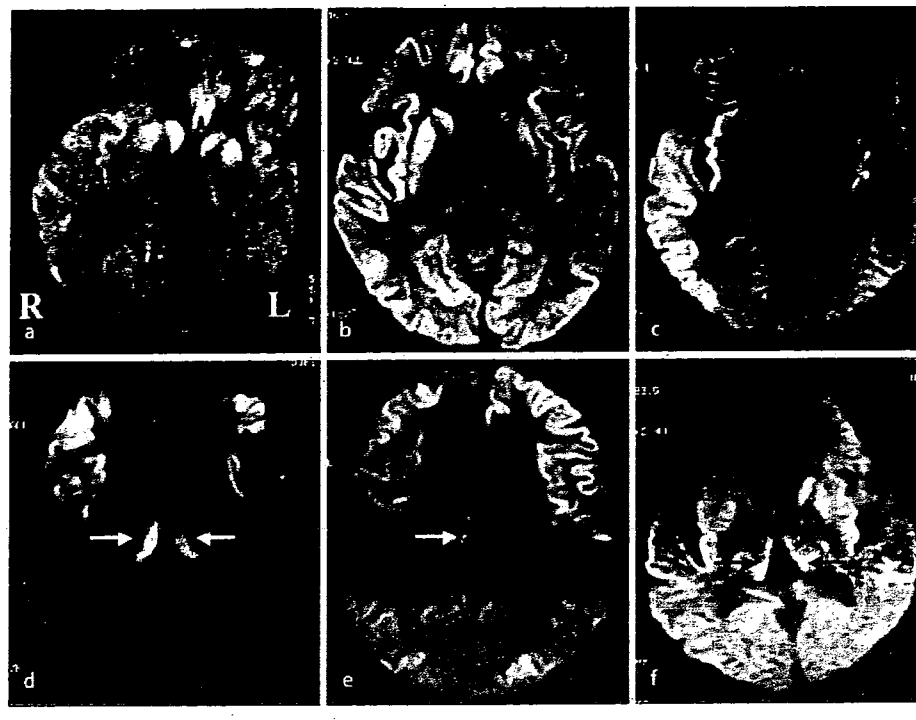

Fig. 3 DWI of the rapid-type group (**A–C**) and the slow-type group (**D–F**). **A** DWI obtained from a 55-year-old woman demonstrating high-intensity lesions mainly in the bilateral striatum. The right temporal cortex demonstrated slightly high-intensity lesions. **B** DWI obtained from a 60-year-old woman demonstrating high intensity lesions in the frontal, temporal, occipital and insular cortex, and the striatum. The right side predominated. **C** DWI obtained from a 62-year-old woman demonstrating high-intensity lesions in the bilateral occipital and insular cortex. The right temporal cortex was also depicted as an area of high intensity. We did not find high-intensity lesions in the striatum. **D** DWI obtained from a 69-year-old woman demonstrating high-intensity lesions in the bilateral frontal and insular cortex. The bilateral caudate head showed slightly high-intensity lesions. Interestingly, the bilateral medial thalami showed high-intensity lesions with the so-called hockey stick sign (white arrows). **E** DWI obtained from a 70-year-old man demonstrating high-intensity lesions in the bilateral frontal, occipital, and insular cortex. The right medial thalamus also showed high intensity (white arrow). **F** DWI obtained from a 52-year-old man demonstrating high-intensity lesions in the right temporal cortex and the left striatum. The bilateral medial thalami also showed high intensity lesion (black arrows)

no family history of prion disease had the M232R substitution: one was previously reported, pathologically confirmed dementia with Lewy bodies [12], one was encephalitis, and one was not diagnosed yet, but was confirmed as not having CJD because his symptoms rather fluctuated. There remains the possibility that the M232R substitution is a rare polymorphism, not a causative point mutation [6], although the M232R substitution was not found among 100 healthy controls [4].

Discussion

In the present study, by reviewing the clinical and laboratory findings of 21 patients, we found that there were two distinct phenotypes in CJD232 in spite of the same genotype of PRNP, M232R, MM129, and GG219. Different phenotypes with the same pathogenic changes of PRNP are known in several types of genetic prion disease [14–21]. Fatal familial insomnia and gCJD with a common point mutation at codon 178 are well-known. However, the different phenotypes are regulated by a polymorphism at codon 129 [14, 15]. Similarly, a phenotypic variant of gCJD with a point mutation of glutamic acid to lysine at codon 200 (CJD200) is coupled with valine at codon 129 [19]. On the other hand, a thalamic variant of CJD200, which has the same polymorphism of MM129 as the vast majority of CJD200, has been reported [17, 21], although it is exceptional. In our results, 15 of the patients were the rapid-type, five were the slow-type. In CJD232, the slow-type, which has uncommon clinical features, is not exceptional and constitutes one of the major phenotypes because 25% of patients with CJD232 belong to the slow-type. Similarly, there are two different major phenotypes that are not influenced by the polymorphism of codon 129 and 219 in Gerstmann-Sträussler-Scherinker disease with a point mutation of proline to leucine at codon 102 of PRNP (GSS102), which is characterized by chronic cerebellar ataxia of long duration (several years or more) associated with neurological signs including dementia [21]. In GSS102, a sCJD-like variant of short duration (less than one year) has been reported [16]. In 27 patients with GSS102 recognized by the Creutzfeldt-Jakob Disease Surveillance

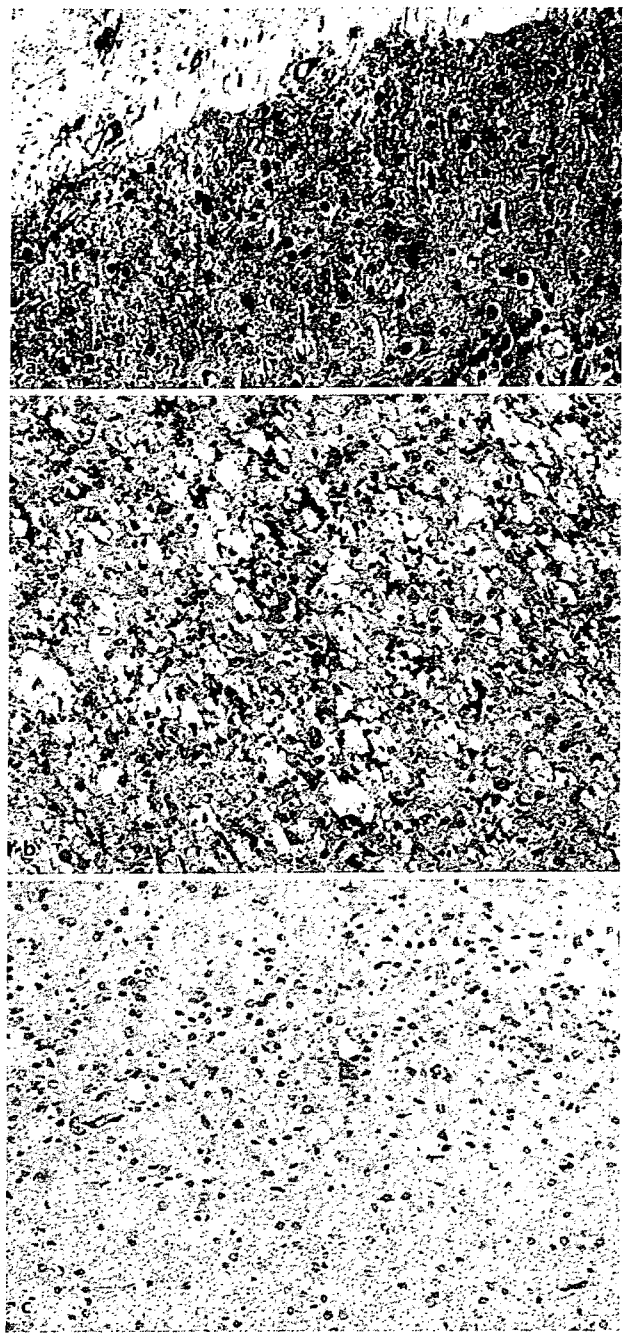

Fig. 4 Immunohistochemical staining of abnormal PrP using monoclonal antibody 3F4. **A** Anti-PrP immunostaining in a 67-year-old woman suffering from the rapid-type of CJD232 with an initial symptom of cerebellar ataxia. The molecular layer of the cerebellum shows a diffuse synaptic-type PrP deposit. Photographed at 200 times magnification. **B** Anti-PrP immunostaining in a 64-year-old woman suffering from the slow-type of CJD 232 with an initial symptom of dressing apraxia. This patient was previously reported by Satoh et al. (1997). The perivacuolar-type PrP deposit is predominantly demonstrated in the temporal cerebral cortex. Photographed at 50 times magnification. **C** Anti-PrP immunostaining in the same patient with Fig. 4B. The synaptic-type PrP deposit is demonstrated in the occipital cerebral cortex. Photographed at 50 times magnification

Committee, Japan until February 2006, five (18.5%) were this sCJD-like variant. It should be emphasized that CJD232 has two major different phenotypes with the completely same genotype of PRNP that is undoubtedly a major factor which influences the clinical phenotype [2, 22–24].

The gender and age at onset influence the disease progression [25]. However, there were no significant differences in the male to female ratio and age at onset between the two types in our series of CJD232. The molecular type of PrP^{Sc} is another factor that is closely associated with the clinical and pathological phenotypes of sCJD [26]. Unfortunately, the molecular type of PrP^{Sc} has not been sufficiently examined. One previously reported patient [27] in the rapid-type group had type 1 and one patient in the slow-type group had type 1 + 2. This difference may be a determinant of the clinical phenotypes of CJD232. More studies are needed to determine the relationship between the clinical phenotype and the molecular type of PrP^{Sc}. Immunohistochemical staining of PrP from four patients with the rapid-type revealed a diffuse synaptic-type deposit similar to that found in sCJD with MMI [28]. The synaptic-type PrP deposit may be an important pathological finding of the rapid-type. If so, we cannot differentiate the rapid-type of CJD232 from sCJD with MM1 based on the pathological findings. PrP immunohistochemical staining of three patients with the slow-type revealed that two had a perivacuolar-type and diffuse synaptic-type PrP deposits and one had only diffuse synaptic-type deposits. These pathological results suggest that the rapid-type might be a homogeneous group and the slow-type might not be. The number of studied patients in the two groups was too small to determine the pattern. If the PrP^{Sc} type 1 + 2 and the perivacuolar-type PrP deposits are key pathological features of the slow-type of CJD232, these may be related to the absence or late occurrence of myoclonus and PSWC on EEG, and the slower progression of the disease.

Diagnosing the rapid-type of CJD232 is not difficult because the patients start with progressing dementia, cerebellar ataxia, and visual problems, rapidly progress to akinetic mutism, demonstrate PSWC, are positive for 14-3-3 protein in the CSF immunoassay, and have characteristic MRI findings. These clinical features including the MRI findings are very similar to those of typical sCJD with MM1 [3] that accounts for the vast majority of sCJD. We can easily suspect CJD when we encounter such patients. Genetic examination of PRNP is necessary to differentiate the rapid-type of CJD232 from sCJD with MM1 [3] since a patient with CJD232 usually has no family history of prion disease or dementia, and differentiating CJD232 from sCJD with MM1 [3] is difficult when based on the clinical and laboratory features alone.

