8935, Japan
[b] Department of Pediatrics, National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, 886 Urushiyama, Aoi-ku, Shizuoka 420-8688, Japan

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ABSTRACT

This report describes a rare case presenting with focal epilepsy and focal dystonia associated with glutamate receptor δ2 antibody. The patient was a 47-year-old male patient with neurosyphilis. He presented with an intractable focal seizure spreading from the right arm, with dystonia of the left leg. The IgG antibody of glutamate receptor δ2 was detected. Ictal and interictal SPECT suggested focal epilepsy in the left frontal cortex. Antibiotic and antiepileptic drugs were ineffective, although steroid pulse therapy effectively diminished the patient's symptoms. Inflammatory mechanisms may have contributed to this disorder.

1. Introduction

The incidence of symptomatic seizures resulting from neurosyphilis has been reported to range from 14 to 60% (Hooshmand, 1976). Among the various types of seizures that can occur under these circumstances, focal seizures are the most common (Timmermans and Carr, 2004). Antibiotic or antiepileptic drugs are typically administered to reduce seizures caused by neurosyphilis (Ances et al., 2004). In contrast, dystonia is an uncommon symptom of neurosyphilis. This paper describes a rare case in which a patient with neurosyphilis developed steroid-responsive focal epilepsy with focal dystonia accompanied by glutamate receptor (GluR) δ2 antibody.

2. Case report

The patient was a 47-year-old man. He suddenly lost consciousness and presented with a clonic seizure spreading from his right arm through his entire body, and was admitted to our hospital. He had a history of syphilitic meningitis treated by antibiotic therapy. His family and life histories were unremarkable.

On admission, his blood pressure was 140/100 Torr, with a pulse of 130 beats/min and a body temperature of 36.9 °C. He had no skin rash. Disturbance of consciousness and clonic seizure spreading from his right arm to his whole body were observed intermittently. Interictal neurological examination showed cognitive impairment (mini-mental state examination score was 20), deep sensation dominant sensory disturbance in the legs with shooting pain, absent deep tendon reflexes in the legs, and dystonia of the left leg. The muscle tone of the left leg was always abnormally increased with co-contraction of antagonistic muscles, whereas the interictal muscle tone of the right leg was decreased. Dystonic posturing of the leg consisting of knee extension and foot inversion was noted. This was relieved with sleep, though not by any clear sensory trick.

Routine blood examination was almost normal except for positive rapid-plasma regain (RPR) and positive *Treponema pallidum* hemagglutination (TPHA). Immunological blood examination was negative for anti-nuclear antibody, anti-thyroglobulin antibody, anti-thyroid peroxidase antibody, anti-neutrophil cytoplasmic antibody, and anti-glutamic acid decarboxylase antibody; interleukin (IL)-6 was also normal. Liquor examination showed a normal cell count (1 cell/mm^3), mildly increased protein (64 mg/dl), increased immunoglobulin (Ig) G index (0.91), negative oligoclonal band, normal IL-6, negative RPR, and positive TPHA.

Whole-body computed tomography, brain magnetic resonance imaging, and brain magnetic resonance angiography yielded no abnormal findings. Right tibial nerve sensory evoked potentials (SEPs) showed severely prolonged central sensory conduction time (CSCT) calculated based on the interval between the N21 and P38 potentials (lumbar potential N21: 23.0 ms, cortical potential P38: 53.8 ms, CSCT: 25.4 ms). Interictal electroencephalography (EEG) showed irregular α waves (8–9 Hz), but neither epileptic discharge nor focal slow wave

* Corresponding author. Tel.: +81 3 3400 1311; fax: +81 3 3409 1604.
E-mail address: hideyukimatsumoto.jp@gmail.com (H. Matsumoto).

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was observed. Ictal single photon emission computed tomography (SPECT) with N-isopropyl (I-123)-iodoamphetamine (IMP) revealed hyperperfusion in the left inferior frontal cortex and right thalamus. Analysis of the three-dimensional stereotactic surface projection (3D-SSP) more clearly revealed the hyperperfusion in the left inferior frontal cortex (Fig. 1). On the other hand, interictal SPECT showed hypoperfusion in the left inferior frontal cortex and hyperperfusion in the right thalamus, and the 3D-SSP analysis likewise indicated hypoperfusion in the left inferior frontal cortex (Fig. 2).

To treat the focal epilepsy with focal dystonia, which was suspected to have been induced by neurosyphilis, an antiepileptic drug (phenytoin 300 mg/day) and an antibiotic drug (iv penicillin G 24 million U/day for 14 days) were administered, but the symptoms remained uncontrolled. Antibiotic therapy was continued and several other antiepileptic drugs were added (zonisamide 300 mg/day and lamotrigine 350 mg/day). Nevertheless, the symptoms did not completely resolve.

To investigate the possibility of another concurrent disease such as immune-mediated encephalitis, GluR antibodies were examined according to a previously reported method (Takahashi et al., 2003, 2005). The GluR δ2-IgG antibody was detected in the patient's serum but not in his cerebrospinal fluid. N-Methyl D-aspartate receptor (NMDAR) antibody and voltage-gated potassium channels (VGKC)-related antibodies were not examined. To determine whether an inflammatory process was contributing to his symptoms, steroid pulse therapy was administered. Thereafter, his focal epilepsy with focal dystonia diminished and his GluR δ2 IgG antibody normalized along with his IgG index (0.73).

3. Discussion

We reported the case of a 47-year-old man with neurosyphilis who presented with steroid-responsive focal epilepsy with focal dystonia. His cognitive impairment, the sensory disturbance in his legs, and the absence of deep tendon reflexes in his legs even after steroid pulse therapy seem to be sequelae caused by his neurosyphilis. Based on SPECT findings, the responsible lesion for the focal epilepsy was considered to be in the left inferior frontal cortex, as cerebral blood flow at the focus of epilepsy increases during the ictal period and decreases during the interictal period (Duncan, 1997). Surface EEG revealed no interictal epileptiform discharges, suggesting that the responsible lesion for frontal lobe epilepsy was located in a small or deep area in the frontal lobe (Salanova et al., 1993; Kellinghaus and Lüders, 2004). It is also possible that the antiepileptic drug may have masked the epileptiform discharges. The focal dystonia was considered to be related to the hyperperfusion in the right thalamus revealed through the SPECT study, because hyperperfusion in the contralateral thalamus has been described in two previous papers on SPECT findings in patients with focal limb dystonia (Fiedler et al., 1999; Hiraga et al., 2003). The fact that steroid pulse therapy

