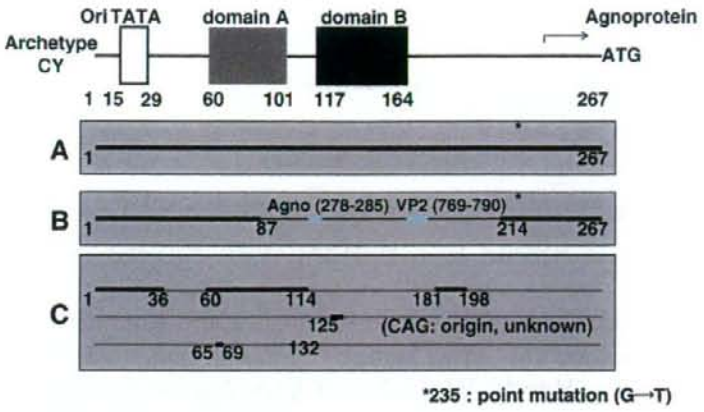

Fig. 1. The nested PCR for transcriptional control region (TCR) of the JCV test detected three types of TCR in the CSF. A: archetype with point mutation (235 G→T) virus. B and C: two different types of rearrangement of TCR virus.

chronic ischemic heart disease, he was not administrated corticosteroid or immunosuppressive therapy for SjS at any time before he visited our hospital.

On admission, he was afebrile, his blood pressure was 130/64 mmHg, his heart rate was 70 bpm and regular, his heart sounds had systolic murmur (Levine 3/IV), his respiratory sounds had fine crackles in the bilateral inferior areas of the chest, and no cervical or axially lymph nodes were palpable. Neurological examination revealed dementia, right hemiparesis, truncal and limb ataxia, normal deep tendon reflex, positive Babinski and Chaddock signs on the right side, and forced grasping in both hands.

Laboratory tests of his peripheral blood showed normal WBC counts with low percentage of lymphocytes; his CD4+ T-lymphocyte count of 272 cells/μl (%CD4; 16.1% (normal, 25–60%)) with a decline to 217 cells/μl (%CD4; 13.6%) within 1 month; and C-reactive protein level was 2.68 mg/dl. The serological tests for antibodies against HIV-1/-2, HTLV-1 and HBs were negative. The serological tests for anti-HCV antibodies were weakly positive; the result of the HCV RNA–PCR was negative. The syphilis serological tests (TPHA, STS) were negative. Autoimmune serological examination demonstrated 1280× antinuclear antibodies. However, the serological tests for other antibodies, including those to Rheumatoid factor, ds-DNA-IgG, Sm, SS-A, SS-B, RNP, and Scl-70, were entirely negative. Other laboratory findings were unremarkable, including the tumor markers. Analysis of his CSF revealed normal pressure and normal parameters with respect to cell count and chemical analysis (cell count; 3 mononuclear cell /μl, protein concentration; 24 mg/dl, glucose concentration; 56 mg/dl). Concentration of soluble IL2-receptor, β2 microglobulin and ferritin were not elevated in CSF. Oligoclonal bands (OCBs) were detected in CSF. Nested PCR revealed 3 types of JCV transcriptional genomes, including an archetype in CSF (Fig. 1). Whole body CT and ^{67}Ga-citrate scintigraphy revealed no malignancy in his body.

Brain MRI showed T1-weighted images with low-intensity lesions, and T2-weighted and FLAIR images with high-intensity lesions in the left frontal white matter, bilateral parietal and left occipital white matter, left thalamus, and right middle cerebellar peduncle, which were not enhanced by Gadolinium (Fig. 2). Spinal cord MRI showed no obvious abnormalities. Serial images of early, 6-hour delayed and 24-hour delayed ^{123}I-IMP SPECT (IMP-SPECT) showed a gradual increase in radioactivity and a gradual increase in amount and a long-term retention of the tracer in the left frontal white matter (Fig. 3). Brain biopsy from the left frontal deep white matter revealed disseminated enlarged oligodendrocytic nuclei that were immunopositive with rabbit polyclonal antibody against JCV-VP1 protein (Fig. 4) [2], JCV-agnoprotein and a large T protein (data not shown).

Treatments with steroid pulse therapy (methylprednisolone 1 g/day, 3 days) and cytarabine (AraC 2 mg/kg/day, 5 days) were not effective, and the patient developed akinetic mutism approximately 3 months after the onset of PML, and

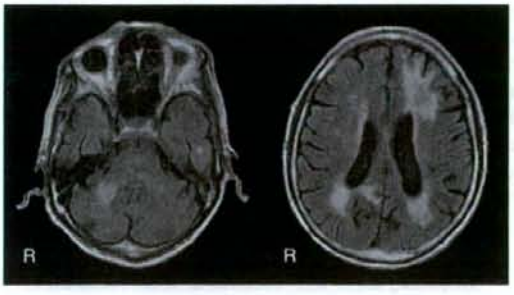

Fig. 2. Axial brain MRI on 1.5-tesla demonstrating multifocal high-intensity areas in left frontal and bilateral periventricular white matters, and right middle cerebellar peduncle on FLAIR (TE 144, TR 8002, TI 2000) images.

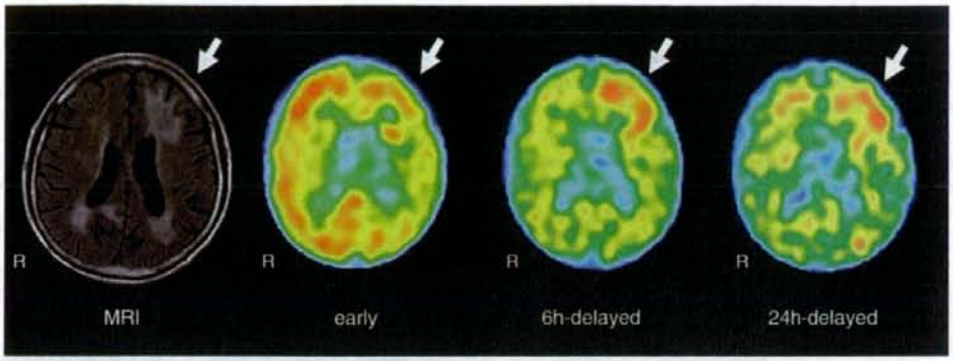

Fig. 3. Axial FLAIR MRI and Serial images of ^{123}I-IMP SPECT: early image, 6-hour delayed image and 24-hour delayed image. These serial images shows a delayed increase of radioactivity, a gradual increase and long term retention of the tracer on the left frontal white matter.

he has been alive for 18 months without any improvement in clinical state.

3. Discussion

We have described a case of PML with SjS, in which progressive dementia, right hemiparesis, and truncal and limb ataxia were the clinical features.

In this case, tumefactive multiple sclerosis (MS), cerebral vasculitis with SjS and primary CNS malignant lymphoma were initially considered as differential diagnoses from the MRI findings.

IMP-SPECT images demonstrated delayed high accumulation of IMP in the left frontal white matter. Primary CNS malignant lymphoma, malignant astrocytoma, and metastatic brain tumors, including malignant melanoma, have been reported to be the lesions to show high accumulation of IMP on delayed IMP-SPECT images [3–6]. Compared with that in normal brain tissues, a small amount of IMP is retained in tumor cells due to the differences in the number of amine receptors, fat distribution and tissue pH [6]. Recently it has been reported that IMP-SPECT shows the delayed accumulation of IMP in tumefactive MS lesions. Therefore, it appears to be a pitfall for distinguishing between MS and malignant lymphoma [7]. The mechanism underlying IMP delayed accumulation is still unclear, but we speculate a small amount of IMP is retained in JCV infected oligodendrocytes as well as in tumor cells.

OCBs were detected in our patient's CSF. OCBs are frequently detected in MS, and in some infectious diseases (meningitis, subacute sclerosing panencephalitis (SSPE), and PML), and other neurological diseases. OCBs observed in SSPE and PML are measles virus-specific [8] and JCV-specific IgG antibodies [9], respectively. Detecting OCBs in CSF is associated with intrathecal synthesis of IgG, and is not pivotal in distinguish between MS and other neurological diseases.

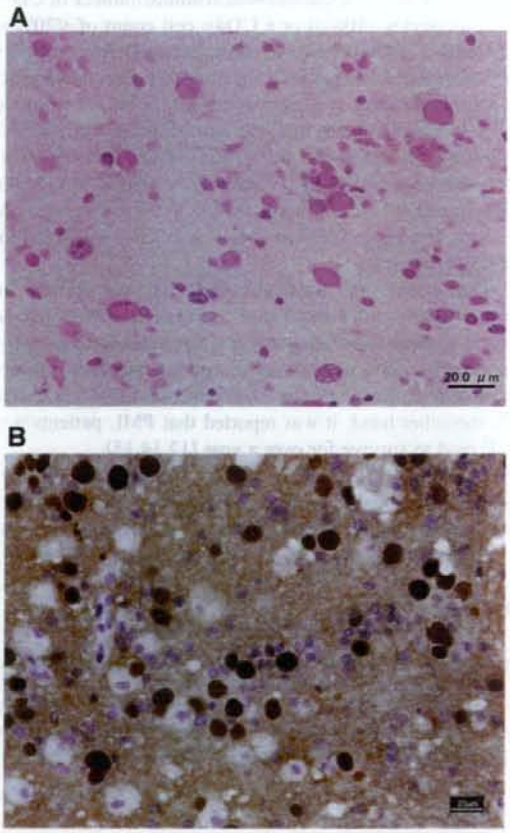

Fig. 4. (A) Brain section stained with hematoxylin-eosin, showing enlarged oligodendrocytic nuclei filled with somewhat basophilic substance in the white matter. Bar 20 μm. (B) Brain section immunostained with antibody against JCV-VP1, demonstrating that such enlarged oligodendrocytic nuclei are clearly positive for this protein. Bar 20 μm.

