

condensed (Figure 2D) and co-localized with the PrP (Figure 2E) accumulated on the complement receptor 1/2 (CR1/CR2)-immunopositive dendrites [27] of FDCs (Figure 2F). No such accentuation of clusterin expression was observed in the spleens of scrapie-inoculated Tg7/263K mice or Tga20/Fu-1 mice (data not shown). The co-localization of clusterin and PrP on the FDCs was also confirmed by double immunofluorescence (Figure 2G and H).

Although the increased clusterin immunoreactivity mostly correlated with the abnormal PrP accumulations on splenic FDCs from scrapie-agent inoculated C57BL/ME7 mice, temporary FDC depletion 14 or 42 days after scrapie-agent inoculation [21,22] did not affect the association of clusterin with abnormal PrP (Table 3). Similarly, in the spleens of scrapie agent-inoculated IL-6-KO mice, immunoreactivity for clusterin was mostly correlated with abnormal PrP accumulation (Table 3) despite the impaired germinal centre development in these mice [23].

In Peyer's patches, increased clusterin expression was seen not only on the PrP-immunopositive FDCs of infected mice but also in the lymphoid follicles without PrP deposition (Table 3). Clusterin-labelled lymphoid follicles were also confirmed in the Peyer's patches of non-infected control mice (data not shown), but this occurred to a lesser extent than in TSE-infected mice.

Increased clusterin expression associated with the abnormal PrP deposition on FDCs was also observed in cases of human variant CJD. The extent and

intensity of clusterin immunoreactivity did not appear to be related to the age of the patient, or the duration of the clinical illness of the five cases examined (patient ages 17, 29, 33, 36, and 39 years; duration of clinical illness 33, 7, 18, 15, and 14 months, respectively). In each case, immunoreactivity for clusterin was increased wherever abnormal PrP accumulation on the FDCs was found, such as in the spleens, lymph nodes, appendices, and tonsils (Figure 3). In appendices, clusterin immunoreactivity was also increased in the lymphoid follicles without PrP deposition (Figure 3C and D), which was consistent with data from analysis of Peyer's patches of mouse TSE models. No increased clusterin immunoreactivity was observed on FDCs from sporadic CJD cases (data not shown) in which no PrP deposition was detected, except on appendices where clusterin immunoreactivity was increased on the FDCs even in non-CJD control cases (Figure 3E and F).

Discussion

Here we show that clusterin expression on FDCs is increased during TSE diseases and occurs in association with abnormal PrP accumulation. The dendritic network pattern of the clusterin immunoreactivity in the lymphoid follicles was associated with PrP accumulation on FDCs in NZW/Fu-1 mice and C57BL/ME7 mice. The increased clusterin immunoreactivity was mostly dependent on the presence of

Table 3. Effect of FDC depletion and impaired germinal centre development on clusterin expression in TSE-inoculated mice

| | Immune deficiency | PrP IR | Clusterin IR* | CR1/CR2 IR | Effect on disease transmission† |
|------------------------|---------------------------|--------|---------------|------------|---------------------------------|
| | | | | | Intraperitoneal |
| Spleens | | | | | |
| Uninfected | | | | | |
| | FDCs depleted | – | – | – | n/a |
| | Control | – | – | + | n/a |
| 14 dpi | | | | | |
| | FDCs depleted | – | – | – | Delayed [21] |
| | Control | +- | – | + | |
| 42 dpi | | | | | |
| | FDCs depleted | + | + | – | Delayed [21] |
| | Control | + | + | ++ | |
| IL-6-KO | | | | | |
| | Impaired germinal centres | ++ | + | ++ | No effect [23] |
| | Control | ++ | + | ++ | |
| | | | | | Oral |
| Peyer's patches | | | | | |
| –3 dpi | | | | | |
| | FDCs depleted | – | +‡ | n.d. | Inhibited [22] |
| | Control | + | +‡ | n.d. | |

FDCs were depleted by treatment with LT β R-Ig, or Hu-Ig as a control [28]. Ig treatment was performed on the indicated days relative to scrapie challenge. Three mice were examined and estimated the immunoreactivity on each immune-deficient group (LT β R-Ig treated or IL-6 KO) or control group.

IR = immunoreactivity; dpi = days post scrapie inoculation; n.d. = not determined; n/a = not applicable; (–) = negative; (+) = faint; (+) = positive; (++) = strongly positive.

* Immunoreactivity in the dendritic-network pattern.

† Data from previous reports [21–23].

‡ Clusterin immunoreactivity does not always correlate with PrP deposition; increased clusterin expression is also seen in some lymphoid follicles without PrP accumulation.

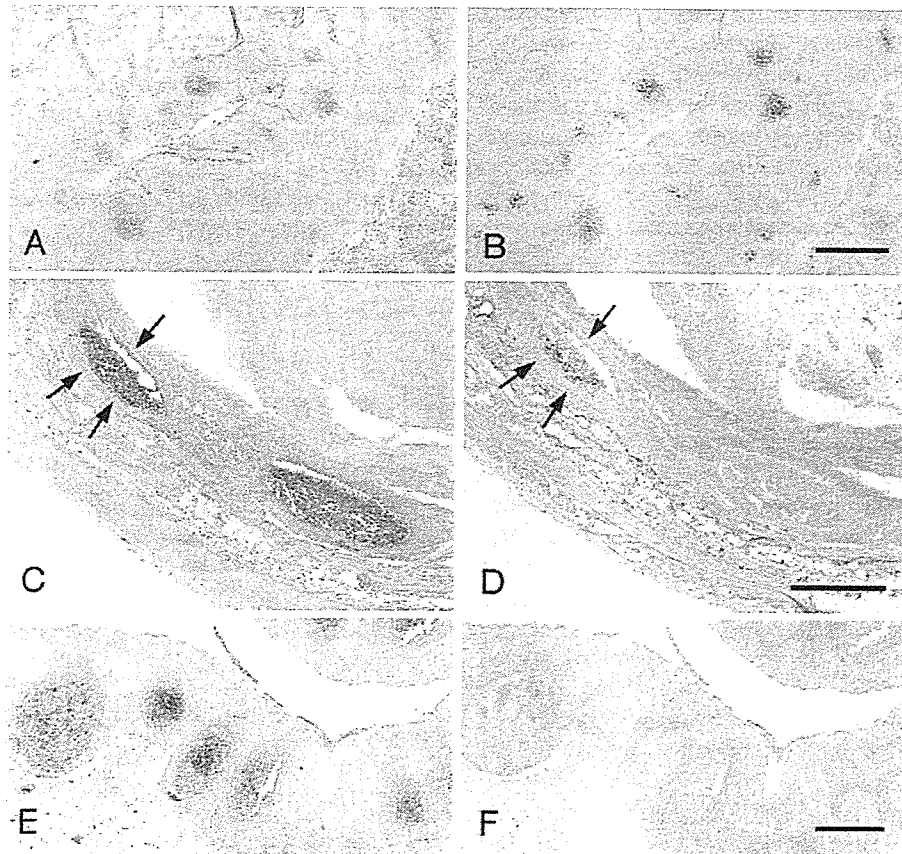


Figure 3. Immunohistochemistry of lymphoreticular tissues from human variant CJD and control (non-CJD) cases. (A, C, E) Clusterin. (B, D, F) PrP. (A, B) Serial sections of the tonsil from a variant CJD case. Clusterin immunoreactivity is increased on FDCs where abnormal PrP deposits are detected. (C–F) Serial sections of appendices from a variant CJD case (C, D) and a control case (E, F). Note that not only the lymphoid follicles with PrP accumulation (arrows) but also those without PrP accumulation show increased clusterin expression. Bars: 500 μ m

abnormal PrP deposition, irrespective of the TSE agent strains, host mouse strains, or various immune deficiencies. Moreover, clusterin accumulation was also seen in human variant CJD cases, which was consistently accompanied by PrP deposition on FDCs in the lymphoreticular system (except for the intestinal lymphoid follicles). We have previously reported that clusterin expression in the cerebrum was increased in association with increased PrP deposition [13]. The results in the present report provide evidence that clusterin accumulation also occurs in peripheral lymphoid tissues. Although a direct interaction between clusterin and PrP remains to be confirmed, these data suggest that clusterin may play an important role in the pathogenesis of TSE diseases in lymphoid tissues.

To determine whether clusterin was associated with abnormal PrP, we analysed the effect of FDC depletion on clusterin accumulation. Signalling through the $LT\beta R$ provides important stimuli for FDC maturation and maintenance. Blockade of this stimulation through treatment with $LT\beta R$ -Ig causes the temporary de-differentiation of FDCs [28]. In this study, CR1/CR2 expression on FDCs was certainly affected by $LT\beta R$ -Ig treatment in comparison with the control mice treated with Hu-Ig (Table 3), confirming that the

FDCs were temporarily de-differentiated. In uninoculated mice, clusterin expression by FDCs is down-regulated by $LT\beta R$ signalling blockade and undetectable by immunohistochemistry within two days of FDC depletion [12]. However, in this study we show that the detection of clusterin in the spleens of scrapie-inoculated mice was unaffected by FDC depletion and remained in close association with abnormal PrP (Table 3). These data imply that clusterin associates directly with abnormal PrP molecules exposed extracellularly on the surface of FDC dendrites.