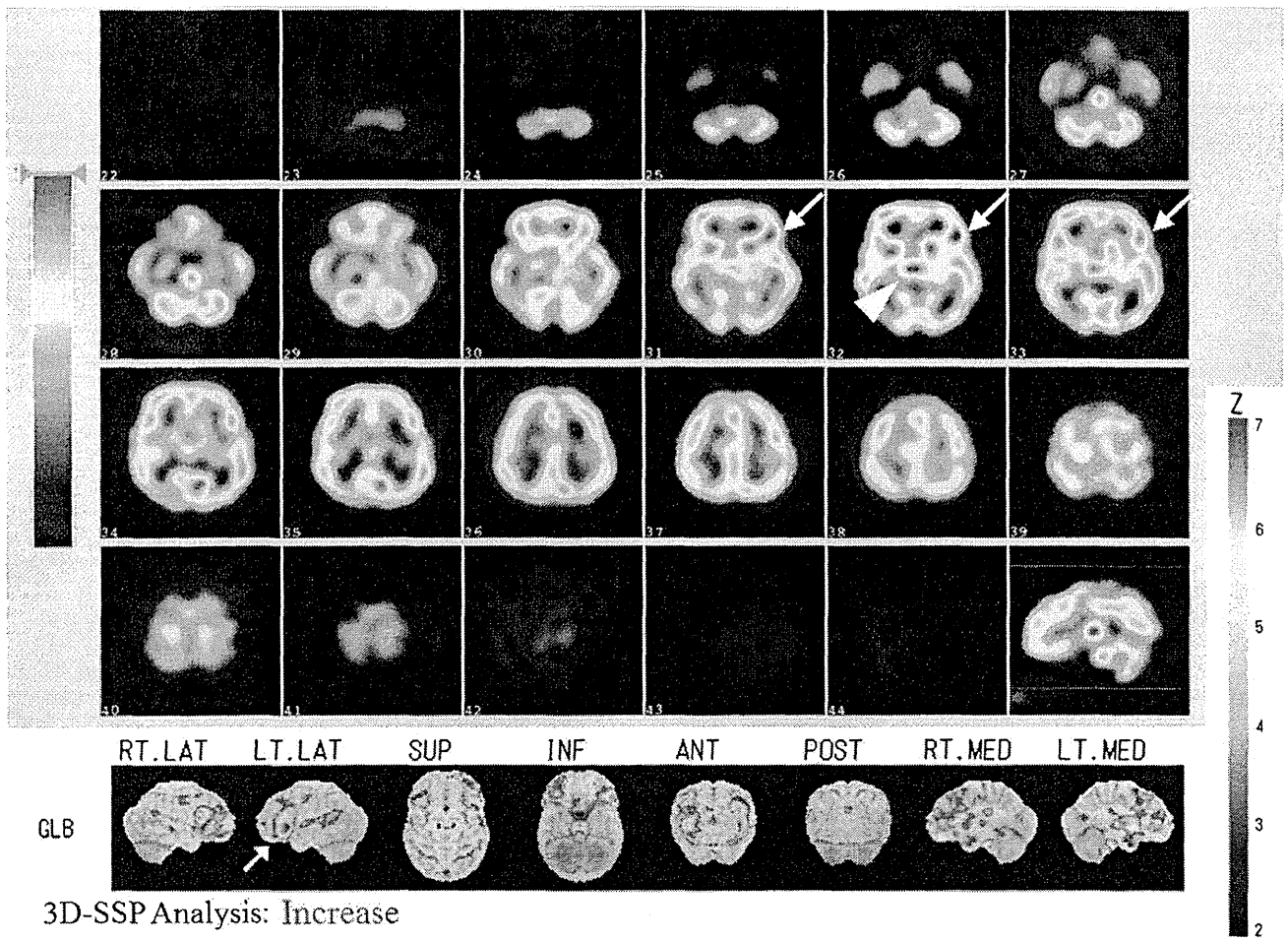

Fig. 1. Ictal SPECT with I-123 IMP and 3D-SSP analysis.Ictal SPECT showed hyperperfusion in the left inferior frontal cortex (large arrow) and right thalamus (arrowhead). The 3D-SSP analysis emphasizes the hyperperfusion in the left inferior frontal cortex (small arrow).

