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Short communication

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

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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

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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

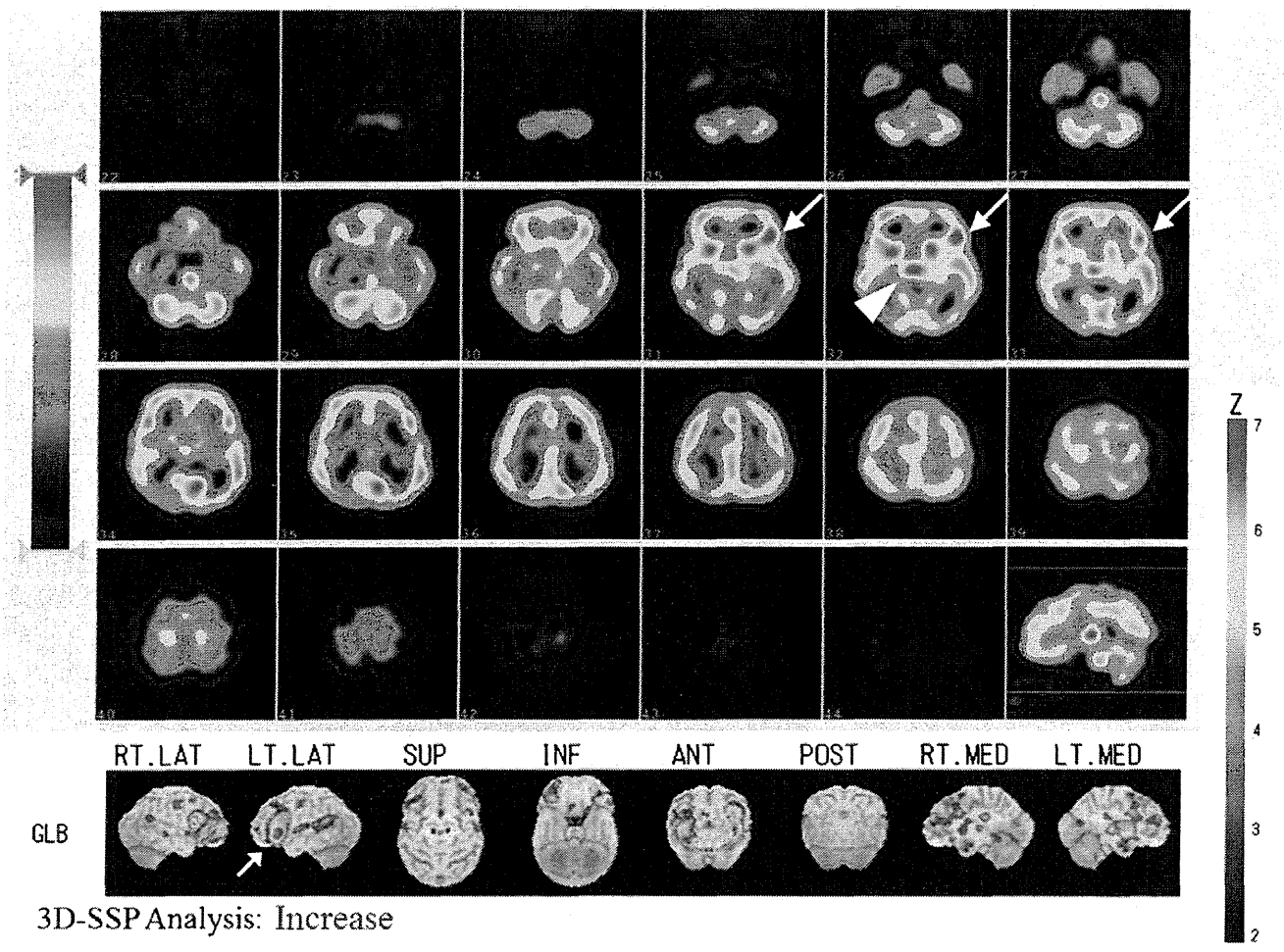

Fig. 1. Ictal SPECT with I-123 IMP and 3D-SSP analysis.Ictal SPECT showed hyperperfusion in the left inferior frontal cortex (large arrow) and right thalamus (arrowhead). The 3D-SSP analysis emphasizes the hyperperfusion in the left inferior frontal cortex (small arrow).

