

Original communication

**Familial Creutzfeldt-Jakob disease with a V180I mutation: comparative analysis
with pathological findings and diffusion-weighted images**

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Abstract

Background: Diffusion-weighted imaging (DWI) has been reported to be a useful technique for the diagnosis of Creutzfeldt-Jakob disease (CJD). The present study reported results of DWI in cases of familial CJD with a V180I mutation in the *prion protein gene*, as well as neurological findings.

Methods: A retrospective analysis of three patients with V180I was performed. CSF analysis, brain MRI, SPECT, and MR spectroscopy (MRS) were included. CSF was analyzed for biochemical markers, and each patient underwent brain MRI, SPECT and MRS. A brain biopsy from the frontal cortex, which corresponded to the area of increased signals on DWI, was utilized for neuropathological analysis.

Results: CSF analysis results revealed elevated total tau protein and the absence of 14-3-3 protein, as well as decreased concentrations of NSE, S-100 protein, and PGE2. All patients presented with unique MRI features. Brain biopsy showed severe spongiform morphology, but comparatively preserved neurons and mild astrocytic gliosis. Accumulations of PrP^{Sc} were not detected using the 3F4 antibody, and microglial activation was subtle. SPECT revealed hypoperfusion throughout both hemispheres. MRS revealed reduced NAA/Cr ratio.

Conclusion: Results from this study suggested that increased signals on DWI could reflect severe spongiform changes in CJD 180 patients.

1. Introduction

Creutzfeldt-Jakob disease (CJD) is a transmissible spongiform encephalopathy associated with accumulation of abnormal prion protein. The disease has been classified into sporadic, familial, and infectious subtypes. Familial CJD comprises approximately 15% of all human prion disease, and is a result of point mutations or insertions in the *prion protein gene (PRNP)*. To date, 30 subtypes of the familial form have been determined, of which 24 are due to mutations, and six are a result of insertions. CJD, with a causative point mutation of valine to isoleucine at codon 180 (V180I) in the prion protein gene (PRPN), is a rare type of familial CJD, with only two cases reported from Europe^{1,2}. However, this mutation is recognized as the most common cause of familial CJD in Japan.

Jin *et al.*³ reported that CJD patients with the V180I (CJD180) mutation exhibit characteristic clinical features, and that the clinical and neuroradiological findings of CJD180 patients are different compared with those of classical CJD patients: 1) older onset age; 2) slower disease progression; 3) unique clinical symptoms, such as greater cortical dysfunction, which is less frequent in sporadic CJD (sCJD) patients, and no visual or cerebellar symptoms, which are frequently observed in sCJD patients; 4) reduced rate of brain-specific proteins, such as NSE and 14-3-3 protein, in CSF samples; and 5) the lack of a periodic sharp discharge (PSD) in EEG throughout the

course of the disease.

Diffusion-weighted MRI (DWI) may be useful in the premortem diagnosis of sporadic CJD, and recent reports have suggested its usefulness in familial CJD cases. Jin *et al.*³ reported that diffuse cortical high-intensity DWI signals are a characteristic feature of CJD180. The exact mechanisms responsible for high-intensity signals on DWI have not yet been established. Many reports have described the relationship between autopsy and DWI findings. However, brain biopsy is rarely performed, and autopsy results are not always consistent with lesions revealed by DWI abnormalities, and do not always reflect the pathogenesis of DWI abnormalities. This confirms the importance of reporting and analyzing biopsy cases for neuropathology. Neuropathological findings in CJD180 patients have revealed significant spongiform changes throughout all cell layers of the gray matter, and neuronal numbers were relatively preserved, with very little abnormal prion protein expression.

A detailed analysis of the pathogenesis, which was reflected in abnormal pathological features in DWI-based neuroimaging and biochemical markers of CSF, was performed. In addition, three CJD180 patients were studied using MRI, magnetic resonance spectroscopy (MRS), and single photon emission-computed tomography (SPECT).

2. Materials and Methods

2.1. Subjects

Case 1. A 70-year-old woman was admitted with progressive forgetfulness. Approximately one year prior to admission, her neighbors began noticing her amnesia. Six months later, she began to forget things and became disoriented. Finally, she was unable to walk without support and was admitted to hospital. DWI demonstrated diffuse bilateral high-intensity signals in the cerebral cortex, caudate nucleus, and putamen, but was predominant in the left hemisphere. The patient was right-handed. Therefore, a brain biopsy from the right frontal cortex was performed in this patient. The area of brain biopsy corresponded to the region of increased DWI signals. Following diagnosis of CJD, she was transferred to our university hospital. Upon admission, she was bed-ridden and exhibited myoclonic jerks and startle reactions. Muscle rigidity was present in all extremities, and the deep tendon reflexes were exaggerated. CSF analysis was normal, except for negative 14-3-3 protein and elevated total tau protein. She showed no PSD on EEG. Genetic analysis revealed a V180I point mutation, and she was diagnosed with familial CJD.