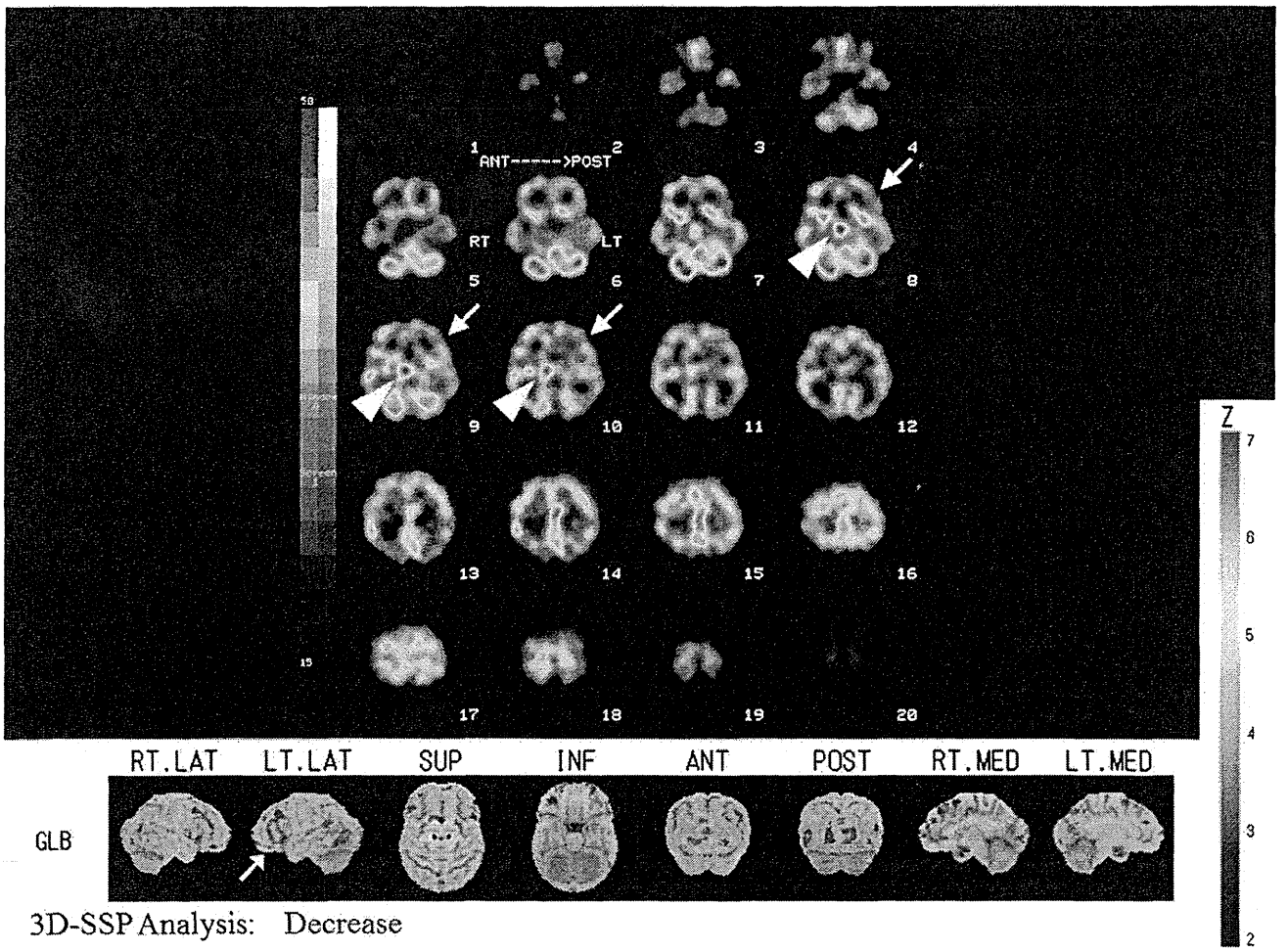

Fig. 2. Interictal SPECT with I-123 IMP and 3D-SSP analysis.Interictal SPECT showed hypoperfusion in the left inferior frontal cortex (arrow) and hyperperfusion in the right thalamus (arrowhead). The 3D-SSP analysis emphasizes the hypoperfusion in the left inferior frontal cortex (small arrow).

improved the intractable focal epilepsy with focal dystonia suggests that not only neurosyphilis but also some immune-mediated mechanism such as auto-immune encephalitis was involved in the pathogenesis of our case. The correlation between the GluR antibody and the patient's symptoms as well as his apparent response to steroid therapy supports an inflammatory process.

To date, it has not been revealed whether the GluR δ2 antibodies play a role in epileptogenesis. It has been revealed, however, that GluR δ2 antibodies can be detected in patients with refractory epilepsy (Wakamoto et al., inpress). In addition, GluR δ2 antibodies have also been detected in patients with cerebellitis (Shiihara et al., 2007; Shimokaze et al., 2007; Kubota and Takahashi, 2008), opsoclonus–myoclonus syndrome (Kubota and Takahashi, 2008; Matsumoto and Ugawa, 2010; Shiihara and Takahashi, 2010) and nonspecific encephalitis (Mochizuki et al., 2006; Kawashima et al., 2010). We cannot prove that the GluR δ2 antibody was pathogenic; in fact, it may have been a byproduct of the seizure activity. We can recommend, however, that if unusual clinical findings such as focal dystonia are observed or if GluR δ2 antibodies are detected in similar cases, a pathologic inflammatory process should be considered.

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Short communication

Antibodies to N-methyl-D-aspartate glutamate receptors in Creutzfeldt–Jakob disease patients

Koji Fujita [a,*], Tatsuhiko Yuasa [b], Yukitoshi Takahashi [c], Keiko Tanaka [d], Wataru Sako [a], Hidetaka Koizumi [a,e], Yasushi Iwasaki [f], Mari Yoshida [f], Yuishin Izumi [a], Ryuji Kaji [a]

[a] Department of Clinical Neuroscience, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan
[b] Department of Neurology, Kamagaya-Chiba Medical Center for Intractable Neurological Disease, Kamagaya General Hospital, 929-6 Hatsutomi, Kamagaya 273-0121, Japan
[c] National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, 886 Urushiyama, Aoi-ku, Shizuoka 420-8688, Japan
[d] Department of Neurology, Kanazawa Medical University, 1-1 Daigaku, Kahoku 920-0293, Japan
[e] Department of Neurology, Kyoto Prefectural University of Medicine, 465 Kaji-cho, Kyoto 602-0841, Japan
[f] Department of Neuropathology, Institute for Medical Science of Aging, Aichi Medical University, Nagakute 480-1195, Japan

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ABSTRACT

Psychiatric symptom can be a prominent feature early in Creutzfeldt–Jakob disease (CJD), which is also common in autoantibody-mediated limbic encephalitis. We hypothesized that anti-neuronal autoantibodies, especially those against N-methyl-D-aspartate glutamate receptors (NMDAR), can also be associated with CJD. Thirteen patients with CJD and 13 patients with limbic encephalitis were enrolled. Immunohistochemistry demonstrated that serum of CJD patients reacted with neuronal components of the rat hippocampus, indicating that those samples contained anti-neuronal antibodies. Enzyme-linked immunosorbent assay revealed that titers of antibodies against peptides of GluN2B subunit of NMDAR were significantly elevated in the serum and cerebrospinal fluid of CJD patients.

1. Introduction

Creutzfeldt–Jakob disease (CJD) shares some clinical features with immune-mediated limbic encephalitis (Chitravas et al., 2011). Of note, psychiatric symptoms are often prominent both in CJD (Wall et al., 2005) and some forms of limbic encephalitis. Autoantibodies detected in limbic encephalitis can play a role in the development of psychiatric features (Dalmau et al., 2011). We have hypothesized that autoantibodies are also produced and potentially contribute to neuropsychiatric symptoms in CJD. In fact, we reported a sporadic CJD patient with antibodies against the GluN2B molecule and native N-methyl-D-aspartate glutamate receptor (NMDAR) (Fujita et al., 2012a). Here, we explored further the association of CJD and antibodies recognizing NMDARs.

2. Materials and methods

2.1. Subjects

Serum and cerebrospinal fluid (CSF) samples of 13 patients who met the World Health Organization criteria for CJD (World Health Organization, 1998) were used (Table 1). Control samples were serum of 13 healthy people, CSF of 19 patients with non-inflammatory epilepsy, and serum and CSF of 13 patients with acute non-infectious limbic encephalitis in whom polymerase chain reaction was negative for herpes simplex virus (HSV)-1, HSV-2, varicella–zoster virus, cytomegalovirus, Epstein–Barr virus, and human herpes virus-6; autoantibodies were not screened. This study was approved by the Ethics Committee of the Tokushima University Hospital, and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Each patient or a legally authorized representative provided written informed consent.