Why clusterin expression is up-regulated during TSE disease is not known. Data from both human and experimental studies demonstrate that TSE infections induce the expression of both early and terminal complement components within the brain. However, the membrane attack complex (C5b–C9) is not involved in TSE pathogenesis [29]. Thus it is plausible that, during TSE diseases within the CNS, clusterin might be expressed as part of an innate response to inactivate the membrane attack complex formed by the terminal complement components [10], and to help protect neurons from potential complement-mediated lysis. It is also plausible that clusterin might be induced to exert anti-amyloidogenic properties [30]. Because

FDCs express clusterin (current study and reference [12]), and its expression appears to be up-regulated during TSE disease, it is plausible that clusterin is likewise released by FDCs as an activating factor for themselves and/or neighbouring germinal centre B cells [12], which might accelerate abnormal PrP accumulation.

In the Peyer's patches and appendices, some lymphoid follicles showed no detectable PrP deposition, but revealed enhanced clusterin immunoreactivity. Although it is possible that small amounts of undetectable abnormal PrP molecules were present in these follicles, enhanced clusterin expression at this site was probably due to non-specific immune stimulation. Clusterin-enhanced lymphoid follicles were confirmed even in the Peyer's patches of non-infected control mice and in the appendices of non-CJD control cases. The continual exposure of the intestinal lymphoreticular system to a variety of antigens or stress conditions might account for the enhanced clusterin expression in non-TSE affected subjects in comparison with the spleens. The reasons why the regulation of clusterin expression on FDCs in the intestine and other lymphoid tissues appears to differ in uninfected hosts are unknown and therefore worthy of further investigation.

We employed a detergent autoclaving method for PrP detection in this study and found that this method significantly improved sensitivity, especially when analysing the lymphoreticular pathology of TSEs. The early detection of PrP accumulation on FDCs might aid the detection of scrapie transmissibility, and the diagnosis of human variant CJD, for example on tonsil biopsy specimens. Detergent autoclaving also provides a less harsh pretreatment for antigen retrieval, avoids tissue damage, and reduces non-specific background staining. Prolonged formalin fixation of tissue samples results in a considerable reduction of PrP immunoreactivity. This effect might be overcome by modifying the concentration of detergents and/or autoclaving time without causing significant tissue damage.

In conclusion, we have demonstrated that clusterin expression was increased in association with abnormal PrP deposits not only in the CNS [13] but also in the peripheral lymphoreticular system. The observed co-localization and correlative expression of these proteins implies that clusterin might have an important role in PrP pathogenesis in the TSEs, perhaps as a chaperone-like molecule. In keeping with this suggestion, Kempster and associates reported that clusterin-deficient mice inoculated i.p. with mouse-passaged BSE agent had an increased incubation time in comparison with wild-type mice [31]. Thus clusterin might influence the accumulation and/or aggregation of PrP on FDCs, and affect disease progression. Comparative transmission studies using clusterin-deficient mice inoculated with a variety of TSE agent strains by various peripheral routes of exposure will provide further insights into the role of clusterin in TSE pathogenesis.

Acknowledgements

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Case Report

Increased asymmetric pulvinar magnetic resonance imaging signals in Creutzfeldt–Jakob disease with florid plaques following a cadaveric dura mater graft

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A 9-year-old Japanese girl received a cadaveric dura mater graft during surgery following a head injury with brain contusion. She continued to do well, but when she became 19-years-old, she gradually showed a violent character and was treated in a psychiatric hospital. Another 6 years later, 200 months after the procedure, she developed a progressive gait ataxia, which subsequently led to her death within 10 months of onset. An autopsy showed she had CJD. This patient represents an atypical case of dura-associated CJD (dCJD) with unusual clinicopathological features including the late occurrence of myoclonus, an absence of periodic synchronous discharges in the electroencephalogram, and the presence of widespread florid plaques. However, our detection of an asymmetrical increase in the MRI-derived images of pulvinar nuclei has not been previously observed in other atypical cases of dCJD. Because atypical dCJD cases share several clinicopathological features with those of vCJD, and because asymmetrical hyperintense signals in the pulvinar have been observed in some neuropathologically confirmed vCJD cases, we had some difficulty in a differential diagnosis between atypical dCJD and vCJD. This is the first atypical dCJD case showing a pulvinar high signal compared with all other basal ganglia on MRI.

Key words: CJD, dura graft, MRI, pulvinar sign, vCJD.

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INTRODUCTION

Most dura-associated CJD (dCJD) cases have similar clinicopathological features to sporadic CJD (sCJD), presenting progressive mental deterioration, ataxia, myoclonus and characteristic EEG findings such as periodic discharges.¹ However, some patients with atypical dCJD exhibit clinicopathological features distinct from those of most dCJD cases have some resemblance to vCJD cases; that is, a slow progressive clinical course with the absence or late occurrence of periodic discharges on EEG, and the presence of widespread florid plaques in addition to widespread spongiform change, neuronal loss and astrogliosis.^{2–8} On the other hand, the radiological features are different between atypical dCJD and vCJD cases. While vCJD cases present pulvinar high signals compared to all other basal ganglia,^{9,10} atypical dCJD cases have not been reported to show such hyperintensity in pulvinar nuclei.^{2–8} Moreover, the pathological features of the pulvinar nuclei are also different between atypical dCJD and vCJD cases. Here, we report an atypical dCJD case presenting asymmetrical pulvinar hyperintensity on MRI with different pathological characteristics of the pulvinar nucleus from those of reported atypical dCJD and vCJD cases.

CLINICAL SUMMARY

In November 1985, a 9-year-old Japanese girl received a cadaveric dural graft (Lyodura, B Braun Melsungen AG, Germany) at left frontotemporal region following a head injury with brain contusion. Six months later, she had a seizure and anti-epileptic drugs were administered. However, she did not take anti-epileptic drugs regularly, and epilepsy

frequently occurred. From 1995, she gradually developed a violent character. She was diagnosed as suffering a post-traumatic psychosis and received treatment in a psychiatric hospital. In June 2002, 200 months after the procedure, she began to develop an ataxic gait and became bed-ridden. By January 2003, she gradually showed memory disturbance and lost her spontaneous speech and was unable to communicate. She also exhibited myoclonic jerks in her upper limbs. She frequently suffered from aspiration pneumonia, and was brought to our hospital for respiratory insufficiency on 9 April 2003. On admission, she was akinetic and mute with decorticate posturing. There was no apparent history of depression, anxiety, apathy or delusions. Neurological examination showed moderate rigidity in bilateral upper and lower extremities, and myoclonus in bilateral upper extremities. The light reflex, corneal reflex and oculocephalic reflex were all normal. The motor and sensory systems could not be examined in detail. Deep tendon reflexes were normal. There was slow activity without any periodic discharge in her EEG. One week before her death, T2-weighted and proton density-weighted brain MRI showed significant hyperintensity in the thalamic pulvinar nuclei, which was predominantly on the left side, and moderate hyperintensity of the basal ganglia (Fig. 1A,B). Diffusion-weighted and gadolinium(III)-diethyltriaminepentaacetic acid (Gd-DTPA)-enhanced MRI revealed hyperintense signals in the pulvinar (Fig. 1C,D). The brain MRI also presented hyperintense signal by T2-weighted and proton density-weighted images and hypointense signal by diffusion-weighted and Gd-DTPA-enhanced images at the left frontal lobe, corresponding to where the brain contusion existed. In addition, Gd-DTPA enhanced images revealed hyperintense signals at the margin of the brain contusion. Based on the findings of the brain MRI, the diagnosis of dCJD was suspected. However, her respiratory insufficiency progressively worsened and she developed septic shock. Therefore, we could not assess further examination including the cerebrospinal fluid level of the 14-3-3 protein. She died on 19 April, 2003, 10 months after the onset of ataxic gait. The patient's family gave informed consent for the genetic and postmortem studies.

PATHOLOGICAL FINDINGS, IMMUNOBLOTTING AND GENE ANALYSIS

On examination, her brain weighed 1120 g, and there was a large defect in the left basal forebrain. While cerebral atrophy was not obvious, there was substantial atrophy of the cerebellum. The right side of the half-brain was deep-frozen for biochemical examination, and the other half was fixed in buffered formalin. Microscopically, most cerebral

cortices had mild to moderate spongiform changes, neuronal loss, and astrocytosis with a predilection for the deep cortical layers. In contrast, the cingulate gyrus and insular cortex exhibited moderate to severe pathological changes throughout the whole layers. The putamen showed moderate spongiform change, neuronal loss and astrocytosis. Anterior nucleus of the thalamus presented mild spongiform change and neuronal loss, and moderate astrocytosis. Ventral lateral nucleus and dorsomedial nucleus of the thalamus displayed mild spongiform change, moderate neuronal loss and astrocytosis. Pulvinar nucleus of the thalamus exhibited the most severe neuronal loss and spongiform change, and greater astrocytosis than the cerebral cortices (Table 1). The hippocampus was almost completely spared. In the cerebellum, moderate atrophy of the molecular layer was associated with mild spongiform change and astrocytosis, while moderate neuronal loss of the granule layer and severe neuronal loss of the dentate nucleus was associated with moderate astrocytosis. The Purkinje cells were almost completely preserved. In HE sections, we frequently noted Kuru plaques in a number of regions of the cerebral cortex, the putamen and the pulvinar nucleus of the thalamus. The cores of the plaques were stained with PAS, and, when stained with Congo red, appeared apple green in polarized light. In addition, many of the Kuru plaques were surrounded by a zone of vacuolar change, consistent with florid plaques, which are known hallmarks of vCJD. Although the pulvinar nucleus of the thalamus presented frequent Kuru plaques, there were only a few plaques in anterior, ventral lateral and dorsomedial nuclei of the thalamus. Immunohistochemistry for PrP revealed widespread synaptic staining and numerous plaque-like deposits, including florid plaques in all areas of the cerebral cortices, putamen, pulvinar nucleus of the thalamus and white matter of the cerebellum. The globus pallidus, the anterior, ventral lateral and dorsomedial nuclei of the thalamus, and molecular layer of the cerebellum mainly showed synaptic staining associated with mild granular prion protein depositions. In addition, there were PrP deposits surrounding blood vessels, neuronal cell bodies and processes in the cerebral cortex and in the pulvinar nucleus (Fig. 2). The region around the head injury with brain contusion showed mild spongiform change with severe astrocytosis. Only a few Kuru plaques were found in this region (Table 1).