In the present case, we eventually considered that brain biopsy was necessary for making an accurate diagnosis of the brain lesions; a small brain tissue was collected from the left frontal white matter, where delayed accumulation of IMP was evident. The biopsy specimen showed many enlarged oligodendrocytic nuclei immunopositive for JCV-VP1 protein in demyelinated lesions. Subsequently, the presence of PML types of JCV DNA was also detected in the patient's CSF. Interestingly, the other Sjögren syndrome's patient with PML was from Japan [10]. This case was associated with acute myelocytic leukemia during the clinical course of SjS, and SjS itself was not considered to be the basic disease [10].

Recently, several reports have shown the development of PML in patients with idiopathic CD4+ T-lymphocytopenia (ICL) [11–16]. ICL is a recently described syndrome characterized by a marked decrease in the number of circulating CD4+ T-lymphocytes in the absence of any identifiable causes of immunologic abnormalities, and can be defined by the presence of a documented absolute number of CD4+ T-lymphocytes $<300/\mu l$ or a CD4+ cell count of <20% of the total T cells on two occasions, no evidence of HIV infection, and the absence of any defined immunodeficiency or therapy leading to the decrease in the CD4+ T cell level [17]. It has also been reported that according to the definition mentioned above, 5.2% of SjS patients have CD4+ T-lymphocytopenia [18]. Therefore, we also studied the presence or absence of this condition in the present case. As a result, the laboratory data obtained fulfilled the definition of this syndrome.

The patient has been alive but has not shown any improvement for 18 months since the onset of the disease: this is an unusually long course for the disease. Generally, the clinical course of PML in the absence of an HIV infection is rapidly progressive, and most patients die within a year. On the other hand, it was reported that PML patients with ICL tend to survive for over a year [12,14,15].

In conclusion, it is now necessary to consider PML as one of the differential diagnoses in patients with SjS showing leukoencephalopathy and also to study the number of CD4+ T cells in such patients.

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ABSTRACT: A 20-year-old woman with selective cauda equina hypertrophy presented with muscle weakness and severe pain in the lower extremities. Serial immunotherapy was not effective. We performed biopsy of the cauda equina, and laminectomy and duraplasty of the thoracolumbar region. Biopsy revealed marked infiltration of small lymphocytes and foamy macrophages in the endoneurium. Three years after decompression surgery, her symptoms have improved slightly without progression or relapse. This is the first case of selective cauda equina hypertrophy with idiopathic inflammation. We propose that this is a new disease entity.

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SELECTIVE CAUDA EQUINA HYPERTROPHY WITH IDIOPATHIC INFLAMMATION

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Selective cauda equina hypertrophy has been reported to be associated with Guillain–Barré syndrome,[5] chronic inflammatory demyelinating polyradiculoneuropathy,[3,8] malignant lymphoma,[9] metastatic tumor,[10] paraneoplastic syndrome,[11,12] sarcoidosis,[4] hereditary motor sensory neuropathies,[2,6] postirradiation neuropathy,[4] and infectious diseases [human immunodeficiency virus (HIV), human T-cell leukemia virus-1 (HTLV-1), cytomegalovirus, *Varicella zoster* virus, cryptococcus, and tuberculosis].[4] In most of these cases, peripheral nerve conduction abnormalities were evident.

Burton et al. first reported two cases of isolated hypertrophic radiculopathy of the cauda equina without any evidence of peripheral nerve conduction abnormalities. A paraneoplastic cause was suspected in one case because of improvement after removal of a bronchial carcinoid tumor. The other case was characterized by delayed-onset external opthalmoplegia, but the etiology remained unclear.[1] Herein we describe a case with selective cauda equina hypertrophy with idiopathic inflammation. In this patient, although opthalmoplegia was not noted, all the clinical features are similar to those described in Burton's idiopathic case.[1]

CASE REPORT

A 20-year-old woman presented with a 4-week history of progressive muscle weakness and severe pain in the lower extremities. The pain worsened in the recumbent position. Severe back pain on coughing commenced in mid-November 2003. At the beginning of December, she experienced severe pain in the left fibular region and at her hips in the recumbent position. On December 24, severe muscle weakness developed in both legs.

The patient became wheelchair-bound and was admitted for further evaluation in mid-January 2004. At admission, her general physical and mental status

Abbreviations: ANA, antinuclear antibody; c-ANCA, cytoplasmic antineutrophil cytoplasmic antibody; CA19-9, carbohydrate antigen; CEA, carcinoembryonic antigen; CSF, cerebrospinal fluid; FDG-PET, ^{18}F-deoxyglucose positron emission tomography; HBs, hepatitis B surface; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTLV-1, human T-cell leukemia virus-1; IgE, immunoglobulin E; Met-PET, ^{11}C-methionine positron emission tomography; *MPZ*, myelin protein zero gene; MRC, Medical Research Council; MRI, magnetic resonance imaging; p-ANCA, perinuclear antineutrophil cytoplasmic antibody; *PMP22*, peripheral myelin protein gene; RNP, ribonucleoprotein antibody; SCC, squamous cell carcinoma; SUV, standardized uptake value; SS-A or -B, Sjögren's syndrome A or B; UCHL-1, ubiquitin C-terminus hydrase-L1

Key words: cauda equina biopsy; cauda equina hypertrophy; FDG-PET; inflammation; lumbar MRI

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Table 1. Nerve conduction studies.

Nerve	MCV	d.CMAP	p.CMAP	F wave occ. (%)	min F lat.	SCV	SNAP
Rt. Median	69.5	20.84	17.91	81.3	21.80	67.4	29.95
Rt. Ulnar	70.7	10.14	9.11			63.3	30.52
Rt. Tibial	43.1	16.35	11.74	18.8	42.75		
Lt. Tibial	55.2	27.19	22.45	37.5	40.75		
Lt. Sural						50.8	28.66

MCV: motor conduction velocity; d.CMAP: distal compound muscle action potential; p.CMAP: proximal compound muscle action potential; F wave occ.: F wave occurrence; min F lat: minimum F latency; SCV: sensory conduction velocity; SNAP: sensory nerve action potential.

was normal. Neurological examination revealed that cognitive functions and cranial nerves were intact. She had symmetrical muscle weakness in both legs without muscle atrophy [Medical Research Council (MRC) scale findings: iliopsoas, right 4/5, left 4/5; quadriceps, right 5/5, left 5/5; gluteus maximus, right 2/5, left 2/5; hamstrings, right 4/5, left 4/5; tibialis anterior, right 4/5, left 4/5; gastrocnemius, right 4/5, left 4/5; peroneus, right 4/5, left 4/5; toe extensors, right 4/5, left 4/5; and toe flexors, right 4/5, left 4/5]. Deep tendon reflexes in the arms were normal; however, patellar and Achilles tendon reflexes were absent. Mild bladder dysfunction was evident. Except for mild dysesthesia over her soles, no sensory abnormalities were detected. She complained of pain in the hips and lower extremities that was more severe in the recumbent position or with motion and urinary retention.

Hematological and biological tests, including tests for autoantibodies associated with collagen dis-