On the other hand, diagnosing the slow-type of

Table 1 Comparison of clinical and laboratory features between the rapid-type (R-type) and the slow-type (S-type) of CJD232

Clinical features	R-type (N = 15)	S-type (N = 5)	p
Age at onset (Year)	65.4 ± 5.2	59.0 ± 12.8	NS
Men: Women	8: 7	2: 3	NS
Family history	0/15 positive	0/5 positive	NS
Initial symptoms	7: progressive dementia 2: visual symptoms 2: cerebellar ataxia 2: involuntary movement 2: others	3: progressive dementia 1: psychiatric symptoms 1: dressing apraxia	
Myoclonus (Mo)[a]	2.4 ± 1.8	15.3 ± 12.3	< 0.005
Positive rate	14/14[b]	4/5*	NS
Akinetic mutism (Mo)[a]	3.1 ± 1.5	20.6 ± 4.4	< 0.001
Positive rate	15/15	5/5	NS
14-3-3 protein	8/8 positive	4/4 positive	NS
PSWC (Mo)[a]	2.8 ± 1.8	13	< 0.01
Positive rate	15/15	1/5**	< 0.01
MRI	8/9 positive	4/5 positive	NS
Codon 129	15: Met/Met	5: Met/Met	
Codon 219	14: Glu/Glu 1: Glu/Lys	5: Glu/Glu	
Autopsied cases	5/15	3/5	
PrP immunostaining	Synaptic: 4	Synaptic + Perivacuolar: 2 Synaptic: 1	
PrP type	Type 1: 1	Type 1 + 2: 1	

Values are means ± SD where applicable

[a] The duration until the appearance of myoclonus, akinetic mutism, and PSWC from the onset; [b] It was uncertain whether myoclonus had appeared or not in one patient

* Mean observation period was 14.8 ± 10.7 months; ** Mean observation period was 21.6 ± 12.8 months

R-type the rapid-type of CJD232; *S-type* the slow-type of CJD232; *PSWC* periodic sharp and wave complexes in EEG; *PRNP* prion protein gene; *Met/Met* methionine homozygosity; *Glu/Glu* glutamic acid homozygosity; *Glu/Lys* heterozygosity of glutamic acid and lysine; *NS* not significant

CJD232 is not easy because the patients initially manifest non-characteristic dementia or memory disturbance, or psychiatric symptoms as in other neurodegenerative disorders, progress relatively slowly, do not become akinetic and mute within a year, and do not demonstrate PSWC. When we diagnose the slow-type of CJD232, we cannot rely on PSWC, the presence of which is the most widely accepted diagnostic marker at the present time. In addition to the slow progression, the lack of a family history may cause this disease to be confused with other neurodegenerative disorders such as Alzheimer's disease, dementia with Lewy bodies, corticobasal degeneration, frontotemporal dementia, etc., especially in the early phase. MRI, especially DWI [11], is very useful to distinguish the slow-type of CJD232 from other neurodegenerative disorders, because the slow-type of CJD232 demonstrates CJD-related high-intensity lesions in DWI, whereas the above-mentioned neurodegenerative disorders do not demonstrate abnormal changes in signal intensities. There has been a report of suspected CJD patients who had M232R and in whom a final pathological diagnosis of dementia with Lewy bodies demonstrated no signal changes in DWI [12]. In our series of three patients with the slow-type examined by DWI, medial thalamic lesions were demonstrated. However, these lesions are not specific for the slow-type of CJD232, and we sometimes encounter them in sCJD [29]. The major differential diagnosis of the slow-type of CJD232 is sCJD with the MM2-cortical type [3], because the slow-type of CJD232 usually fulfills the previously advocated diagnostic criteria for sCJD with the MM2 cortical type [30]. It is hardly possible clinically to distinguish the slow-type of CJD232 from sCJD with the MM2 cortical type. However, the molecular type of PrP^{Sc} in one patient of the slow-type CJD232 was type 1 + 2, not type 2. The molecular types of PrP^{Sc} in each group may be different, although the presence of perivacuolar-type PrP deposits is also a finding of sCJD with the MM2-cortical type [3]. PRNP study is indispensable to distinguish between the two groups and molecular typing may be able to distinguish between them. We did not find any peculiar lesions of the slow-type such as a remarkable high intensity lesion in the cerebral cortex except for those in the medial occipital and cerebellar cortices which are characteristic of fCJD with a point mutation of valine to isoleucine at codon 180 (CJD180),

which is an unusual type of fCJD [13]. The degree of the abnormalities in MRI did not correlate with the disease severity. To diagnose the slow type of CJD232, recognizing the clinical phenotype that demonstrates uncommon clinical and laboratory features found in other neurodegenerative disorders with dementia and performing genetic examination of PRNP are important.

Other characteristics of CJD232 are that CJD232 patients have no family history of CJD or dementia in either type and are reported only in Japan. More than half of genetic prion disease patients with various PRNP mutations lack family histories and the lack of family histories is not restricted to CJD232 [1]. De novo mutations [31] and very low penetration [32] are considered as the reasons. Individual PRNP mutations also show variable geographical distributions [1]. The M232R substitution may influence the disease progression because the M232R substitution extended the incubation time in an experimental transmission study using humanized knock-in mice [33]. Three suspected patients with M232R substitution but with a final diagnosis of diseases other than CJD have been reported to the Creutzfeldt-Jakob Disease Surveillance Committee, Japan because they had the M232R substitution, not because they had clinical symptoms suspecting CJD. Therefore, we think that the prevalence of 6 % in 50 non-CJD patients is not the same as that of the normal Japanese population. At least, it cannot be said that all patients having the M232R substitution demonstrate the symptoms of CJD232, and it does not seem to be supported that M232R substitution is a causative mutation. On the other hand, two probable CJD patients with M232R substitution in one family have been reported [6]. We cannot overlook these patients based on the fact that M232R substitution is very rare [4]. Whether M232R is really a causative mutation or only a rare polymorphism is another issue that needs to be resolved. We need more studies of CJD patients with M232R substitution, and especially the correlation between the pathological findings including the molecular type of PrP^{Sc} and immunohistochemical staining of PrP and the clinical findings should be clarified to determine whether it influences the disease progression. We need to study the morbidity of a population having the M232R substitution to determine whether it is a causative mutation or not.

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References

1. Kovács GG, Puopolo M, Ladogana A, et al. (2005) Genetic prion disease: the EUROCJD experience. Hum Genet 118:166–174
2. Kovács GG, Trabattoni G, Heinfellner JA, Ironside JW, Knight RSG, Budka H (2002) Mutations of the prion protein gene: Phenotypic spectrum. J Neurol 249:1567–1582
3. Parchi P, Giese A, Capellari S, et al. (1999) Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 46:224–233
4. Kitamoto T, Ohta M, Doh-ura K, Hitoshi S, Terao Y, Tateishi J (1993) Novel missense variants of prion protein in Creutzfeldt-Jakob disease or Gerstmann-Sträussler Syndrome. Biochem Biophys Res Commun 191:709–714
5. Shimizu T, Tanaka K, Tanahashi N, Fukuuchi Y, Kitamoto T (1994) Creutzfeldt-Jakob disease with a point mutation at codon 232 of prion protein – A case report. Clin Neurol 34: 590–592
6. Hoque MZ, Kitamoto T, Furukawa H, et al. (1996) Mutation in the prion protein gene at codon 232 in Japanese patients with Creutzfeldt-Jakob disease: a clinicopathological, immunohistochemical and transmission study. Acta Neuropathol 92:441–446
7. Satoh A, Goto H, Satoh H, et al. (1997) A case of Creutzfeldt-Jakob disease with a point mutation at codon 232: Correlation of MRI and neurological findings. Neurology 49:1469–1470
8. Saito T, Isozumi K, Komatsumoto S, Nara M, Suzuki K, Doh-ura K (2000) A case of codon 232 mutation-induced Creutzfeldt-Jakob disease visualized by the MRI-FLAIR images with atypical clinical symptoms. Clin Neurol 40: 51–54
9. Tagawa A, Natsuno T, Suzuki M, Ono S, Shimizu N (2001) Creutzfeldt-Jakob disease with codon 232 point mutation and showing myoclonus and PSD in the early stage. A case report. Neurol Med 54:161–165
10. Hitoshi S, Nagura H, Yamanouchi H, Kitamoto T (1993) Double mutations at codon 180 and codon 232 of the PRNP gene in an apparently sporadic case of Creutzfeldt-Jakob disease. J Neurol Sci 120:208–212
11. Shiga Y, Miyazawa K, Sato S, et al. (2004) Diffusion-weighted MRI abnormalities as an early diagnostic marker for Creutzfeldt-Jakob disease. Neurology 63:443–449
12. Koide T, Ohtake H, Nakajima T, et al. (2002) A patient with dementia with Lewy bodies and codon 232 mutation of PRNP. Neurology 59:1619–1621
13. Jin K, Shiga Y, Shibuya S, et al. (2004) Clinical features of Creutzfeldt-Jakob disease with V180I mutation. Neurology 62:502–505
14. Goldfarb LG, Peterson RB, Tabaton M, et al. (1992) Fatal familial insomnia and familial Creutzfeldt-Jakob disease: disease phenotype determined by a DNA polymorphism. Science 258: 806–808

15. Monari L, Chen SG, Brown P, et al. (1994) Fatal familial insomnia and familial Creutzfeldt-Jakob disease: different prion proteins determined by a DNA polymorphism. Proc Natl Acad Sci 91:2839–2842
16. Barbanti P, Fabbrini G, Salvatore M, et al. (1996) Polymorphism at codon 129 or 219 of PRNP and clinical heterogeneity in a previously unreported family with Gerstmann-Sträussler-Scherinker disease (PrP-P102L mutation). Neurology 47:734–741
17. Chapman J, Arlazoroff A, Goldfarb LG, et al. (1996) Fatal insomnia in a case of familial Creutzfeldt-Jakob disease with the codon 200^{Lys} mutation. Neurology 46:758–761
18. Young K, Clark HB, Piccardo P, Dlouhy SR, Ghetti B (1997) Gerstmann-Sträussler-Scherinker disease with the PRNP P102L mutation and valine at codon 129. Molecular Brain Research 44:147–150
19. Hainfellner JA, Parchi P, Kitamoto T, Jarius C, Gambetti P, Budka H (1999) A novel phenotype in familial Creutzfeldt-Jakob disease: Prion protein gene E200K mutation coupled with valine at codon 129 and type 2 protease-resistant prion protein. Ann Neurol 45:812–816
20. Yamada M, Itoh Y, Inaba A, et al. (1999) An inherited prion disease with a PrP P105L mutation: Clinicopathologic and PrP heterogeneity. Neurology 53: 181–188
21. Teratuto AL, Piccardo P, Reich EG, et al. (2002) Insomnia associated with thalamic involvement in E200K Creutzfeldt-Jakob disease. Neurology 58:362–267
22. Doh-ura K, Tateishi J, Sasaki H, Kitamoto T, Sasaki Y (1989) Pro-leu change at position 102 of prion protein is the most common but not the sole mutation related to Gerstmann-Sträussler syndrome. Biochem Biophys Res Commun 163:974–979
23. Furukawa H, Kitamoto T, Tanaka Y, Tateishi J (1995) New variant prion protein in a Japanese family with Gerstmann-Sträussler-Scherinker syndrome. Brain Res Mol Brain Res 30:385–388
24. Shibuya S, Higuchi J, Shin RW, Tateishi J, Kitamoto T (1998) Codon 219 Lys allele of PRNP is not found in sporadic Creutzfeldt-Jakob disease. Ann Neurol 43:826–828
25. Pocchiari M, Puopolo M, Croes EA, et al. (2004) Predictors of survival in sporadic Creutzfeldt-Jakob disease and other human transmissible spongiform encephalopathies. Brain 127: 2348–2359
26. Hill AF, Joiner S, Wadsworth JD, et al. (2003) Molecular classification of sporadic Creutzfeldt-Jakob disease. Brain 126:1333–1346
27. Satoh K, Muramoto T, Tanaka T, et al. (2003) Association of an 11–12 kDa protease-resistant prion protein fragment with subtypes of dura graft-associated Creutzfeldt-Jakob disease and other prion diseases. J General Virol 84:2885–2893
28. Kitamoto T, Shin RW, Doh-ura K, et al. (1992) Abnormal isoform of prion proteins accumulates in the synaptic structures of the central nervous system in patients with Creutzfeldt-Jakob disease. Am J Pathol 140:1285–1294
29. Young GS, Geschwind MD, Fischbein NJ, et al. (2005) Diffusion-weighted and fluid-attenuated inversion recovery imaging in Creutzfeldt-Jakob disease: High sensitivity and specificity for diagnosis. Am J Neuroradiol 26: 1551–1562
30. Hamaguchi T, Kitamoto T, Sato T, et al. (2005) Clinical diagnosis of MM2-type sporadic Creutzfeldt-Jakob disease. Neurology 64:643–648
31. Dagvadorj A, Peterson RB, Lee HS, et al. (2003) Spontaneous mutations in the prion protein gene causing transmissible spongiform encephalopathy. Ann Neurol 52:355–359
32. Mitrova E, Belay Gl (2002) Creutzfeldt-Jakob disease with E200K mutation in Slovakia: characterization and development. Acta Virol 46:31–39
33. Taguchi Y, Mohri S, Ironside JW, Muramoto T, Kitamoto T (2003) Humanized knock-in mice expressing chimeric prion protein showed varied susceptibility to different human prions. Am J Pathol 163:2585–2593