The sequence analysis of prion protein gene (*PRNP*) derived from the brain tissue showed no mutation with homozygous for methionine at codon 129 or for glutamate at codon 219, which are polymorphic sites of the *PrP* gene. Immunoblotting for the abnormal prion protein in the brain demonstrated a type 1 prion protein glycoform pattern (Parchi's classification)¹¹ (Fig. 3). The final diagnosis was atypical dCJD.

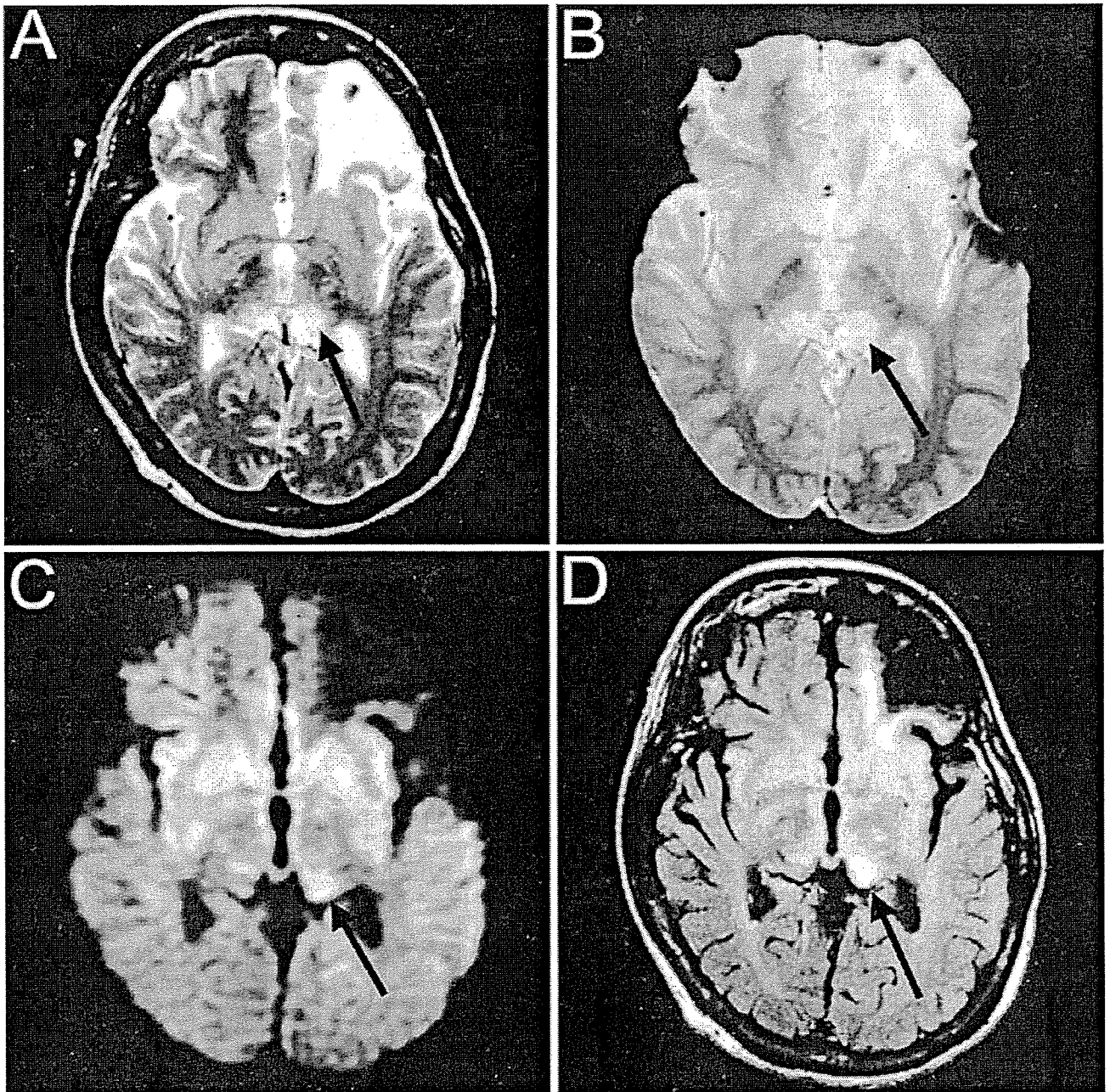


Fig. 1 Neuroradiological images. (A) T2-weighted image, (B) proton density-weighted image, (C) diffusion-weighted image, and (D) gadolinium(III)-diethyltriaminepentaacetic acid (Gd-DTPA)-enhanced brain MRI obtained 1 week prior to death. The images reveal hyperintensity in the pulvinar of the thalamus, and is more apparent in the left side (arrows). Images also show intense bilateral signals in the caudate nuclei and putamen.

DISCUSSION

We present the first atypical dCJD case to show a high pulvinar signal compared with all other basal ganglia on MRI, prominent lesions with severe spongiform changes,

neuronal loss and numerous florid plaques in the posterior thalamus at autopsy.

Most dCJD cases take the form of typical sCJD.¹ Most dCJD cases are clinically evident as a progressive mental deterioration, ataxia and myoclonus. Periodic discharges

are often noted in the EEG before the terminal stage of the clinical course. The disease often progresses rapidly and the clinical course lasts less than 2 years. MRI of dCJD cases has previously shown either normal or non-specific and diffuse brain atrophy.¹² T2-weighted MRI has also shown sCJD cases frequently exhibit symmetrical and hyperintense changes in the putamen and caudate head compared with the thalamus and cerebral cortex.^{13,14} In cases of sCJD, high-intensity signals in the cerebral and cerebellar cortex are also occasionally seen in fluid-attenuated inversion recovery, proton density-weighted and diffusion-weighted MRI-derived images.¹³ Pathologically, the cerebral cortex and cerebellum of dCJD cases are substantially affected. Severe spongiform changes, astrocytosis and neuronal loss are found throughout the brain and cause substantial atrophy, resulting in brain weights of less than 1000 g. Immunohistochemistry reveals diffuse synaptic type deposition of PrP. None or few amyloid plaques are found in the brain.

However, among more than 110 alleged dCJD cases,¹⁵ there have been some atypical dCJD patients with clinicopathological features which are different from those of sCJD and most dCJD cases but share several features with those of vCJD.²⁻⁸ These atypical dCJD and vCJD cases show a slowly progressive clinical course reaching the state of akinetic mutism. The reported duration of atypical dCJD were ranged 5–24 months.²⁻⁸ This duration resembled cases of vCJD, whose duration ranged 8–38 months.¹⁶

In addition, periodic discharges on EEG were commonly absent in both atypical dCJD and vCJD cases. On the other hand, there are several different clinical features between these cases. While most atypical dCJD cases present initially with ataxia and mental deterioration, such as disorientation or memory disturbance often following the ataxia,²⁻⁸ many vCJD cases initially suffer from sensory symptoms including persistent limb pain and/or persistent psychiatric symptoms as depression, anxiety, apathy and withdrawal.¹⁶ Ataxia was reported to develop about 6 months after the onset of psychiatric and sensory symptoms.¹⁶ Moreover, although myoclonus is absent or occurred at the end stage of the disease in atypical dCJD cases, involuntary movements including myoclonus are commonly noted during the clinical course of vCJD cases.¹⁶ The duration of the disease of our case and the absence of periodic discharges on EEG were similar to those features of atypical dCJD and vCJD cases. On the other hand, because our case presented progressive cognitive impairment after the onset of ataxia, and because myoclonus was absent until the end stage of the disease, the clinical course of our case more resembled those of atypical dCJD than vCJD cases.