Case 2. A 67-year-old woman was admitted with a six-month history of progressive aphasia. Her past medical and family histories were unremarkable, except that she had received a cholecystectomy 17 years earlier. Upon examination, she was disoriented and

forgetful of recent events. She exhibited no cerebellar ataxia or myoclonus. She was diagnosed with dementia of Alzheimer's type (DAT) in another hospital, but her symptoms progressed more rapidly than typical DAT. She was able to follow simple commands, but gradually lost the ability to walk.

T2-weighted imaging (T2WI) and DWI revealed increased signals in the bilateral tempo-parietal cortex, predominantly in the left hemisphere. EEG revealed no PSD. Detection of the codon 180-point mutation in *PRNP* confirmed diagnosis of familial CJD.

Case 3. A 74-year-old man became disoriented while returning home from shops to which he was accustomed to traveling back and forth. He was unable to walk by himself, developed urinary incontinence, and began to exhibit progressive memory disturbances one month later. He was admitted to our hospital, and DWI abnormalities were noted. A double mutation at codons 180 and 232 (Met/Arg) of *PRNP* was detected. He was diagnosed with familial CJD.

Since Nagasaki City was hit by an A-bomb on August 9, 1945, during World War II, and because many people died as a result of this tragedy, the detailed family history of these CJD patients remains unknown.

2.2. Biochemical analysis of CSF samples

CSF samples from all three patients were analyzed by ELISA for total tau (t-tau)

protein, phosphorylated tau protein, S100 protein, neuron-specific enolase (NSE), and prostaglandin E2 (PGE2) concentrations, as well as by Western blot analysis for 14-3-3 protein expression. A polyclonal antibody specific for the α -isoform of 14-3-3 protein (sc-639; Santa Cruz, CAA) was used in combination with an enhanced chemiluminescence (ECL) detection kit (Amersham Buchler). ELISAs were performed according to the manufacturer's instructions, using an identical standard in all experiments.

2.3. Brain MRI procedure

MRI was performed on all subjects using a 1.5-T MR unit (General Electric Medical System, Milwaukee, WI, USA) with T1WI (TR = 400 ms, TE = 9/Fr ms), T2WI (TR = 3000 ms, TE = 97 ms), fluid attenuation inversion recovery (FLAIR) (TR = 8002 ms, TE = 104/Ef ms), and DWI sequences of 5-mm slice thickness. The acquired data were analyzed using Digital Imaging and Communications in Medicine (DICOM) format.

2.4. SPECT image analysis using eZIS and ^{123}I -IMP

SPECT was performed using $^{99\text{m}}\text{Tc}$ -ethyl cysteinate dimer ($^{99\text{m}}\text{Tc}$ -ECD) and *N*-isopropyl-*p*-[^{123}I] iodoamphetamine (^{123}I -IMP) as a tracer in all subjects. Obtained images were anatomically standardized with an original $^{99\text{m}}\text{Tc}$ -ECD template using the easy Z-score imaging system (eZIS) established by Masuda *et al.*⁴

2.5. MR spectroscopic analysis

Single-voxel ^1H -MRS was performed. Spectra were acquired from an 8-ml cubic volume of interest centered on the right cerebral cortex.

2.6. Neuropathological investigation

The right frontal lobe brain biopsy from Case 1 corresponded with the increased DWI signals. The time interval between brain biopsy and DWI was one day.

Formalin-fixed, paraffin-embedded sections of brain biopsy tissue were subjected to histological analyses, including hematoxylin-eosin (HE) staining, and PrP^{Sc} immunohistochemistry with 3F4 monoclonal antibody. Glial activation (astrocytic gliosis and microglia activation) was assessed by immunohistochemistry on tissue sections using S100 protein and CD68. The neuropathological findings (HE staining, S-100 protein, CD68, and 3F4 immunohistochemistry) were assessed in biopsy tissue from Case 1 and compared to autopsy tissue from four typical, sporadic, CJD cases.

2.7. Statistical analysis

Comparisons of clinical symptoms between the present cases, seven cases described in previous reports, and pooled data from sporadic CJD patients were performed using the chi-squared test and Student's *t*-test.

The Medical Ethics Committee of Nagasaki University School of Medicine approved this study, and the participants provided written informed consent.

3.Results

3.1. Clinical findings in CJD180 patients

Clinical features from the three cases are summarized in **Table 1**.