2.2. Immunofluorescent study

Adult Sprague–Dawley rats were administered an i.p. injection of a lethal dose of pentobarbital and were perfused transcardially with 0.01 M phosphate-buffered saline (PBS, pH 7.4) and cold 0.1 M phosphate buffer (PB, pH 7.4) containing 4% paraformaldehyde. The brain was removed, post-fixed overnight in the same fixative at 4 °C, and stored in 0.1 M PB containing gradient (10–30%) sucrose at 4 °C for cryoprotection. Sections of 25 μm-thickness were cut on a cryostat and stored in PBS/0.05% NaN_3 until use. Serum (1:500) and CSF (1:10) from CJD patients or controls and rabbit polyclonal anti-GluN2B IgG (ab65875, 1:1000, Abcom; generated against a peptide mapping the N-terminus [NT] of GluN2B) were used as the primary antibodies. The

* Corresponding author. Tel.: +81 88 633 7207; fax: +81 88 633 7208.
E-mail address: kof@clin.med.tokushima-u.ac.jp (K. Fujita).

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Table 1
Clinical characteristics of Creutzfeldt–Jakob disease patients.

No.	Patient age/sex	Diagnosis Codon 129 (PrP^{Sc} type)	Duration before serum/CSF	Psychiatric symptoms	Past history or prodromal features
1	69/M	Pro, Spo MM[a]	NA[b]/4 wk	No	No
2	74/F	Def[c], Spo[d] MM (NA)	NA/17 wk	Yes	Schizophrenia
3	67/M	Pro[e], M232R[f] MM	NA/9 wk	Yes	No
4	76/M	Pro, Spo MM	NA/11 wk	Yes	Asthma
5	60/M	Def, M232R MM (1)	25 wk/14 wk	Yes	Diabetes
6	70/F[g]	Pro, Spo MM	6.8 yr/27 wk	No	No
7	84/M	Pos[h], V180I[i] MV[j]	48 wk/13 wk	No	Influenza
8	83/F	Pro, Spo MM	6 mo/6 mo	Yes	No
9	65/F	Pro, Spo MM	8 mo/8 mo	Yes	Depression
10	80/M	Def, Spo MM (1)	22 mo/22 mo	Yes	Cerebral infarction
11	74/M	Def, Spo MM (1)	NA/28 mo	Yes	No
12	74/F	Def, Spo MM (1)	10 mo/10 mo	Yes	No
13	64/F	Def, Spo MM (1)	30 mo/NA	Yes	No

[a] MM – homozygous for methionine.
[b] NA – not available.
[c] Def – definite.
[d] Spo – sporadic.
[e] Pro – probable.
[f] M232R – a substitution of methionine to arginine at codon 232.
[g] Previously described (Fujita et al., 2012a).
[h] Pos – possible.
[i] V180I – a point mutation of valine to isoleucine at codon 180.
[j] MV – heterozygous for methionine and valine.

sections were blocked with 3% bovine serum albumin (BSA) in PBS (pH 7.2) for 1 h and then incubated overnight at room temperature in 3% BSA–PBS containing the primary antibodies. Immunoreactivity was detected using fluorescent secondary antibodies conjugated with Alexa 568 or Alexa 488 (1:1000, Invitrogen), respectively.

2.3. Enzyme-linked immunosorbent assay

Serum and CSF samples of CJD patients and controls underwent enzyme-linked immunosorbent assay (ELISA) studies. Peptides were synthesized from the sequences of GluN2B: amino acids 369 to 382 (KERKWERVGKWKDK) from the extracellular NT and amino acids 1153 to 1166 (DIYKERSDDFKRDS) from the intracellular C-terminus (CT). Maxisorb plates (#468667, Nalge Nunc International) were coated overnight with peptide (1 μg/well) in PBS (pH 7.2) and blocked with BSA (5% w/v) in PBS-Triton X-100 (PBST; 0.05% v/v) for 2 h. Serum (100 μL, diluted 1:10 in PBST containing 1% BSA) or CSF (100 μL, undiluted) was then incubated at 37 °C for 2 h. After washing (PBST), plates were incubated with a protein A-horseradish peroxidase conjugate (1:10,000) for 2 h, and developed using TMB Microwell Peroxidase Substrate System (#50-76-00, KPL). Optical densities (450 nm) were measured using a microplate reader.

2.4. Statistical analysis

The study groups were compared by Kruskal–Wallis test, followed by Dunn's multiple comparison post hoc analysis. $P<0.05$ was considered statistically significant. All data were analyzed using GraphPad Prism 5.

3. Results

3.1. Immunoreactivity with the rat hippocampus

To characterize the immunoreactivity of the serum and CSF samples from CJD patients, we performed immunofluorescence in the adult rat hippocampus. Patients' samples reacted with the neuronal components and this immunoreactivity was comparatively stronger in the pyramidal layer. Control samples showed no immunoreactivity in the rat brain. Double immunostaining with patients' serum and anti-GluN2B IgG demonstrated that the reactivity of the patients' serum partly colocalized with the expression of GluN2B (Fig. 1).

3.2. Quantitative analysis of antibodies to glutamate receptor peptides

The titers of serum and CSF antibodies to the synthesized peptides of NT and CT of GluN2B measured by ELISA were significantly elevated in the CJD group (Fig. 2).

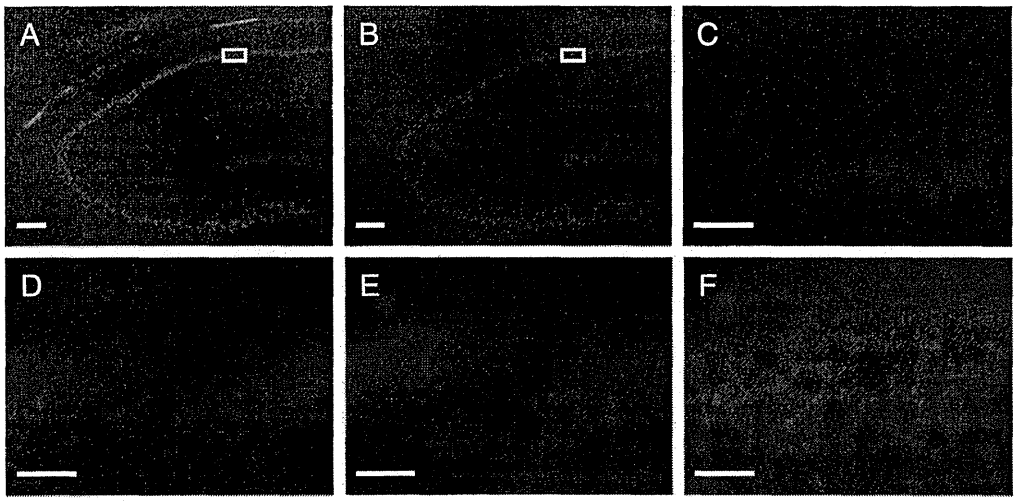

Fig. 1. Hippocampal immunoreactivity of serum samples. (A, B) The same rat brain slice is immunostained with the patient's serum (A, case 5) and polyclonal anti-GluN2B IgG (B). (C) Control serum does not react with the rat hippocampus. (D, E) At higher magnification of A and B, the reactivity of the patient's serum (D) partly corresponds to GluN2B expression (E). (F) Merge of D and E. Scale bars: 200 μm (A, B); 20 μm (C–F).