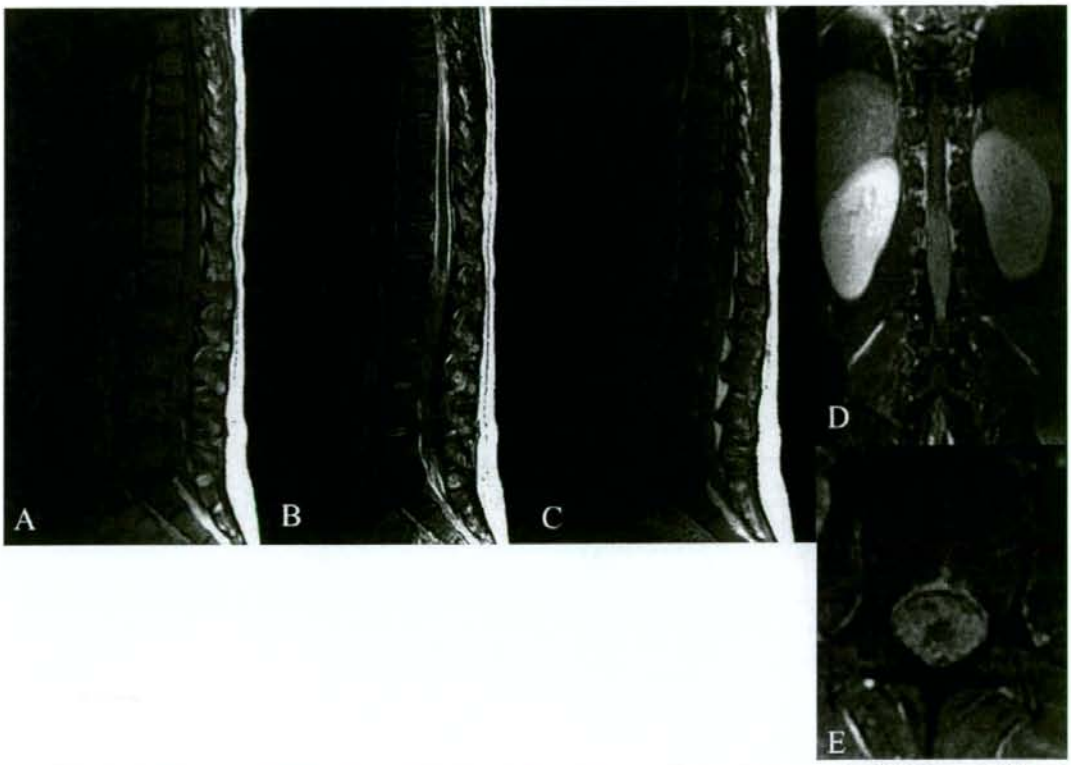

FIGURE 1. Cauda equina nerve roots were enlarged and slightly enhanced by gadolinium according to sagittal **(A–C)**, coronal **(D)**, and axial **(E)** MRI images. **(A)** T1-weighted image, **(B)** T2-weighted image, and **(C–E)** T1-weighted image with enhancement by gadolinium.

ease [antinuclear antibody (ANA), rheumatoid factor, Sjögren's syndrome A antibody (SS-A), Sjögren's syndrome B antibody (SS-B), ribonucleoprotein antibody (RNP), perinuclear antineutrophil cytoplasmic antibody (p-ANCA), and cytoplasmic antineutrophil cytoplasmic antibody (c-ANCA) levels] and autoantibodies to gangliosides revealed no abnormality except for high levels of immunoglobulin E (IgE) and tick-specific IgE. Serological tests for antibodies against HTLV-1, HIV-1/2, hepatitis B surface (HBs), and hepatitis C virus (HCV) were negative. The results of the DNA analyses for myelin protein zero (*MPZ*) and peripheral myelin protein 22 (*PMP22*) genes were normal.

In the upper and lower extremities, sensory nerve action potential and compound muscle action potential amplitudes and nerve conduction velocities were within normal ranges. The velocities and occurrence of the F-waves were decreased in the lower limbs (Table 1). In summary, nerve conduction abnormalities were only found in proximal nerve roots. Needle EMG studies of the legs were not performed.

Only a few drops of highly viscous cerebrospinal fluid (CSF) were collected. The CSF sample was dark yellow and contained 1605 lymphocytes. The lymphocytes appeared to be reactive lymphocytes, and flow-cytometric analysis of surface antigens of these cells showed no monoclonality. The CSF also revealed markedly elevated protein levels (3200 mg/dl) and β_2-microglobulin (8.88 mg/dl) levels and decreased glucose levels (25 mg/dl). Tumor marker levels (CEA, CA19-9, and SCC) were not elevated in the serum or CSF. No malignancies were detected on whole-body computed tomography (CT).

Spinal magnetic resonance imaging (MRI) revealed the cauda equina to be swollen with slight enhancement by gadolinium. The intradural CSF space was completely occupied by the swollen cauda equina (Fig. 1). ^{18}F-deoxyglucose positron emission tomography (FDG-PET) and ^{11}C-methionine positron emission tomography (Met-PET) were performed twice. FDG-PET revealed an extremely high standardized uptake value (SUV) (maximum 10.3 in March 2004) in the cauda equina, and no abnormal uptake in other organs (Fig. 2). Met-PET did not exhibit any accumulation in the body.

No change was noted after three series of steroid pulse therapy (methylprednisolone 1000 mg/day, 3 days), two series of intravenous immunoglobulin therapy (0.4 g/kg/day, 5 days), and two series of simple plasma exchange (50 ml/kg/day, on 4 alternate days). The patient's symptoms progressed gradually. She developed increased leg weakness and vesicorectal dysfunction.

FIGURE 2. According to FDG-PET, this lesion has a high uptake of glucose, with a maximum SUV of 10.3; however, there was no uptake of methionine (data not shown).

To obtain a pathological diagnosis, biopsy of the cauda equina was performed twice (in February and September 2004 the right and left S2 nerve roots were sampled). During surgery, the swollen cauda

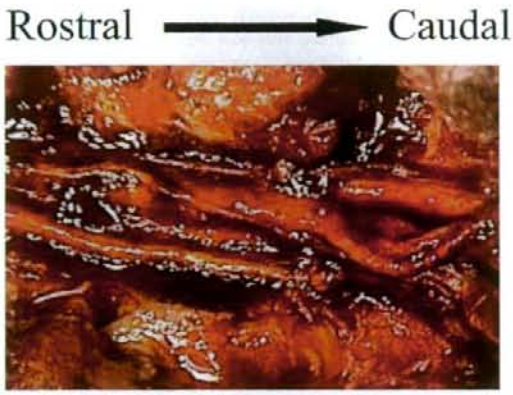

FIGURE 3. Enlargement of the cauda equina nerve roots gives the appearance of Japanese *Udon* noodles (Φ3.5 mm).

equina nerve roots had the appearance of Japanese noodles and were a maximum of 3.5 mm in diameter (Fig. 3). Motor and sensory nerve roots could not be distinguished from each other. At the second biopsy, decompressive laminectomy and duraplasty of the thoracolumbar region (T10–L5) were performed. Both biopsy specimens of the nerve roots demonstrated essentially the same findings. There was marked infiltration of small lymphocytes and foamy macrophages in the endoneurium (Fig. 4A). Occasional multinucleated giant cells were also observed, and their clear cytoplasm contained a number of small lymphocytes (emperipolesis) (Fig. 4B). Most of the lymphocytes were ubiquitin C-terminus hydrase-L1 (UCHL-1)-immunopositive (CD45RO, an anti–T-lymphocyte antibody). The macrophages and multinucleated giant cells were immunopositive for

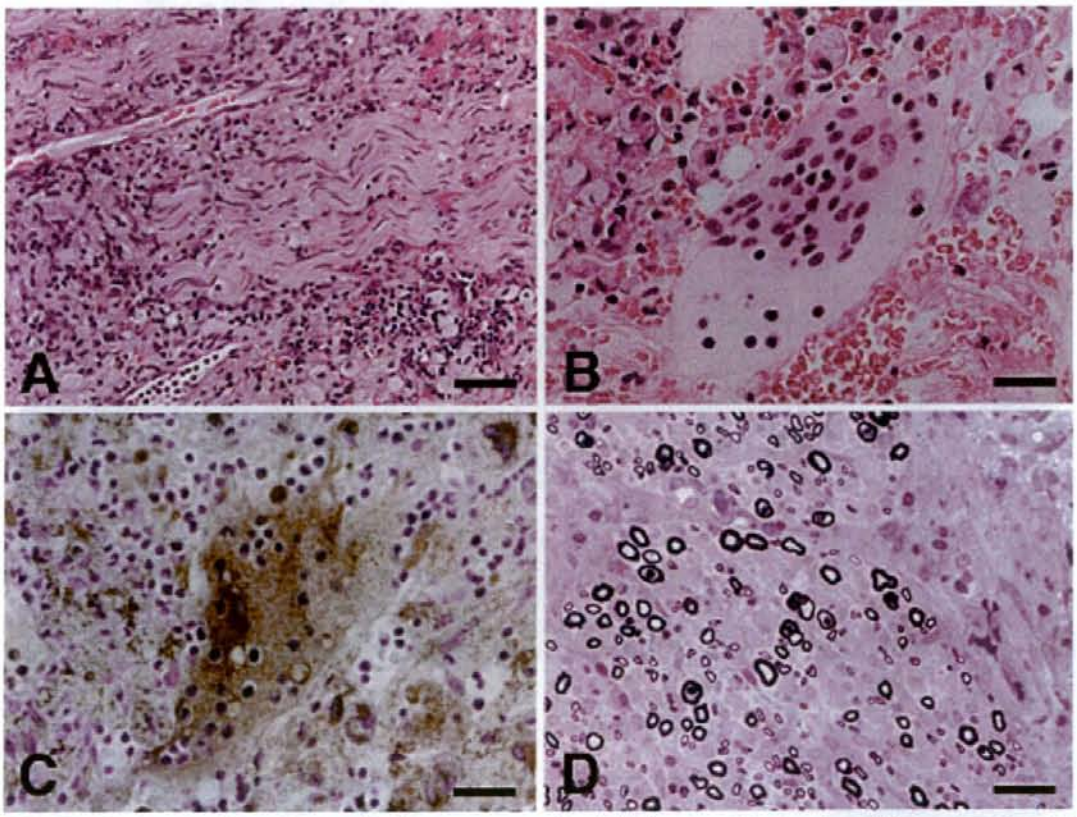

FIGURE 4. Cauda equina biopsy histology. **(A–C)** In transverse section, infiltration of small lymphocytes and foamy macrophages is evident in the endoneurium **(A)**. A multinucleated giant cell (center) has, within its cytoplasm, several intact lymphocytes (emperipolesis) **(B)**. Such a giant cell (center) shows cytoplasmic CD68 immunoreactivity **(C)**. **(D)** In cross-section, diffuse loss of myelinated fibers is evident. **(A, B)** Hematoxylin–eosin staining. **(C)** CD68 immunostaining. **(D)** Epon-embedded semi-thin section stained with toluidine blue. Bars: **(A)** 50 μm; **(B–D)** 25 μm.