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Total Tau Protein in Cerebrospinal Fluid and Diffusion-Weighted MRI as an Early Diagnostic Marker for Creutzfeldt-Jakob Disease

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Key Words
Creutzfeldt-Jakob disease · Cerebrospinal fluid · Magnetic resonance imaging

Abstract
Background: We have recently begun to doubt the effectiveness of periodic sharp wave complexes observed on electroencephalographs and the detection of 14-3-3 protein in cerebrospinal fluid (CSF) as diagnostic criteria for Creutzfeldt-Jakob disease (CJD). Diffusion-weighted magnetic resonance imaging (DWI) and the detection of total tau (t-tau) protein in CSF may be more sensitive diagnostic criteria. ***Methods:*** Among 44 CJD patients, we selected 21 subjects that suffered from early-stage CJD, which was defined as cases in the 6 weeks following the onset of the disease. The sensitivities of DWI and electroencephalographs, as well as those of t-tau protein, 14-3-3 protein, neuron-specific enolase (NSE), and S-100b protein in CSF were compared as diagnostic markers for early-stage CJD. ***Results:*** NSE, S-100b protein, t-tau protein, and 14-3-3 protein were detected in the samples from 57.1, 4.8, 95.2, and 76.2% of the 21 early-stage CJD patients, respectively. Additionally, DWI was used to positively identify 90.5% of these cases. ***Conclusion:*** We concluded that t-tau protein was the most sensitive of the diagnostic markers for CJD. Moreover, the data in this study showed that detection of t-tau protein combined with DWI identified 98% of the early-stage cases, and these tests should be included as diagnostic criteria for CJD.

In the past, a diagnosis of Creutzfeldt-Jakob disease (CJD) depended on clinical findings and electroencephalographic criteria. Because abnormal prion proteins cannot presently be detected without a brain biopsy, supplementary methods are required for the diagnosis of CJD. A clinical diagnosis of CJD can be supported by the detection of biochemical markers in the patient's cerebrospinal fluid (CSF). Moreover, periodic sharp wave complexes (PSWC) observed on an electroencephalograph (EEG) and the presence of 14-3-3 protein in the CSF, both of which are included in the diagnostic criteria for CJD supplied by the World Health Organization (WHO), are considered to be reliable diagnostic markers for CJD [1]. However, it has been reported that 14-3-3 protein cannot be detected in the CSF of some patients in the early or late phases of disease progression.

Otto et al. [2] reported that total tau (t-tau) protein is a diagnostic marker in patients with CJD. Additionally,

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Table 1. Profiles of 44 CJD patients

	Definite cases	Probable cases	Possible cases	Total cases
Sporadic cases				
Male	2	13	0	15
Female	4	15	0	19
Familiar cases				
Male	0	1	2	3
Female	3	0	1	4
Iatrogenic cases				
Male	0	1	0	1
Female	1	1	0	2

We analyzed 44 CJD patients that were classified according to the criteria by the WHO.

we confirmed that t-tau protein was more prominent than other diagnostic markers, including 14-3-3 protein, in Japanese CJD patients [3]. On the other hand, Shiga et al. [4] reported that diffusion-weighted magnetic resonance imaging (DWI), a sensitive and noninvasive test, can be used to diagnose CJD. Not all hospitals, however, have magnetic resonance imaging (MRI) scanners, and even if a hospital has an MRI scanner, it is often not suitable for DWI.

Because some therapies, such as a cerebroventricular infusion of pentosan polysulfate or orally administered quinacrine, are effective for patients at a very early stage of CJD, it is clear that the identification of a sensitive and reliable for the early stage of CJD is required. To ascertain which of the potential CJD markers is the most sensitive for CJD in the first 6 weeks following the onset of the disease, we compared the results of DWI with the presence of t-tau protein and 14-3-3 protein, as well as S-100b protein and neuron-specific enolase (NSE), in CSF as diagnostic markers of CJD.

Methods

Patients

Forty-four subjects were admitted as CJD patients to the neurology departments at Nagasaki University, the Nagasaki Kita Hospital, and the Nagasaki Medical Center of Neurology. Patients or their families agreed with the aims and significance of our research and gave appropriate informed consent. We examined all of the subjects using DWI and obtained CSF from the patients at all stages of the disease. All 44 patients fulfilled the WHO diagnostic criteria for CJD during the time course of this study (table 1). We collected CSF samples from 92 patients who suffered from one of the following disorders: Alzheimer's disease (n = 54: male = 33, female = 21), cerebrovascular disorders (n = 7: male = 5, female = 2), Pick's disease (n = 1: male = 1, female = 0), Parkinson's disease (n = 5: male = 4, female = 1), corticobasal degeneration (n = 2: male = 0, female = 2), Huntington's disease (n = 1: male = 1, female = 0), frontotemporal dementia (n = 1: male = 1, female = 0), progressive supranuclear palsy (n = 3: male = 2, female = 1), Wernicke's encephalopathy (n = 2: male = 2, female = 0), limbic encephalopathy (n = 3: male = 2, female = 1), and amyotrophic lateral sclerosis (n = 3: male = 2, female = 1). We also obtained CSF and DWI data from 4 healthy volunteers (male = 2, female = 2). We analyzed the DWI data and screened the CSF samples for biochemical markers (14-3-3 protein and t-tau protein).

Detection of the β-Isoform of 14-3-3 Protein, T-Tau Protein, S-100b Protein, and NSE in CSF Samples

CSF samples were collected, divided into aliquots, and stored at −80°C until they were assayed. All of the assays were performed at the same time to avoid repeatedly freezing and thawing the samples. Immunoassays for 14-3-3 protein in the CSF were performed as described previously [3]. Polyclonal antibodies specific for the β-isoform of 14-3-3 protein were obtained from Santa Cruz Biotechnology (sc-639; Santa Cruz, Calif., USA) or Immuno-Biological Laboratories (IBL; Gunma, Japan); all samples were analyzed with both antibodies to compare their sensitivities. Detection of the protein was performed using an enhanced chemiluminescence detection kit (Amersham Buchler).

The detection of t-tau protein in the CSF samples was performed as previously reported [3]. ELISA kits included plates with anti-hTAU antibody-coated wells. In each well, 25 μl of the CSF sample was mixed with 75 μl of conjugate diluents containing biotinylated anti-hTAU antibodies and incubated for 20 h at 25°C. Each well was then washed four times with wash buffer. We then added 100 μl of conjugated elusion containing peroxidase-conjugated streptavidin to each well, incubated the samples for 30 min at 20–25°C, and washed the wells four times with wash buffer. One hundred microliters of phosphate buffer containing stabilizing agents was added to each well, and the samples were incubated for 30 min at 20–25°C. Finally, we stopped the reaction by adding 100 μl of 2 N sulfuric acid to each well. The resulting signals were measured and quantified by absorbance at 450 nm using a Labry system image station and the accompanying software. These measurements were used to calculate the ratio of each signal to a standard.

Analysis of S-100b protein in the CSF samples was performed using a chemiluminescent enzyme immunoassay. Antibodies against S-100b protein (DAKO Japan) were fixed on 96-well microplates, and anti-rabbit IgG peroxidase-linked species-specific F(ab') fragments were used as the secondary antibodies (DAKO Japan). The range of S-100b levels detected using this method was 0.001–25 ng/ml and CJD patients with values >35 ng/ml were considered to be positive according to receiver operating characteristic (ROC) curve analysis performed using SPSS software. For NSE, final measurements were obtained using an NSE protein radioimmunoassay measurement kit (EIKEN Chemical Company, Japan), which, according to the manufacturer's protocol, was applicable for NSE levels in the range of 2–35 ng/ml. The range of NSE levels in this study was 2–200 ng/ml, and CJD patients with values >2.2 ng/ml, were considered to be positive according to ROC curve analysis performed using SPSS software. The levels of NSE and S-100b protein in the CSF samples were measured commercially using ELISA-based methods (SRL Laboratory, Tokyo, Japan).

MRI Protocol

Scans were performed at two hospitals using various units. For each of the patients, a 1.0-tesla or 1.5-tesla magnetic resonance system was used and T_1-weighted, fast spin-echo T_2-weighted, and FLAIR images were obtained. The DWI data were obtained as DECOM data and analyzed using a standardized method [4, 5]. We also used our standard diagnostic protocol for neurodegenerative diseases and our standard acquisition parameters: for sagittal T_1-weighted spin-echo images, the parameters were TR/TE/NEX = 600/minimals/2, and for axial and/or coronal and axial FLAIR images, the parameters were TR/TE/TI/NEX = 1,000/140/2,200/1. Both coronal and axial FLAIR imaging and DWI were performed in most of the subjects, whereas axial DWI and FLAIR imaging were performed in all of the subjects [4–6]. Cases were judged to be positive when high-intensity signals were observed at two different lesions in the cortex and basal ganglia. Only cases that were determined to be positive by two experienced neuroradiologists and/or neurologists were diagnosed as CJD. We focused on lesions in the striatum (caudate and/or putaminal), in the thalamus, including the pulvinar, and along the cerebral cortical ribbon.

PSWC on EEGs

EEGs were recorded using the international 10–20 system for electrode placement. PSWC were defined as diffuse biphasic or triphasic sharp wave complexes with durations between 100 and 600 ms and intercomplex intervals between 500 and 2,000 ms.

Disease Control Group

We retrospectively reviewed the clinical records of three new patients who suffered from CO poisoning, herpes encephalitis, or meningoencephalitis, and those of 87 previously examined patients [3].

Statistical Analysis

Standard measures of diagnostic test validity were used to identify true-positive, true-negative, false-positive, and false-negative results. For these calculations, 44 CJD patients and 21 CJD patients at an early stage of the disease were examined.

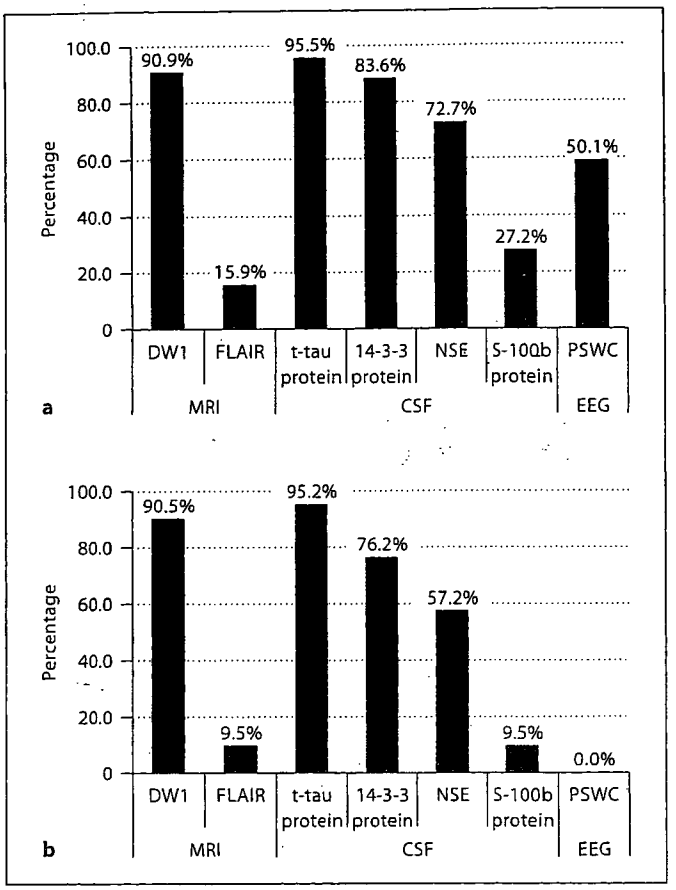

Fig. 1. a The percentages of the 44 all-stage CJD patients diagnosed using MRI (DWI and FLAIR); the detection of t-tau protein, 14-3-3 protein, NSE, or S-100b protein in CSF samples, and PSWC on EEGs. **b** The percentages of the 21 early-stage CJD patients diagnosed using MRI (DWI and FLAIR); the detection of t-tau protein, 14-3-3 protein, NSE, or S-100b protein in CSF, and PSWC on EEGs.