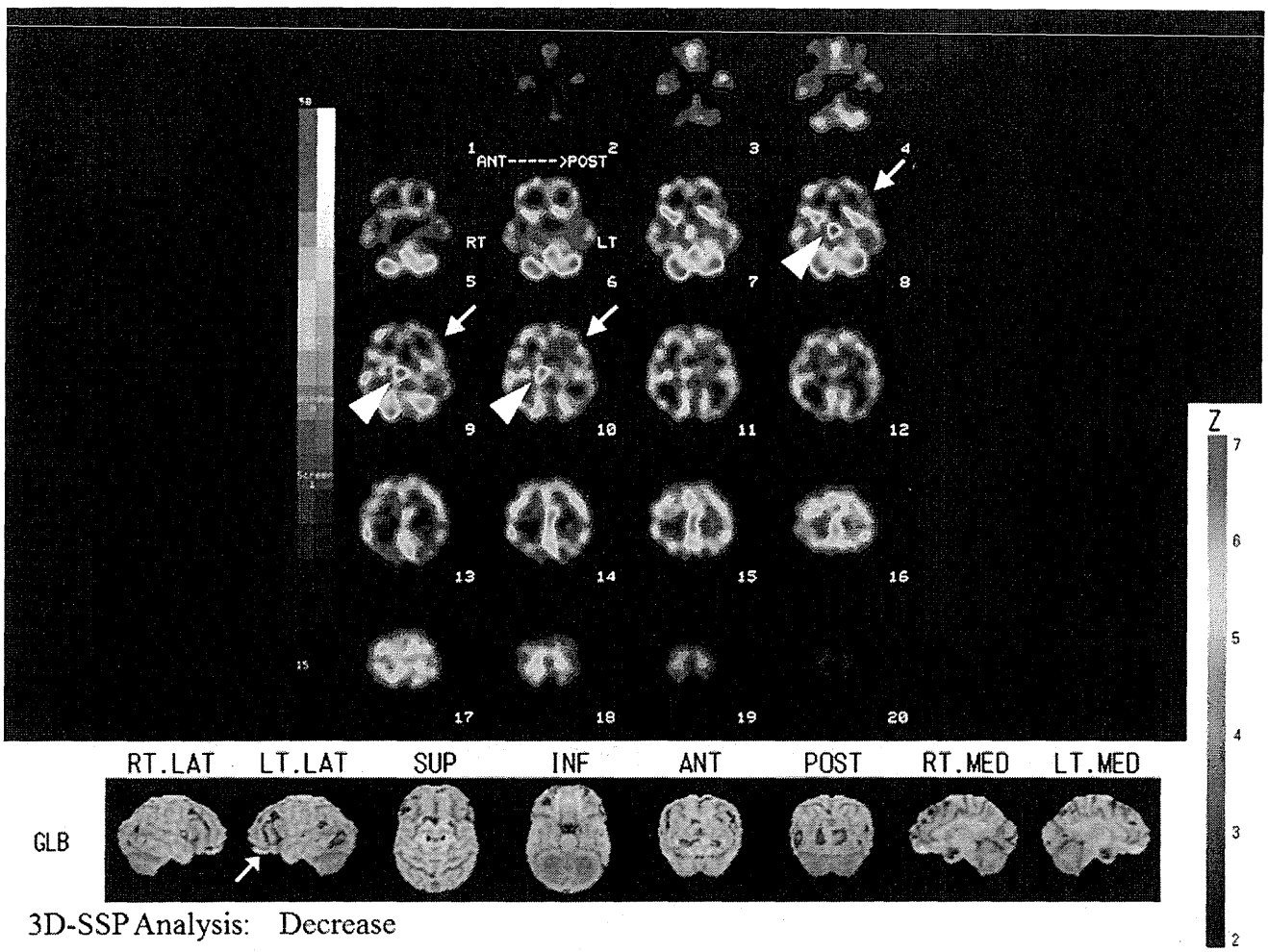

Fig. 2. Interictal SPECT with I-123 IMP and 3D-SSP analysis.Interictal SPECT showed hypoperfusion in the left inferior frontal cortex (arrow) and hyperperfusion in the right thalamus (arrowhead). The 3D-SSP analysis emphasizes the hypoperfusion in the left inferior frontal cortex (small arrow).

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

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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

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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).

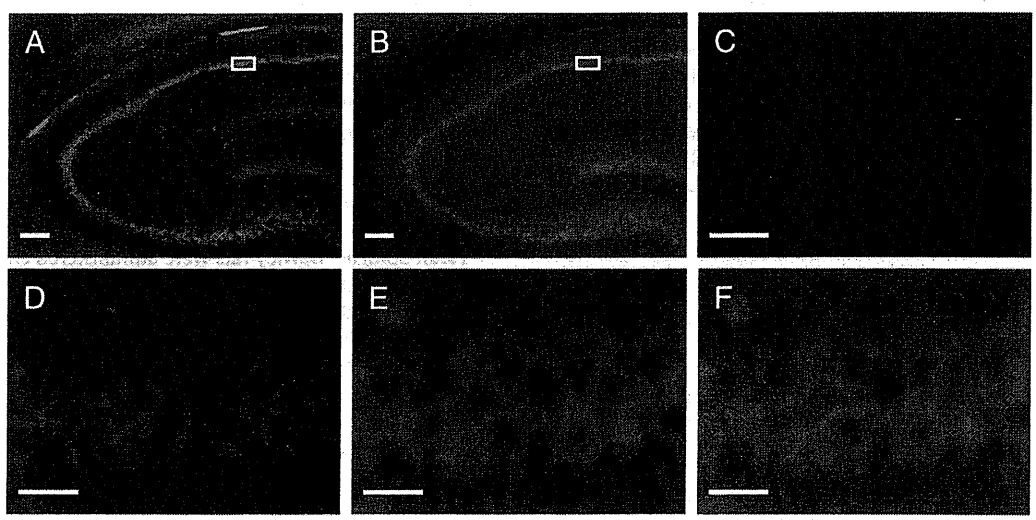

Fig. 1. Hippocampal immunoreactivity of serum samples. (A, B) The same rat brain slice is immunostained with the patient's serum (A, case 5) and polyclonal anti-GluN2B IgG (B). (C) Control serum does not react with the rat hippocampus. (D, E) At higher magnification of A and B, the reactivity of the 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).