The age at onset was 69 ± 1.41 years in the three CJD180 patients. When the four CJD180 patients described in previous reports were combined with this analysis, the age at onset of the seven CJD180 patients was 70.3 ± 3.9 years. There was no statistically significant difference in age at onset between the present CJD180 patients and the previously described sporadic CJD patients (65.3 ± 11.6 years old; $n = 128$).

Appearance of myoclonic jerk from disease onset was 5.33 ± 0.93 months in the present three CJD180 patients, and 8.0 ± 4.5 months in all seven CJD180 patients (including four previously described patients). Myoclonic jerk was identified in all cases, but the duration to appearance was longer in CJD180 patients compared to sporadic CJD patients (2.7 ± 2.4 months). The myoclonic jerks were less remarkable in the present patients compared to the sporadic CJD patients, and the myoclonic jerks of all CJD180 patients exhibited similar frequency to Parkinson's disease tremors (5-9 Hz).

Time to appearance of akinetic mutism from disease onset was 12.3 ± 4.50 months in the present three CJD180 patients, and 11.7 ± 3.34 months in all seven CJD180 patients (including four previously described patients). The time to appearance of akinetic

mutism was longer in CJD180 patients compared to sporadic CJD patients ($p < 0.01$).

Survival time of the present CJD180 patients was 30.3 ± 4.78 months.

3.2. Biochemical analysis of CSF

The biochemical markers used for CSF analysis are listed in **Table 1**.

CSF from all patients was negative for 14-3-3 protein (present and previously described cases). 14-3-3 protein was detected in 87.7% of sporadic CJD patients. Therefore, 14-3-3 protein expression in CSF was not used as a diagnostic marker for CJD180.

In patients from this study, t-tau protein titers were above the cut-off level (1300 pg/ml), but much lower than the sporadic CJD cases (5689 ± 169 pg/ml; $n = 128$; data not shown). NSE concentrations in all three cases were less than the cut-off value (35 ng/ml), but greater than concentrations in individuals with neurodegenerative disorders (10.35 ± 4.35 ng/ml; $n = 100$). S-100 protein and PGE2 protein concentrations were below the detection thresholds.

3.3. Neuroimaging (MRI, MRS, SPECT)

According to MRI results, all cases demonstrated a wide range of cerebral cortical ribbons, which were depicted as low-intensity areas in the bilateral putamen, caudate head, and cerebral cortex by T1WI, and as high-intensity areas by T2WI, FLAIR, and DWI. The cortical lesion was not always symmetric (Cases 1 and 2). Basal ganglia

lesions were detected, and the caudate head was detected in all cases (**Figure 1**). The cerebellum and brain stem were not abnormal.

MRS revealed decreased N-acetyl aspartate (NAA)/creatine (Cr) and choline (Cho)/Cr ratios in cerebral cortices of all patients (**Table 2**), whereas there were no changes in myo-inositol (MI) levels (**Figure 2**). SPECT images from Case 2 revealed widespread decreased perfusion in both cerebral hemispheres, in particular the left temporal cortex (**Table 2**). Highly insensitive DWI regions were throughout the cerebral cortex in the CJD180 cases, which were similar to the smaller volume area on SPECT (**Figure 3**). Regional cerebral blood flow (rCBF) in the cerebral cortex was less in the CJD180 cases than in the sporadic CJD cases.

3.4. Diagnosis of CJD

Three cases of V180I CJD patients were misdiagnosed as of Alzheimer's-type dementia. We analyzed CSF samples from 100 patients with various neurodegenerative disorders by Western blot for expression of 14-3-3 protein, quantification of total tau (t-tau) protein, and phosphorylated tau (p-tau) protein. The concentration of t-tau protein in CSF of CJD patients was >1,200 pg/ml. However, the level of t-tau protein in CSF of neurodegenerative disorder patients was 200-500 pg/ml, and the level of t-tau protein from CSF of dementia of Alzheimer's type (DAT) was 400-1400 pg/ml. Elevated t-tau protein was also detected in three patients from the non-CJD group. Elevated t-tau

protein levels were observed in two patients with DAT and in one patient with cerebrovascular disease in the acute phase. To distinguish CJD patients from non-CJD patients with elevated t-tau protein in CSF, we compared the ratio of p-tau and t-tau proteins. The p-/t-tau ratio was dramatically and significantly greater in DAT patients, compared to CJD patients. Some diseases revealed >2000 pg/ml t-tau protein in CSF of CJD patients. When it was difficult to distinguish CJD from DAT patients, the ratio of p-tau and t-tau proteins was compared.

The highest level of t-tau protein in FTD patients was 670 pg/ml, and there were no FTD patients with CSF values > 1000 pg/ml t-tau protein, which was consistent with previous reports⁵. Results from CSF, clinical disease course, and neuroradiological findings in the V180I CJD patients were different from the DAT or FTLD patients.