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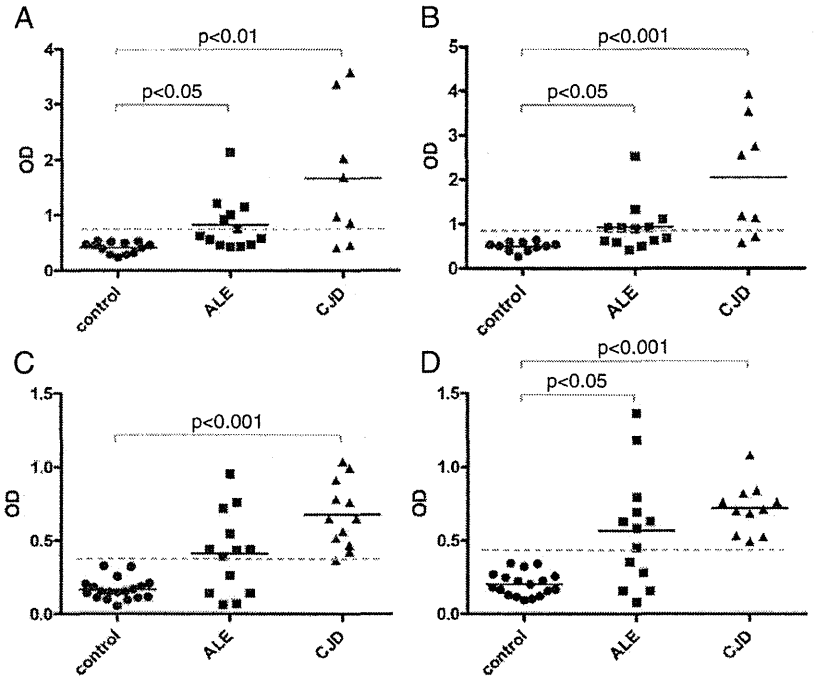

Fig. 2. Enzyme-linked immunosorbent assay. Titers of antibodies to synthesized peptides of the N-terminus (NT) and C-terminus (CT) of GluN2B in the serum and cerebrospinal fluid (CSF) are plotted. (A) Serum antibodies to the NT peptide. (B) Serum antibodies to the CT peptide. (C) CSF antibodies to the NT peptides. (D) CSF antibodies to the CT peptide. Solid horizontal bars indicate mean values. Dotted lines indicate mean + 3SD of control. ALE = acute limbic encephalitis, CJD = Creutzfeldt–Jakob disease, OD = optical density.

4. Discussion

Immunohistochemistry indicate that samples of CJD patients contain antibodies against neuronal components. Some of the antigens seem spatially associated with GluN2B, although it does not necessarily mean that GluN2B is the major antigen. ELISA show that some antibodies react with synthesized GluN2B peptides. Together, we speculate that CJD patients have antibodies against various neuronal molecules and one of the antigens is the NMDAR molecule.

The mechanisms of autoantibody induction in CJD patients may not be identical to those in encephalitis patients. First, prodromal viral infection was observed in only 1 of 13 CJD patients and thus virus-induced molecular mimicry would not be a good explanation. Second, it is unlikely that most of our CJD patients had teratoma and neuronal antigens including NMDAR that were ectopically expressed. If the antigens are denatured peptides of NMDAR, the antigens may be released from damaged neurons and be recognized by microglia and B cells. Microglia can play a role in the presentation of these self antigens to helper T cells. Subsequently, autoreactive helper T cells may activate self antigen-binding B cells (Goverman, 2009). Epitope spreading, in which epitopes other than the inducing epitope become the chief targets of an ongoing immune response (Kaufman et al., 1993; Drayton et al., 2006), may also occur. ELISA findings showing antibodies to NT and CT peptides of the NMDAR molecule may be consistent with intramolecular epitope spreading. These immune responses can take place in the peripheral circulation or the central nervous system (CNS). The potential role of helper T cells, which are observed near or around cerebral blood vessels and in the CNS parenchyma in CJD (Lewicki et al., 2003), warrants investigation.

The remaining concern is the antigens other than NMDAR recognized by the antibodies in CJD. Some antibodies reported in CJD (Sotelo et al., 1980; Toh et al., 1985) and CJD-mimicking encephalitis (Seipelt et al., 1999; Geschwind et al., 2008) are worth considering. First, antibodies against axonal neurofilament were documented in CJD and kuru (Sotelo et al., 1980; Toh et al., 1985). However, the immunostaining pattern of our CJD cases did not correspond to the distribution of axonal neurofilament. Second, the phenotype of Hashimoto encephalopathy can resemble that of CJD (Seipelt et al., 1999). Antibodies to NT of α-enolase, reported to be specific to Hashimoto encephalopathy (Yoneda et al., 2007), need to be investigated in CJD. Third, the clinical features of limbic encephalitis with antibodies against voltage-gated potassium channel (VGKC) complex can also be similar to those of CJD (Geschwind et al., 2008). Although anti-VGKC complex antibodies were first reported to be absent in CJD patients (Geschwind et al., 2008), we do not exclude the association of anti-VGKC complex antibodies and CJD, as recently documented (Fujita et al., 2012b). Detection of antigens that are specifically targeted by the antibodies in CJD patients will lead to further understanding of the pathogenesis of the disease.

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LETTER TO THE EDITORS

Detection of anti-glutamate receptor ε2 and anti-*N*-methyl-D-aspartate receptor antibodies in a patient with sporadic Creutzfeldt–Jakob disease

Koji Fujita · Tatsuhiko Yuasa · Yukitoshi Takahashi · Keiko Tanaka · Shuji Hashiguchi · Katsuhito Adachi · Yuishin Izumi · Ryuji Kaji

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Dear Sirs,

Creutzfeldt–Jakob disease (CJD) is the most common type of human prion disease. CJD, particularly sporadic CJD (sCJD), is not completely related to immunological responses. However, immune abnormalities such as elevated pro- and anti-inflammatory cytokines have been reported in patients with sCJD [1, 2], warranting investigation of the immunological aspects of this disease. Here we present an sCJD case with autoantibodies against the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor.