Results

We examined 44 patients with CJD, as determined using DWI, and classified the cases as sporadic CJD (n = 34), familial CJD (n = 7; 5 cases resulting from a V180I mutation in the prion protein and 2 cases resulting from an M232R mutation in the prion protein) or iatrogenic CJD (dura-associated CJD; n = 3). We obtained CSF from these patients at all stages of the disease (table 1). Because the period considered to be the 'early stage' of CJD had not been determined, we defined early-stage CJD as the first 6 weeks following the onset of the disease. Among the 44 CJD patients, we selected 21 cases that were within 6 weeks of the onset of the disease (table 2). It has previously been reported that the most sensitive and specific results for the diagnosis of CJD using the level of t-tau protein were obtained with a cutoff value of 1,260 pg/ml determined on the basis of ROC analysis [3]. Using this value, 42 of the 44 CJD patients were positive for t-tau protein in their CSF samples (>1,260 pg/ml in 95.5% of the patients). The patients that were negative for CSF t-tau protein included 1 case that resulted from a V180I mutation and 1 case of sporadic CJD. At each stage of illness in the CJD patients, detection of t-tau protein in CSF was a more sensitive marker of CJD than imaging (DWI and FLAIR), detection of 14-3-3 protein, NSE, or S-100b protein in CSF, and PSWC on EEGs (fig. 1). Using antibodies against the β-isoform of 14-3-3 protein, we detected this protein in the CSF samples from 39 of the 44 CJD patients (88.6%). In assays of the CSF samples for NSE and S-100b

Table 2. Summary of the detection of t-tau, 14-3-3 protein, NSE, and S-100b protein in CSF, and the results from DWI for 21 patients with early-stage CJD

Patient No.	Age	Sex	CJD type	d.l.	d.w. weeks	CSF				MRI		EEG
						t-tau protein pg/ml	14-3-3 protein	NSE ng/ml	S-100b protein, ng/ml	DWI	FLAIR	PSWC
Cutoff data in CJD patients						>1,260	> ±	>35	>2.5	> ±	> ±	–
1	64	f	sp	probable	4	3,414	+	–	4.73	+	–	–
2	73	m	sp	probable	4	2,068	+	–	1.64	+	–	–
3	67	m	sp	probable	4	9,055	+	–	0.86	+	–	–
4	76	m	sp	probable	4	4,645	+	–	0.60	+	–	–
5	80	f	sp	probable	4	8,766	+	–	0.84	+	–	–
6	77	m	sp	probable	2	10,671	+	–	0.60	+	–	–
7	63	f	sp	probable	4	1,814	–	–	1.28	+	–	–
8	69	f	sp	definite	6	4,917	+	+	0.91	+	–	–
9	54	f	sp	definite	6	1,317	–	–	1.76	+	+	–
10	67	m	sp	probable	4	3,055	+	–	1.96	+	–	–
11	70	f	sp	probable	4	2,841	–	–	1.91	+	–	–
12	70	f	ia	probable	6	9,787	+	–	0.85	–	–	–
13	67	f	sp	probable	5	2,657	–	+	1.33	–	–	–
14	70	f	fa	probable	6	3,358	+	–	0.60	+	+	–
15	63	m	sp	probable	4	3,125	+	–	0.84	+	–	–
16	63	m	sp	probable	4	3,530	+	–	0.90	+	–	–
17	64	f	sp	probable	6	2,630	+	–	0.90	+	–	–
18	51	f	ia	probable	6	3,930	+	–	0.62	+	–	–
19	74	m	sp	probable	5	4,574	+	–	0.98	+	–	–
20	74	f	sp	probable	6	3,666	+	–	1.86	+	–	–
21	71	m	sp	probable	0	8,66	–	2/21	0.22	+	–	–
Total number of patients						20/21	16/21	12/21	1/21	18/21	2/21	0/21
Sensitivity, %						95.2	76.2	57.1	4.8	90.5	9.5	0

sp = Sporadic CJD; ia = iatrogenic CJD; fa = familiar CJD; d.l. = diagnostic level based on the WHO and the Masters criteria; d.w. = duration from the onset of the disease to the diagnostic examination. In all 21 cases, codon 129 of the gene coding for the prion protein was Met/Met homozygous, whereas codon 219 was Glu/Glu homozygous. Total protein contents of all the patients stayed within the normal range. All patients in this study were Asian.

protein, 32 of the 44 CJD patients were clearly positive for NSE (72.7%), whereas 12 of the 44 subjects were S-100b positive (27.2%) (fig. 1a).

During the period that we defined as the early stage of CJD, all 21 of the selected patients were found to be abnormal according to at least one of the diagnostic criteria (table 2). On the other hand, all 21 patients with early-stage CJD were able to speak to other people and walk without assistance. On Western blots, the antibodies produced by Santa Cruz Biotechnology against the β-isoform of 14-3-3 protein detected the protein in 14 of the 21 early-stage cases, whereas the IBL antibodies detected the protein in 17 of the 21 cases (fig. 1b). Analyses of the 21 patients detected NSE, S-100b protein, t-tau protein, and 14-3-3 protein in the CSF from 12 (57.1%), 1 (4.8%), 20 (95.2%), and 16 (76.2%) of the early-stage cases, respectively. In addition, DWI identified 18 of the 21 early-stage CJD cases (90.5%), whereas the sensitivity of FLAIR MRI was 9.5% (2/21) (fig. 1b).

Finally, the gene that codes for the prion protein was sequenced from each of the early-stage patients; each of the 21 patients was found to be homozygous for the codons coding for the amino acids at positions 129 (Met) and 219 (Glu).

Standard measures of the validity of the diagnostic tests, including the identification of true-positive, true-negative, false-positive, and false-negative patients, were calculated. For these calculations, 44 CJD patients and 21 early-stage CJD patients were used (table 3). In the 44 all-stage CJD patients, statistical analyses showed that there

Table 3. Comparison of the biomarkers (14-3-3 protein and t-tau protein) and DWI results using the number of true-positive, true-negative, false-positive, and false-negative patients

	TP	TN	FP	FN	Sens., %	Specific., %	PPV, %	NPV, %	LR	NLR	Accur., %
44 CJD patients											
t-tau protein	42	88	4	2	95.5	95.7	91.3	97.8	22.0	0.048	95.6
14-3-3 protein	39	88	4	5	88.6	95.7	90.7	94.6	20.4	0.119	93.4
t-tau protein and 14-3-3 protein	39	88	4	5	88.6	95.7	90.7	94.6	20.4	0.119	93.4
DWI	40	90	2	4	90.9	97.8	95.2	95.7	41.8	0.093	95.6
DWI and t-tau protein	38	90	2	6	86.4	97.8	95.0	93.8	39.7	0.139	94.1
DWI and 14-3-3 protein	38	90	2	6	86.4	97.8	95.0	93.8	39.7	0.139	94.1
21 early-stage CJD patients											
t-tau protein	20	88	1	2	90.9	98.9	95.2	97.8	80.9	0.092	97.3
14-3-3 protein	16	88	5	5	76.2	94.6	76.2	94.6	14.2	0.252	91.2
t-tau protein and 14-3-3 protein	16	88	5	5	76.2	94.6	76.2	94.6	14.2	0.252	91.2
DWI	18	90	3	4	81.8	96.8	85.7	95.7	25.4	0.188	93.9
DWI and t-tau protein	17	90	4	6	73.9	95.7	81.0	93.8	17.4	0.272	91.5
DWI and 14-3-3 protein	15	90	6	6	71.4	93.8	71.4	93.8	11.4	0.305	89.7

TP = True positive; TN = true negative; FP = false positive; FN = false negative; Sens. = sensitivity; Specific. = specificity; PPV = positive predictive value; NPV = negative predictive value; LR = likelihood ratio; NLR = negative likelihood ratio; Accur. = accuracy.

were no significant differences in the specificities and the sensitivities of t-tau protein, 14-3-3 protein, and DWI. In the 21 early-stage CJD patients, on the other hand, there were significant differences between the specificities and the sensitivities of these diagnostic criteria.

Discussion

Before we were able to examine the utility of these markers for the diagnosis of CJD, we had to define the period of time in which the patients suffered from early-stage CJD. Because many CJD patients visit a hospital in the first 6 weeks following the onset of the disease in Japan, we defined the early stage of CJD as the 6 weeks after the disease onset. This is an important period of time for the treatment of CJD patients, particularly because CJD patients must be diagnosed before the onset of the symptoms associated with disease progression, such as akinetic mutism or gait disturbance. Therefore, the level of activities of daily living was very important for determining that the early stage of the disease occurred during the first 6 weeks following the disease onset. In the future, it will be very important to research into the diagnostic markers in order to be able to find drugs to successfully treat CJD patients.

The sensitivity of t-tau protein in CSF was 95.2% in this study, so we were satisfied with the data as diagnostic markers of early-stage CJD patients.

Several researchers have reported that measuring the levels of t-tau protein, 14-3-3 protein, and NSE in CSF had high diagnostic impacts for the differential diagnosis of CJD. We have recently begun to doubt the significance of PSWC on EEGs and the detection of 14-3-3 protein as diagnostic criteria for CJD; the sensitivities of these tests in CJD patients are lower than the sensitivities of DWI and the detection of t-tau protein in CSF. The qualitative results from Western blots for 14-3-3 protein can also result in false positives, which make the diagnosis of CJD difficult. Moreover, although the antibody against the β-isoform of the 14-3-3 protein (sc-639) that is produced by Santa Cruz Biotechnology is commonly used as the WHO standard throughout the world, this antibody has two problems. First, it can recognize both the β- and γ-isoforms of 14-3-3 protein. Second, in early-stage CJD patients, the sensitivity of this antibody for the β-isoform of 14-3-3 protein was lower than that of the anti-14-3-3 β protein-specific antibody produced by IBL (fig. 2). So, we concluded the sensitivity of anti-14-3-3 β produced by IBL was higher at the early stage.