3.5. Neuropathology

HE staining revealed severe spongiform changes in the cerebral cortex, which were more moderate in the CJD180 patients than the sporadic CJD cases. The CJD180 patients exhibited relatively less neuronal loss than the sporadic CJD patients (**Figure 4-a**).

Immunostaining with 3F4 antibody in this patient was not detected, but all control cases of sporadic CJD were positive for 3F4 expression. CD68 expression was positive, but the number of CD68-positive microglia in the present sporadic CJD cases was

greater than the CJD180 patients (**Figure 4-b**). S-100 immunohistochemistry demonstrated mild astrocytic gliosis in the CJD180 patients. However, two sporadic CJD (early stage and middle stage) and two late-stage patients exhibited severe and moderate astrocytic gliosis, respectively (**Figure 4-c, d**).

3.6. Typing of protease-resistant prion protein

According to Western blot analysis, the abnormal prion protein in the V180I cases was type 1+2, according to Parchi's classification⁶, which was similar to the variant CJD.

4. Discussion

Neuropathological examination of tissue biopsies from Case 1 demonstrated unusual presentation compared to biopsy sections from sporadic CJD cases. First, all cell layers of the gray matter exhibited marked spongiform changes. Second, neuronal numbers were relatively preserved. Third, no abnormal prion protein expression was detected. Fourth, microglial activation was subtle. These results were compatible with unique characterizations from previous reports^{7,8,9}.

More than 30 CJD patients have undergone brain biopsies, but only three reports (Heinemann *et al.*¹⁰, Kim *et al.*¹¹, and See *et al.*¹²) discussed the correlation between neuropathological and DWI findings in brain biopsy tissue. In other studies, brain biopsies were used only as a diagnostic method to rule out progressive dementia.

Heinemann *et al.*¹⁰ suggested that the correlation between DWI and neuropathological findings in brain biopsies could be a result of neuronal loss and spongiform changes. Spongiform neuronal degeneration was demonstrated to underlie increased DWI signals in sporadic CJD patients, as shown by Kim *et al.*¹¹ In addition, See *et al.*¹² suggested that fluid accumulation within cytoplasmic vacuoles of the neuropil, or astrogliosis, contributes to a restricted diffusion range that underlies DWI abnormalities. Microglial activation has been suggested as another possible cause of DWI abnormalities. A relationship between DWI abnormalities and accumulation of

PrP^{Sc} has been suggested as a possible causative mechanism.^{6, 13} Results from the present study demonstrated very low abnormal prion protein expression. These results suggested that there is not a strong relationship between DWI abnormalities and PrP^{Sc} accumulation.

It is difficult to determine the mechanisms underlying DWI abnormalities based on results from a single brain biopsy. Nevertheless, the mechanism underlying DWI abnormalities was assumed to be a result of severe spongiform changes, as revealed by neuropathological findings.

Due to ethical considerations, brain biopsies were not performed in all cases in the present study. Therefore, the present hypothesis was addressed by supplementary biochemical analysis of CSF and neuroimaging.

Several reports have described MRS results in CJD patients^{14, 15}. NAA is produced exclusively in neuronal mitochondria. Reduced levels of NAA are considered to reflect neuronal loss or dysfunction. In turn, Cho levels reflect membrane synthesis and graduation. MI is thought to be located only in glial cells and is, therefore, considered to be a glial marker. A previous report described that reduced levels of NAA correlate with histological neuronal loss and astrocytic gliosis in CJD patients. Some studies have reported reduced NAA levels as a feature of CJD, followed by spongiform changes accompanying neuronal loss and gliosis. Although some studies have reported increased

levels of MI in sporadic CJD, results from the present study demonstrated reduced NAA/Cr and Cho/Cr ratios, but normal MI levels, in the right frontal lobe. These results were assumed to reflect the severe spongiform change and neuronal loss, as revealed by MRS.

SPECT imaging revealed marked CBF reduction, predominantly in cerebral cortical regions corresponding to brain areas with high-intensity DWI signals. This was mostly like due to severe neuronal loss and/or severe spongiform change. However, one study reported preserved CBF, or increased perfusion, and marked high-intensity DWI changes¹⁶. Autopsy findings revealed mild neuronal loss, which most likely was responsible for the preserved perfusion. Moreover, SPECT results revealed hypoperfusion due to spongiform change and mild neuronal loss.