A 70-year-old right-handed woman developed a hand tremor, right-predominant rigidity in the upper limbs, and Myerson's sign. Two months later, she developed left hemineglect. Her memory, insight, and judgment were normal at that time, but dressing apraxia, acalculia, and Balint's syndrome were observed. She developed idiopathic hyponatremia, but her cognitive impairment progressed even after treatment of the hyponatremia. She underwent tracheostomy (without mechanical ventilation) 6 months after onset. Subsequently, she developed myoclonus of the left upper limb. About 2 years after onset, she developed akinetic mutism. Her disease duration was more than 8 years.

Serum anti-thyroid peroxidase, anti-thyroglobulin, and anti-voltage-gated potassium channel complex antibodies were found to be negative. The cell count was 1/mm^3, protein level was 63 mg/dl, 14-3-3 protein was strongly positive, and total tau protein was 8,860 pg/mL (cut-off value 1,300 pg/mL) in the cerebrospinal fluid (CSF) collected 6 months after onset. Diffusion-weighted imaging performed 7 months after onset showed widespread cortical hyperintensity (Fig. 1a), but the signal changes were less evident on fluid-attenuated inversion recovery (Fig. 1b). An electroencephalogram showed diffuse slow waves at early stages, and periodic sharp wave complexes (PSWCs) were evident about two and a half years after onset. She was homozygous for methionine at prion protein gene codon 129, and no mutations were identified. Probable sCJD was diagnosed as per the World Health Organization criteria [3]. Accordingly, she did not receive any immunotherapy. Real-time quaking-induced conversion (RT-QUIC) [4] of CSF performed later was positive for PrP^{Sc}, validating the diagnosis of CJD. This study has been approved by the Ethics Committee of the Tokushima University Hospital and has, therefore, been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Immunoblot analysis, as described previously [5], detected IgG antibody to the entire NMDA-type glutamate receptor ε2 molecule (GluRε2, also called NR2B) in CSF.

K. Fujita (✉) · Y. Izumi · R. Kaji
Department of Clinical Neuroscience,
The University of Tokushima Graduate School,
3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan
e-mail: kof@clin.med.tokushima-u.ac.jp

T. Yuasa
Department of Neurology, Kamagaya-Chiba Medical Center for Intractable Neurological Disease, Kamagaya General Hospital, Kamagaya 273-0121, Japan

Y. Takahashi
National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka 420-8688, Japan

K. Tanaka
Department of Neurology, Kanazawa Medical University, Kahokugun 920-0293, Japan

S. Hashiguchi · K. Adachi
Department of Neurology, Tokushima National Hospital, National Hospital Organization, Yoshinogawa 776-8585, Japan

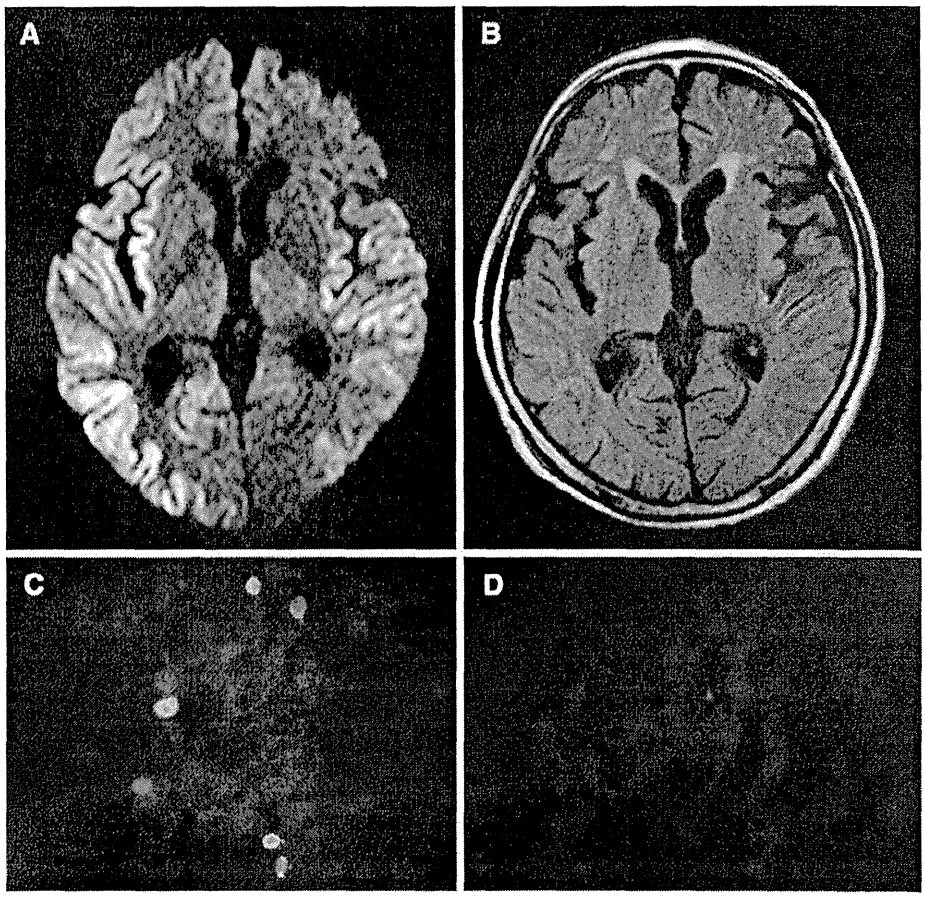

Fig. 1 **a** Diffusion-weighted imaging showing widespread cortical hyperintensity. **b** Abnormal signals were less evident on fluid-attenuated inversion recovery. **c**, **d** Immunocytochemical demonstration of anti-*N*-methyl-D-aspartate receptor antibody. Human embryonic kidney 293 cells co-transfected with NR1 and NR2B reacted with cerebrospinal fluid of the patient (**c**) but not with that of a control case (**d**)

We also analyzed anti-NMDA receptor (NMDAR) antibody using a cell-based assay with human embryonic kidney 293 (HEK293) cells co-transfected with NR1 and NR2 cDNA. The detailed method has been described previously [6]. In brief, the NMDAR subunit genes (NR1/NR2A or NR1/NR2B in an equimolar mixture) were transfected with Lipofectamine reagent (Invitrogen, Carlsbad, CA, USA) into HEK293 cells in media containing 10 μM MK-801 (Wako Pure Chemicals, Tokyo, Japan) for neuroprotection. Twelve hours after transfection, the HEK293 cells were fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4) for 20 min. Non-specific binding was blocked using 10% goat serum/PBS, and the cells were incubated with CSF or sera in 0.02% Triton X-100, 10% goat serum in PBS overnight at 4°C, followed by incubation with FITC-conjugated anti-human IgG (DAKO, Glostrup, Denmark) for 1 h. *SlowFade*® Gold anti-fade reagent (Invitrogen) was then applied to the slides, and staining was observed through a fluorescence microscope, Axiovision (Carl Zeiss, Jena, Germany). The antibody was detected in CSF (Fig. 1c, d).