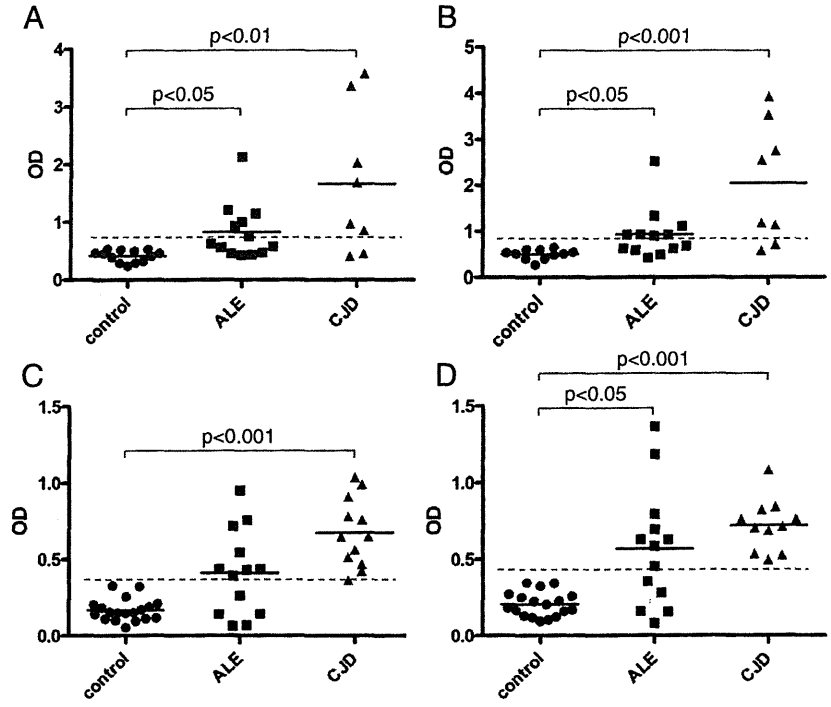

Fig. 2. Enzyme-linked immunosorbent assay. Titers of antibodies to synthesized peptides of the N-terminus (NT) and C-terminus (CT) of GluN2B in the serum and cerebrospinal fluid (CSF) are plotted. (A) Serum antibodies to the NT peptide. (B) Serum antibodies to the CT peptide. (C) CSF antibodies to the 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

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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.

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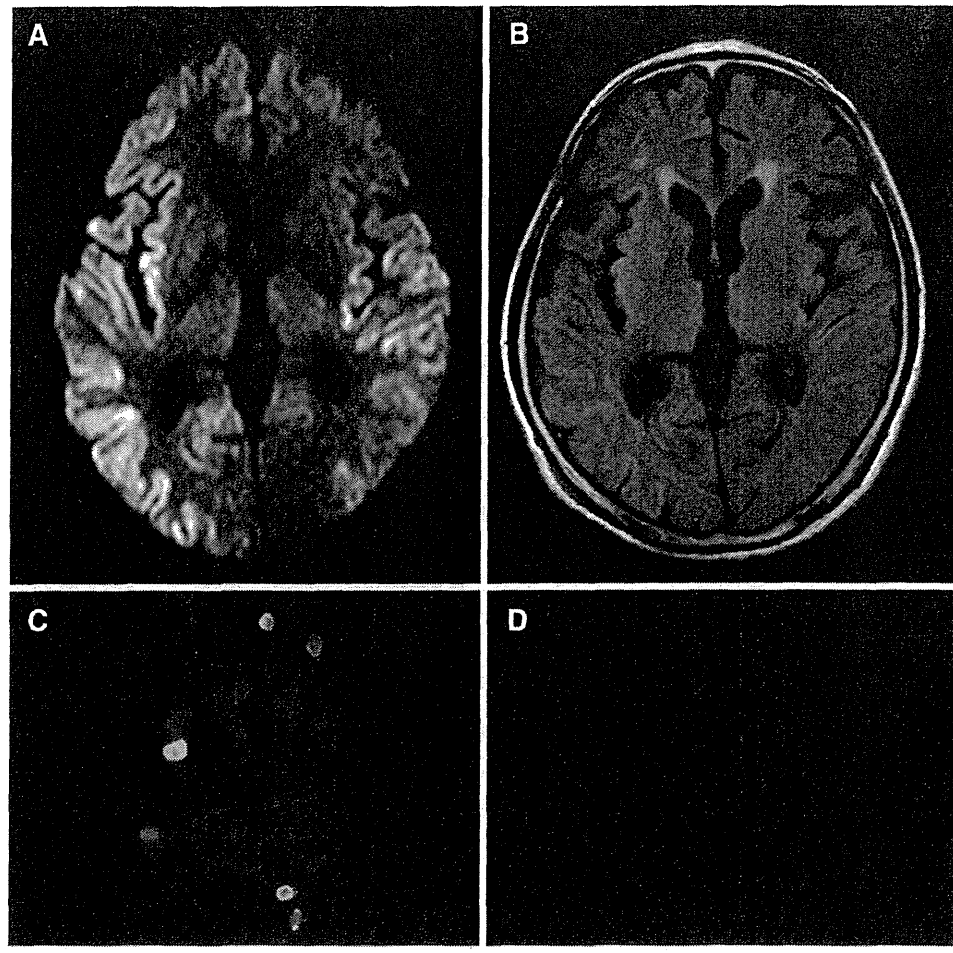