CD68 (Fig. 4C) and immunonegative for both S-100 protein and CD1a. The nerve fascicles demonstrated an obvious loss of myelinated fibers with no onion-bulb formation (Fig. 4D). The lesion appeared to be inflammatory in nature with secondary demyelination. Diagnoses of Rosai–Dorfman disease, Langerhans cell hystiocytosis, and sarcoidosis were excluded. Three years after decompression surgery, the patient showed slight improvement in her symptoms without disease progression or relapse.

DISCUSSION

We have described a rare case of selective cauda equina hypertrophy with idiopathic inflammation diagnosed by cauda equina biopsy. PET studies revealed accumulation of ^{18}FDG, but no accumulation of ^{11}C-methionine in the cauda equina. Usually, in FDG, an SUV of more than 10 is observed in the case of malignant tumors and occasionally in special inflammatory conditions, such as tuberculosis[7] or non-tuberculous mycobacterial infection.[13] A previous study revealed that ^{11}C-methionine frequently accumulated in malignant lymphoma.[14] In the present case, our systemic study revealed no malignancy.

With regard to the therapies administered, serial immunotheraphies were not effective; however, laminectomy and duraplasty of the thoracolumbar region for decompression appeared to be effective in preventing progression.

Sarcoidosis has been reported to present with selective cauda equina hypertrophy and CSF pleocytosis.[4] Pathologically, the diagnosis of sarcoidosis was excluded.

Burton et al. first reported a case of idiopathic cauda equina hypertrophy, but they did not have pathological findings.[1] We believe that the diagnosis of selective cauda equina hypertrophy with idiopathic inflammation was highly likely in our patient. Therefore, we propose that this is a new disease entity: selective cauda equina hypertrophy with idiopathic inflammation. The biopsy findings suggest that the disease pathology may be mainly pronounced focal inflammation in the cauda equina. Pathological analysis and medical treatment were not done in the case presented by Burton et al., although the symptoms of the patient in their study gradually progressed.[1]

In our case, serial immunotherapy did not work, and time was lost with this medical therapy. Surgical decompression was an important intervention. In our opinion, in the case of severe cauda equina hypertrophy, when immunotherapy is not effective, both surgical decompression and pathological analysis should be performed as soon as possible.

This is the first case of selective cauda equina hypertrophy with idiopathic inflammation, which was diagnosed by cauda equina biopsy. Further reports of similar cases with pathological findings are required to establish the disease concept and to clarify the underlying pathophysiology.

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Autoantibodies against Glutamate Receptor ε_2-Subunit Detected in a Subgroup of Patients with Reversible Autoimmune Limbic Encephalitis

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Key Words
Autoantibody · Glutamate receptor ε_2-subunit · Immunotherapy · Limbic encephalitis · N-methyl-D-aspartate glutamate receptor · Paraneoplastic syndrome

Abstract
We investigated the presence of autoantibodies against glutamate receptor (GluR) ε_2 in serum and cerebrospinal fluid (CSF) samples from 12 consecutive patients with acute encephalitis/encephalopathy by immunoblotting using recombinant GluRε_2 as antigen. In 4 patients, IgM autoantibodies against GluRε_2 were detected in CSF in the early phase of the disease but were not detectable after several months. Seizures and psychiatric symptoms were noted during the acute phase of the disease in these 4 patients, who showed various degrees of residual amnesia. Immunotherapy was performed on 3 patients (patients 1, 3 and 4), and they showed marked improvements. Immunohistochemistry using these patients' sera showed that immunoreactivity is specifically detected in the cytoplasm of rat hippocampal and cortical neurons. The clinical features and neuroimaging findings of patients with IgM autoantibodies against GluRε_2 in CSF resemble those of patients with reversible autoimmune limbic encephalitis.

Introduction

There are some reports that indicate the production of autoantibodies in patients with encephalitis [1–3] and encephalopathy [4]. Recently, autoantibodies against the N-methyl-D-aspartate (NMDA)-type glutamate receptor (GluR) ε_2 have been detected in patients with epilepsia partialis continua (EPC) causally related to Rasmussen syndrome [5], nonparaneoplastic limbic encephalitis [6] and acute encephalitis [5, 7]. The NMDA receptor, which is one of the three major ionotropic GluRs, is a heterodimer composed of ε- and ζ-subunit families [8]. There are 4 members in the ε-subunit family (ε_1–ε_4) [9]. The NMDA receptor channel is unique in terms of its functional properties [10, 11]. After birth, the expression of GluRε_2 mRNA becomes restricted to the forebrain, which includes the cerebral cortex and limbic system [12]. GluRε_2 is associated with memory and learning [10, 11]. Therefore, we investigated autoantibodies against GluRε_2 in serum and cerebrospinal fluid (CSF) samples from patients with acute encephalitis/encephalopathy to clarify its clinical features and immunological aspects.

Materials and Methods

We obtained serum and CSF samples from 12 consecutive patients with acute encephalitis/encephalopathy in our department from August 2003 to January 2005 (n = 12; male:female = 6:6; age

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Table 1. Autoantibodies against GluRε_2

Patient	Age/sex	Clinical diagnosis	Anti-GluRε_2 antibody	
			CSF IgM/IgG	serum IgM/IgG
1	45/F	ILE	+/+	+/+
2	62/M	ILE	+/+	−/−
3	53/F	ILE	+/+	+/+
4	30/M	ILE	+/−	+/+
5	22/F	ILE	−/−	−/−
6	68/M	bacterial meningoencephalitis	−/−	−/−
7	57/F	cryptococcal meningoencephalitis	−/−	−/−
8	18/F	MELAS	−/−	−/−
9	59/F	neuro-Sweet disease	−/−	−/−
10	65/M	PLE (anti-Hu antibody-positive)	−/−	−/+
11	26/M	etiology-unknown meningoencephalitis	−/−	+/−
12	56/M	brainstem encephalitis	−/−	−/−

ILE = Idiopathic limbic encephalitis; MELAS = mitochondrial encephalopathy with lactic acidosis and stroke-like episodes; PLE = paraneoplastic limbic encephalitis.

range = 18–68; mean age = 47 years; idiopathic limbic encephalitis, 5; etiology-unknown meningoencephalitis, 1; anti-Hu-antibody-positive paraneoplastic limbic encephalitis, 1; bacterial meningoencephalitis, 1; mitochondrial encephalopathy with lactic acidosis and stroke-like episodes, 1; cryptococcal meningoencephalitis, 1; brainstem encephalitis, 1; neuro-Sweet disease, 1).

Detection of Autoantibodies against GluRε_2

The method used has previously been reported [5]. The supernatants of cell extracts from stable NIH3T3-transformant cell lines expressing full-length GluRε_2 were subjected to SDS-PAGE, and the separated proteins on the gels were transferred to nitrocellulose membranes. The membranes were reacted with diluted sera or CSF and stained with alkaline-phosphatase-labeled secondary antibodies (IgG or IgM; Jackson Immunoresearch, West Grove, Pa., USA). Anti-GluRε_2 autoantibodies were detected as a band corresponding to approximately 180 kDa.

Immunohistochemistry Using Patient's Serum

Under ether anesthesia, adult Sprague-Dawley rats were sacrificed. The cerebrums were immediately removed and frozen in dry-ice powder. Frozen sections (8 μm thick) of the cerebrums were fixed in 4% paraformaldehyde. The sections were incubated with serially diluted serum from a patient or with an anti-NMDAε_2 antibody (1:500; Santa Cruz Biotechnology, USA). Then, the sections were incubated with a goat biotinylated anti-human IgM (μ-chain-specific) antibody (Vector, USA) or a rabbit biotinylated anti-goat IgG (H+L) antibody (Chemicon, USA). After washing, the sections were reacted with a streptavidin-peroxidase complex (Nichirei, Japan). The reactions were finally developed with 3,3′-diaminobenzidine tetrahydrochloride (Wako, Japan) and 0.01% H_2O_2 in PBS. For adsorption tests, frozen sections after blocking were immunostained with a patient's serum (1:2,000) or with the anti-NMDAε_2 antibody (1:500) that was incubated for 24 h with extracts from transformant cells expressing full-length GluRε_2.