We report an sCJD patient who had anti-GluRε2 and anti-NMDAR antibodies. The patient presented with parkinsonism, parieto-occipital symptoms, and slowly progressive dementia. Two years after onset, akinetic mutism and PSWCs were observed. The disease course and laboratory findings were compatible with MM2 cortical-type sCJD [7]. Although we could not initially confirm the diagnosis of CJD, RT-QUIC, which has >80% sensitivity and 100% specificity [4], was positive, thus validating the diagnosis.

Anti-GluRε2 antibody has been reported in patients with Rasmussen's encephalitis [5], acute reversible limbic encephalitis [8], and other encephalitis/encephalopathies. Anti-NMDAR antibody was originally reported as a specific marker for ovarian teratoma-associated encephalitis [9]. To date, this antibody has also been detected in encephalitis patients without tumors or epilepsy patients [10, 11]. The presence of these antibodies indicates autoimmune mechanisms; moreover, anti-NMDAR antibody is related to pathogenic processes [12].

Detection of these antibodies in our case with sCJD raised some critical issues. First, CJD and autoimmune limbic encephalitis, two important differential diagnoses of rapidly progressive dementia [13, 14], may not be reliably distinguished by the presence of autoantibodies reported

for limbic encephalitis. Magnetic resonance imaging and RT-QUIC are useful for diagnosing suspected CJD patients [4, 15]. However, when these laboratory findings are unavailable immediately or these markers are negative and non-diagnostic, immunological therapies may be applied. On the other hand, the possibility of CJD must be considered in cases of suspected encephalitis, even if the antibodies are present.

Second, CJD and autoimmune limbic encephalitis share not only some clinical features but also some pathomechanisms. In fact, serum from patients with anti-NMDAR encephalitis decreases the number of NMDAR clusters and inhibits NMDAR function in vitro [12]. It is speculated that the antibody blocks NMDAR in presynaptic γ-aminobutyric acid (GABA) neurons, thereby causing decrease in GABA release that results in disinhibition of postsynaptic glutamatergic transmission and excessive glutamate release [16]. The glutamate hypofunction model was originally hypothesized for schizophrenia, mainly based on observations that NMDAR antagonists exacerbate psychiatric symptoms. On the other hand, a neuropathological study showed that NMDAR expression decreases in CJD patients [17]. Taken together, NMDAR dysfunction may have an effect on neuropsychiatric features of CJD, although the exact physiological relevance of the antibody remains elusive.

Several autoantibodies have been demonstrated in human and animal prion diseases. Gajdusek's group first reported antibodies against axonal neurofilaments in kuru and CJD [18, 19]. Later, antibodies to prion and *Acinetobacter* peptide sequences were identified in bovine spongiform encephalopathy (BSE) [20]. It was surmised that exposure to *Acinetobacter*, which carries epitopes similar to brain antigens such as prions, may lead to autoantibody production [20], suggesting that molecular mimicry may contribute to antibody production. Furthermore, antibodies to glial fibrillary acidic protein (GFAP) have been reported in cases with BSE [21], suggesting that autoantibody is produced against GFAP that has entered the peripheral circulation from a BSE-affected brain [21].

We postulated some mechanisms for antibody production in the present case. As in anti-GFAP antibodies in BSE [21], autoantibody production may occur against molecules that have entered the peripheral circulation from the brain. Alternatively, antigen presentation and antibody production may be initiated by immune cells already residing in the central nervous system. On the other hand, virus-induced molecular mimicry or ectopic expression of the receptor is less likely because prodromal infection or associated tumors were not observed. Recently, evidence for roles of native PrP (PrP^C) in the immune system has been accumulating [22]. Furthermore, several studies have indicated that loss of PrP^C function induces autoimmune responses. For example, knockout or pharmacological silencing of PrP^C has been found to exacerbate or prolong neuroinflammation in experimental autoimmune encephalomyelitis [23, 24]. We speculate that loss of PrP^C function leads to aggravation of autoimmune responses in CJD patients.

It might be argued that our results are non-specific and only a secondary phenomenon. However, based on the accumulating evidence, we suggest that the immune system plays a pivotal role in the pathogenesis of prion disease.

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Conflict of interest The authors declare that they have no conflict of interest.

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CASE REPORT

Subacute cerebellar ataxia and atrophy developed in a young woman with systemic lupus erythematosus whose cerebrospinal fluid was positive for antineuronal cell antibody

Y Iwasaki[1], A Okamoto[1], H Shoda[1], Y Takahashi[2], K Fujio[1], K Kawahata[1] and K Yamamoto[1]
[1]Department of Allergy and Rheumatology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; and
[2]National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan

Subacute cerebellar ataxia in combination with cerebellar atrophy has rarely been reported as one of the manifestations of lupus in the central nervous system (CNS). We describe a 27-year-old woman with systemic lupus erythematosus who developed subacute cerebellar ataxia. Computed tomography and magnetic resonance imaging of her brain showed cerebellar atrophy in both hemispheres, particularly on the right side. Moreover, increased antineuronal cell antibody levels were detected in her cerebrospinal fluid. The cerebellar ataxia improved markedly following high-dose corticosteroid administration. This suggests that a relationship exists between autoantibodies and subacute atrophic processes in CNS lupus. *Lupus* (2012) **21,** 324–328.

Key words: APS; cerebellar ataxia; cerebellar atrophy; CNS lupus

Introduction

Although neuropsychiatric manifestations are present in 50–70% of patients with systemic lupus erythematosus (SLE),[1] cerebellar involvement occurs in less than 2% of cases.[2] Among them, cerebellar atrophy has rarely been reported, although cerebral cortical atrophy has been detected by magnetic resonance imaging (MRI) in no less than 70% of patients with SLE.[3] In the case of cerebellar ataxia, cerebellar symptoms have often been associated with signs of brainstem or corticospinal tract disease.[2] This suggests that crossed cerebellar diaschisis plays an important role in these symptoms. In the present report, we describe a young woman with SLE who developed cerebellar ataxia and bilateral cerebellar atrophy. An MRI of her brain showed no lesions other than cerebellar atrophy. Treatment with high dose (1 mg/kg) oral prednisolone was effective, resulting in an improvement in her neurological symptoms, although her atrophied cerebellar hemisphere did not change.