Neuroradiologists in America and Europe have shown the effectiveness of FLAIR MRI as a diagnostic proce-

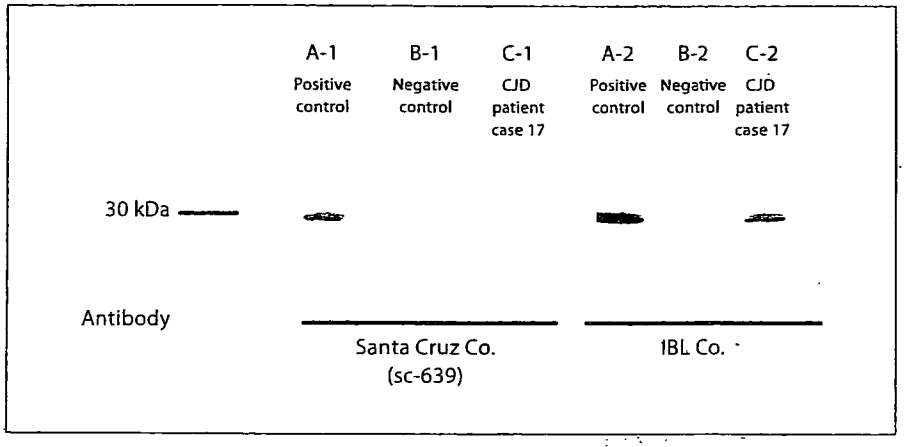

Fig. 2. Detection of 14-3-3 protein using antibodies for CSF obtained from a positive control (CJD patient defined by WHO criteria), a negative control (healthy volunteer) and case 17. The 1st antibody that was used in lane A-1, B-1 and C-1 was produced by Santa Cruz Biotechnology (sc-639; Santa Cruz, Calif., USA), and the 1st antibody that was used in lane A-2, B-2 and C-2 was produced by IBL (Gunma, Japan).

dure for CJD with a sensitivity and specificity between 91 and 94% [5, 6]. We, however, did not detect abnormalities in the CJD patients examined in the present study with FLAIR MRI. This may have been because many of the changes that we were able to detect with FLAIR MRI were identified in patients with late-stage CJD. In the present study, the sensitivity of DWI was 90% for the early stage of CJD, and 100% for the final stage of the disease.

A study by Shiga et al. [4] reported that the sensitivity of DWI was 92.3% for the diagnosis of the final stage of CJD. In addition, Mendez et al. [7] used DWI and the detection of 14-3-3 protein in CSF for the diagnosis of CJD. Neither of these reports, however, discussed the difference between the sensitivities of the detection of t-tau protein in CSF and DWI. In addition, although Young et al. [6] reported that the sensitivity of DWI and FLAIR MRI for the diagnosis of CJD was 91%, their report did not clarify the patient profiles or the stages of the disease that they were examining.

In conclusion, we have clearly shown that, during the period we defined as the early stage of CJD, the sensitivity of the detection of t-tau protein in CSF was greater than the sensitivity of DWI for the diagnosis of CJD. It is important to note that the sensitivity of these two diagnostic criteria was 98% in cases that were positive for t-tau protein and had abnormal DWI results; in fact, these two methods strongly complement each other (table 3). Thus, our results demonstrate that the combination of elevated levels of t-tau protein and abnormal DWI results is useful for the diagnosis of early-stage CJD.

References

1 Brandel JP, Delasnerie-Laupretre N, et al: Diagnosis of Creutzfeldt-Jakob disease: effect of clinical criteria on incidence estimates. Neurology 2000;54:1095–1099.

2 Otto M, Wiltfang J, et al: Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease. Neurology 2002; 58:192–197.

3 Satoh K, Shirabe S, et al: 14-3-3 protein, total tau and phosphorylated tau in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease and neurodegenerative disease in Japan. Cell Mol Neurobiol 2006;26:45–52.

4 Shiga Y, Miyazawa K, et al: Diffusion-weighted MRI abnormalities as an early diagnostic marker for Creutzfeldt-Jakob disease. Neurology 2004;63:443–449.

5 Collie DA, Sellar RJ, et al: MRI of Creutzfeldt-Jakob disease: imaging features and recommended MRI protocol. Clin Radiol 2001;56: 726–739.

6 Young GS, Geschwind MD, et al: Diffusion-weighted and fluid-attenuated inversion recovery imaging in Creutzfeldt-Jakob disease: high sensitivity and specificity for diagnosis. Am J Neuroradiol 2005;26:1551–1562.

7 Mendez OE, Shang J, et al: Diffusion-weighted MRI in Creutzfeldt-Jakob disease: a better diagnostic marker than CSF protein 14-3-3? J Neuroimaging 2003;13:147–151.

Original Research Article

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Chronological Changes in MRI and CSF Biochemical Markers in Creutzfeldt-Jakob Disease Patients

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Key Words
Creutzfeldt-Jakob disease · Total tau protein · Diffusion-weighted magnetic resonance imaging

Abstract
Background: There are currently no markers for evaluating chronological changes in Creutzfeldt-Jakob disease (CJD). We examined if chronological changes in biochemical markers in cerebrospinal fluid (CSF) and diffusion-weighted magnetic resonance imaging (DWI) were utilizable for this purpose. ***Methods:*** Ten independent patients were divided into two groups of 5 patients each. We analyzed CSF biochemical markers, DWI and the clinical course in one group. In the remaining group, only the CSF biochemical markers were analyzed before and after the onset of akinetic mutism. ***Results:*** The level of total tau (t-tau) protein in CSF in the early phase after disease onset was 2,655 ± 423.9 pg/ml, reaching a mean peak of 14,675 ± 1,240 pg/ml in the middle phase and gradually declining after that. Just before patients deteriorated into akinetic mutism, t-tau protein titers reached a maximum (8,786 ± 2,975 pg/ml). There were dramatic changes in t-tau protein levels throughout the clinical course, unlike the other markers. DWI was not always utilizable, because of discordance with clinical symptoms seen in this study. Four cases exhibited peaks in t-tau protein levels while the patients fell into akinetic mutism except 1 case. ***Conclusion:*** Our results suggest that t-tau protein is the most sensitive marker of disease progression in CJD patients.

Introduction

While Creutzfeldt-Jakob disease (CJD) is a rapidly progressive disease, clinical findings in the early stages of the disease may resemble the symptoms of other neurodegenerative diseases. Previously, we have used cerebrospinal fluid (CSF) levels of 14-3-3 protein and periodic sharp-wave complexes on electroencephalography as diagnostic markers [1]. We have recently evaluated total tau (t-tau) protein, excluding 14-3-3 protein, in CSF as a diagnostic marker of CJD [2, 3]. CSF levels of t-tau protein are now considered to be a diagnostic biochemical marker of CJD; as yet, however, no studies have demonstrated a correlation between CSF biochemical markers and clinical features.

Magnetic resonance imaging (MRI) is also used as a diagnostic procedure for CJD. Diffusion-weighted MRI (DWI) is more diagnostic in the early phases of CJD than

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CSF levels of 14-3-3 protein [4–6]. But there were no reports about clear links between abnormal lesions of MRI and clinical features.

As a number of drugs have recently been reported as useful potential agents for the treatment of CJD, we had to analyze the efficacy of these drugs in patients with CJD. There are currently no biochemical markers or measurements applicable to patients with CJD who have been administered drugs to gauge responses to therapy. We sought to investigate such markers for clinical monitoring. To determine the usefulness of treatment in the time course of CJD, we compared biochemical markers, clinical symptoms and MRI findings between treated and untreated patients. Therefore, we can elucidate the point in the disease time course of CJD in which treatment is efficacious by following the chronology of biochemical markers, clinical symptoms and MRI findings. The end point of treatment for patients with CJD is akinetic mutism.

We attempted to determine the MRI findings and CSF biochemical markers that would be most suitable to predict the onset of akinetic mutism.

Patients and Methods

Patients

We examined the first 10 patients (5 men; 5 women) with CJD from 1999 to 2005 who were seen at one of four hospitals (Nagasaki University, Nagasaki Kita Hospital, Matsue Red Cross Hospital and Nagasaki Medical Center of Neurology). This study protocol was approved by the Nagasaki University Ethics Committee. The clinical diagnosis of CJD was made using standard criteria. Patients were classified using the World Health Organization (WHO) criteria, identifying 3 'definite' cases and 7 'probable' cases.

Not all patients or families agreed with the serial collections during the disease course. Only 5 of the 10 CJD patients or families agreed to follow the full chronology of our study protocol. The patients were divided into two groups of 5 patients each: one group completed the full chronological time course study (cases 1–5), and the other group took part in a 2-point analysis study (cases 6–10). We analyzed the CSF, MRI and clinical findings of the first group of 5 patients throughout the disease course. Of these 5 patients, 3 had sporadic CJD (cases 1, 2 and 3), 1 had dura-graft-associated CJD (case 4) and 1 had the Heidenhain variant of CJD (case 5). These 5 cases were admitted to the hospital every 3–4 weeks, at which points CSF was obtained for the measurement of biochemical markers, including t-tau and 14-3-3 proteins. DWI, fluid-attenuated inversion recovery (FLAIR), T_1-weighted and T_2-weighted MRI images were also obtained every 3 weeks throughout the disease time course.

We collected CSF from the remaining 5 patients at admission and 8 weeks later upon succumbing to akinetic mutism. The levels of t-tau protein were determined at these time points. Of these 5 patients, 2 had a V180I mutation (cases 6 and 7), 1 possessed V180/M232R mutations (case 8), and 2 patients had sporadic CJD (cases 9 and 10).

Methods

All patients were subjected to a standard neurological evaluation, including cognitive testing. Additional clinical and laboratory tests were performed according to the discretion of the evaluating neurologists.

CSF was obtained using a standard clinical procedure. All samples were free of blood contamination. Samples were stored between –30 and –80°C until examination by the t-tau protein assay.

Detection of the β-Isoform of 14-3-3 Protein in CSF and Measurements of Biochemical Markers in CSF (Total Tau Protein, S-100b Protein and Neuron-Specific Enolase)

The 14-3-3 protein immunoassay of CSF was performed as previously reported [4]. Immunodetection of the protein utilized a polyclonal antibody against the β-isoform of 14-3-3 (IBL Co.) and an enhanced chemiluminescence detection kit (Amersham Buchler).

Measurement of t-tau protein in CSF was performed as previously reported [3]. Briefly, t-tau protein levels were measured with commercially available assays from Innogentics NV (Ghent, Belgium). Detection of t-tau protein by ELISA ranges from 70 to 1,120 pg/ml in human CSF. We measured t-tau protein levels by ELISA according to the manufacturer's instructions and using identical standards in all experiments. The resulting signal intensities were measured and quantitated using the Labry system image station at 450 nm with the accompanying software. These measurements were used to calculate the ratio of each signal intensity to the standard.

The quantities of neuron-specific enolase (NSE) and S-100 protein in CSF samples were measured using a commercially available ELISA (SRL Laboratory, Tokyo, Japan). S-100 protein levels could be measured in the range of 0.001–25 ng/ml; values greater than 35 ng/ml were considered positive in CJD patients. The measurement range for NSE was 2–200 ng/ml, with values greater than 2.2 ng/ml considered positive.

MRI scans were performed using various units. Using a 1.0- or 1.5-tesla MR, we acquired T_1-weighted, fast spin echo T_2-weighted and FLAIR images. The DWI technique has previously been described [5]. We also used a standard diagnostic protocol for neurodegenerative diseases with standard acquisition parameters: sagittal T_1-weighted spin echo (TR/TE/NEX = 600/minimals/2) and axial and/or coronal FLAIR (TR/TE/TI/NEX = 1,000/140/2,200/1). Both coronal and axial FLAIR imaging and DWI were acquired for most subjects, while axial DWI and FLAIR images were obtained for all patients.

Molecular Genetic Analysis

Genomic DNA extracted from peripheral blood leukocytes was used to amplify the open reading frame of the prion protein gene by polymerase chain reaction. The resulting products were analyzed for polymorphisms at codons 129 and 219 by sequencing as previously described [3].