CSF S-100 protein levels are affected by astrocytic gliosis, and CSF PGE2 levels are influenced by microglial activation. The present results clearly identified reduced levels of S-100 in CSF and decreased PGE2 titers. CSF t-tau protein concentration was increased due to neuronal loss, resulting in lower t-tau protein levels in CJD180 patients compared to classical CJD patients. One of the neuropathological features in classical CJD patients is severe neuronal loss in the cerebral cortex. Accordingly, neuropathological findings and CSF analysis (t-tau protein) revealed that neuronal loss in CJD180 patients was less than in classical CJD patients. Biochemical CSF markers

do not necessarily reflect the clinical condition, but CSF biomarker levels can be used as a pathological index. CSF analysis from the present study demonstrated mild astrocytic gliosis or spongiform change in the CJD180 patients.

Biochemical CSF analysis and neuroimaging results suggested that the cause of abnormal DWI signals was spongiform change and neuronal loss. However, this is difficult to prove. Indeed, results demonstrated that neurons in CJD180 patients were better preserved than in sporadic CJD patients, and the spongiform change in CJD180 patients was more moderate than in the four typical sporadic CJD cases examined.

In conclusion, results from the present study suggested that high-intensity DWI signals in V180I patients were influenced by spongiform changes.

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Figure Legends

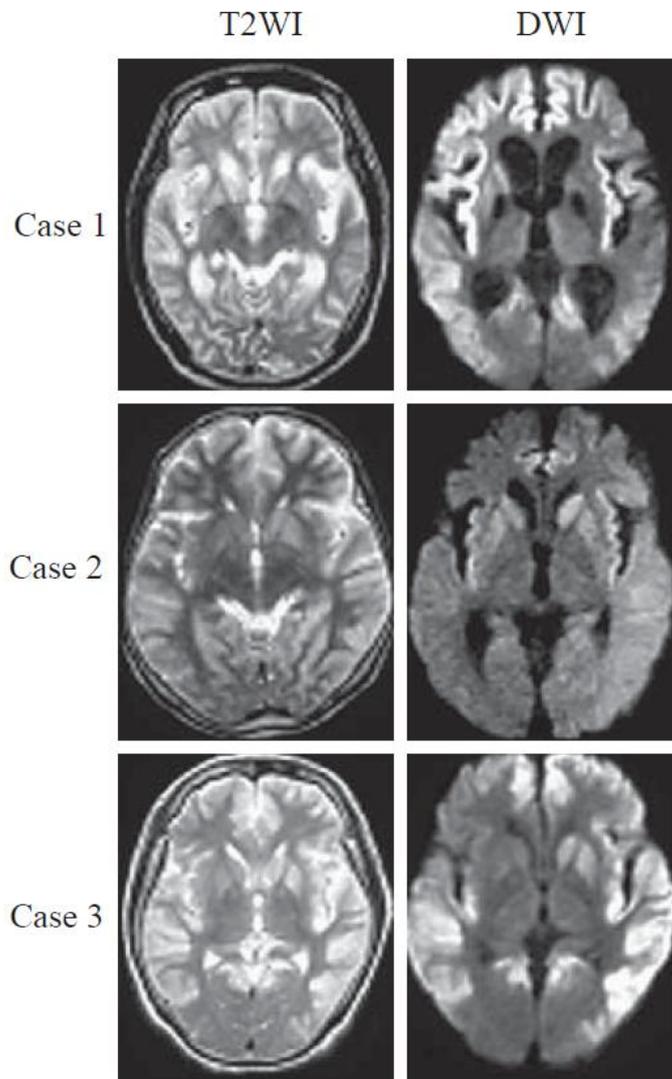


Figure 1.

Axial T2- (A) and diffusion-weighted (B) MRI images from three CJD cases with the V180I mutation, revealing extensive cortical hyperintensity lesions. Hyperintense signals in the bilateral caudate nucleus and putamen are also demonstrated, but these are subtle compared to the cortical lesions. Medial regions of the occipital lobes are not involved (arrowheads).

