Creutzfeldt-Jakob Disease With Florid-Type Plaques After Cadaveric Dura Mater Grafting

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Background: Many reported cases of iatrogenic Creutzfeldt-Jakob disease (CJD) developed after grafting cadaveric dura mater contaminated with CJD prions (dura-associated CJD). They are known to be clinicopathologically similar to sporadic CJD. We report herein 2 autopsy cases of dura-associated CJD with atypical clinicopathological features.

Patients: Two patients presented with progressive ataxia and mental deterioration 10 or 11 years after neurosurgical treatment with cadaveric dural grafting, which led to their deaths at 8 and 17 months, respectively, after onset.

Results: The cases were clinically atypical in exhibiting no or late occurrence of myoclonus and periodic synchronous discharges on electroencephalographic studies. They were pathologically unique in several aspects. The most striking feature was the presence of many prion protein (PrP) plaques in multiple areas in the brain. Some of them were the "florid" type surrounded by a zone of spongiform changes known to be a hallmark for the new variant CJD. The distribution of spongiform degeneration was also unique in that it was intense in the thalamus, basal ganglia, and the dentate nuclei of the cerebellum but milder in the cerebrum. There were no mutations in the PrP gene of the patients. There was no major difference in the size and glycoform pattern between the abnormal isoform of PrP extracted from the brain tissue from the dura-associated cases of CJD and that from a sporadic case of CJD.

Conclusions: These 2 cases are clinicopathologically distinct from typical dura-associated cases of CJD. They may be a subtype with florid-type plaques in dura-associated CJD.

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REUTZFELDT-JAKOB disease (CJD) manifests as a sporadic, familial, or iatrogenically transmitted form. Iatrogenic transmission of CJD occurs via several routes that include the therapeutic use of human cadaveric pituitary-derived growth hormone or gonadotropin and dura mater or corneal grafting. Dura-associated CJD develops after cadaveric dural grafting and has been reported as clinicopathologically similar to sporadic CJD.\(^1\)\(^-\)\(^5\) It presents with progressive mental deterioration, ataxia, and myoclonus. Periodic activities are often recorded on electroencephalograms (EEGs) before the terminal stage in the clinical course. Disease progression is often rapid with the entire clinical course shorter than 2 years. Pathologically, the cerebral cortex and cerebellum are intensely affected, which results in a severe atrophy of the brain, while no or a few amyloid plaques are found in the brain. No mutations in the prion protein (PrP) gene are known to predispose one to or inhibit the occurrence of dura-associated CJD, while glutamate/lysine heterozygosity at polymorphic codon 219 could inhibit the occurrence of sporadic CJD.\(^6\)\(^,\)\(^7\)

We report herein 2 cases of dura-associated CJD. Although they are unique in several aspects, the most striking feature is the presence of florid-type plaques in the brain, known to be a pathological hallmark for the new variant CJD (nvCJD).\(^8\) This report may establish CJD with florid-type plaques as a clinicopathologically distinct subtype in dura-associated CJD.

RESULTS

CLINICAL COURSE

Case 1

A 68-year-old man visited a hospital in June 1996 because of unsteady gait that had started 1 month before. His medical history revealed that he had had left hemifacial spasm treated with a cadaveric dural...
**MATERIALS AND METHODS**

**PRNP GENOTYPE DETERMINATION**

Genomic DNA was extracted using standard techniques from white blood cells and brain tissues. The open reading frame of the PrP gene was amplified by polymerase chain reactions. The products were analyzed for insertion, point mutations, and polymorphisms at codons 129 and 219 by restriction fragment length polymorphism (RFLP) as described. To confirm the results obtained by the RFLP analyses, the amplified PrP gene were cloned and sequenced bidirectionally.

**TISSUE PREPARATIONS**

One half of each brain removed at autopsy from the 2 patients with dura-associated CJD and 1 with sporadic CJD with codon 129 M/M was immediately frozen and stored at −80°C. The remaining tissue was fixed in 10% formalin. Blocks of the fixed tissue were then immersed in 90% formic acid for 2 hours or less at room temperature to attenuate the infectivity and then embedded in paraffin. Five-micrometer-thick sections of the paraffin-embedded tissues were deparaffinized and stained with hematoxylin-eosin or used for PrP immunohistochemical analysis. The frozen tissues from the cerebral cortex from the 3 patients were used to prepare the samples for Western immunoblots. The brain from an nvCJD case was processed as described previously.

**PrP IMMUNOHISTOCHEMICAL ANALYSIS**

Paraffin sections were processed as described previously except that goat antirabbit immunoglobulins–peroxidase-labeled dextran polymer conjugate (Envision; Dako, Carpinteria, Calif) was used as the secondary antibody. Sections were probed with an anti-PrP polyclonal antiserum that was raised against synthetic polypeptides corresponding to the N-terminal residues 25-49 of human PrP.