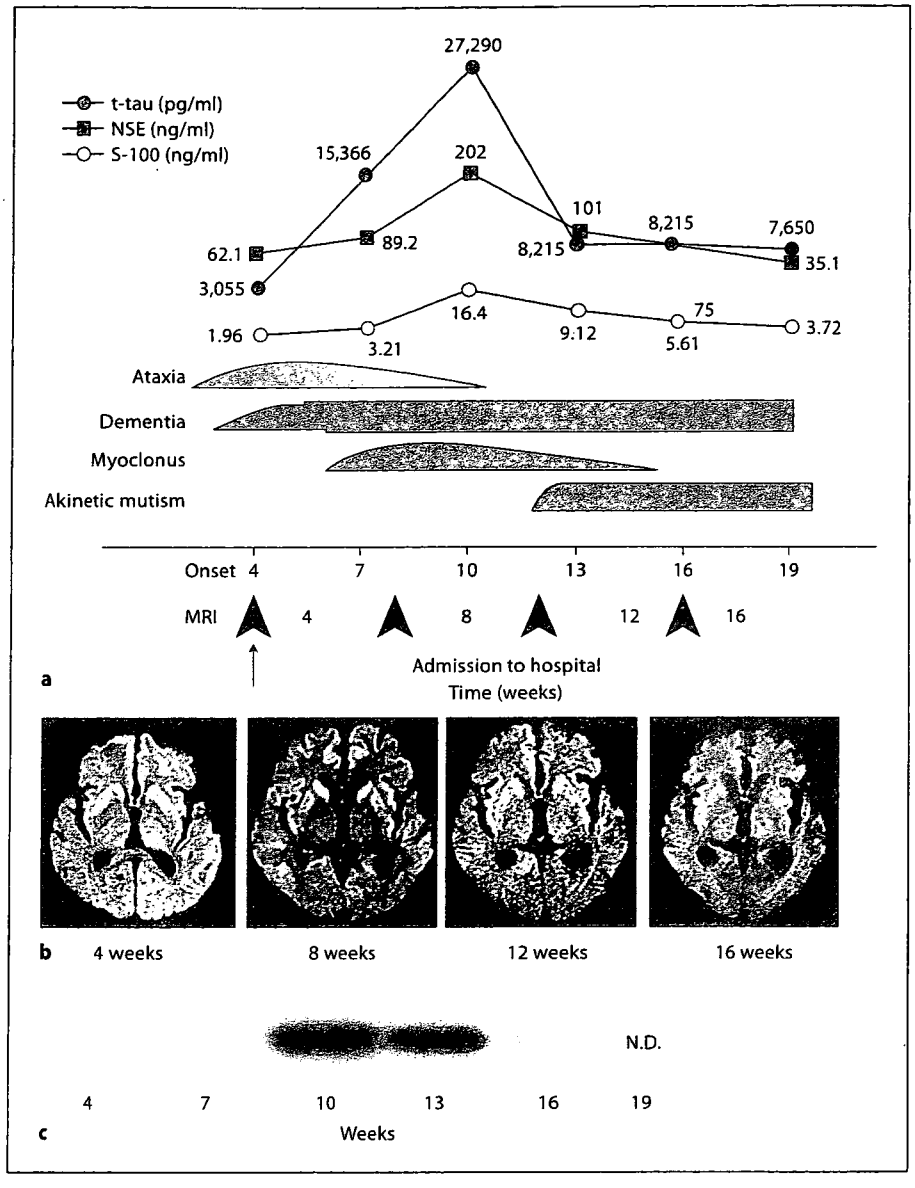

Fig. 1. Clinical time course, DWI findings and biochemical markers in CSF in a patient with CJD (case 1). **a** Chronological data of biochemical markers, t-tau protein, S-100b protein and NSE in CSF, obtained chronologically at 6 time points (4, 7, 10, 13, 16 and 19 weeks). **b** Chronological changes in DWI of case 1. DWI was acquired at 4 time points (4, 8, 12 and 16 weeks). **c** The detection of the β-isoform of 14-3-3 protein (IBL Co.) by Western blotting of CSF from case 1 was performed using an enhanced chemiluminescence detection kit (Amersham Buchler). CSF was collected at 6 time points (4, 7, 10, 13, 16 and 19 weeks). We could not detect the β-isoform of 14-3-3 by 19 weeks; N.D. = not detected.

Results

Clinical Time Course, DWI Findings and CSF Biochemical Markers

Case 1. Case 1 was a 71-year-old male who first presented with an unstable gait in September 2002. After developing this symptom, he immediately became disoriented. He developed progressive dementia and an ataxic gait within 3 weeks of the onset of symptoms. He was admitted to our hospital 4 weeks after the onset of symptoms. Upon admission, he could no longer walk without assistance (fig. 1a). The patient was diagnosed as having CJD by clinical features, DWI findings and CSF profile. Myoclonic seizures began in the sixth week of the disease. In the tenth week after disease onset, frontal signs, such as the snout reflex, appeared. He developed akinetic mutism at 12 weeks and died in October 2004.

DWI at admission exhibited high-intensity areas within both putamina and caudate nuclei and abnormal densities in regions of the left occipital lobe at 4 weeks. The abnormal intensities on DWI extended into the frontal lobes by 8 weeks after onset. After the sixteenth week, the cerebral atrophy gradually improved; the high-intensity areas that had been present on DWI became roughly restricted to the temporal lobes (fig. 1b).

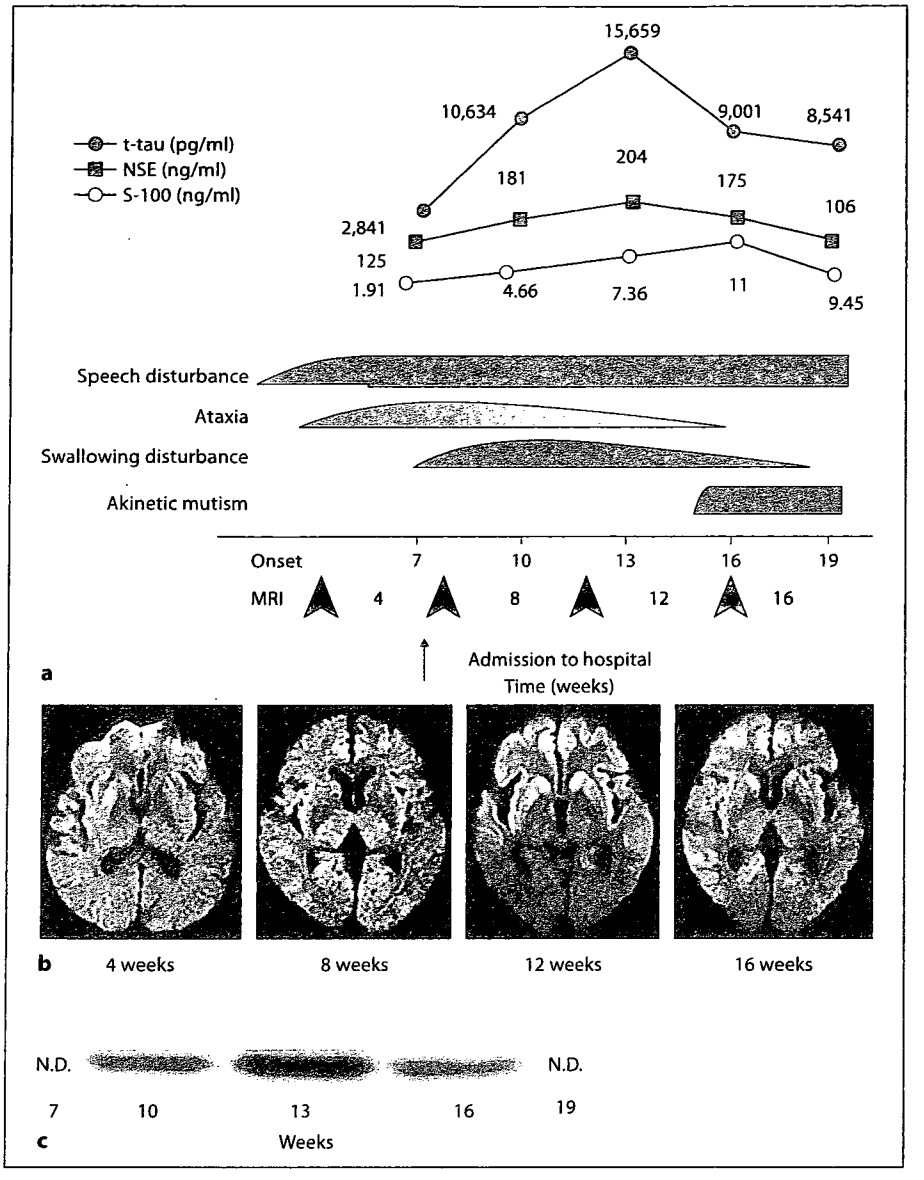

Fig. 2. Clinical time course, DWI findings and CSF biochemical markers in a CJD patient (case 2). **a** Chronological data of biochemical markers, t-tau protein, S-100b protein and NSE in CSF, obtained chronologically at 5 time points (7, 10, 13, 16 and 19 weeks). **b** Chronological changes in DWI of case 2. MRI was acquired at 4 time points (4, 8, 12 and 16 weeks). **c** The detection of the β-isoform of 14-3-3 protein (IBL Co.) was performed using an enhanced chemiluminescence detection kit (Amersham Buchler) by Western blotting of the CSF from case 2. CSF was obtained at 5 time points (7, 10, 13, 16 and 19 weeks). We could not detect the β-isoform of 14-3-3 at 7 or 19 weeks; N.D. = not detected.

Of the biochemical markers measured, t-tau protein and NSE levels reached peaks of 27,290 pg/ml and 202 ng/ml just prior to the onset of akinetic mutism. In contrast, 14-3-3 and S-100b protein levels remained unchanged (fig. 1a, c).

Case 2. Case 2 was a 65-year-old man who initially presented with speech disturbances. Ataxic speech began in August 2002 and progressed rapidly. His neurological examination at 4 weeks after the onset of symptoms revealed ataxic speech, limb ataxia and truncal ataxia. These neurological abnormalities were reflected by abnormalities on DWI detected at 4 weeks. At 6 weeks, he was admitted to our hospital with action myoclonus of the limbs and dysphasia. At 7 weeks, he was diagnosed as having possible CJD based on the WHO diagnostic criteria (fig. 2a). He could neither walk without assistance nor swallow at 10 weeks. He developed akinetic mutism at 15 weeks.

Although abnormalities were detected on DWI as early as 4 weeks after onset, we determined that these observations were artifacts within the basal ganglia. Until the eighth week, despite a rapid decline in the patient's activities of daily living, DWI failed to reveal any abnormality. At 12 weeks, DWI exhibited abnormal densities primarily in the frontal lobes. At 16 weeks, at which point the patient had developed akinetic mutism, DWI did not