Fig. 1 **a** Diffusion-weighted imaging showing widespread cortical hyperintensity. **b** Abnormal signals were less evident on fluid-attenuated inversion recovery. **c**, **d** Immunocytochemical demonstration of anti-*N*-methyl-D-aspartate receptor antibody. Human embryonic kidney 293 cells co-transfected 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

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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

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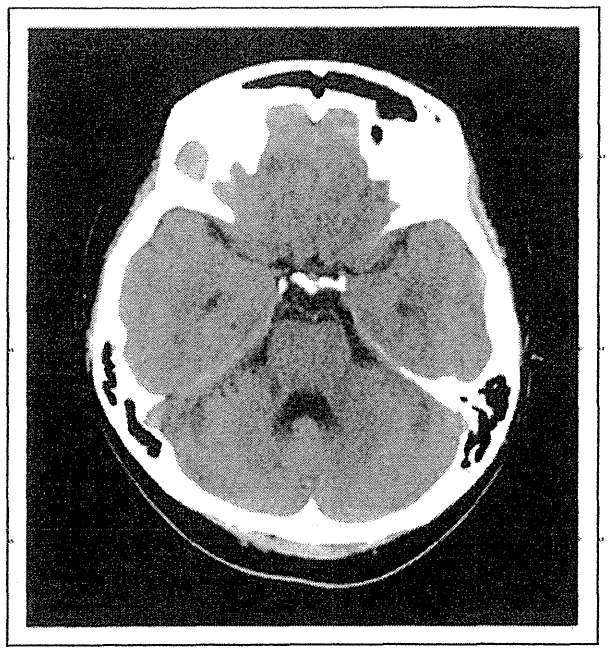

Figure 1 Coronal computed tomography at onset of symptoms in patient showing a lesion in the right middle cerebellar peduncle.

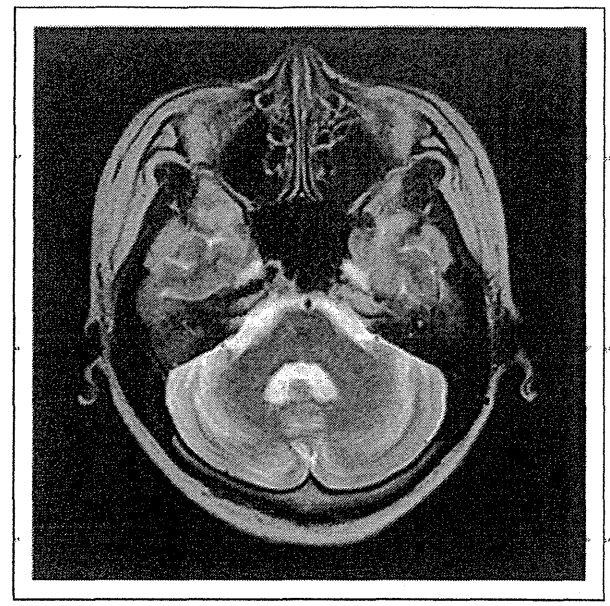

Figure 2 Coronal magnetic resonance imaging at onset of symptoms in patient. Bilateral cerebellar atrophy, dominant in the right side, was detected. No lesion was observed in the right middle cerebellar peduncle.

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

no anti-NR2 antibody was detected in the CSF. No anti-Yo, anti-Hu, or anti-Ri antibodies were found in her serum or CSF.

Lupus activity was suspected, because her anti-ds DNA antibody showed continuous elevation from less than 10 IU/ml in 2003 to 31 IU/ml in 2008. A diagnosis of central nervous system (CNS) lupus was made, and 1 mg/kg body weight per day prednisolone (55 mg/day) was started. As a result, she showed gradual improvements in her signs and symptoms, and her dysdiadochokinesia and gait improved markedly. After 6 weeks, she was placed on 45 mg/day prednisolone, and a further spinal fluid examination revealed improvement, a cell count of 2 cells/ml, a protein concentration of 61 mg/dl, an IgG index of 1.22, and an interleukin 6 level of 1.6 pg/ml. Her anti-dsDNA and anti-ssDNA antibody levels were also decreased to 5 IU/ml and 18 AU/ml, respectively. However, a repeat MRI of the brain showed no change in her bilateral cerebellar atrophy (Figure 3). In 2011, about 2 years after the onset of her cerebellar signs, she is currently on low-dose prednisolone (6 mg/day) and remains well. She displays minimal cerebellar signs.