Results

Detection of Autoantibodies against GluRε_2

The IgM autoantibody against GluRε_2 in CSF was detected in 4 out of 12 consecutive patients in the early phase of the disease but was not detectable after several months (table 1). The IgG autoantibody against GluRε_2 in CSF was detected in 3 of these 4 patients (patients 1–3). No autoantibodies against GluRε_2 were detected in the CSF of the other patients. In the early phase of the disease, patients 1 and 4 had IgM and IgG autoantibodies against GluRε_2 in their serum. Patient 3 had only the IgM autoantibody but became positive for the IgG autoantibody 2 months later.

Clinical Features

All the patients who had the IgM autoantibody against GluRε_2 in CSF presented with seizures (i.e. partial seizures evolving to secondary generalized seizures) and psychiatric symptoms (i.e. hallucination, behavioral changes and agitation) in the early phase of the disease and developed prolonged consciousness disturbances with status epilepticus (table 2). None of these patients presented with paralysis or disturbances in sensation in the chronic stage. However, all of them presented with various degrees of recent memory disturbance and amnesia. Patient 3 showed residual psychiatric symptoms after treatment. Three patients (patients 1, 3 and 4) received intravenous methylprednisolone pulse therapy and showed improvement in their seizures and consciousness levels.

Table 2. Clinical features of patients with IgM autoantibody against GluRε_2 in CSF

Patient	Age/sex	Clinical diagnosis	Initial symptoms	Sequelae	Steroid treatment
1	45/F	ILE	convulsion, visual and olfactory hallucinations	disorientation, amnesia, recent memory disturbance	responsive
2	62/M	ILE	convulsion, auditory hallucination	disorientation, amnesia, recent memory disturbance	not performed
3	53/F	ILE	convulsion, behavioral changes	disorientation, amnesia, recent memory disturbance, psychiatric symptoms	responsive
4	30/M	ILE	convulsion, behavioral changes	amnesia, recent memory disturbance	responsive

ILE = Idiopathic limbic encephalitis.

Case Presentation

Here, we describe a representative patient with IgM autoantibodies against GluRε_2 in CSF. Patient 1 has been described previously [6].

Patient 4 was a 30-year-old male initially complaining of headaches with fever. Throughout 1 week, he showed no improvement in these symptoms and presented with agitation and behavioral disturbance. He was admitted to a nearby hospital because he suddenly suffered a generalized tonic seizure. After admission to the hospital, he was treated with antiepileptic drugs and intravenous acyclovir. However, he developed consciousness disturbance and status epilepticus. Then, he was transferred to our hospital. He had an unremarkable medical history, a temperature of 37.7°C, a pulse of 74/min and a blood pressure of 105/68 mm Hg. On neurological examination, he exhibited somnolence. There was lateral gaze-evoked nystagmus. The deep tendon reflexes of all four limbs were slightly hypoactive. There were no pathological reflexes. No meningeal signs were observed. After his consciousness level had improved, muscle strength, sensation and coordination became normal. Laboratory tests revealed leukocytosis and elevated levels of myogenic enzymes: white blood cell count, 13.8 × $10^3/\mu l$; creatine kinase level, 3,285 IU/l; aspartate aminotransferase level, 80 IU/l, and lactate dehydrogenase level, 394 IU/l. The serum C-reactive protein level was slightly elevated to 1.89 mg/dl. The serum antinuclear antibody was absent. IgM and IgG autoantibodies against GluRε_2 were present in serum on the day of admission. Analysis of CSF showed 34 cells/mm^3 (mononuclear cells only), 36 mg/dl total protein and 68 mg/dl glucose. He had a mildly elevated IgG index (0.73). IgM and IgG antibodies against herpes simplex virus, cytomegalovirus and varicella-zoster virus were absent in paired sera and CSF tested at 2-week intervals. PCR analysis showed negativity for herpes simplex virus, cytomegalovirus and human herpesvirus 6/7 DNA in CSF. The IgM autoantibody against GluRε_2 was present, but the IgG autoantibody was absent in CSF on the day of admission. EEG revealed diffuse θ-waves with small spikes in the left temporal lobe. Brain MRI revealed no abnormal intensity changes but diffuse cortical edema. ^{99m}Tc-ECD SPECT performed on the 14th day of hospitalization revealed hypoperfusion in the diffuse cerebral cortex and bilateral mesial temporal lobes. After admission, he was treated with phenytoin. However, the seizures were difficult to control and required treatment with anesthetic agents (pentobarbital sodium and midazolam) under respiratory management. He was treated with an intravenous infusion of 1 g of methylprednisolone for 3 consecutive days. He showed improvement in consciousness level and the frequency of the convulsions decreased following the treatment. However, approximately 2 weeks after steroid therapy, his seizures increased in frequency again, and intravenous immunoglobulin (400 mg/kg/day for 5 consecutive days) was administered. His condition slowly improved. On the 36th day of hospitalization, he required no respiratory management. Afterwards, we carried out the administration of intravenous immunoglobulin and steroid pulse therapy, and his seizures disappeared completely. However, he showed disorientation and severe amnesia. Revised Hasegawa Dementia Scale (HDS-R) and Mini Mental State Examination scores determined approximately 3 months after admission were 10/30 and 13/30, respectively. His Revised Wechsler Adult Intelligence Scale (WAIS-R) full-scale IQ was less than 40, and his verbal and performance subscale scores were 51 and less than 45, respectively. All Revised Wechsler Memory Scale (WMS-R) indexes showed significantly low scores (gen-

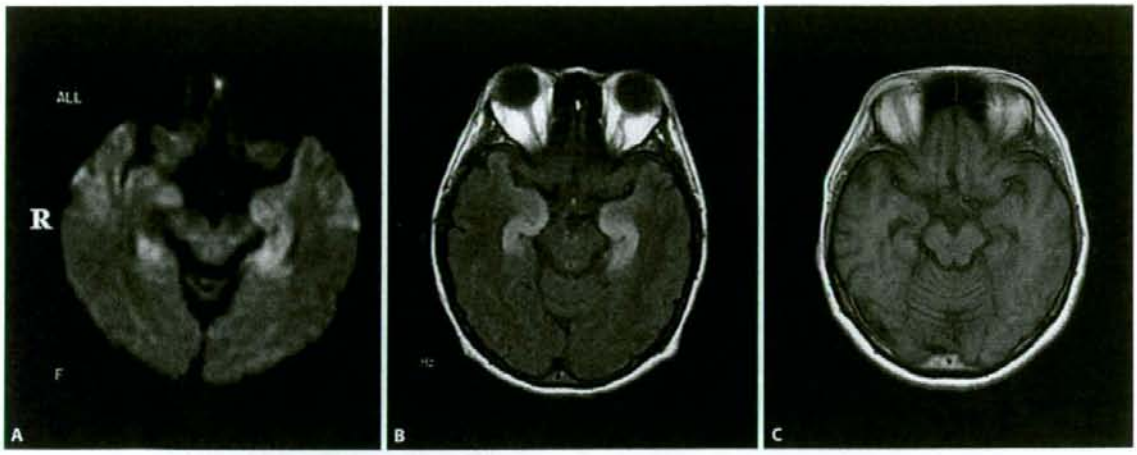

Fig. 1. MR images of patient 1. An axial diffusion-weighted image (**A**) and a fluid-attenuated inversion recovery image (**B**) show hyperintensity in the bilateral mesial temporal lobes in the acute phase (2 days after admission). **C** A follow-up T_1-weighted MR image shows the disappearance of signal abnormalities and the appearance of bilateral hippocampal and mild cortical atrophies (1 year after the disease onset).

Table 3. Laboratory and neuroimaging findings of patients with IgM autoantibody against GluRε_2 in CSF

Patient	WBC n/mm^3	CRP mg/dl	CSF cell n/mm^3	CSF protein mg/dl	IgG index	Other autoantibodies	Initial brain MRI
1	13,800	1.30	81 (M 81)	30	0.53	TPO Ab	hyperintensity in bilateral mesial temporal lobes
2	3,500	0.42	10 (M 9, P 1)	67	0.80	–	hyperintensity in left mesial temporal lobe
3	18,240	<0.05	7 (M 1, P 6)	38	n.e.	ANA	hyperintensity in bilateral mesial temporal lobes, insulae and cingulate gyri
4	17,090	1.89	34 (M 34)	36	0.73	–	normal

WBC = White blood cells; CRP = C-reactive protein; MRI comprised diffusion-weighted, T_2-weighted and fluid-attenuated inversion recovery imagings; ANA = antinuclear antibody; TPO Ab = thyroid peroxidase antibody; M = mononuclear cell; P = polynuclear cell; n.e. = not examined.

eral memory <50; verbal memory = 54; delayed memory <50; visual memory <50; attention and concentration <50). It was difficult for him to immediately recall things related to logical memory after hearing them. He showed no higher functional impairments such as aphasia, apraxia and agnosia. His memory and cognitive state slowly improved, and he was discharged 4 months after admission. Eight months after the disease onset, his memory and cognitive scale scores improved significantly [HDS-R score 25/30, WAIS-R IQ (total IQ, 70; verbal IQ, 63; performance IQ, 87), WMS-R scores (general memory, 53; verbal memory, 57; delayed memory, 60; visual memory, 70; attention and concentration, 61)]. Brain MRI revealed no intensity changes; however, mild cortical atrophy was observed 6 months after the disease onset.