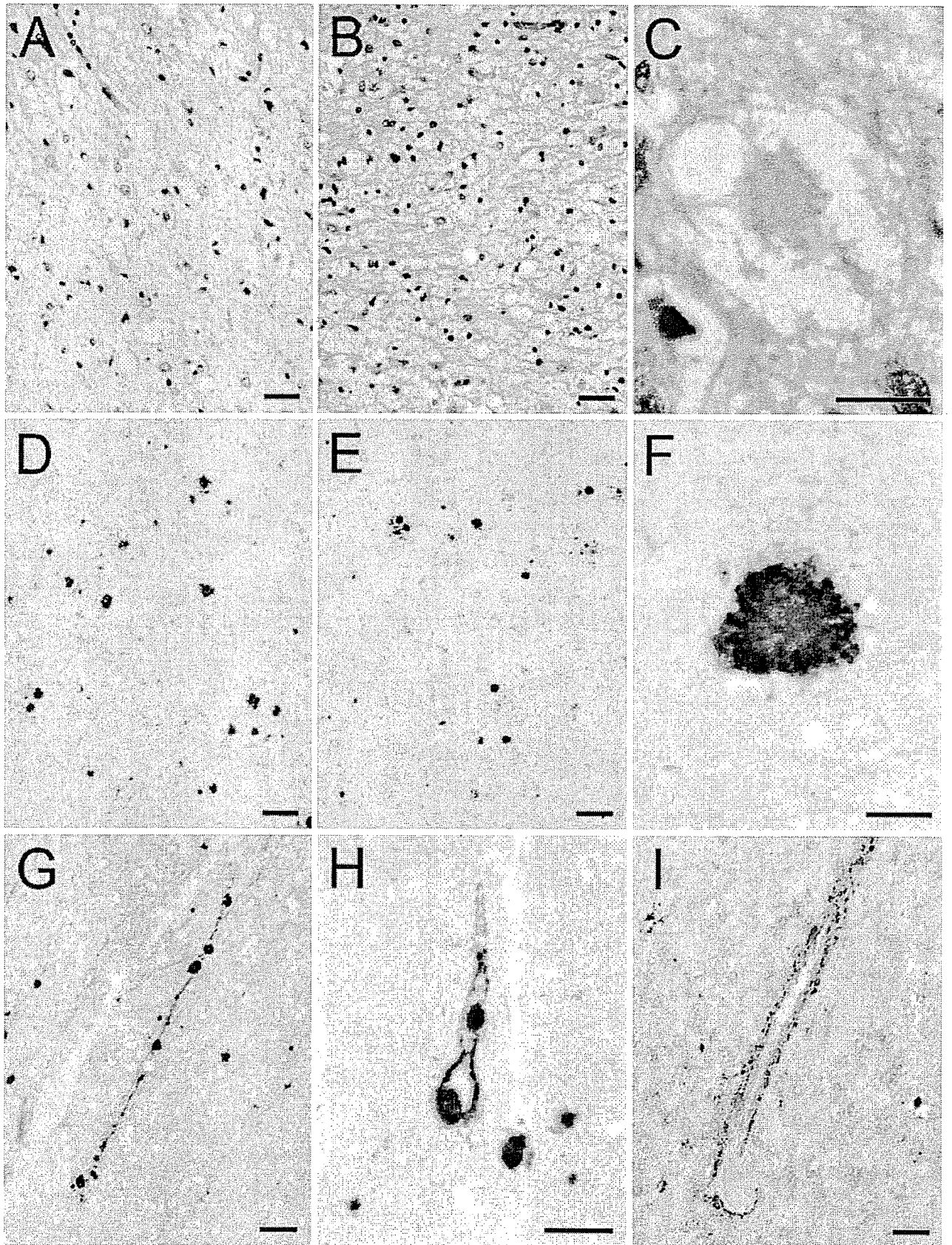
Pathologically, most atypical dCJD and vCJD cases show widespread spongiform change, neuronal loss and astrocytosis. These pathological changes are mild in the cerebral cortex and severe in caudate nucleus, putamen and cerebellum in both atypical dCJD and vCJD cases.²⁻⁸

Table 1 Neuropathological findings of left side of the half brain

| | Spongiform change | Neuronal loss | Astrocytosis | Kuru plaques |
|-----------------|-------------------|---------------|--------------|--------------|
| MFG | +++ | + | ++ | +++ |
| MTG | + | + | ++ | ++ |
| IPG | + | + | ++ | + |
| PVC | + | + | + | ++ |
| Cingulate gyrus | +++ | ++ | ++ | ++ |
| Insular cortex | ++ | ++ | ++ | +++ |
| Hippocampus | - | - | - | - |
| Globus pallidus | + | + | + | - |
| Putamen | ++ | ++ | ++ | ++ |
| Thalamus AN | + | + | ++ | + |
| DMN | + | ++ | ++ | + |
| VLN | + | ++ | ++ | + |
| PN | +++ | +++ | +++ | +++ |
| Contusion area | + | | +++ | + |
| Cerebellum ML | + | | + | - |
| PCL | | - | | |
| GL | | ++ | ++ | |
| WM | | | ++ | + |
| DN | | +++ | ++ | - |

MFG, middle frontal gyrus; MTG, middle temporal gyrus; IPG, inferior parietal gyrus; PVC, primary visual cortex of occipital lobe; AN, anterior nucleus; DMN, dorsomedial nucleus; VLN, ventral lateral nucleus; PN, pulvinar nucleus; ML, molecular layer; PCL, purkinje cell layer; GL, granular layer; WM, white matter; DN, dentate nucleus.

Spongiform change and neuronal loss are absent (-), mild (+), moderate (++) , severe (+++) on HE sections. Astrocytosis and Kuru plaques are absent (-), mild (+), moderate (++) , severe (+++) on sections of immunohistochemistry probed with an anti-GFAP antibody (polyclonal, DAKO, Glostrup, Denmark) and antiprion protein antibody (monoclonal, clone 3F4, Senetek, Maryland Heights, MO, USA), respectively.



The florid plaques, which are the pathological hallmark of vCJD, are also found in atypical dCJD cases, although they are more abundant in number in vCJD than atypical dCJD cases. These florid plaques distribute widely in the cerebrum and cerebellum but are only occasionally seen in the thalamus in both CJD cases. However, the pathological features of the thalamus are different between these CJD cases. In atypical dCJD cases, spongiform change, neuronal loss and astrocytosis were reported to be intense especially in anterior and medial nuclei.⁵ In vCJD cases, while spongiform change is focally seen in anterior and medial thalamic nuclei and pulvinar nuclei are relatively spared from spongiform change, there is extensive neuronal loss with marked astrocytosis in the pulvinar nuclei.¹⁷ In our case, the neuronal loss and astrocytosis were markedly noted in pulvinar nucleus rather than anterior and medial nuclei. Thus, because the pulvinar nucleus of our case presented marked neuronal loss and astrocytosis as well as severe spongiform change and numerous florid plaques, the pathological features of our case was considered to be distinct from those of reported atypical dCJD and vCJD cases.

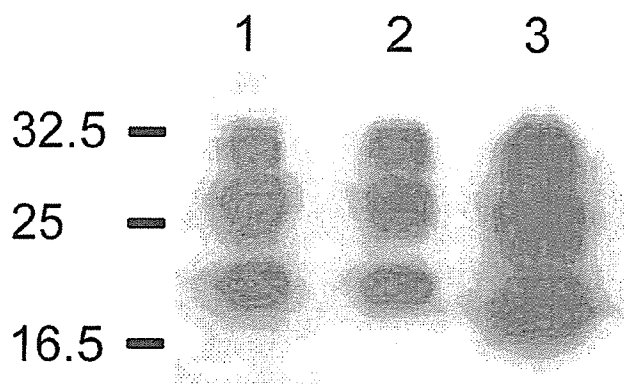


Fig. 3 Immunoblotting. Lane 1, positive control showing type 1 PrP glycoform pattern; lane 2, present study; lane 3, positive control showing type 2 PrP glycoform pattern. The abnormal PrP molecules in the brain homogenates of this case (lane 2) exhibit a type 1 PrP glycoform pattern. Molecular sizes (kDa) are indicated on the left.

Pulvinar increased signals greater than all other basal ganglia on T2-weighted, proton density-weighted, and diffusion-weighted MRI found in our case were unique features and have not been observed in other atypical dCJD cases. The hyperintensity of these images is thought to correlate with the astrocytosis and neuronal loss observed in histological examination.⁹ On the other hand, the Gd-DTPA enhancement of the pulvinar nuclei has not been reported in any type of CJD cases. Because the left frontal lesion of the contusion as well as the pulvinar nuclei were enhanced with Gd-DTPA, and because the increased pulvinar signals were asymmetrical and more intense on the left side, these hyperintense signals might reflect damage of the pulvinar nuclei due to a contusion in the left basal forebrain. However, although the thalamus has direct reciprocal connection to the cerebral cortices, the pulvinar nucleus make connections not with the frontal lobe but with the occipital cortex including the striate cortex.¹⁸ In addition, the common pathological findings of the Gd-DTPA-enhancing lesions, the left pulvinar nucleus and the rim of the contusion in the left basal forebrain, was severe astrogliosis. While Gd-DTPA enhancement indicates vascular leakage in general, Gd-DTPA enhancement associated with astrogliosis has also been reported.¹⁹ Therefore, although the precise reason was not clear, we considered that the increased pulvinar signals of our case would reflect pathological changes including severe astrogliosis caused not by traumatic brain injury but by CJD.