Discussion

The cerebellar ataxia and atrophy in our case can be explained by an autoimmune mechanism associated with the exacerbation of SLE because these symptoms occurred in combination with serological abnormalities compatible with SLE flare and improved after treatment with high-dose prednisolone therapy. The increases in the CSF antineuronal cell antibody level and in the serum anti-ribosomal P antibody level were compatible with CNS lupus.[4] The anti-NR2 antibody level was increased in the serum, but not in the CSF. The significance of the increase in the serum anti-NR2 antibody level is unclear because serum NR2 autoantibodies are detectable in more than a third of patients with SLE, and the correlation between the antibody and neuropsychiatric complications of SLE remains controversial.[5,6] It is also possible that antiphospholipid antibodies directly damage neuronal cells by cross-reacting with epitopes on CNS phospholipids.[7,8] Considering her good response to corticosteroid therapy, it is unlikely that her antiphospholipid antibodies are related to cerebellar atrophy through microvascular occlusion.

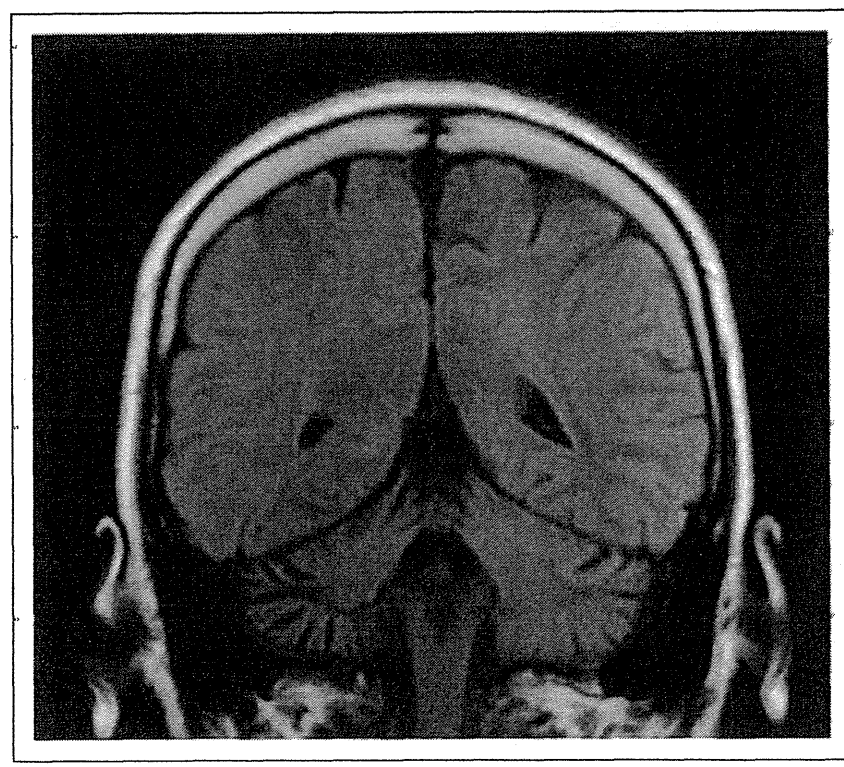

Figure 3 Axial magnetic resonance imaging at follow-up of patient showing no improvement of her atrophic cerebellar hemisphere.

Table 1 Comparison of SLE cases involving both cerebellar ataxia and atrophy

Age	*Sex*	*CT/MRI scan*	*APS*	*Neuronal cell specific antibodies*	*Treatment*	*Treatment effect*	*Ref.*
56	F	Diffuse cerebellar atrophy	NE	NE	no treatment	(improved)	10
47	F	No abnormal lesions in the cerebellum at the onset of ataxia	No	Antibody to human Purkinje cells, antibody against a 75 kDa protein in the cerebellar cortex	mPSL pulse therapy followed by 50 mg of oral PSL	Slight improvement in ataxia, progression of cerebellar atrophy	11
40	F	Mild cerebellar atrophy in both hemispheres combined with moderately severe atrophy of the vermis	No	NE	1 mg/kg/day oral PSL and 150 mg/day AZA	Improvement of ataxia, unchanged atrophy	12
27	F	Pancerebellar atrophy without evidence of demyelination	No	Negative for serum and CSF anti-Yo, anti-Hu, and anti-Ri	mPSL pulse therapy followed by 1 mg/kg/day of oral PSL, 150 mg/day AZA was added when PSL was tapered to 5 mg daily	Improvement of ataxia, unchanged atrophy	13

NE, not examined; mPSL, methylprednisolone pulse therapy; AZA, azathioprine; PSL, prednisolone