Laboratory Findings

Virological examinations (table 3) showed negativity for IgM and IgG antibodies against the herpes simplex virus, cytomegalovirus and varicella-zoster virus in paired sera and CSF tested at 2-week intervals. PCR analysis showed negativity for the herpes simplex virus and human herpesvirus 6/7 DNA in CSF. Patient 3 had the antinuclear antibody (ELISA: 34.4, normal <20.0) in her se-

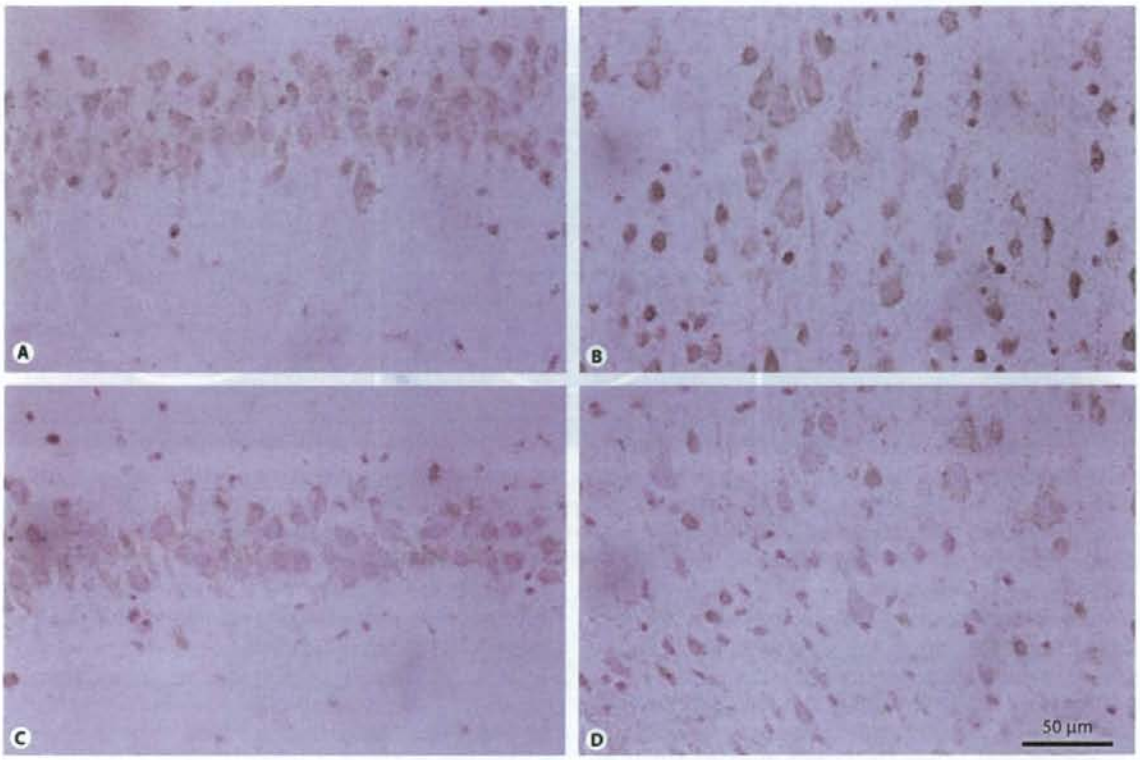

Fig. 2. Immunohistochemistry. **A**, **B** Coronal sections of rat brain immunoreacted with serum samples from patient 1. **C**, **D** Coronal sections immunoreacted with serum samples from patient 4. The diluted sera (1:2,000) of these patients reacted with the cytoplasm of rat hippocampal (**A**, **C**) and cortical (**B**, **D**) neurons. The same pattern of reactivity was observed for the serum samples from patient 3 and the anti-NMDAε_2 antibody. The sections are slightly counterstained with hematoxylin. Magnification ×400.

rum. Patient 1 showed an elevated level of the antithyroperoxidase antibody in the serum, which was not detected in CSF. We measured the titers of voltage-gated potassium channel (VGKC) antibodies by radioimmunoassay using whole rabbit brain homogenate as described previously [13]. The titers of VGKC antibodies showed normal levels in these 4 patients (patient 1: 0 pM, patient 2: 21 pM, patient 3: 0 pM, patient 4: 18 pM, normal range <100 pM).

Neuroimaging and Physiological Examination

Brain MRI at disease onset showed signal abnormalities in the bilateral mesial temporal lobes in patients 1 and 3 and on one side of the mesial temporal lobe in patient 2. In patient 4, brain MRI showed no signal abnormalities. However, several months (range, 6 months to 1 year) after the disease onset, cerebral atrophy of various degrees, including atrophy in the mesial temporal lobe, was common (fig. 1). SPECT was performed in the acute or subacute phase. All of the patients except patient 1 showed hypoperfusion in the mesial temporal lobe. In patient 1, ^{99m}Tc-HMPAO SPECT performed the day after admission revealed hyperperfusion in the bilateral mesial temporal lobes; however, hypoperfusion was observed 17 months later. All the patients showed irregular diffuse cortical hypoperfusion. In 3 patients, EEG revealed small focal spikes. In all the patients, a mixture of diffuse θ-waves was observed. No tumors were detected in any of the patients using various methods [chest, abdomen and pelvic CTs (all patients), whole-body FDG-PET (patient 1) and gallium scintigraphy (patient 2)].

Immunohistochemical Findings

The diluted serum samples from patients 1, 3 and 4 reacted with the cytoplasm of rat hippocampal and cerebral cortical neurons (fig. 2). The most appropriate dilution of serum for immunohistochemical staining was 1:2,000–4,000. The sections incubated with the anti-NMDAε_2 antibody showed the same pattern of immunoreactivity as those incubated with the patients' sera. The serum of the healthy control did not significantly immunoreact with the sections. The immunoreactivity of sera of the 3 above-mentioned patients to the anti-GluRε_2 antibody was markedly decreased by prior incubation with the supernatants of extracts from stable transformant cells expressing GluRε_2. Patients 1, 2 and 3 were negative for serum paraneoplastic anti-Yo, anti-Hu, anti-Ri, anti-CV2, anti-Tr, anti-Ma-2 and antiamphiphysin antibodies.

Discussion

We presented 4 patients with acute encephalitis who had IgM autoantibodies against GluRε_2 in CSF in the early phase of the disease; however, these antibodies were not detectable after several months. Other acute encephalitis/encephalopathy patients (patients 5–12) had neither the IgG nor IgM autoantibody against GluRε_2 in CSF. Four antibody-positive patients had the characteristic clinical features of reversible autoimmune limbic encephalitis such as intractable convulsion, psychiatric symptoms, recent memory disturbance and sufficient responsiveness to immunotherapy. Concerning the cause of limbic encephalitis, there are many reports in which limbic encephalitis is associated with cancer, most commonly a small-cell carcinoma of the lung [14–20]. The 4 antibody-positive patients had no findings of viral infection or cancer. Several reports have been published concerning the immunotherapy response form of nonparaneoplastic limbic encephalitis [21–26]. Recently, a VGKC antibody has been detected in patients with reversible autoimmune limbic encephalitis [27–32]. However, the titers of VGKC antibodies were normal in our 4 antibody-positive patients' sera. We speculate that reversible autoimmune limbic encephalitis is heterogeneous. Some forms of this disease may be mediated by autoantibodies against antigens such as ion channels or ionotropic receptors in the limbic system.

In our immunohistochemical analysis of the sera of patients with the IgM autoantibody against GluRε_2, immunoreactivity was detected in the cytoplasm of neurons in the hippocampus and cerebral cortex. The immunoreactivity was specifically demonstrated using an immunoadsorption test. The NMDA receptor is one of the ionotropic glutamate receptors essential for excitatory neurotransmission and synaptic plasticity, which underlie memory and learning [10, 11]. An antibody-mediated disturbance of NMDA-type GluR function might influence synaptic plasticity in the hippocampus and cortical neuronal excitability. The clinical response to immunotherapy and the results of immunoblotting and immunohistochemistry suggest that IgM autoantibodies may be related to pathogenesis in a subgroup of patients with reversible autoimmune limbic encephalitis.

In a previous study, autoantibodies against GluRε_2 were detected in patients with chronic EPC causally related to Rasmussen syndrome [7]. There are some reports that these antibodies have also been detected in patients with encephalitides other than chronic EPC [6, 7]. In this study, we detected autoantibodies against GluRε_2 in patients with reversible autoimmune limbic encephalitis. We suggest that autoantibodies against GluRε_2 contribute to the onset of localized encephalitides such as EPC and reversible autoimmune limbic encephalitis [7].