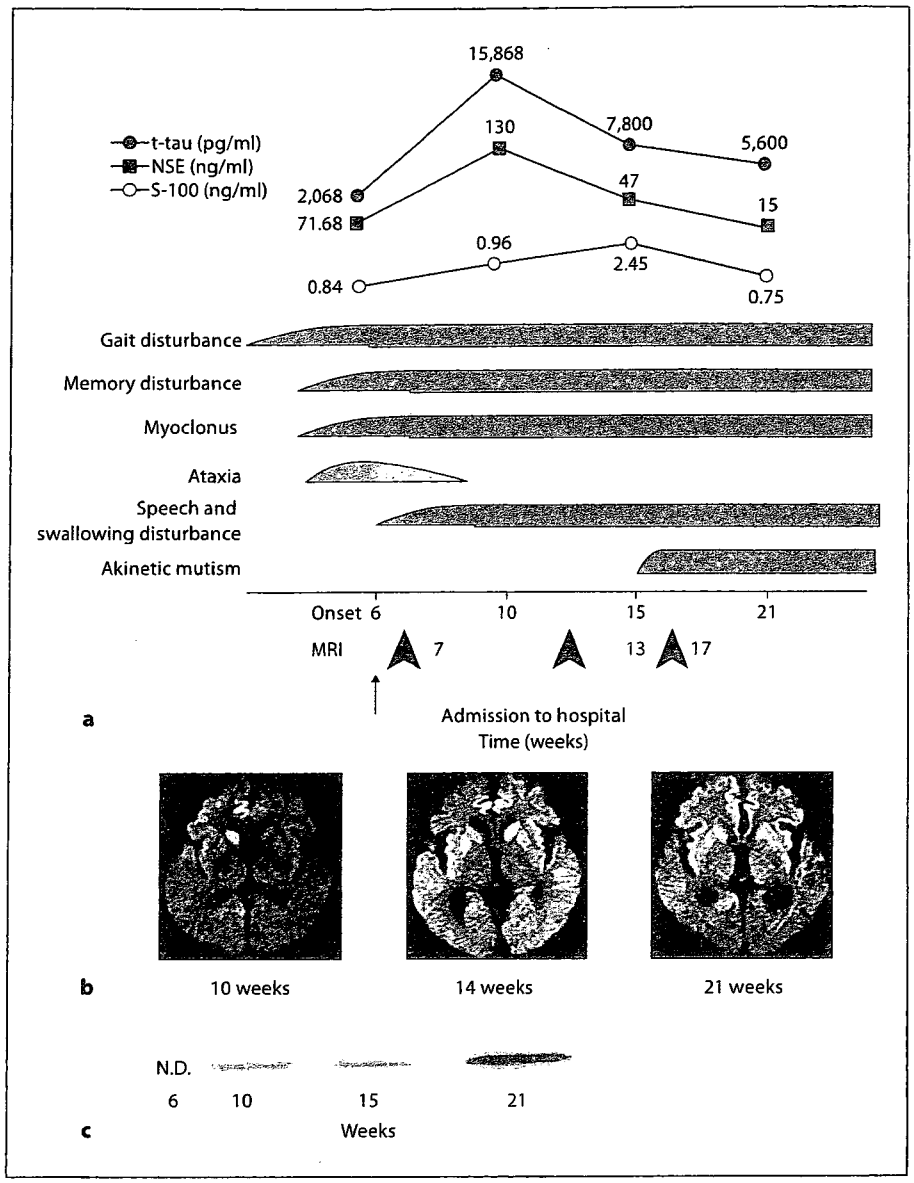

Fig. 3. Clinical time course, DWI findings and CSF biochemical markers in a CJD patient (case 3). **a** Chronological data of biochemical markers, t-tau protein, S-100b protein and NSE in CSF, obtained at 4 time points (6, 10, 15 and 21 weeks). **b** Chronological changes in DWI of case 3. MRI was acquired at 3 time points (7, 13 and 17 weeks). **c** The detection of the β-isoform of 14-3-3 protein (IBL Co.) was performed by Western blotting of CSF from case 3 using an enhanced chemiluminescence detection kit (Amersham Buchler). CSF was sampled at 4 time points (6, 10, 15 and 21 weeks). N.D. = Not detected.

demonstrate any cerebral atrophy but did reveal extensive areas of abnormal signal intensity in the right frontal, temporal and occipital lobes (fig. 2b).

Of the biochemical markers examined, t-tau protein and NSE levels peaked at 16,959 pg/ml and 60 ng/ml, respectively, just prior to the patient developing akinetic mutism; 14-3-3 and S-100b protein levels, however, increased progressively with time (fig. 2a, c).

Case 3. Case 3 was a 67-year-old male whose gait became unstable in April 2004. Memory disturbances followed shortly thereafter. Four weeks after the onset of symptoms, he developed myoclonus and cerebellar ataxia. At 6 weeks, he developed speech and swallowing disturbances. From his suggestive clinical symptoms, we diagnosed the patient as having CJD at 10 weeks; the patient succumbed to akinetic mutism at 16 weeks (fig. 3a).

We detected an abnormal density in the right caudate nucleus at 6 weeks. Over time, the area of abnormal intensity expanded to the frontal and temporal lobes (fig. 3b).

At 6 weeks, CSF contained 2,068 pg/ml of t-tau protein; 14-3-3 protein was undetectable. Of the biochemical markers examined, t-tau protein peaked at 15,868 pg/ml, while NSE reached a maximum of 130 ng/ml. The levels of both 14-3-3 and S-100b proteins gradually decreased with time (fig. 3a, c).

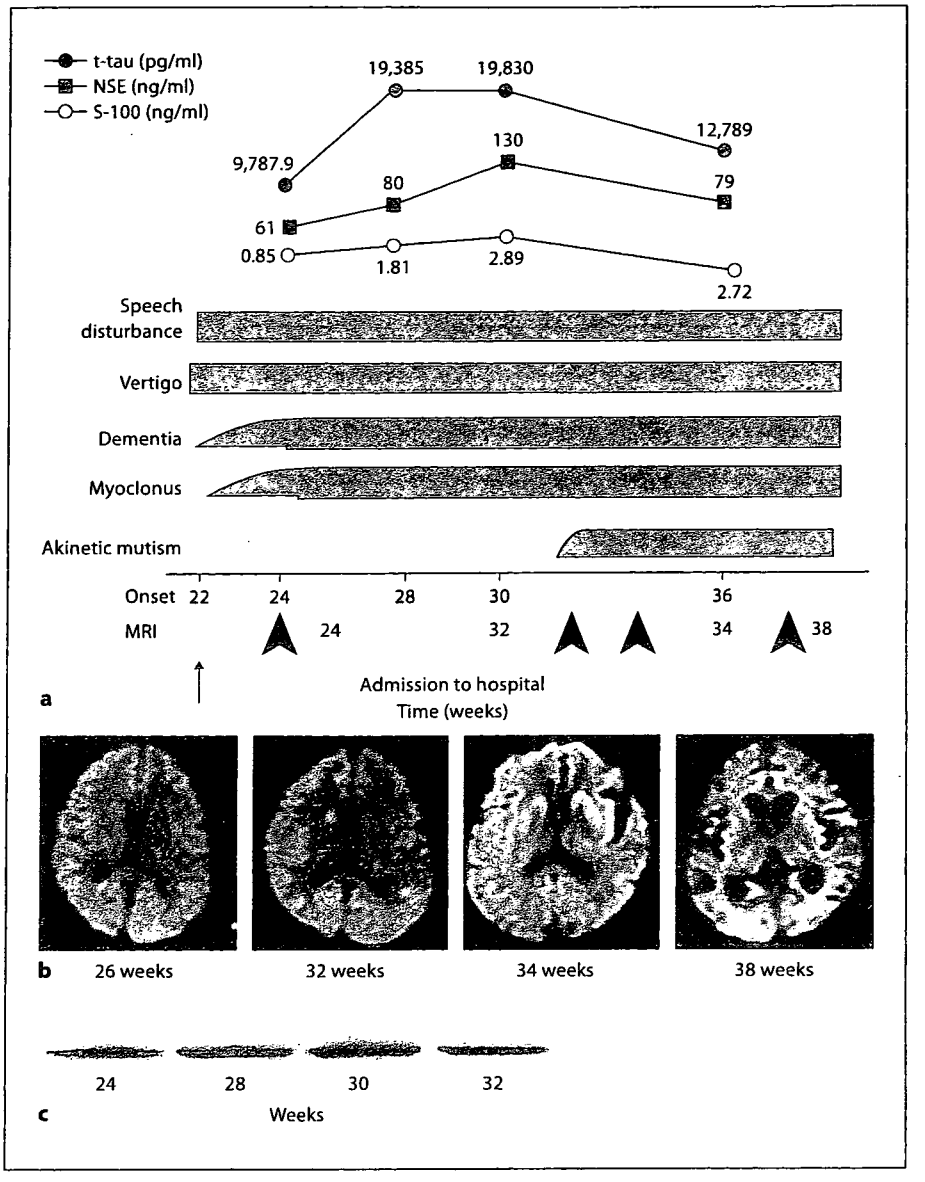

Fig. 4. Clinical time course, DWI findings and CSF biochemical markers in a patient with dura-graft-associated CJD (case 4). **a** Chronological data of biochemical markers, t-tau protein, S-100b protein and NSE in CSF, obtained at 4 time points (24, 28, 30 and 32 weeks). **b** Chronological changes in DWI of case 4. MRI was acquired at 4 time points (26, 32, 34 and 38 weeks). No abnormal intensities were detected by DWI at 26 weeks and 32 weeks in case 4. **c** The detection of the β-isoform of 14-3-3 protein (IBL Co.) was performed by Western blotting of CSF from case 4 using an enhanced chemiluminescence detection kit (Amersham Buchler). CSF was obtained at 4 time points (6, 10, 15 and 21 weeks).

Case 4. Case 4 was a 40-year-old woman. At 27 years old, a malignant ependymoma at the ventricular quarters of the brain was removed neurosurgically with a desiccated dura. At 40 years of age, she developed vertigo and slurred speech. She was admitted to the hospital at 22 weeks after the onset of symptoms, then diagnosed as having CJD by clinical features such as myoclonus and the presence of periodic sharp-wave complexes on the electroencephalogram. She developed akinetic mutism at 32 weeks (fig. 4a).

MRI performed at 26 and 32 weeks failed to reveal any abnormal intensities; however, MRI at 34 weeks detected an abnormal intensity along the cerebral cortex. Cerebral atrophy was also more marked at this point, with the abnormal intensities primarily identified in the parietal lobes (fig. 4a, b).

Of the biochemical markers examined, t-tau protein and NSE peaked at values of 19,385 pg/ml and 130 ng/ml, respectively. But in this case (case 4) the period in the peak of t-tau protein was different from that of the peak of NSE. Both 14-3-3 and S-100b protein levels gradually declined over time (fig. 4a, c).

Case 5. Case 5 was a 64-year-old woman who presented with progressive visual impairment over a period of 4 weeks. Despite the absence of additional clinical features than her cortical blindness, we diagnosed CJD based on

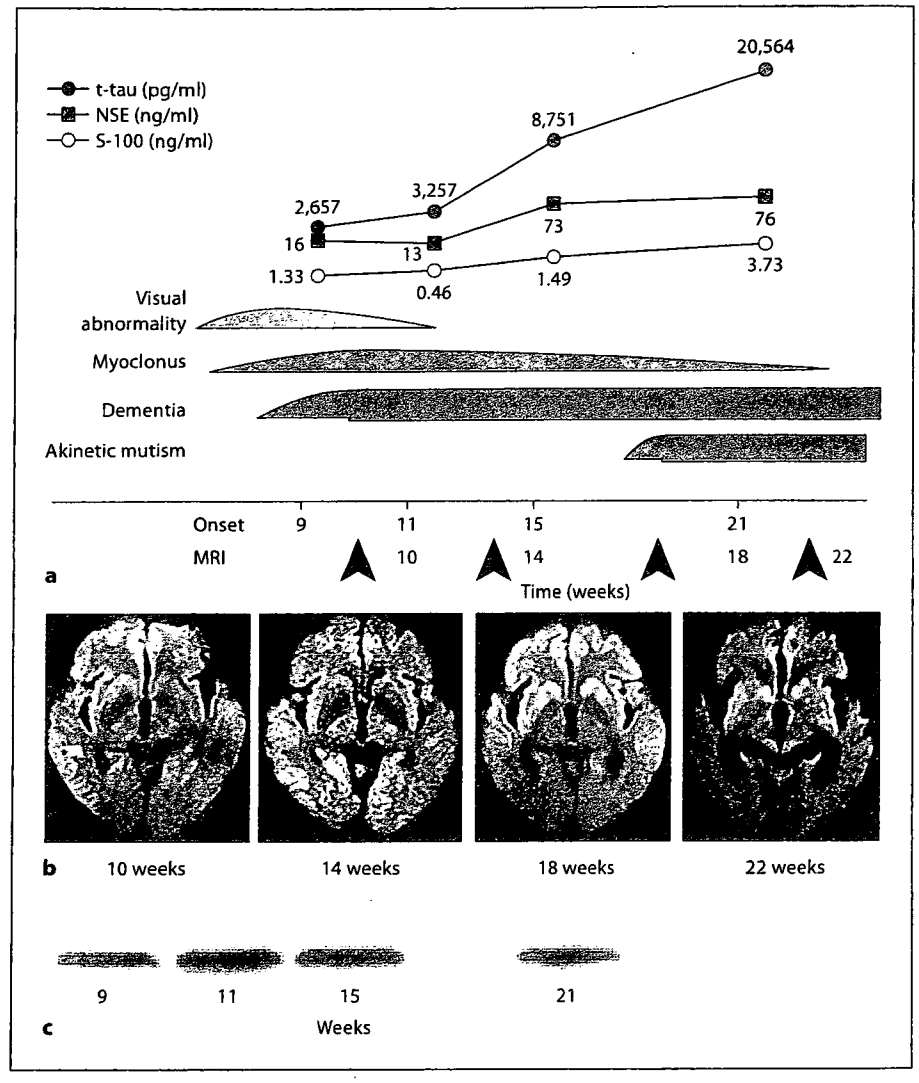

Fig. 5. Clinical time course, DWI findings and CSF biochemical markers in a Heidenhain variant of CJD (case 5). **a** Chronological data of biochemical markers, t-tau protein, S-100b protein and NSE in CSF, obtained at 4 time points (9, 11, 15 and 21 weeks). **b** Chronological changes in DWI of case 5. MRI was acquired at 4 time points (9, 11, 15 and 21 weeks). No clearly abnormal intensities were detected in case 5 at 9 weeks by DWI. **c** The detection of the β-isoform of 14-3-3 protein (IBL Co.) was performed by Western blotting of CSF from case 5 using an enhanced chemiluminescence detection kit (Amersham Buchler). CSF was obtained at 4 time points (9, 11, 15 and 21 weeks).

the presence of a giant visual evoked potential and elevated CSF biochemical markers. An electroencephalogram did not reveal any periodic sharp-wave complexes (fig. 5a). Myoclonus appeared at 7 weeks; myoclonus and dementia developed rapidly, becoming evident as early as 8 weeks after the onset of symptoms. She developed akinetic mutism at 17 weeks and died within 2 years.