Although cerebellar ataxia is an uncommon symptom in patients with SLE, cases of cerebellar ataxia presenting with cerebellar atrophy are much rarer. We found only four such cases after searching the PubMed database (Table 1). In these cases, moderate to high doses of corticosteroid therapy including methylprednisolone pulse therapy (intravenous methylprednisolone 1 g daily for 3 days) were the main treatments and obtained a relatively good response. Previous reports revealed that brain MRI of acute cerebellar ataxia SLE patients showed reversible edematous lesions in the brain, suggesting acute blood–brain barrier changes secondary to small-vessel vasculopathy.[9] There might be pathogenetic differences between acute and subacute cerebellar ataxia. In our case, the clinical response to corticosteroid therapy was rapid, although MRI showed no changes in the patient's cerebellar atrophy. We achieved reductions in the patient's CSF IgG index and serum anti-dsDNA and anti-ssDNA levels. It has been suggested that antibody-mediated reactions could explain her cerebellar symptoms. Although cerebellar ataxia may occur secondary to paraneoplastic syndromes associated with anti-Hu and anti-Yo antibodies, the clinical course is usually acute. Our case is characteristic in that autoantibody-mediated subacute cerebellar ataxia was suggested. We could not confirm the binding of her antineuronal cell antibody to cerebellar tissues by immunohistochemical analysis. The autoantigen of her antineuronal cell antibody should be identified to clarify the processes of observed subacute cerebellar ataxia.

Our case highlights the importance of performing MRI for SLE patients with cerebellar signs. Although the significance of specific antineuronal antibodies in manifestations of CNS lupus is still unclear, high-dose corticosteroid therapy is expected to improve the neurological symptoms of patients suffering from this condition, probably by suppressing the production of pathogenic autoantibodies or cytotoxic processes mediated by cellular and humoral immunity.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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INVITED REVIEW

Serine racemase: an unconventional enzyme for an unconventional transmitter

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Abstract The discovery of large amounts of D-serine in the brain challenged the dogma that only L-amino acids are relevant for eukaryotes. The levels of D-serine in the brain are higher than many L-amino acids and account for as much as one-third of L-serine levels. Several studies in the last decades have demonstrated a role of D-serine as an endogenous agonist of *N*-methyl-D-aspartate receptors (NMDARs). D-Serine is required for NMDAR activity during normal neurotransmission as well as NMDAR overactivation that takes place in neurodegenerative conditions. Still, there are many unanswered questions about D-serine neurobiology, including regulation of its synthesis, release and metabolism. Here, we review the mechanisms of D-serine synthesis by serine racemase and discuss the lessons we can learn from serine racemase knockout mice, focusing on the roles attributed to D-serine and its cellular origin.

Keywords D-serine · NMDA · Glutamate · Astrocytes · Gliotransmission · Glia

D-Serine in the brain and discovery of serine racemase

D-Serine was identified in the brain by Nishikawa, Hashimoto and coworkers (Hashimoto et al. 1992) when they were trying to develop brain-penetrating drugs to enhance NMDAR function, like *N*-myristoyl-D-serine. Serendipitously, they found large amounts of endogenous D-serine in the brains of control rats when trying to detect D-serine-derived compounds in injected animals (Nishikawa 2005). The surprising discovery of D-serine in the brain led some laboratories to embark on the task of unraveling D-serine origin and function.

Despite the existence of high levels of D-serine in the brain, the lack of knowledge about its origin hampered much progress in the field. One major question was whether D-serine was an endogenous or an exogenous amino acid. Experiments employing intraperitoneal injection of L-serine showed that it might be a precursor for D-serine synthesis (Dunlop and Neidle 1997; Takahashi et al. 1997). Moreover, Esaki and co-workers found that partially purified extracts from silkworm *Bombyx mori* (a eukaryote) could convert L- into D-serine (Uo et al. 1998). On the other hand, another study suggested that D-serine was synthesized by the glycine cleavage system (Iwama et al. 1997). The levels of D-serine were greatly reduced in patients lacking activity of the glycine cleavage system, while glycine levels in postmortem brains were several folds higher than controls (Iwama et al. 1997).

Using conventional biochemical purification, the D-serine biosynthetic enzyme was isolated and cloned from rat brain (Wolosker et al. 1999a, b). The enzyme, dubbed serine racemase (SR), converts L- into D-serine and does not use glycine as a substrate. SR is inhibited by high levels of glycine in a competitive manner (Dunlop and Neidle 2005; Strisovsky et al. 2005), which may explain the reduction of D-serine observed in patients exhibiting non-ketotic hyperglycemia due to mutations in the glycine cleavage system (Iwama et al. 1997).

The discovery of the D-serine biosynthetic enzyme preceded our understanding about its role in brain function.

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SR belongs to the fold type II of pyridoxal 5′-phosphate (PLP)-dependent enzymes (De Miranda et al. 2000). The cofactor binds to a lysine at the catalytic site of SR (Lys56) to form an internal aldimine, like in other members of the PLP-dependent family of enzymes (Wolosker et al. 1999a). Interestingly, SR has striking similarity to the serine/threonine dehydratase enzyme of *E. coli*, rather than to classical amino acid racemases, like alanine racemase (Wolosker 2011).