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Clinical and Genetic Characterizations of 16q-Linked Autosomal Dominant Spinocerebellar Ataxia (AD-SCA) and Frequency Analysis of AD-SCA in the Japanese Population

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Abstract: Autosomal dominant spinocerebellar ataxias (AD-SCAs) form a clinically and genetically heterogeneous group of neurodegenerative disorders. Recently, a single nucleotide substitution in the 5′-untranslated region of the *puratrophin-1* gene was found to be associated with one type of AD-SCA linked to chromosome 16q (16q-SCA). To obtain further insight into the contribution of the C-to-T substitution in the *puratrophin-1* gene to the clinical and genetic characteristics of patients with 16q-SCA, we analyzed 686 families with 719 individuals diagnosed with progressive ataxia. We found C-to-T substitution in the *puratrophin-1* gene in 57 unrelated families with 65 affected individuals. The mean age at onset in the patients with 16q-SCA was 59.1 (range, 46–77). Ataxia is the most common initial symptom. The elderly patients over 65 occasionally showed other accompanying clinical features including abnormalities in tendon reflexes, involuntary movements, and reduced vibration sense. We also examined the frequency of the AD-SCA subtype, considering the effects of age at onset. In the 686 AD-SCA families, SCA6 and Machado-Joseph disease/SCA3 are frequent subtypes, followed by dentatorubral-pallidoluysian atrophy and 16q-SCA. 16q-SCA is not a rare subtype of Japanese AD-SCA, particularly in patients with ages at onset over 60. © 2007 Movement Disorder Society

Key words: autosomal dominant cerebellar ataxia; 16q-SCA; genetic testing; common haplotype; frequency analysis.

Autosomal dominant spinocerebellar ataxias (AD-SCAs) form a clinically and genetically heterogeneous group of neurodegenerative disorders, characterized by progressive cerebellar signs and symptoms.[1,2] Recent advances in molecular genetics have elucidated at least 13 genes and 15 additional loci responsible for AD-SCA.[3,4] The mutational basis of most causative genes for AD-SCAs is a CAG repeat expansion in the coding region of the genes for SCA1, SCA2, Machado-Joseph disease (MJD)/SCA3, SCA6, SCA7, SCA17, and dentatorubal-pallidolyusian atrophy (DRPLA).[3,4] Although SCA8 was initially proposed to be caused by a CTG repeat expansions in the 3′-untranslated region (UTR) of the *SCA8* gene,[5] it remains controversial whether the CTG repeat expansion is casual or only polymorphisms. In SCA12, a CAG repeat expansion in the 5′ region of

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the *PPP2R2B* gene, which does not encode a polyglutamine tract, was found in a German family.[6] In addition to mutations with repeat expansions, mutations resulting in the change in amino acid composition of the protein encoded by the fibroblast growth factor 14 gene for SCA27,[7] the protein kinase C γ gene for SCA14,[8] the voltage-gated potassium channel, KCNC3, for SCA12,[9] and β-III spectrin for SCA5[10] have been reported to cause AD-SCA in a few families. The detection of these mutations has enabled the classification of AD-SCAs by molecular analysis.

SCA4, originally described in a large pedigree of Scandinavian origin in Utah, is characterized by progressive cerebellar ataxia with sensory axonal neuropathy, and was mapped to 16q22.1.[11] Another SCA4 patient with a similar clinical phenotype was subsequently reported in a German family.[12] Although Japanese AD-SCA families were also mapped to the same loci of SCA4 and termed 16q-linked SCA,[13] 16q-SCA is clinically distinct from the SCA4 in the Utah and German families. Clinical presentations of 16q-SCA are characterized by pure cerebellar ataxia, without signs of peripheral neuropathy. In addition, the ages at onset of patients with 16q-SCA are later than those reported in SCA4 patients. Recently, a single-nucleotide substitution (C-to-G), in 5′ UTR of the *puratrophin-1* gene, was identified in patients with 16q-SCA.[14] These families share a single common haplotype between D16S421 and CATG003 at 16q22.1.[14] Although microscopic aggregates in the cytoplasm of Purkinje cells in brains of the 16q-SCA patients are strongly stained by the puratrophin-1 antibody,[14] the pathological relevance of the altered anti-puratrophin-1 immunoreactivity in the cerebellum remains unclear.

Here, we report detailed analyses of clinical and genetical characteristics of 16q-SCA patients with the single-nucleotide substitution of the *puratrophin-1* gene by screening a large data set of AD-SCA patients. Furthermore, we present a frequency analysis using one of the largest cohorts of 686 AD-SCA pedigrees on the basis of genetic testing.

PATIENTS AND METHODS

Patients

In our study, 686 unrelated families with 719 affected individuals presenting progressive ataxia as a cardinal clinical feature, who were referred to our institute (Brain Research Institute, Niigata University) on a consecutive basis from January 1995 to December 2005, were enrolled. Pedigrees were considered to have a dominant SCA when affected individuals with progressive ataxia were observed in at least two generations. Sporadic cases with ataxia were not included in this study. Geographically, collected families are residents in Honshu Island, which is the main island of Japan. Age at onset and family history were determined on the basis of historical information provided by the patients or their close relatives. All the patients who enrolled in the study gave their written informed consent before molecular genetic investigations.

Molecular Studies

Genomic DNA was extracted from peripheral blood leukocytes according to standard procedures. Screening for CAG repeat expansion of SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA17, and DRPLA was performed as described previously.[15,16] In this study, SCA10 and SCA12 expansions were not analyzed. The C-to-T substitution in the 5′ UTR of the *puratrophin-1* gene was analyzed by PCR-RFLP using EcoNI, as described previously.[14] The nucleotide substitution was further analyzed by direct sequencing using a standard procedure using a BigDye terminator on an ABI PRISM 3100 Genetic analyzer (Applied Biosystems, Foster City, CA).

For 16q-SCA families in which at least two patients were available for genetic analysis, we determined the genotype of the patients in the region tightly linked to 16q-SCA using markers on 16q22.1, which include D16S3086, GATA01, D16S421, TA001, GA001, and 17msm. Presumed haplotypes were constructed assuming the least number of recombinations.

RESULTS

C-to-T Substitution in *puratophin-1* Gene and Haplotype Analysis of 16q-SCA

Among 686 AD-SCA families in our cohort, 57 families were identified to have 65 affected individuals, who carried the C-to-T substitution of the *puratrophin-1* gene. Of the 65 affected individuals, 34 were men and 31 were women. We were able to determine presumed haplotype cosegregating with the C-to-T substitution in the *puratrophin-1* gene in the families, in which more than one affected individual were available for genetic analysis. The pedigrees (Peds 1154, 1440, 1432, 2498, and 2946) shared a common genotype of 183–157-213-T-142–200-191 at D16S3086-GATA01-D16S421-*puratrophin-1*-TA001-GA001–17msm (Table 1).

Clinical Features of 16q-SCA Having C-to-T Substitution of *puatrophin-1* Gene

The mean age at onset of the 65 affected individuals with 16q-SCA was 59.1 (range, 46–77 years). This mean

TABLE 1. *Haplotype analysis in families with 16q-SCA*

	D16S3086	GATA01	D16S421	*puratrophin-1*	TA001	GA001	17msm
Ped 1154							
II-1	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	185	161	213	C	157	206	193
II-2	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	185	161	213	C	157	206	193
Ped 1432							
I-1	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	187	149	213	C	152	206	191
I-2	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	185	153	213	C	152	206	195
II-1	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	187	149	213	C	152	206	191
Ped 1440							
I-1	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	183	157	213	C	140	202	197
I-2	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	183	157	213	C	142	203	195
Ped 2498							
I-1	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	183	161	213	C	142	202	195
I-2	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	183	161	213	C	142	202	195
Ped 2946							
I-1	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	183	157	215	C	154	202	197
I-2	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	183	157	213	C	156	206	193
Ped 3946							
I-1	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	185	157	213	C	150	206	195
I-2	**183**	**157**	**213**	**T**	**142**	**200**	**191**
	185	157	213	C	156	206	195
Ped 2333							
II-3	185	**157**	**213**	C	151	206	193
	185	153	221	C	155	206	195
III-1	183	161	**213**	**T**	**142**	**200**	**191**
	185	161	213	C	156	208	195

Numbers in bold indicate the shared genotype patients in the pedigrees.

age at onset of the 16q-SCA patients is much later than those of other AD-SCAs, including SCA1 (mean age at onset, 40.9 years; range, 16–65 years, n = 22), SCA2 (36.1, 19–60, n = 29), MJD/SCA3 (39.5, 7–75, n = 221), SCA6 (52.5, 25–76, n = 209), SCA17 (37.0, 27–47, n = 2), and DRPLA (30.4, 0–71, n = 170) in our cohort. The clinical characteristics of 16q-SCA patients in our cohort are summarized in Table 2. The initial clinical features were gait ataxia (70%) or dysarthria (28%). On neurological examination, the affected individuals commonly exhibited limb and truncal ataxia (100%) and dysarthria (94%), which were invariably the most prominent features throughout the disease course. Other accompanying clinical features were less frequent, which include gaze-evoked nystagmus (41%), hyperactive (33%) and reduced (11%) muscle stretch reflex, decreased vibration sense (10%), involuntary movements (8%), and dementia (5%). These extracerebellar features

TABLE 2. *Clinical characteristics of patients with 16q-SCA*

Patients examined (n)	65
Men/women (n)	34/31
Age at onset (yr)	59.1 ± 6.9 (range, 46–77)
Age at examination (yr)	67.3 ± 8.4 (range, 47–94)
Duration (yr)	8.2 ± 6.1 (range, 1–23)
Initial symptoms (%)	
Unsteadiness of gait	70
Dysarthria	28
Clinical characteristics (%)	
Limb and truncal ataxia	100
Dysarthria	94
Gaze nystagmus	41
Muscle stretch reflex	
Hyperactive	33
Reduced	11
Reduced vibration sense	10
Involuntary movements	8
Dementia	5

were observed more frequently in elderly patients aged over 65 years. Hearing impairments were inconspicuous in our cohort.