The marked pulvinar hyperintensity compared with all other basal ganglia recognized in our case raised the concern of vCJD because the asymmetrical pulvinar hyperintensity has previously been detected in some neuropathologically confirmed vCJD cases.²⁰ However, the radiological hallmark of vCJD, the pulvinar sign, is defined as symmetrical hyperintensity. Thus, the lack of symmetry in our case was not identical to the radiological features of vCJD cases. In addition, the proposed diagnostic criteria excluded the classification of our case as vCJD after possible iatrogenic exposure.¹⁶ Moreover, the PrP glycoform pattern of our case was type 1 according to Parchi's classification, and was identical to those of reported atypical dCJD cases but was completely different from that of vCJD.¹

Fig. 2 Verification of abnormal PrP deposition by histological examination. (A–C) HE staining. (D–I) Immunohistochemistry probed with an anti-prion protein antibody (monoclonal, clone 3F4, Senetek, Maryland Heights, MO, USA). Severe spongiform change, neuronal loss, and astrocytosis occurred in the left middle frontal lobe (A) and left pulvinar nucleus of the thalamus (B). (C) A prion protein plaque is surrounded by spongiform changes, to give the appearance of a florid plaque in the pulvinar nucleus. There are many prion plaques in the left middle frontal lobe (D) and in the left pulvinar nucleus of the thalamus (E). (F) The core of the florid plaque is intensely immunostained. (G) Unique prion protein depositions are linearly aligned with the neuronal axon in the left cingulate gyrus. (H) Prion protein deposition occurred around the neuronal cell body and process in the left cingulate gyrus. (I) Granular prion protein deposits are present around the wall of a blood vessel in the left pulvinar nucleus of the thalamus. Bars (A,B,D,E,H) 30 μ m, (C,F) 15 μ m, (G,I) 60 μ m.

Therefore, we concluded our case to be atypical dCJD with radiological and pathological characteristics distinct from those of any dCJD cases.

ACKNOWLEDGMENTS

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Original Article

Brain stem lesions in sporadic Creutzfeldt–Jakob disease: A histopathological and immunohistochemical study

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Lesions of the brain stem in sporadic CJD were histopathologically and immunohistochemically investigated using an anti-PrP antibody on ten consecutive autopsy cases. Three major histopathological changes, spongiform changes, neuronal loss and hypertrophic astrocytosis, were employed as parameters of the alterations. The quadrigeminal plate and pontine nuclei were the most severely and consistently affected structures, and immunoreactivity against PrP was seen in these structures. There existed some discrepancies between the severity of the lesions and the intensity of the immunoreactivity against PrP. The medulla oblongata essentially remained normal on histopathological examination, but the inferior olivary nucleus showed prominent PrP deposition. Although the general view that pathological alterations in the brain stem are relatively mild in sporadic CJD was confirmed in this study, lesions of variable degrees which might influence a patient's clinical course were still observed in many structures in the brain stem.

Key words: brain stem, CJD, histopathology, immunohistochemistry, PrP.

INTRODUCTION

The principal neuropathological features seen in a brain affected by sporadic CJD include spongiform changes of the neuropil, loss of neurons, proliferation of hypertrophic astrocytes and, in a subset of cases, the formation of amyloid plaque.^{1,2} These changes are most prominent in the

neocortex of the cerebrum and are also found in various degrees in the basal ganglia and thalamus. In many cases, the cerebellar cortex is also severely affected and, especially in the panencephalopathic type, the white matter of the cerebrum exhibits remarkable pathological alterations.³ On the other hand, it is believed that pathological alterations in the brain stem below the level of the mesencephalon are mild in sporadic CJD, although involvement of the pontine base or inferior olivary nucleus has been occasionally described.^{1,3–5} This is in contrast to other human prion diseases, such as kuru,^{6,7} and also prion diseases of animals, such as scrapie,⁸ and bovine spongiform encephalopathy (BSE),⁹ in which the brain stem is consistently and severely affected by similar pathological processes.

There exist few reports which detail brain stem lesions in sporadic CJD.¹⁰ We investigated such lesions by histopathological and immunohistochemical methods using an anti-PrP antibody on consecutive autopsy cases of sporadic CJD.

MATERIALS AND METHODS

From the autopsy files of the departments of pathology of the Osaka Red Cross Hospital and the National Cardiovascular Center, ten consecutive autopsy cases of sporadic (non-familial and non-iatrogenic) CJD were retrieved. No case of the variant CJD^{11,12} was investigated. A summary of the clinicopathological findings of these cases is presented in Table 1. Analysis of the *PrP* gene had been only performed for the recently autopsied cases (cases 8–10), and in all of these the gene was the wild type and codon 129 was homozygous for methionine.

In all cases, the brain had been routinely examined after formalin fixation. Representative sections taken from many regions of the cerebrum and cerebellum were

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Table 1 Clinicopathological findings of the cases studied

| Case | Age (years) | Gender | Duration (months) | Brain weight (grams) | Prion typing |
|------|-------------|--------|-------------------|----------------------|--------------|
| 1 | 70 | F | 20 | 830 | |
| 2 | 77 | F | 7 | 1110 | |
| 3 | 59 | M | 24 | 850 | |
| 4 | 75 | F | 35 | 900 | |
| 5 | 66 | M | 45 | 860 | |
| 6 | 67 | M | 18 | 1010 | |
| 7 | 71 | M | 16 | Unknown | |
| 8 | 71 | M | 7 | 1140 | MM1 |
| 9 | 61 | F | 7 | 900 | MM1 |
| 10 | 56 | F | 16 | 790 | MM1 |

reviewed, and the neuropathological diagnosis of sporadic CJD was confirmed. Each brain stem had been cut in planes vertical to the long axis in all cases, and tissue sections had been taken from four to six different levels extending from the rostral mesencephalon to the caudal medulla oblongata. Paraffin sections had then been stained with HE, luxol fast blue-periodic acid-Schiff, modified Bielschowsky, and Nissl stains. We evaluated the lesions of the gray matter of the brain stem employing the following three histopathological parameters:^{1,2} (i) spongiform changes of the neuropil; (ii) loss of neurons; and (iii) the proliferation of hypertrophic astrocytes. Other specific pathological alterations were also recorded. Because there was a subtle difference in the examined levels of sections between the cases because of the retrospective nature of this study, only easily identifiable, distinct nuclear groups were selected for evaluation. Changes in the white matter of the brain stem were excluded, because they were considered to largely represent secondary alterations caused by lesions in the cerebral or cerebellar cortex.

In eight cases for which preserved paraffin blocks were available, sections were recut and immunohistochemical investigations were performed using a monoclonal antibody against PrP (clone 3F4, DakoCytomation, Glostrup, Denmark; 1:100) and by employing the Envision Plus detection system (DakoCytomation) after pretreatment of the sections by hydrolytic autoclaving in 1.5 mmol hydrochloric acid at 121°C for 10 min.¹³ Paraffin sections of the medulla oblongata were available only for cases 7–10.

The severity of individual lesions and the intensity of the immunohistochemical reactions were evaluated using the four-tiers grading system: (–) absent or negative; (±) equivocal or very weakly positive; (+) definitely present or positive; and (++) severe or intensely positive.

RESULTS

For the overall neuropathological features of the cerebral hemispheres and the cerebellum, in all cases the brain showed gross atrophy, and the thinning of the cerebral cor-

tex was marked. The cerebellum also exhibited severe cortical atrophy along with the marked depopulation of granule cells and thinning of the molecular layer. No amyloid plaque was observed in any case. The white matter of the cerebral hemispheres showed diffuse myelin pallor and axonal loss. In addition, peculiar, localized spongiform changes of the subcortical white matter, which is a characteristic of panencephalopathic type CJD,³ were noted in cases 9 and 10.

On gross examination, the mesencephalon and pons showed diffuse atrophy of mild to moderate degree in every case, and the pontine base had lost its bulge on the ventral surface. On the other hand, the medulla oblongata appeared almost normal on macroscopic observation. A summary of the brain stem lesions is presented in Table 2.

Mesencephalon

The superior and inferior colliculi were the most distinctly and consistently affected sites among the structures present in the mesencephalon. Spongiform changes were seen chiefly in the superficial layers, and neuronal loss and astrocytosis were found diffusely throughout these structures (Fig. 1). The periaqueductal gray matter was also involved, but less distinctly than in the quadrigeminal plate, and neuronal loss was not apparent. In case 1, a few senile plaques were observed in this region. Neurons of the oculomotor nucleus were well preserved, but spongiform changes were noted in cases 9 and 10. Of special interest, multiple large vacuoles were occasionally found in the neuronal perikarya in the oculomotor nucleus of these two cases (Fig. 2). In another case (case 3), similar vacuoles in the perikarya were found in several pigmented neurons of the substantia nigra. Pathological alterations in the red nucleus and reticular formation were mild except for case 9, in which spongiform changes and astrocytosis were noted to a moderate degree. In the substantia nigra, neuronal loss to a mild degree was noted in the zona compacta in most cases. Spongiform changes were clearly observed in four cases, and a few “foamy spheroids” were