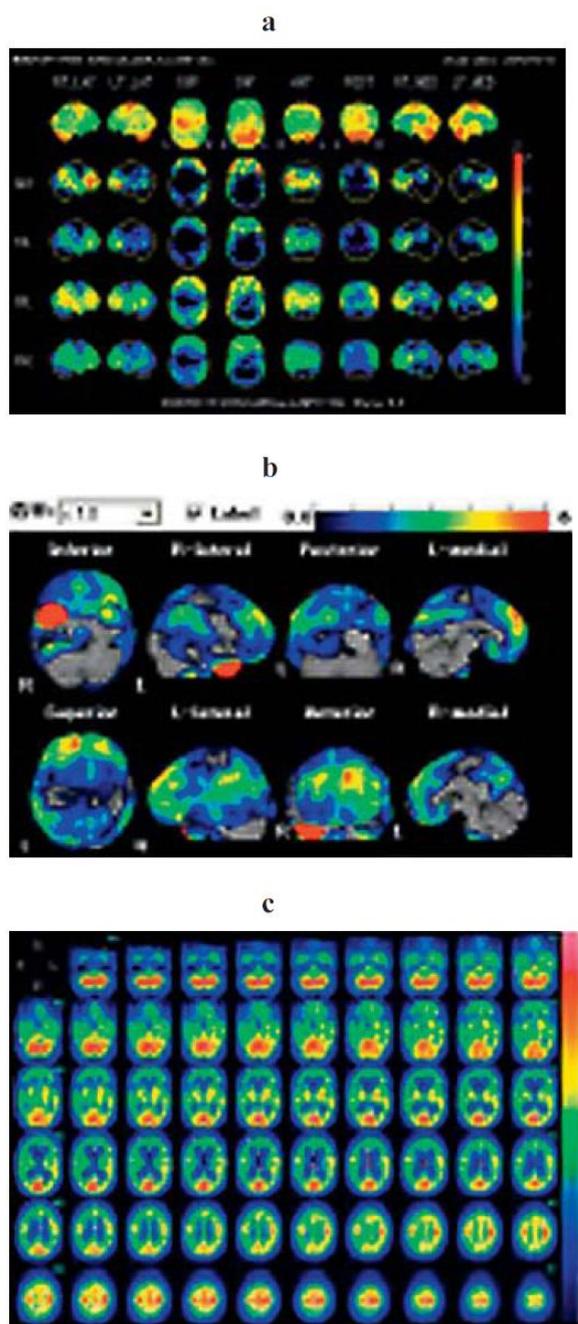


Figure 2. SPECT analysis in case 1 (CJD180)

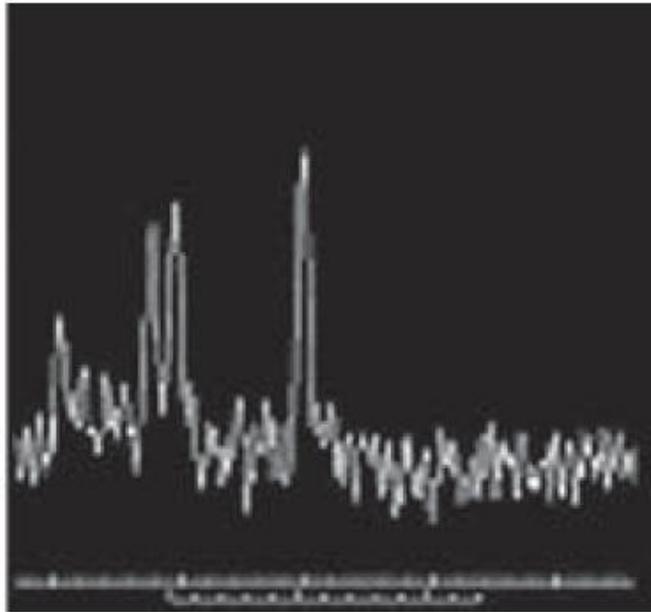
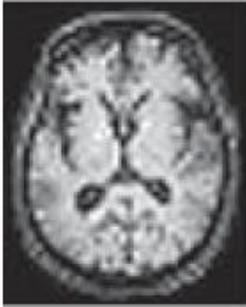
2-a. 123I-IMP SPECT-demonstrated decreased uptake.

2-b. eZIS analysis in SPECT performed using ^{99m}Tc -ethyl cysteinate dimer (^{99m}Tc -ECD)

2-c. 3DSRT analysis in SPECT performed using ^{99m}Tc -ECD.

sporadic CJD

MRS



V180I CJD

MRS

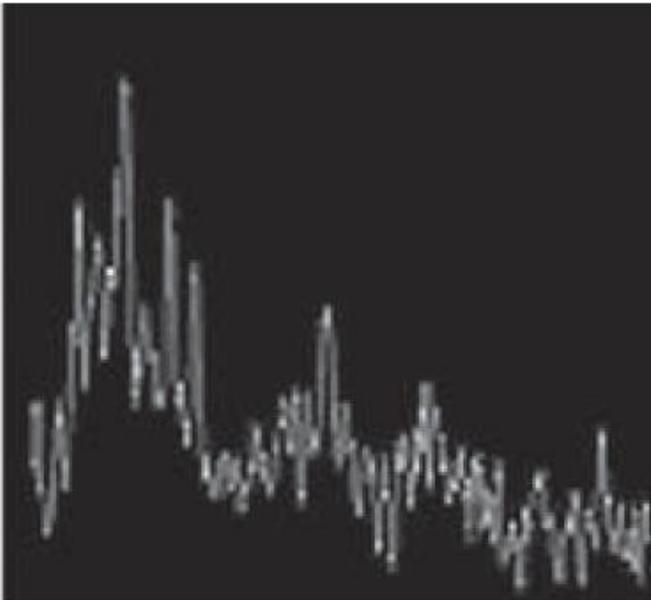


Figure 3.