In addition to PLP, SR binds divalent cations (mainly Mg^{2+}, but also Ca^{2+}), and also has a nucleotide binding site which binds the complex Mg.ATP with high affinity. Mg^{2+} binds at a cation binding site located outside the catalytic site. Chelating Mg^{2+} greatly reduces the enzyme activity (Cook et al. 2002; De Miranda et al. 2002), and it is likely that the Mg^{2+}-binding site is important for proper folding. The crystal structure of the yeast homolog of mammalian SR shows that Mg^{2+} coordinates with the carboxylic groups of Glu-208 and Asp-214, residues that are conserved in mammalian SR as well (Goto et al. 2009). Nucleotides (mainly the complex Mg.ATP) also stimulate enzyme activity (De Miranda et al. 2002; Neidle and Dunlop 2002). ATP is not hydrolyzed by the enzyme and binds to a groove formed at the intersection between the domain interface and the subunit interface (Goto et al. 2009; Smith et al. 2010). Like divalent cations, Mg.ATP seems to stabilize the folding of SR.

The discovery of the physiological cofactors of SR also disclosed the main chemical reaction catalyzed by SR: the α, β-elimination of water from L-serine to form pyruvate and NH_4 (De Miranda et al. 2002). This reaction is reminiscent of the homology of SR to serine dehydratases. For each D-serine generated, about four molecules of pyruvate are produced. Lower, but significant elimination with D-serine and L-threonine are also detectable (Foltyn et al. 2005). α, β-Elimination with L-threonine, however, is not accompanied by any racemization. Several artificial substrates for α, β-elimination have been identified (e.g., L-serine *O*-sulfate, L-threo-3-hydroxyaspartate), but none of them are epimerized, indicating that the racemization reaction is more selective toward serine (Panizzutti et al. 2001; Strisovsky et al. 2005).

The physiological roles of SR α, β-elimination are not clear. Pyruvate (from L-serine) and 2-oxobutyrate (from L-threonine) are important metabolites. However, the rate of pyruvate formation from other sources (e.g., glycolysis) is several orders of magnitude faster than the rate of α, β-elimination of L-serine, making it unlikely that SR-derived pyruvate plays an important metabolic role.

The α, β-elimination by SR provides some indication on how this enzyme evolved. Its structural similarity with eliminases, rather than racemases, likely reflects convergent evolution. SR probably originated from a serine dehydratase gene that partially lost its eliminase activity and acquired serine racemization; both activities are well known to be catalyzed by the cofactor PLP.

Another possible function of the α, β-elimination is to regulate D-serine levels (Foltyn et al. 2005). SR also eliminates with D-serine, and this results in partial consumption of synthesized D-serine. It is possible that this activity limits D-serine production in cells and may be especially relevant in forebrain regions that lack significant levels of D-amino acid oxidase enzyme, a peroxisomal protein that degrades D-amino acids (Hashimoto et al. 1993; Nagata et al. 1999). SR mutants lacking α, β-elimination activity more efficiently produce D-serine in vitro and in intact cells. However, the role of α, β-elimination with D-serine has not yet been demonstrated in vivo.

SR reaction mechanisms

The rate of racemization by SR is about 100-fold lower than the bacterial alanine racemase. The catalytic constant (Kcat) of SR racemization ranges between 3 and 45 min^{-1}. These values indicate that it takes more than 1 s for SR to release one molecule of D-serine. This relatively low efficiency of racemization fits the slow D-serine turnover in the brain (about 17 h half-life) (Dunlop and Neidle 1997).

SR reactions involve a combination of two classical PLP-mediated reactions, through the formation of a common intermediate, the resonance-stabilized carbanion (Fig. 1) (Foltyn et al. 2005). Racemization is attained by deprotonation of the external aldimine (L-serine-PLP complex) leading to the formation of a planar carbanion. The proton abstraction from the α-carbon is made possible by the special orientation of the neutral amino group of Lys56 toward the α-proton of L-serine-PLP and the deprotonated hydroxyl group of Ser84 toward the α-proton of D-serine-PLP, in a so-called two-base mechanism (Fig. 1) (Goto et al. 2009). D-Serine-PLP is generated by the reprotonation of the carbanion at the opposite side of the molecule.

β-Elimination is attained by protonation on the substrate β-hydroxy group which results in removal of water (hence, a dehydratase reaction). This is associated with the formation of aminoacrylate–PLP, an unstable intermediate that spontaneously breaks down into pyruvate and ammonia (Fig. 1).

SR regulation

A major unanswered question in the field is with regard to the regulation of D-serine dynamics. Several regulatory mechanisms and protein interactions have been proposed to