**WESTERN IMMUNOBLOTS**

Purification and Western immunoblot analyses of proteinase-resistant PrP from brain tissue were performed as described. Proteins were separated on a 13% polyacrylamide gel. Anti-PrP monoclonal antibody 3F4 was used as a probe. For deglycosylation of proteinase-resistant PrP with peptide N-glycosidase F (PNGase F; Boehringer Mannheim GmbH, Tutzing, Germany), proteinase-resistant PrP denatured in Laemmli’s sample buffer was incubated overnight at 37°C in the reaction mixtures containing 8.8-mol/L Tris-hydrochloride (pH 7.6), 0.5% Triton X-100, 1 X protease inhibitor cocktail (Boehringer Mannheim), and 2 U/mL of PNGase F. Proteins in the reaction mixtures were precipitated by adding a methanol-chloroform mixture and then analyzed by Western immunoblot assays as described above.

**PATHOLOGICAL AND PrP IMMUNOHISTOCHEMICAL ANALYSES**

**Case 1**

The brain weighed 1260 g. Mild atrophy of the cerebellar hemisphere and mild dilatation of the third ventricle and cerebral aqueduct were noticed on inspection. The cerebral cortex was not atrophic. Microscopically, there was severe spongiform degeneration in the thalamus, especially in the anterior and medial nuclei, caudate/putamen, and the dentate nucleus of the cerebellum (Figure 1, A and B). It was associated with moderate to marked neuronal loss and astrocytic gliosis. There were moderate lesions in the hippocampus, globus pallidus, red nucleus, periaqueductal gray matter, pontine tegmentum, and cerebellar...
cortex, and milder ones in the cerebral cortex, substantia nigra, and inferior olivary nucleus. Marked degeneration was also observed in the cerebellar white matter and superior cerebellar peduncle. There were many plaques in the gray matter areas with spongiform changes and in the cerebellar white matter which stained intensely with anti-PrP antibody (Figure 2, A and B). Plaques in the cerebral cortex were often surrounded by a zone of spongiform changes, giving them a florid appearance. Prion protein plaques of this type were quite similar, morphologically and

Figure 1. Degeneration and prion protein (PrP) deposition in the brain tissue of patients with dura-associated Creutzfeldt-Jakob disease (CJD) with florid-type plaques. A, B, D, and E, Brain sections from the patient in case 1; C, section from the brain of the patient in case 2. A, Severe spongiform degeneration with astrogliosis in the putamen (original magnification x200). B, Marked neuronal loss and spongiform changes in the dentate nucleus of the cerebellum (original magnification x100). C, Deposition of PrP around neuronal cell bodies and processes (arrows) in the cerebral cortex (original magnification x200). D, Prion protein depositions of plaque type aligning linearly (arrowheads) or clustering around a vessel (arrow) in the cerebellar white matter (original magnification x179). E, Diffuse synaptic-type PrP deposition in the dentate nucleus of the cerebellum (original magnification x25). F, Prion protein synaptic-type deposition in the dentate nucleus of the cerebellum in a case of sporadic CJD (original magnification x42); note that intensity of staining is much weaker than in E. A and B, hematoxylin-eosin; C through F, PrP immunostain.
in their immunoreactivity to anti-PrP antibodies, to those found in the brain of a patient with nvCJD (Figure 2, C and D). Some plaques in the cerebellar white matter were found to align linearly or cluster around vessels (Figure 1, D). The synaptic type of diffuse deposition of PrP was also detected by PrP immunohistochemical testing in most gray matter areas, including the dentate nucleus of the cerebellum and brainstem nuclei (Figure 1, E). It was in contrast that the synaptic type of PrP staining was much weaker in the dentate nucleus of the cerebellum in a case of a sporadic CJD (Figure 1, F). Occasionally, PrP was found to deposit around neuronal cell bodies and processes in the gray matter, especially in the cerebral cortex.

Case 2

The brain weighed 1055 g. Cerebellar vermis was markedly atrophic. The brainstem was also mildly atrophic, but the other parts of the brain were not. Microscopically, spongiform degeneration was severe in the thalamus, especially in the anterior and medial nuclei, caudate/putamen, and cerebellar hemisphere and vermis; moderate in the hippocampus, globus pallidus, periaqueductal gray matter, and the dentate nuclei of the cerebellum; and milder in the cerebral cortex and inferior olivary nucleus. There were many plaques in the areas with spongiform changes, which stained with anti-PrP antibody. Some of them were of the florid type. Other abnormal findings revealed by PrP immunohistochemical testing included PrP plaques in the cerebellar white matter, which aligned linearly or clustered around vessels, synaptic type of diffuse PrP deposition in most gray matter areas in the brain, and PrP deposition around cell bodies and processes of some neurons (Figure 1, C).

PRNP GENOTYPE

The RFLP analyses revealed that both dura-associated CJD cases were homozygous for methionine (M/M) at codon 129 and for glutamate (E/E) at codon 219. No mutations in the PrP gene open reading frame were detected by the RFLP analyses or sequencing of 10 alleles from either case.