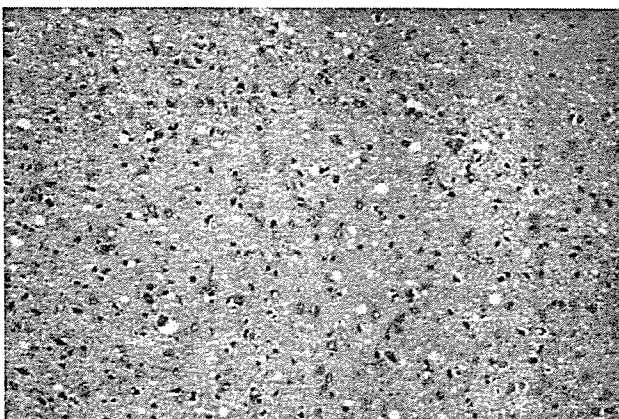
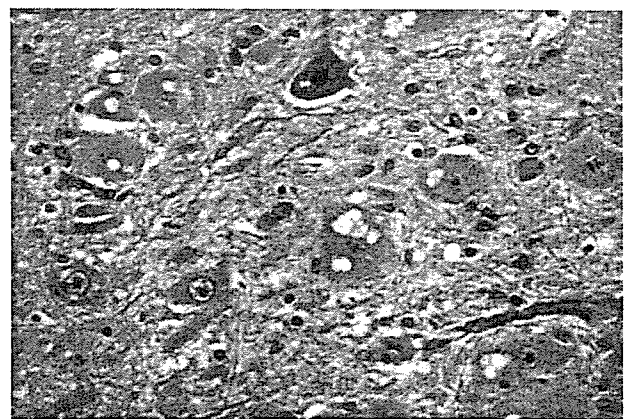
Table 2 Summary of the histopathological and immunohistochemical findings of the brain stem

| | | Case 1 | | | Case 2 | | | Case 3 | | | Case 4 | | | Case 5 | | | | | | | |
|---------------------|----------------------------|--------|----|----|--------|----|----|--------|----|----|--------|-----|----|--------|----|-----|----|----|----|----|----|
| | | SC | NL | AS | PrP | SC | NL | AS | SC | NL | AS | PrP | SC | NL | AS | PrP | | | | | |
| Mesencephalon | Superior colliculus | - | + | ++ | + | NE | NE | NE | - | ± | ++ | - | NE | NE | NE | NE | + | ± | + | + | |
| | Inferior colliculus | NE | NE | NE | NE | + | ± | + | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE |
| | Periaqued. gray matter | - | - | + | ± | + | - | - | - | - | + | - | NE | NE | NE | NE | ± | - | ± | + | |
| | Oculomotor nucleus | - | - | + | - | ± | - | - | - | - | - | - | NE | NE | NE | NE | ± | - | ± | ± | |
| | Red nucleus | - | - | + | - | NE | NE | NE | - | - | + | - | ± | - | + | - | - | - | ± | - | |
| | Reticular formation | - | - | + | + | ± | - | ± | - | - | + | - | NE | NE | NE | NE | - | - | ± | ± | |
| | Subst. nigra. Z. comp. | - | + | + | - | - | - | - | + | + | + | - | ++ | + | + | - | ++ | ± | ± | ± | |
| | Subst. nigra. Z. reticu. | - | + | + | - | - | - | - | + | + | + | - | NE | NE | NE | NE | ++ | ± | ± | ± | |
| Pons | Locus ceruleus | - | ± | + | + | NE | NE | NE | NE | NE | NE | NE | ± | ± | - | - | - | - | - | + | |
| | Raphe nucleus | - | - | - | + | NE | NE | NE | - | - | + | ± | ± | ± | - | - | - | - | - | + | |
| | Reticular formation | - | - | - | + | NE | NE | NE | - | - | + | - | ± | ± | - | - | - | - | - | + | |
| | Pontine nuclei | - | ++ | ++ | + | - | - | - | + | ++ | + | - | ++ | ++ | - | ++ | ++ | ++ | ++ | ± | |
| Medulla oblongata | Nucl. N. hypogloss. | - | ± | + | NE | - | - | - | - | ± | NE | - | - | + | NE | - | - | ± | NE | | |
| | Nucl. dors. n. trigem. | - | - | - | NE | NE | NE | NE | - | ± | NE | - | - | + | NE | - | - | ± | NE | | |
| | Nucl. tr. spin. n. trigem. | - | - | - | NE | - | - | - | - | ± | NE | - | - | + | NE | - | - | ± | NE | | |
| | Raphe nucleus | - | - | - | NE | NE | NE | NE | - | - | ± | NE | - | - | NE | - | - | ± | NE | | |
| | Reticular formation | - | - | ± | NE | - | - | - | - | - | ± | NE | - | - | + | NE | - | - | ± | NE | |
| | Olivary nucleus | - | - | + | NE | - | - | - | - | + | NE | - | - | + | NE | - | - | + | NE | | |
| Nucl. fasc. poster. | - | - | ± | NE | - | - | - | - | - | NE | - | - | + | NE | NE | NE | NE | NE | NE | | |

(continued)

| | | Case 6 | | | Case 7 | | | Case 8 | | | Case 9 | | | Case 10 | | | | | | |
|---------------------|----------------------------|--------|----|----|--------|----|----|--------|----|----|--------|-----|----|---------|----|-----|---|----|----|----|
| | | SC | NL | AS | SC | NL | AS | PrP | SC | NL | AS | PrP | SC | NL | AS | PrP | | | | |
| Mesencephalon | Superior colliculus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + |
| | Inferior colliculus | + | + | + | + | + | + | - | NE | NE | NE | NE | NE | NE | NE | NE | - | + | + | ++ |
| | Periaqued. gray matter | - | ± | + | + | - | + | + | + | - | + | ± | + | ± | + | + | - | - | + | + |
| | Oculomotor nucleus | - | - | - | - | - | - | - | ± | - | ± | ± | + | - | + | ± | + | - | - | + |
| | Red nucleus | - | - | + | - | - | - | - | ± | - | ± | ± | + | - | + | ± | - | - | - | - |
| | Reticular formation | - | - | + | + | - | + | - | ± | - | ± | ± | + | - | ± | ± | ± | - | - | ± |
| | Subst. nigra. Z. comp. | - | + | - | - | + | ± | - | ± | + | + | ± | - | - | ± | + | + | ± | ± | + |
| | Subst. nigra. Z. reticu. | - | + | + | - | + | ± | - | - | - | + | - | - | - | ± | + | + | ± | ± | + |
| Pons | Locus ceruleus | - | - | - | - | - | - | - | ± | - | ± | + | - | - | ± | + | - | - | - | + |
| | Raphe nucleus | - | - | - | - | - | - | + | - | - | + | + | ± | + | ± | - | - | - | - | + |
| | Reticular formation | - | - | - | - | - | - | ± | ± | ± | + | + | ± | + | ± | ± | - | - | - | + |
| | Pontine nuclei | - | + | + | - | + | + | ± | ± | ± | + | + | ± | ± | + | + | + | ++ | ++ | + |
| Medulla oblongata | Nucl. N. hypogloss. | - | - | - | - | - | - | - | - | - | - | ± | - | ± | - | - | - | - | - | + |
| | Nucl. dors. n. trigem. | - | - | - | - | - | - | - | - | - | - | ± | - | ± | - | - | - | - | - | + |
| | Nucl. tr. spin. n. trigem. | - | - | - | - | - | - | - | - | - | - | ± | + | - | + | - | - | - | - | + |
| | Raphe nucleus | - | - | - | - | - | - | - | - | - | ± | ± | - | ± | - | - | - | - | - | + |
| | Reticular formation | - | - | - | - | - | - | - | - | - | ± | ± | + | - | + | + | - | - | + | + |
| | Olivary nucleus | - | + | + | - | + | + | + | - | + | + | + | + | + | + | + | - | - | + | ++ |
| Nucl. fasc. poster. | - | - | - | - | - | - | - | - | - | + | - | + | - | + | - | - | - | - | - | ++ |

AS, astrocytosis; NE, not examined; NL, neuronal loss; PrP, prion protein; SC, spongiform change.

**Fig. 1** Spongiform changes of moderate degree associated with neuronal loss and proliferation of hypertrophic astrocytes in the superior colliculus (case 8, HE stain).**Fig. 2** Multiple large vacuoles found in the neuronal perikarya in the oculomotor nucleus (case 9, HE stain).

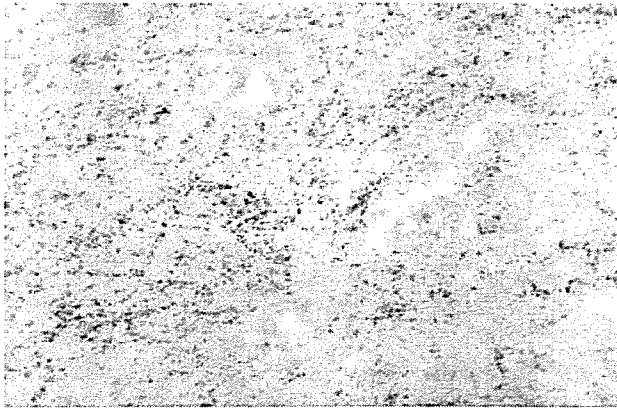


Fig. 3 Diffuse or synaptic type immunoreactivity against PrP in the neuropil of the superior colliculus (case 1, immunostain for PrP).