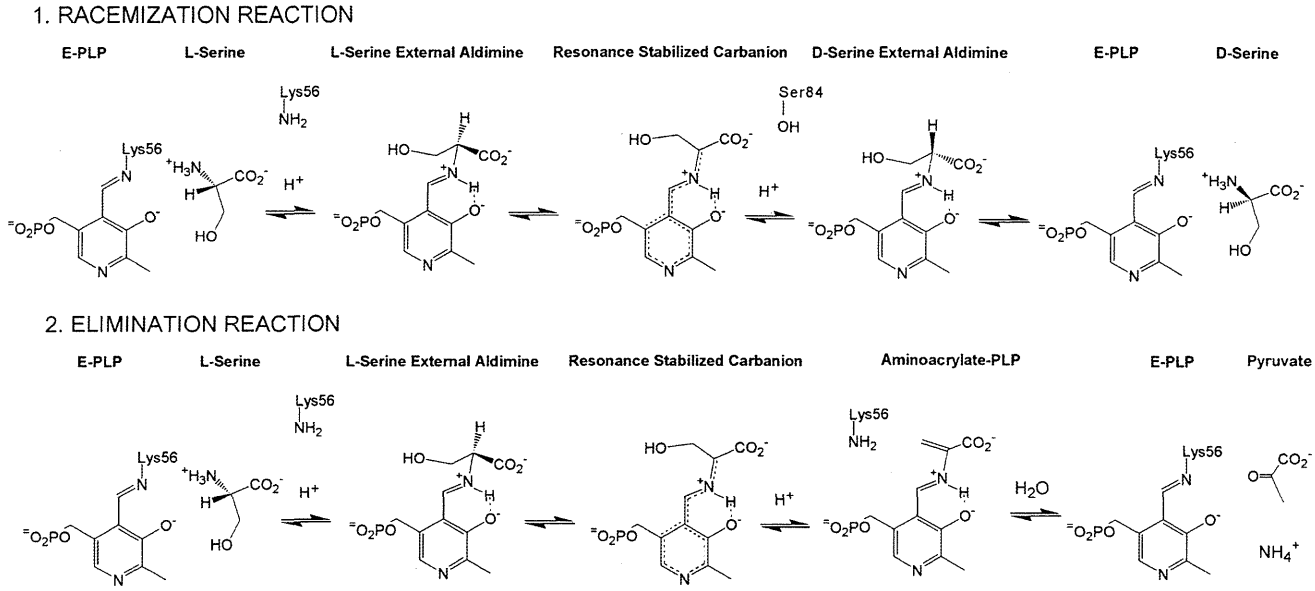

Fig. 1 Reactions catalyzed by SR. For further details, see Foltyn et al. (2005) and Goto et al. (2009)

play a role in modulating SR activity and D-serine release, but the in vivo relevance of the large majority of proposed mechanisms is not known. One complicating factor is that there is no consensus on the cell types that produce D-serine. In this context, some studies focused on astrocytes or microglia, while others investigated D-serine synthesis in neurons. It is not clear which mechanisms are cell specific, but some forms of regulation might be similar in different types of cells.

In astrocytes, mouse SR binds to Grip-1 and Pick-1 (Baumgart et al. 2007; Fujii et al. 2006; Hikida et al. 2008; Kim et al. 2005). The binding occurs through the C-terminal region of mouse SR, which contains a PDZ binding consensus. Binding of SR to Grip-1 increases D-serine production (Kim et al. 2005). Snyder and co-workers proposed that glutamate acting on α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) causes Grip-1 dissociation from the receptors, with consequent binding to SR and enhancement of D-serine production/release (Kim et al. 2005). A direct effect of Pick-1 in SR activity has not been demonstrated, but Pick-1 deficient mice display lower brain D-serine, indicating that it may regulate the mechanisms of D-serine synthesis (Hikida et al. 2008). Of note, the last four amino acids of SR required for binding to the PDZ domains of Grip-1 and Pick-1 are absent in rat and bovine SR, indicating that this interaction may modulate species-specific behaviors (Dumin et al. 2006; Konno 2003).

Another interactor of SR is Golga-3, a protein that binds to the cytosolic face of the Golgi apparatus (Dumin et al. 2006). Interaction with Golga-3 also disclosed a pool of SR that was strongly attached to the membrane fraction. Golga-3 stabilizes SR levels through inhibition of its ubiquitination which leads to slower degradation by the ubiquitin–proteasome system (Dumin et al. 2006). The ubiquitin ligase enzyme (E3) that attaches ubiquitin chains to SR, however, has not been identified yet.

In addition to regulation by protein interactors, SR is regulated by glutamate receptors by different mechanisms. SR binds to and is inhibited by phosphatidylinositol (4,5)-bisphosphate (PIP2) in membranes (Mustafa et al. 2009). Activation of the metabotropic glutamate receptors (mGluR5) increases D-serine synthesis by promoting degradation of PIP2 via activation of phospholipase C. This relieves the inhibition of SR and activates D-serine synthesis (Mustafa et al. 2009). PIP2 affects the nucleotide site of SR, as its inhibition of the enzyme is competitive with Mg.ATP. SR mutants that are unable to bind to PIP2 display higher activity when transfected into cells and are insensitive to mGluR5 activation, confirming that interaction with PIP2 mediates mGluR5 effects on SR.

On the other hand, activation of NMDARs inhibits SR activity. Following NMDAR stimulation, SR translocates from the cytosol (where it resides) to dendritic membranes. This is associated with SR inactivation toward D-serine production (Balan et al. 2009). SR is palmitoylated at serine/threonine residues (*O*-palmitoylation), which is likely to contribute to membrane binding (Balan et al. 2009). Another biochemical mechanism that may control SR interaction with membranes is Thr227 phosphorylation, found only in the membrane-bound SR. Mutation of Thr227Ala decreases the levels of membrane-bound SR under non-stimulated conditions (Balan et al. 2009). SR translocation to the membrane may constitute a feedback