Although abnormal intensities on DWI were observed within the occipital lobe early in the patient's course, MRI did not reveal a clearly abnormal density at 10 weeks. Markedly abnormal densities were first observed primarily in the occipital lobes at 14 weeks; abnormal densities within the basal ganglia and frontal and temporal lobes developed at 18 weeks. Cerebral atrophy became marked at 22 weeks, accompanied by decreases in the abnormal densities (fig. 5b).

Between 9 and 11 weeks, there were no significant changes in the levels of t-tau protein, NSE or S-100 protein. Of the biochemical markers examined, t-tau protein levels peaked at 20,564 pg/ml after the appearance of akinetic mutism, then decreased to 12,659 pg/ml at 26 weeks. NSE levels exhibited a similar profile. 14-3-3 and S-100b protein levels remained stable throughout the disease course (fig. 5a, c).

Analysis of the Relationships between Biochemical Markers of CSF and Clinical Features

Serial analysis of biochemical markers, including t-tau protein, in CSF demonstrated marked peaks at 9–18 weeks of the clinical course in 4 cases. Based on the clinical findings and biochemical markers, the time course of CJD can be divided into three phases: an early phase of

the first 8 weeks after the onset of illness, a middle phase beginning after approximately 9 weeks to akinetic mutism, and a late phase after the development of akinetic mutism. Most cases of CJD presented to the hospital within 8 weeks, as suggested by a basic search of the Japanese CJD surveillance committee.

The t-tau protein levels on the lumbar first puncture in the early phase of disease was 2,655 ± 423.9 pg/ml (mean ± SD). Peak levels were observed at approximately 10–16 weeks after the onset of symptoms (14,675 ± 1,240 pg/ml), after which the titers of t-tau decreased steadily. Analysis of the CSF at the point at which all patients exhibited akinetic mutism, the mean t-tau protein titer was 8,786 ± 2,975 pg/ml. S-100b protein levels exhibited a similar time course.

Of the biochemical markers examined, t-tau and S-100b proteins displayed similar concentration profiles throughout the time course of CJD. The pattern of NSE expression differed from that of t-tau and S-100b protein, peaking at a different time from t-tau protein in case 4.

In multiple cases, the myoclonus and dementia were identified before the first clinical examination or admission to a hospital. The CSF samples did not reveal a relationship between CSF biochemical markers and the duration of the myoclonus or dementia. The progression to myoclonus and dementia did not correlate with the transitions in biochemical markers. The only biochemical marker that correlated with disease progression was the peak in t-tau protein levels that preceded the onset of akinetic mutism.

We did not quantitatively assess 14-3-3 protein levels using either ELISA or RIA, instead using the qualitative results of Western blotting. 14-3-3 protein levels were highest at the point at which t-tau protein levels peaked (cases 1, 2 and 3). 14-3-3 protein was undetected at the early stages of disease in most cases. S-100b protein and NSE exhibited a similar concentration pattern in early disease.

Analysis of the Relationships between DWI and Clinical Features

Case 5 complained of cortical blindness and visual impairment, which correlated well with the DWI findings of abnormal intensities primarily in the occipital lobes. Cases 1 and 5 presented with symptoms of dementia; however, DWI did not reveal any abnormal intensities in the hippocampus or frontal lobes. In addition, both patients exhibited cerebellar ataxia but did not show any abnormal intensities of the cerebellum on DWI. Indeed, none of these 10 patients displayed abnormal intensities within the cerebellum by DWI. Thus, the clinical symptoms did not correlate with the areas of abnormal signal intensity on DWI that would be suggested by the focal neurological signs.

Analysis of the Extent of Abnormal Intensities on DWI

The extent of the abnormal intensities on DWI varied widely among the 5 patients. Cases 1 and 3 exhibited abnormal intensities that were first detected within the basal ganglia and temporal lobes, which then extended to the frontal or occipital lobes. In cases 2 and 5, the abnormal intensities began in the frontal or occipital lobes, then extended to the basal ganglia. DWI imaging of case 4 revealed abnormal intensities in the basal ganglia only. These findings led to the conclusion that the extent of abnormal intensities on DWI does not correlate with clinical symptoms or severity.

Analysis of Total Tau Protein at 2 Time Points Alone in Patients Other than the Above 5

The analysis of CSF biochemical markers (t-tau protein, 14-3-3 protein, NSE and S-100b protein) suggested that t-tau protein was the most accurate pathological marker. We therefore analyzed t-tau protein levels in 2 patients with sporadic CJD, 2 CJD patients with a V180I mutation and a CJD patient with the V180I/M232R mutations before and after the onset of akinetic mutism. The 2 sporadic CJD patients and 3 CJD patients bearing V180I mutations (including the patient with both V180I/M232 mutations) exhibited clearly different patterns in t-tau concentrations over time. The levels of t-tau protein, however, tended to decrease between the 2 time points, particularly rapidly in the sporadic CJD patients (fig. 6). Before the onset of akinetic mutism, the mean titer of CSF t-tau protein was 6,455 ± 4,462 pg/ml, decreasing to 3,211 ± 1,607 pg/ml after the development of akinetic mutism.

Discussion

Previous studies have compared biochemical markers in CSF from patients with CJD at 2 time points [2, 7–10]. As yet, no studies have analyzed the detailed chronological changes in CSF biochemical markers, making this study valuable for the clinical management of CJD patients [2, 7–10].

At first, we divided the time course of CJD illness into 3 phases. The t-tau protein level on the first lumbar punc-

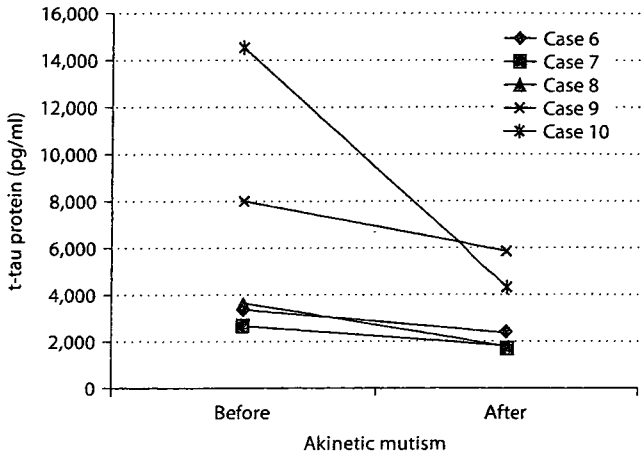

Fig. 6. Comparative analysis of t-tau protein in CSF measured before and after the onset of akinetic mutism (cases 6–10). Cases 6–8 were familial cases. Cases 6 and 7 exhibited point mutations in the prion protein gene (Val/Ile at codon 180), while case 8 possessed 2 mutations in the prion protein gene (Val/Ile at codon 180 and Met/Arg at codon 232). Cases 9 and 10 were sporadic cases. All cases were analyzed for the codon 129 (Met/Met) and codon 219 (Glu/Glu) polymorphisms.

ture in the early phase of disease was 2,655 ± 423.9 pg/ml (mean ± SD). Peak protein levels were observed at approximately 10–16 weeks after disease onset (14,675 ± 1,240 pg/ml). Titers of t-tau decreased thereafter in 4 cases. Samples of CSF acquired when all patients exhibited akinetic mutism had a mean t-tau titer of 8,786 ± 2,975 pg/ml. S-100b protein levels exhibited a similar time course of concentration fluctuations, although these changes were not dramatic. S-100b protein levels peaked later than those of t-tau protein, which may reflect the fact that S-100b protein is secreted from astrocytes, but not from neurons.

A number of studies have reported changes in t-tau protein during 1 or 2 periods, but these studies have not analyzed the chronological progression [2, 9, 11]. t-tau protein levels peaked in Everbroeck's middle stage, corresponding to the clinical symptom of akinetic mutism. The mean value of t-tau protein found in CSF was 14,850 pg/ml in Everbroeck's middle stage.

Otto et al. [2] and Kropp et al. [9] also demonstrated changes in NSE and S-100 in CSF at 2 points during the disease time course in 16 patients. Fifteen of these patients exhibited elevations in both markers which persisted until 30 weeks, although the levels decreased in 1 patient after 30 weeks. Whereas in our study we analyzed CSF every 3–4 weeks, they only analyzed the data at 2 points, which were separated by a variable number of weeks. We speculate that peaks in t-tau levels may have occurred in the interim between measurements.

CSF analysis before and after the onset of akinetic mutism demonstrated that t-tau protein levels tended to decrease over this time (fig. 6). The absolute difference between these 2 measurements was minimal, however, as this group included 3 CJD patients with V180I mutations who have a slowly progressive course and in whom the onset of akinetic mutism is difficult to assess. CSF levels of t-tau protein are thought to reflect neuronal death. On the assumption that the akinetic mutism associated with changes in the sporadic CJD patients corresponds to neuronal death, we hypothesized that neuronal death should peak around the time of onset of akinetic mutism. Therefore, t-tau protein in CSF is a pathological marker that should predict the progression of the patient to akinetic mutism.

Recently, several groups have reported that DWI is diagnostic for CJD. Chronological changes in DWI have only been examined in case studies, as reported by Tomita et al. [12], Matoba et al. [13] and Ukisu et al. [14]. Our study revealed that the localization of pathological sites and areas of abnormal signal intensity on DWI did not correlate with the potential sites of localization suggested by focal neurological signs. In addition, we identified that the extent of abnormal intensities on DWI did not predict the severity of the disease, supporting the results of Ukisu et al. [14]. As evidenced by case 5, however, DWI can agree with the clinical symptoms. Thus, DWI does not correlate with the clinical features of disease and cannot be used to predict the extent of CJD lesions. The manner of lesion reflected in the abnormal signal intensity areas on DWI remains unclear, because it is difficult to examine such areas shown by DWI histologically. Neurologists and neuroradiologists agree that it is difficult to explain why these abnormal densities on DWI persist for several months, if they were to be explained in terms of edematous changes and rapid neuronal death.

This study demonstrated that clinical symptoms did not necessarily correlate with abnormal densities visualized on DWI; these abnormal intensities have no definite pattern of extension. t-tau protein levels, however, are an effective pathological marker that can be used to predict progression of the patient to akinetic mutism.