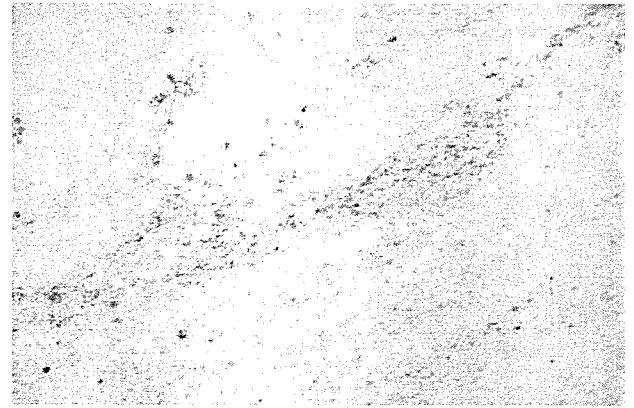


Fig. 5 Prominent deposition of PrP of diffuse or synaptic type in the pontine nuclei (case 8, immunostain for PrP).

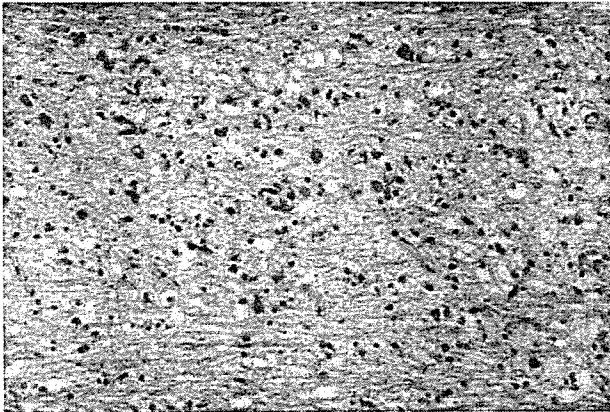


Fig. 4 Severe neuronal loss associated with spongiform changes and astrocytosis in the pontine nuclei. The sizes of the perikarya of the remaining neurons are reduced (case 10, HE stain).

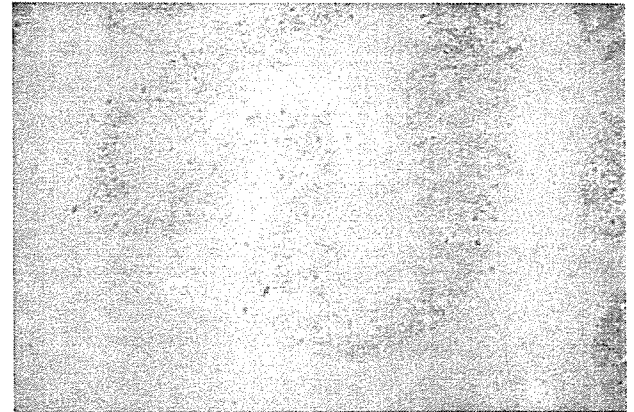


Fig. 6 Neurons of the inferior olivary nucleus were well preserved, but marked deposition of PrP was found in the neuropil (case 10, immunostain for PrP).

occasionally found in the zona reticulata, showing mild astrocytosis.

Diffuse or synaptic-type immunoreactivity against PrP was consistently found in the quadrigeminal plate (Fig. 3), while it was weak or absent in other regions of the mesencephalon, and it roughly paralleled neuronal loss and astrocytosis.

Pons

The histopathological alterations in the pons were almost restricted to the pontine base except for case 9, and the tegmentum was well preserved as a whole. In case 9, spongiform changes accompanied by astrocytosis were seen in the reticular formation and raphe nucleus without apparent neuronal loss. The pontine base was affected in nine of the

ten cases, and the loss of neurons of moderate to marked degree in association with hypertrophic astrocytosis was observed in the pontine nuclei (Fig. 4), while spongiform changes in the pontine nuclei were observed only in cases 5 and 10. The sizes of the perikarya of the remaining neurons in the pontine nuclei were reduced as a whole.

Diffuse or synaptic-type immunoreactivity against PrP was noted throughout all gray matter structures in the pons except in case 4, but was most prominent in the pontine nuclei (Fig. 5).

Medulla oblongata

The medulla oblongata was essentially histopathologically normal in all cases. In case 9, only mild spongiform changes accompanied by astrocytosis were noted throughout all

gray matter structures of the medulla oblongata, but no neuronal loss was observed in the tegmentum. The inferior olivary nucleus showed mild neuronal loss and astrocytosis in six cases, but these changes were considered most likely to be an age-related phenomenon and not of pathological nature. Axonal spheroids (dystrophic axon terminals) were consistently found in the gracile nucleus.

The results of anti-PrP immunohistochemistry, which was performed in four cases, were not anticipated based on the histopathological findings. Distinct, diffuse or synaptic-type immunoreactivity was noted throughout the inferior olivary nucleus in every case (Fig. 6). In addition, the tegmental structures showed diffuse immunoreactivity in case 10.

DISCUSSION

In sporadic CJD, the brain stem is comparatively spared from pathological alterations, and this is a characteristic of sporadic CJD,^{1,4,5} a condition which is distinct from kuru^{6,7} or prion diseases in animals such as scrapie⁸ and BSE⁹ in which the brain stem is severely affected. A previous western immunoblot analysis of the intracerebral distribution of infectious amyloid protein (PrP) in human spongiform encephalopathy revealed the almost complete absence of amyloid protein in the brain stem in sporadic CJD.¹⁴ On the other hand, in variant CJD spongiform changes are occasionally found in the periaqueductal gray matter and pontine nuclei, and neuronal loss with astrocytosis can be seen in structures such as the superior and inferior colliculi, periaqueductal gray matter, inferior olivary nucleus and dorsal vagal nucleus.¹² It is unknown at present why this difference in the distribution of lesions occurs among various kinds of prion disease. In sporadic CJD, the brain stem is less vulnerable or resistant to lesions caused by PrP. It is therefore intriguing to observe in full detail the pathological alterations of the brain stem in sporadic CJD, because progression of the disease might be slower in the brain stem than in the cerebral or cerebellar cortex. By close observation of the brain stem lesions, we might be able to determine the pathological changes in the early stages of the disease.

The results obtained in the present study supported the general view that the brain stem lesions are of mild degree in sporadic CJD. However, on closer examination, some specific and non-specific lesions of variable degree were found at various sites, and this is a phenomenon which is similar with the lesions seen in the hippocampus in sporadic CJD.¹⁵ We discuss some of the brain stem lesions observed and make brief comments on each topic below.

1 We adopted three histopathological parameters^{1,2} to evaluate the brain stem lesions, spongiform changes of the neuropil, neuronal loss and hypertrophic astrocytosis, but

they did not necessarily occur in parallel. While the proliferation of astrocytes was noted in almost all lesions, it did not accompany neuronal loss in some regions, and spongiform changes were not apparent in the brain stem in most cases except for in the quadrigeminal plate and substantia nigra. Whereas spongiform changes in CJD are generally regarded an early event which precedes neuronal loss and astrocytosis,⁴ astrocytosis was observed to occur before the appearance of spongiform changes in experimentally transmitted scrapie.¹⁶ The proliferation of hypertrophic astrocytes therefore might not simply be a secondary change. (It should be also noted that the intensity of immunoreactivity against PrP did not parallel the histopathological alterations. In many regions, for example the pontine tegmentum and inferior olivary nucleus, the deposition of PrP was noted without accompanying spongiform changes or neuronal loss. This might indicate that these brain stem structures are resistant to the pathological processes induced by PrP.)

2 In the mesencephalon, the superior and inferior colliculi are consistently vulnerable in sporadic CJD, and similar involvement of the quadrigeminal plate was previously noted by some authors.⁴ While the substantia nigra was affected in most cases, the changes were relatively mild. A few senile plaques were found in the periaqueductal gray matter in one case (case 1). Although the occurrence of amyloid plaques with a wide morphological spectrum has been previously documented in the mesencephalon of some cases of sporadic CJD,⁵ the plaques in our case were immunohistochemically negative for PrP and probably represented a senile change.

3 The formation of large vacuoles in the perikarya of neurons has previously been consistently observed in the brain stem, especially in the medulla oblongata, in BSE^{9,17} and scrapie,⁸ and is pathognomonic for these disorders. Perikaryal vacuole formation is not a prominent feature in the cerebral or cerebellar cortex in sporadic CJD. On the other hand, it is commonly observed in the brain stem in kuru.^{6,7} In the present study, this was seen in the oculomotor nucleus in two cases and in the substantia nigra in another case, without being accompanied by neuronal loss.

4 In the pontine base, although spongiform changes of the neuropil were found in only two cases, neuronal loss with astrocytosis of moderate to marked degree was noted in the pontine nuclei in most. The deposition of PrP was also observed in five of eight cases. It is certain that the pontine nuclei are sites which are consistently affected by the pathological processes that occur in sporadic CJD, and this is in accordance with the observations of Tateishi *et al.*⁵ Recently Iwasaki *et al.*¹⁰ also described gross atrophy of the pontine base, neuronal loss and prominent PrP immunoreactivity in the pontine nuclei in most cases of sporadic CJD, and similar results were also obtained for variant CJD.¹²

The sizes of the perikarya of the remaining neurons in the pontine nuclei appeared to be generally reduced in our series. Because the sizes of the neuronal perikarya show considerable regional variation within the pontine nuclei,¹⁸ a more strict quantitative study is needed to confirm this observation. Neurons of the pontine nuclei send their axons to the cerebellar granule cell layer and also receive many fibers from the broad regions of the cerebral cortex.¹⁸ Therefore, the possibility is considered that the atrophy of the neuronal perikarya in the pontine nuclei was partly caused by anterograde and/or retrograde trans-synaptic degeneration, as suggested by Tateishi *et al.*⁵

5 The medulla oblongata was preserved in an approximately normal state histopathologically except for one case. In thalamic type of CJD, severe degeneration of the inferior olivary nucleus constantly occurs.^{19,20} In the present series, no case of thalamic type CJD was included and degeneration of the inferior olivary nucleus was not noted. However, in spite of the absence of pathological neuronal loss, the deposition of PrP was consistently found in the inferior olivary nucleus.