Case report

A 27-year-old Japanese woman was diagnosed with SLE in 2002 (at 19 years old) on the basis of arthritis, photosensitivity, and positivity for antinuclear and anti-double-stranded DNA (anti-dsDNA) antibodies. Shortly before her SLE diagnosis, she developed deep vein thrombosis in her left lower leg and was diagnosed with antiphospholipid syndrome and displayed positivity for lupus anticoagulant (LAC) and anti cardiolipin (aCL) and β2 glycoprotein-I (aβ2GPI) antibodies. Soon after her SLE diagnosis, she received oral corticosteroid therapy (starting with 30 mg/day of prednisolone) and has remained well after daily treatment with 5 mg of prednisolone since 2006. She was also given warfarin, and her PT-INR was controlled at 2–3.

In December 2008, she complained of gait instability and was admitted to our hospital in April 2009. On examination, she was found to have truncal ataxia. Bilateral dysdiadochokinesia

Correspondence to: Dr Yukiko Iwasaki, Department of Allergy and Rheumatology, The University of Tokyo Hospital, 7-3-1 Hongou, Bunkyo-ku, Tokyo, 113-8655, Japan
Email: yunyan-todai@umin.ac.jp
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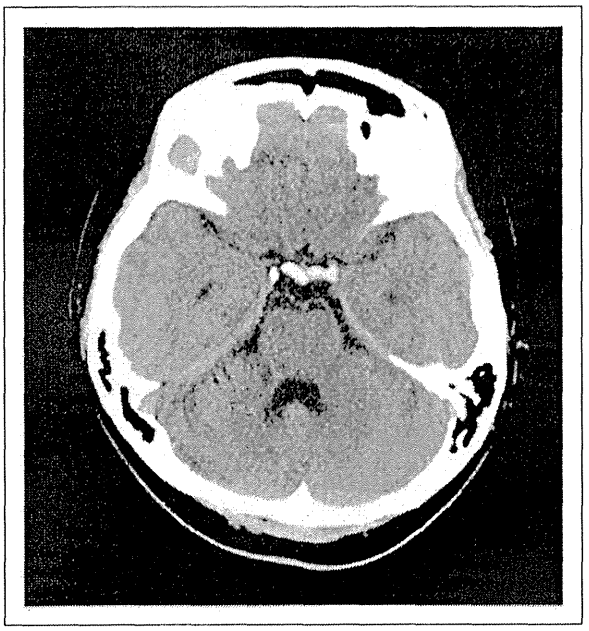

Figure 1 Coronal computed tomography at onset of symptoms in patient showing a lesion in the right middle cerebellar peduncle.

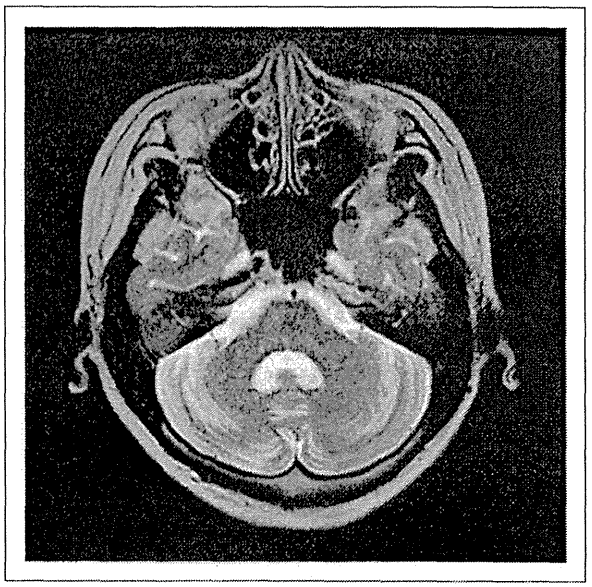

Figure 2 Coronal magnetic resonance imaging at onset of symptoms in patient. Bilateral cerebellar atrophy, dominant in the right side, was detected. No lesion was observed in the right middle cerebellar peduncle.

was detected, and heel–shin test impairment was found to be more marked on the right side. She also displayed fixation nystagmus to the right. No scanning speech was detected. Computed tomography of the brain showed bilateral (although it was dominant on the right side) cerebellar atrophy and a low-density area of about 9 mm in diameter in the right middle cerebellar peduncle (Figure 1). An MRI of the brain also showed bilateral cerebellar atrophy, but no lesion was detected in the right middle cerebellar peduncle (Figure 2). There was no evidence of demyelination. A magnetic resonance angiography of the brain did not reveal any evidence of arterial occlusive lesions or arterial stenosis, although a single photon emission computed tomography of the brain showed decreased vascular flow, which ranged from mild to severe in the right cerebellum and was slight on the left side. An electroencephalogram showed mild diffuse slow waking activity with occasional high voltage delta activity on either side.

The laboratory work-up included a sedimentation rate of 83 mm/h and a C-reactive protein level of 0.18 mg/dl (normal: 0–0.3 mg/dl). A complete blood count revealed lymphopenia (white blood cell count: 6500/mm^3, neutrophils: 79.5%, lymphocytes: 13.6%). Serological tests revealed an IgG level of 3146 mg/dl (normal: 870–1700 mg/dl) and an IgM level of 89 mg/dl (normal: 35–220 mg/dl). Her antinuclear antibody titer was 1:2560 (speckled) (normal: less than 1:40), her anti-dsDNA antibody level was 31 IU/ml (normal: less than 10 IU/ml), and her anti-ssDNA antibody level was 104 AU/ml (normal: less than 20 AU/ml). The anti-RNP antibody index was 153.5 (normal: less than 21), while the anti-Sm antibody was not detected. The anti-SS-A antibody index was 137.8 (normal: less than 29) and the anti-SS-B antibody index was 20.4 (normal: less than 24). Although she had a high level of IgG and positive anti-SS-A/B antibody indexes, she had no sicca syndrome. The serum anti-ribosomal P antibody was present (normal: negative). The glutamate receptor autoantibody, anti-NR2 antibody, was detected. The levels of C3, C4, and CH50 were normal. At this time, tests for LAC and aCL antibody were negative, although aβ2GPI antibody was weakly positive (16 U/ml; normal: less than 10 U/ml). An examination of her cerebrospinal fluid (CSF) revealed a cell count of 8 cells/ml (monocytes: 99%), a protein concentration of 67 mg/dl, a glucose concentration of 52 mg/dl, an IgG level of 345.5 mg/μl (IgG index: 1.38), and an interleukin 6 level of 2.9 pg/ml (normal: less than 4.3 pg/ml). The CSF culture did not reveal any signs of infection. An enzyme-linked immunosorbent assay (ELISA) showed that the antineuronal cell antibody level in her CSF was elevated to 1.27 U/ml (normal: less than 0.27 U/ml), although