Family in Which C-to-T Substitution in *puratrophin-1* Gene Was Not Cosegregated

We found a unique case (II-3) in Ped 2333, who lacks the C-to-T substitution of the *puratrophin-1* gene; another affected member (III-1) was found to carry this substitution. The patient lacking the substitution developed gait disturbance at age 59, and cerebellar symptoms gradually progressed. Brain MRI at age 69 revealed atrophy of cerebellar hemisphere without brainstem involvement. Both clinical and radiological findings of the patient (II-3) were indistinguishable from those of other 16q-SCA patients. On genotype analysis using markers on 16q22.1, the patient (II-3) without the substitution carried only a limited common haplotype at GATA01 and D16S421, whereas the patient (III-1) with the substitution carried the common haplotype of 16q-SCA between D16S421 and 17msm (Table 1).

Coexistence of C-to-T Substitution in *puratrophin-1* Gene and SCA1 Expansion in Patients

In the genetic screening of AD-SCA, we identified a patient who carried both a CAG repeat expansion (44 repeats) in the *SCA1* gene and 16q-SCA mutations, indicating that this patient has two different types of genetic mutation causing cerebellar dysfunction. He developed mild dysarthria and swallowing disturbance at age 57, followed by unsteadiness of gait at age 58. On neurological examination at age 63, the patient exhibited dysarthia, dysphagia, limb and truncal ataxia, and hyperreflexia. MRI of the patient showed mild atrophy of cerebellar vermis and hemisphere, whereas the brainstem appears to be preserved. He now remains well ambulatory 6 years after the onset.

Frequency Analysis of AD-SCA in Japanese Families

The relative frequenies of AD-SCAs in our cohort are summarized in Figure 1A. From the family-based frequency analysis, SCA6 was found to be the most frequent (28%), followed by MJD/SCA3 (27%), DRPLA (20%), 16q-SCA (8.3%), SCA2 (3.5%), and SCA1 (2.8%). The patient-based frequency analysis revealed that MJD/SCA3 is the most frequent subtype, probably because MJD/SCA3 affects a larger number of subjects within pedigrees than does SCA6. Genetically undetermined AD-SCDs still remain at 10% (Fig. 1A).

We next examined whether the relative frequency of AD-SCAs varies with age at onset (Fig. 1B). Among the patients with juvenile ages at onset earlier than 20 years, DRPLA was by far the most prevalent (71%), followed by MJD/SCA3 (16%). In the patients with early-adult ages at onset ranging from 20 to 39 years, MJD/SCA3 was the most predominant subtype (46%), followed by DRPLA (21%). In the patients with mid-adult ages at onset ranging from 40 to 59 years, SCA6 was the most frequent (35%), followed by MJD/SCA3 (29%). In the patients with late-adult ages at onset over 60 years, SCA6 is the most frequent (46%), followed by 16q-SCA (24%). These results suggest that subtype frequency is considerably variable among groups classified by age at onset.

DISCUSSION

In the present study, we found 57 pedigrees with 65 affected individuals, who carry the C-to-T single-nucleotide substitution in the *puratrophin-1* gene among 686 AD-SCA families. Haplotype analysis revealed that these families with the *puratrophin-1* gene substitution share a common haplotype. These results were consistent with a previous report that showed a strong founder effect in 16q-SCA patients.[14] Recently, Wieczorek et al. reported that neither the C-to-T substitution in 5′-UTR nor mutations in the coding regions of the *puratrophin-1* gene was found in 537 European patients with cerebellar ataxia.[17] Thus, 16q-SCA carrying the C-to-T substitution was exclusively found in Japanese.

The clinical features of the 16q-SCA patients in our study are characterized by late-onset ataxia, which are similar to those reported previously.[13,14,18,19] However, the patients in our cohort showed extracerebellar accompanying symptoms, including nystagmus, hyperactive muscle stretch reflex, or reduced vibration sense, compared with previous reports.[13,19] Since SCA6 is an AD-SCA subtype presenting late-adult onset cerebellar ataxia similarly to 16q-SCA,[20] differential diagnosis between 16q-SCA and SCA6 by clinical presentations is often difficult. Although no single clinical sign can reliably distinguish between these two diseases, there are some differences as follows. The age at onset in the SCA6 patients is younger than that in the 16q-SCA patients. SCA6 patients frequently exhibit gaze-evoked nystagmus (90%),[20] which is less frequent (39%) in 16q-SCA.

In Ped 2333, the proband (III-2) carried the single-nucleotide substitution in the *puratrophin-1* gene, whereas another affected patient (II-3) was found to lack this substitution. However, they presented indistinguishable clinical phenotypes. This finding raises the question of whether this substitution is the actual causative mutation of 16q-SCA. One possible answer lies in the fact that two patients with progressive ataxia in the same family

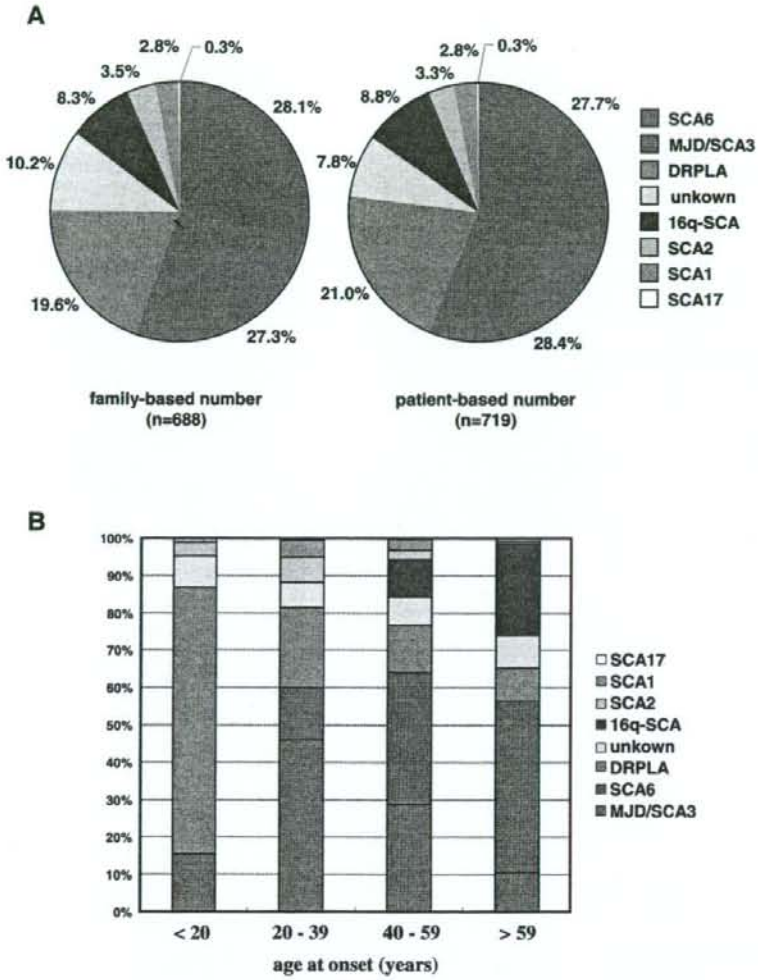

FIG. 1. A. Frequency of SCA subtype in 686 AD-SCA pedigrees determined by family- and patient-based numbers. Each subtype is depicted using different colors, as indicated. Numbers indicate the percentage of each subtype. B. Frequency of AD-SCA subtype among groups, which were classified by age at onset as follows: Group I, under 20 years; Group II, 20 to 39; Group III, 40 to 59; and Group IV, 60 and over.

have suffered from two different cerebellar diseases, and another possible answer is that this substitution is not the causative mutation, but is rather tightly linked to the causative mutation in the surrounding region. Ohata et al. have recently reported the case of a similar 16q-SCA family in which one affected individual lacked the C-to-T substitution of the *puratrophin-1* gene, whereas the other affected individuals carried this substitution.[21] Taken together, our results and those of others indicate the need to further investigate the pathological relevance of the C-to-T substitution in the *puratrophin-1* gene.

The frequency of dominant SCA subtypes is considerably variable within different ethnic groups. Moreover, the previous report suggested the existence of a large geographical difference in the frequency of AD-SCA subtype even in Japan.[22] In this study, we confirmed previous observations that SCA6, MJD/SCA3, and DRPLA are more frequent subtypes in the Japanese population than in other ethnic populations.[15,22] The high prevalence of the SCA3/MJD mutation in Japan is most likely caused by founder effects in the Japanese population.[23] Our study indicates that ~90% of patients with AD-SCA are found to have known mutations, causing either polyglutamine diseases or 16q-SCA in the Japanese population. We also provided the first evidence showing that the relative frequency of AD-SCA consid-