1H MRS in Case 1 showing decreased cortical gray matter NAA/Cr.

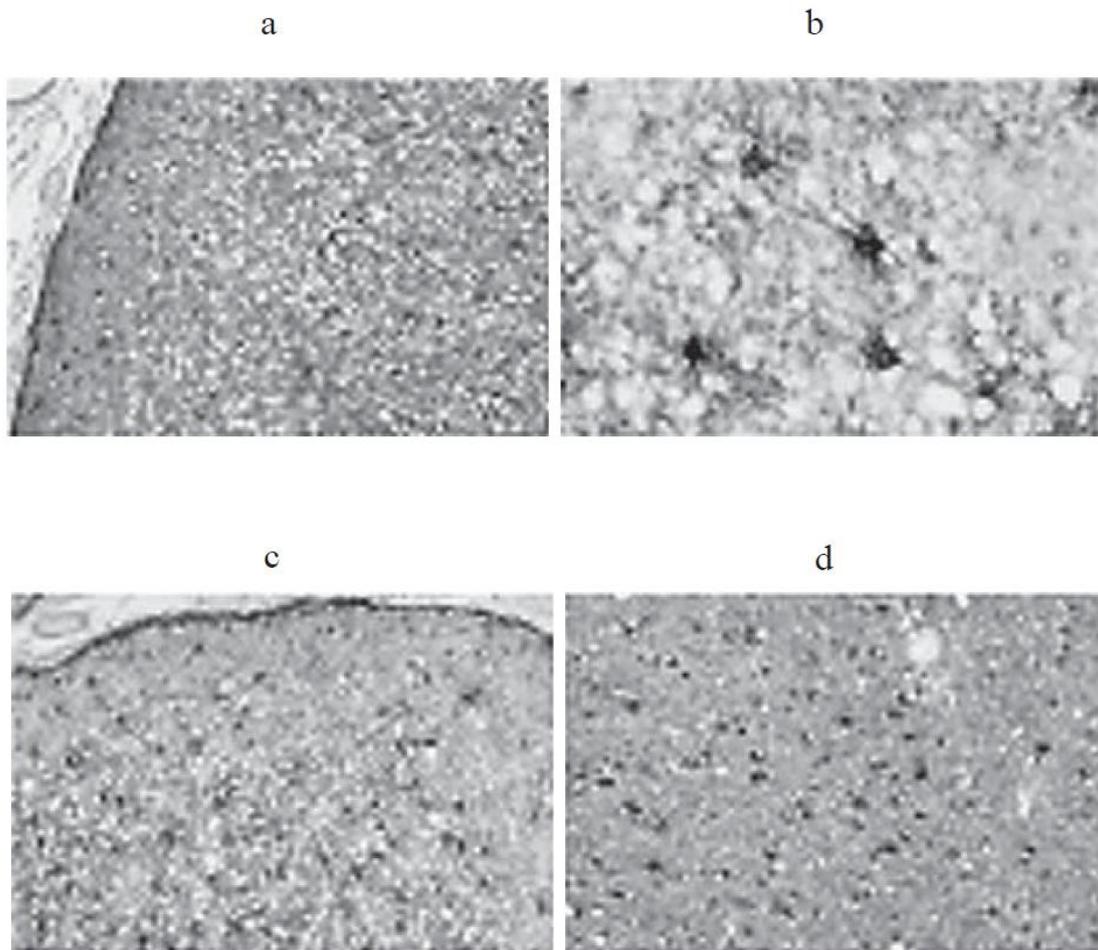


Figure 4

Pathological findings in the right frontal lobe of Case 1 reveal severe spongiform changes and neuronal loss. However, astrocytic gliosis, accumulation of PrP^{Sc}, and microglial activation are less apparent in the cerebral cortex.