WESTERN IMMUNOBLOTS

The presence of proteinase-resistant PrP in samples from 1 case of sporadic CJD and 2 of dura-associated CJD was...
observed on Western immunoblots as 3 major bands (low, middle, and high glycoforms) at 20 to 32 kd (Figure 3, lanes 1-3). The apparent molecular sizes of proteinase-resistant PrP from all 3 cases were almost the same. For all 3 cases, the middle glycoform band gave the strongest signal while the high glycoform band gave the weakest signal among 3 major bands. Taken together, there was no major difference in the size and glycoform pattern of proteinase-resistant PrP between sporadic CJD and dura-associated CJD. Deglycosylated proteinase-resistant PrP from all 3 cases migrated as a single major band at 20 kd (Figure 3, lanes 4-6). Although critical typing of proteinase-resistant PrP using a series of control samples was not performed, the data suggested that deglycosylated proteinase-resistant PrP from cases of dura-associated CJD might be different in size from that from cases of nvCJD cases that had been shown to migrate faster than deglycosylated proteinase-resistant PrP from cases of sporadic CJD.13,14

**COMMENT**

We have presented 2 cases of dura-associated CJD with florid-type PrP plaques in the brain. There have been 61 reported cases of dura-associated CJD, including 43 reported recently by the Japanese National Committee for CJD.1-5 Although many of the reported cases were pathologically studied in detail, only 4 cases, including ours, have been found to have florid-type plaques (Table).15,16 Only 1 of the other cases was reported to have amyloid plaques in the brain.17 However, the morphology of plaques was not described in detail. In contrast, 3 of the 4 cases with florid-type plaques had numerous plaques in multiple areas in the brain while the distribution of plaques was unknown in the French case because only a prefrontal biopsy specimen was studied (Table).15 The plaques were stained with anti-PrP antibody in all 3 cases studied (Table). In addition, our 2 cases were unique in the distribution of the degenerative changes. The changes were intense in the thalamus, basal ganglia, dentate nucleus of the cerebellum, and the upper brainstem, but milder in the cerebrum, which is in agreement with an unusually widespread distribution of synaptic PrP staining that involves the deep cerebellar and brainstem nuclei. A relatively mild degeneration in the cerebrum was also suggested by normal radiographic findings in the French case15 and confirmed in the other Japanese case.
This idea may have some support in the clinical uniqueness of the 4 cases. It is highlighted by the absence or late occurrence of periodic synchronous discharges that have been frequently observed in dura-associated cases of CJD.\(^1,5\) Periodic synchronous discharges were recorded at the terminal stage of only 1 of the 4 cases with florid-type plaques.\(^1,5\) The latency after dura mater grafting might be relatively long (9-11 years).

Some of the features of the 4 cases, especially the presence of florid-type plaques, could be problematic in differentially diagnosing dura-associated cases of CJD with florid-type plaques from nvCJD. Other features that are shared by both conditions and that may hamper correct diagnosis include the absence or rareness of periodic synchronous discharges and myoclonus and more intense degeneration in the thalamus and basal ganglia relative to the cerebral cortex.\(^5,18\) The problem in diagnosis became real in the French case because nvCJD is endemic in France.\(^15\)

Convincing diagnosis was based on the typing of proteinase-resistant PrP that matched the French case with the type that is characteristic to sporadic or iatrogenic CJD, including dura-associated CJD, but not with the type that is unique to nvCJD.\(^2,4,10\) This finding was confirmed in our cases in which the size and glycoform pattern of proteinase-resistant PrP were similar to that of a case of sporadic CJD. It argues that the typing of proteinase-resistant PrP may be mandatory for the diagnosis of nvCJD as already pointed out.\(^19\) It is important and remains to be determined whether dura-associated cases of CJD are all homogeneous on the typing of proteinase-resistant PrP. There is some evidence that proteinase-resistant PrP accumulates in the lymphoid tissues in cases of nvCJD but not of sporadic CJD.\(^20,21\) Spleen and lymph nodes from our case 2 were proved immunohistochemically to contain no proteinase-resistant PrP (data not shown). Further studies might verify the usefulness of this assay in differentiating nvCJD from dura-associated CJD with florid-type plaques. Polymorphic codon 129 was M/M in all 4 cases of dura-associated CJD with florid-type plaques. However, the importance of this finding is currently unclear considering that 3 of the 4 cases are Japanese and 92% of the Japanese population have M/M at codon 129.\(^22\)

It remains to be determined what factors lead to the formation of florid-type plaques in dura-associated CJD and whether the infectious agents from the cases with florid-type plaques and those from the cases without florid-type plaques behave similarly in experimental transmissions to animals. The cases presented here, combined with the previously reported ones, may provide convincing evidence that a clinicopathologically distinct subtype with florid-type plaques exists in dura-associated CJD. To recognize this new entity may be an important step in analyzing these unfortunate but valuable cases of the prion disease that had been transmitted from human to human. Such analyses should contribute to further understanding of the relationships among the strains of the infectious agents, hosts, and pathological characteristics, including amyloidogenesis in the pathogenesis of the prion diseases.

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