In recent years, the variability of clinicopathological phenotypes in patients with sporadic CJD has been demonstrated to depend on a polymorphism at codon 129 of the *PrP* gene as well as a pattern on Western immunoblot analysis of PrP, and a new classification scheme based upon these data is widely being employed.²¹ Because the present study is a retrospective one that was performed on archival autopsy materials, the results of molecular analysis of PrP and its gene were not available for most of the cases. This is a major limitation of our study, and an investigation for the correlation of the molecular characteristics of PrP and pathological findings of brain stem lesions in sporadic CJD should be performed in future studies. At this stage, very limited inference can be made on the genotypes of *PrP* in the present series. Judging from the fact that in more than 90% of the Japanese population codon 129 of the *PrP* gene is homozygous for methionine (MM type) and also judging from the clinicopathological features of each patient in our series (periodic synchronous discharge was observed on electroencephalogram in all patients), it is supposed that most of our cases 1–7 are probably the MM1 type.

The relationship between the histopathological and immunohistochemical findings of the brain stem and the duration of the clinical course is another problem that remains to be elucidated. In the present series, the severity of the histopathological findings and the regional distribution of the immunohistochemical reactivity of PrP varied from case to case, and we could not find a clear correlation between the duration of the clinical course and these findings in the brain stem lesions.

In summary, we showed that in sporadic CJD pathological alterations of variable degree were observed in the

brain stem, in particular in the quadrigeminal plate and pontine nuclei, although spongiform changes were found only infrequently. The medulla oblongata did not show any significant histopathological lesions. The deposition of PrP was mainly observed in the quadrigeminal plate and pontine nuclei. We also noted this in the pontine tegmentum and inferior olivary nucleus, which appeared to be approximately normal by histopathological examination. The remaining problems include the relationships between these changes in the brain stem and the lesions in the cerebellum, the possibility of trans-synaptic degeneration of neurons in the pontine nuclei, the clinical significance of these brain stem lesions, and the reasons for the differences between the lesions of the brain stem in sporadic CJD and those in kuru or variant CJD.

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プリオン病の治療

—その現状と展望—

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プリオン病は、いまだ有効な治療法がない、発症すると確実に死に至る進行性の神経変性疾患である。現在、プリオン本体およびその感染のメカニズムについて急速に理解が進んでいるが、治療薬・治療法についてはいまだ有効なものが見出されていない。英国を中心に欧州で新たに出現した変異型クロイツフェルト・ヤコブ病は、現在小康状態にあるものの、血液製剤を介して二次感染の可能性も考えられている。プリオン病の発症前での診断・検査法、そして予防薬・治療薬の早急な開発が求められている。

はじめに

プリオン病あるいは伝達性海綿状脳症(transmissible spongiform encephalopathy; TSE)は進行性の神経変性疾患であり、有効な治療法が確立されていない現在、治療不可能の致死性の難病となっている。孤発性クロイツフェルト・ヤコブ病(Creutzfeldt-Jakob disease; CJD)を代表とするヒトプリオン病は、年間100万人に1人の割合で発症し、その多くのものは数週間から数ヶ月で急激に症状が進行し、死に至る。発症すると、脳内に海綿状の神経変性とともプリオンと呼ばれる感染性を持つ蛋白質(PrP^{Sc})の蓄積を生じる。ヒトプリオンが実験動物へと感染し得ることは以前よりよく知られていたが、1980年代に英国を中心に蔓延していた牛海綿状脳症(bovine spongiform encephalopathy; BSE)が、1996年、ヒトへも経口感染し、

新たなヒトプリオン病、変異型CJDを引き起こすことが報道されると、欧州全体を巻き込んだ大きなパニックを引き起こした。わが国でもBSE感染牛が2001年に発見され、2005年には英国旅行者の邦人1人が変異型CJDであることが確認された。また、米国や日本ではプリオンに感染した硬膜などの生体材料の移植によって医原性CJDが発生し、特にわが国では対策の遅れもあり、100人を超える症例が報告され、薬害ヤコブ病として大きな社会問題となっている。

現在、プリオン病に対する理解が進み、また感染防止対策がうまく機能していることもあり、BSEや変異型CJDの発生は小康状態あるいは減少へと転じている。しかし、発症していないにもかかわらず感染性を有する、いわゆるキャリアの存在と、血液製剤を介した変異型CJDの二次感染の例が報告され、今後、変異型CJDの発生が拡大していく可能性も出てきた。

Key words

プリオン病
クロイツフェルト・ヤコブ病
治療
予防

近年、プリオン病の基礎研究や治療薬のスクリーニング・検出技術に大きな進展があり、いくつかの薬剤についてはすでに臨床試験にて検討されているが、有効なプリオン病予防薬および治療薬の開発までには至っていない。

プリオン病とは

プリオン病は通常のウイルスや細菌の感染とは異なり、プリオンと呼ばれる蛋白質によって感染が引き起こされる¹⁾。プリオンはプロテアーゼや高熱に耐性を持つ、不溶性の異常感染型プリオン蛋白(PrP^{Sc})である。生体には同じアミノ酸配列からなる正常プリオン蛋白(PrP^C)と呼ばれる膜蛋白が存在する。これらの蛋白質の高次構造(コンフォメーション)は大きく異なり、PrP^{Sc}はβシートに富み、アミロイドと呼ばれる不溶性の多量体を形成するが、PrP^Cはαヘリックスに富む可溶性の単量体として存在する。ヒトにおいては、特定のアミノ酸置換や挿入を持つPrP^C変異体が知られており、PrP^{Sc}の感染の有無にかかわらずプリオン病を自然発症する(遺伝性プリオン病)、外科手術などの医療行為よりPrP^{Sc}に汚染された生体材料より感染する場合もある。しかし、ヒトプリオン病の8割は孤発性であり、その原因は不明である。動物実験では、感染体と同じアミノ酸配列を持つPrP^Cを導入することや、PrP^Cを過剰発現させることによって、感染しやすくなることが知られている。プリオン病は、人

獣共通感染症として知られるが、PrP^{Sc}と同じアミノ酸配列を持つPrP^Cを発現させることによって、異なる種間の感染をより簡単に成立させることも可能になっている。PrP^C、PrP^{Sc}以外の宿主因子の存在も指摘されているが、現在のところ不明である。PrP^Cの存在はプリオン感染の成立だけでなく、神経変性にも必須であり、感染に付随する神経変性の原因は脳、神経に蓄積するPrP^{Sc}というよりも、PrP^CからPrP^{Sc}へと構造変換する過程で生じるという説が有力である。

治療薬スクリーニング法の開発

プリオン病の治療薬の開発には、プリオン持続感染培養細胞を用いてPrP^{Sc}の産生量を測定するアッセイ法と、プリオンを接種したハムスターやマウスの潜伏期の長さを指標にしたアッセイ法が主に用いられている。最近になって、酵母プリオンを用いた方法や、*in vitro*で起こるPrP^CからPrP^{Sc}様のプロテアーゼ耐性産物を測定する方法も開発され、治療薬スクリーニングに利用されている。

培養細胞を用いる方法²⁾は、多数の検体を安価に迅速に処理することが可能である。最近では、サブクローニングによって高いプリオン感染能を持つ細胞を使用し、さらにメンブレンフィルターに転写することによって、多検体を迅速に定量的に測定できるように改良されている³⁾。しかし、培養細胞で認められた治療効果が、動物実験で

は必ずしも再現できない場合が多く、多検体からの一次スクリーニングとして使用されることが多い。

動物を用いたバイオアッセイは、感染価をエンドポイント希釈することによって発症を指標に測定する方法(IC₅₀)や、感染価と潜伏期の長さが反比例関係にあることを利用して感染価を測定する方法を用いて、感染価を推定することができる。これまで開発されたプリオン検出系の中で最も感度が良いことが知られているが、確定するのに時間と費用がかかることが欠点である。ハムスターを用いたバイオアッセイ⁴⁾は、プリオン仮説の提唱者である Prusiner によって採用され、プリオンの精製の際に使用された。このハムスターを用いたバイオアッセイは、最短60日で感染を検出できるバイオアッセイであったが、PrP^Cを過剰発現するトランスジェニックマウスの開発によって、ほぼ同等に潜伏期を短縮することに成功している。加えて、PrP^{Sc}と同じアミノ酸配列を有するPrP^Cを発現するように作製されたトランスジェニックマウスによって、種の異なるプリオン感染も再現できるようになった。また、腹腔内にプリオンを接種後、感染初期にPrP^{Sc}の蓄積が認められる脾臓中のPrP^{Sc}の産生を測定することによって、感染を30日で検出することも可能になっている⁵⁾。

その他に、[PSI+]や[URE3]という酵母プリオンを用いたスクリーニング系⁶⁾や、PrP^CとPrP^{Sc}の相互作用を蛍光相関分光法を用いて測定するハイスループットスクリーニング法⁷⁾や、