A: HE staining, B: CD68 staining, C: S-100b protein staining (×10) D: S-100b protein staining (×200)

Table 1. Profiles of the three cases with CJD180

Case	Our cases			previously reported	
	Case 1	Case 2	Case 3	<i>CJD180*</i>	<i>sporadic CJD*</i>
Age/sex	70/F	67/F	70/M	70.3±3.9	65.3±11.6
Family history	-	-	-	-	-
Myoclonic jerk (month)	6	6	4	8.0 ± 4.5	2.7 ± 2.4
Visual symptoms	-	-	-	0% (0/4)	24% (23/96)
Cerebellar symptom	-	-	-	0% (0/4)	12.5% (12/96)
Akinetic mutism (month)	12	18	7	11.3 ± 2.2	3.5 ± 2.8
Total tau pg/ml	3,811	2,325	3,675	N.E.	89.6% (86/96)
Phosphorylated tau pg/ml	39.4	40.8	36.1	N.E	N.E
NSE ng/ml	34	22	13	50% (2/4)	72.7% (48/66)
S-100 protein (ng/ml)	0.23	0.35	0.13	N.E.	N.E.
14-3-3 protein	-	-	-	0%	87.7% (57/65)
PSD	-	-	-	0%	94% (110/116)
Codon 129 in PRNP	M/M	M/M	M/M	MM 40% MV60%	MM 91% MV9%
Codon 219 in PRNP	E/E	E/E	E/E	E/E 100%	E/E 92% E/K8%
Type of PrP ^{Sc}	type 1+2	N.E.	N.E.	N.E.	type1 or type2

M: methionine, G: glutamic acid, V: valine, K: lysine, E: Glutamic acid

Range of total tau protein levels; 800–15,000 ng/ml, CJD with values > 1180 pg/ml

Range of phosphorylated tau protein levels; 8–120 ng/ml

Range of NSE levels; 2–200 ng/ml, CJD with values > 35 ng/ml

Range of S-100 protein levels; 0.001–25 ng/ml, CJD with values > 2.2 ng/ml

We analyzed the cut-off data of total tau protein, NSE and S-100 protein of CSF among

128 CJD patients and 100 non-CJD patients (dementia of Alzheimer type, vascular

dementia, Pick's disease, Parkinson's disease, corticobasal degeneration, Huntington's disease, frontotemporal dementia, progressive supranuclear palsy, mild cognitive impairment, amyotrophic lateral sclerosis, temporal epilepsy, limbic encephalopathy, paraneoplastic cerebellar degeneration/ Lambert-Eaton myasthenic syndrome, MELAS and encephalopathy owing to unknown etiology). the most appropriate cut-off levels of biomarkers (total tau protein, NSE and S-100 protein of CSF) in CJD patients were evaluated using the Receiver Operating Characteristics (ROC) curve method. The sensitivities for total tau protein, 14-3-3 protein, NSE and S-100 protein of CSF in classical CJD patients (n=128) were 95.9%, 88.7% and 81.5% and 33.1%, respectively (not data shown).

In all three patients the polymorphism in prion protein gene (PRNP) at codon 129 was homozygous for methionine (M/M), while the polymorphism in PRNP at codon 219 was homozygous for glutamic acid (E/E), N.E= not examined

*: previously reported data by Jin et al³ publication, the average level of total tau protein of classical CJD patients (n=128) was 5689 ± 169 pg/ml (average \pm 1 S.D).

Table 2. MRS in three cases with CJD180 and four cases with sporadic CJD

	Case 1	Case 2	Case 3	Sporadic CJD (n=4)
NAA/Cr	0.79	1.1	1.3	1.83± 0.20
Cho/Cr	1.18	1.02	0.91	1.49± 0.20
M.I.	30	28	11	<30

NAA= N-acetyl aspartate, Cr= Creatine, Cho= Choline, M.I.= Myo-inositol

Sporadic CJD (n=4) patients were definite cases and the molecular type of the abnormal prion protein in sporadic CJD cases was type 1 on Parchi's classification ⁴

Table 3. Neuropathological findings in one case with CJD180 (Case 1) and four cases with sporadic CJD

	CJD180 case 1	sCJD case 1	sCJD case 2	sCJD case 3	sCJD case 4
neuronal loss	+	+	+	+++	+++
astrocytic gliosis	+	++	++	+	+
spongiform change	+++	++	++	+	+
microglia activation	+	+++	++	++	++
PrP deposition	-	++	++	+++	++

Spongiform change and neuronal loss are described as absent (–), mild (+), moderate (++) , severe (+++) on HE sections.

Astrocytosis is described as absent (–), mild (+), moderate (++) , severe (+++) on sections labeled immunohistochemically with an anti-S-100 antibody (polyclonal, DAKO,Glostrup, Denmark) or anti-prion protein antibody (monoclonal, clone 3F4, Senetek, Maryland Heights, MO, USA).

Degree of prion protein deposition is described as absent (–), mild (+), moderate (++) , strong (+++) on sections labeled immunohistochemically with an anti-prion protein antibody (monoclonal, clone 3F4, DAKO, Japan).

Microglia activation (the number of CD68-positive microglia) is described as follows: –, no staining; +, slightly staining; ++, moderate staining; +++ , strong staining.

sCJD: sporadic CJD