Creutzfeldt-Jakob Disease, Prion Protein Gene Codon 129VV, and a Novel PrPSc Type in a Young British Woman

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Background: Variant Creutzfeldt-Jakob disease (vCJD) is an acquired prion disease causally related to bovine spongiform encephalopathy that has occurred predominantly in young adults. All clinical cases studied have been methionine homozygotes at codon 129 of the prion protein gene (PRNP) with distinctive neuropathological findings and molecular strain type (PrPSc type 4). Modeling studies in transgenic mice suggest that other PRNP genotypes will also be susceptible to infection with bovine spongiform encephalopathy prions but may develop distinctive phenotypes.

Objective: To describe the histopathologic and molecular investigation in a young British woman with atypical sporadic CJD and valine homozygosity at PRNP codon 129.

Design: Case report, autopsy, and molecular analysis.

Setting: Specialist neurology referral center, together with the laboratory services of the MRC [Medical Research Council] Prion Unit.

Subject: Single hospitalized patient.

Main Outcome Measures: Autopsy findings and molecular investigation results.

Results: Autopsy findings were atypical of sporadic CJD, with marked gray and white matter degeneration and widespread prion protein (PrP) deposition. Lymphoreticular tissue was not available for analysis. Molecular analysis of PrPSc (the scrapie isoform of PrP) from cerebellar tissue demonstrated a novel PrPSc type similar to that seen in vCJD (PrPSc type 4). However, this could be distinguished from the typical vCJD pattern by an altered protease cleavage site in the presence of the metal ion chelator EDTA.

Conclusions: Further studies will be required to characterize the prion strain seen in this patient and to investigate its etiologic relationship with bovine spongiform encephalopathy. This case illustrates the importance of molecular analysis of prion disease, including the use of EDTA to investigate the metal dependence of protease cleavage patterns of PrPSc.

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These models also predict that infection of VV and MV genotypes with BSE or vCJD prions may result in propagation of distinct prion strain types and that patients with VV or MV genotypes might present with clinical, pathological, and molecular phenotypes distinct from that of vCJD.12-14

To date, we know of no reported cases of clinical vCJD occurring in the VV or MV genotypes. However, PrPSc has been reported in lymphoid tissues, but not in the brain, of a patient with PRNP 129 MV who had received blood from a person with preclinical vCJD and who died of an unrelated cause.15 In addition, abnormal PrP immunoreactivity has been reported in anonymous archived lymphoid tissue from 2 individuals with PRNP 129 VV.16 It is unknown whether the individual with the MV genotype would have gone on (or if those with VV will go on) to develop clinical disease and, if so, whether the phenotype will fit the case definition of vCJD.

**METHODS**

Brain homogenates (10% w/v) were prepared in Dulbecco phosphate buffered saline lacking Ca2+ or Mg2+ ions. Aliquots were analyzed with or without proteinase K digestion (30 µg/mL final protease concentration, 1 hour, 37°C) by immunoblotting with anti–PrP monoclonal antibody 3F417 as described previously.7,18 Metal ion–dependent conformations of PrP were determined as previously described.19 Genomic DNA was extracted from peripheral blood, and the entire PRNP open reading frame was amplified by polymerase chain reaction and sequenced as described previously.20

**REPORT OF A CASE**

A 39-year-old woman presented to an optician in January 1999 with episodes of blurred vision and photophobia, but no abnormality was found. Two months later, she noted memory impairment, diplopia, dysarthria, and an unsteady gait of fluctuating severity. Five months after onset, the gait and limb ataxia had progressed, although walking was still possible, and the memory loss became more profound. The patient then developed paranoid ideation, aggression, restless nocturnal behavior, anorexia, and mood disturbance. By 5½ months after onset, she could not walk and was unsteady sitting, and limb movements were clumsy.

Examination showed dysarthria, broken pursuit eye movements without nystagmus, impaired upgaze, and stereotyped involuntary movements of the legs. However, limb power, vibration, proprioception, tendon reflexes, and plantar responses were normal. During the ensuing 4 weeks, speech ceased and incontinence and jerky involuntary limb movements became evident. Eight months after onset, the patient was mute but could follow some commands. She was able to visually fixate and follow moving objects but also had abnormal, spontaneous horizontal roving eye movements with a supranuclear vertical gaze palsy. Her face was impasse with occasional twitching movements, brisk facial reflexes, and trismus. There were prominent jerking movements of all limbs brought out by use; power was relatively preserved and the plantar responses were extensor.

There was a strong family history of late-onset cerebellar ataxia consistent with autosomal dominant inheritance. A polyglutamine expansion in ataxin 3 associated with spinocerebellar ataxia type 3 was found in a symptomatic family member, but our patient did not share this expansion.

Normal results of the following investigations were found: complete blood cell count, erythrocyte sedimentation rate, C-reactive protein, electrolytes, liver function, thyroid function, enzyme-linked immunosorbent assay for syphilis, vitamin B12, folate, ferritin, vitamin E, and serum ceruloplasmin. Tests for antinuclear, anti-neuronal, anti–Purkinje cell, and antiganglioside antibodies were negative. Nerve conduction studies showed no evidence of a peripheral neuropathy. The electroencephalogram 6 months after onset was reported as normal, but at 7 and 8 months electroencephalograms showed diffuse slow-wave activity, without epileptiform changes or periodic discharges typical of CJD. Cerebrospinal fluid examination showed a normal cell count, protein level, and glucose level, and oligoclonal immunoglobulin bands were absent. The protein S100b level of 4.39 ng/mL (reference cutoff, <0.38 ng/mL), neuron-specific enolase level of 20 ng/mL, and 14-3-3 protein were all abnormal values.

A magnetic resonance image of the brain (Figure 1) showed diffuse cerebellar atrophy and diffuse increased signal within both caudate nuclei and each putamen.

**Figure 1.** T2-weighted axial magnetic resonance image showing diffuse increased signal within both caudate nuclei and each putamen.
results. Sequencing of the PRNP open reading frame was normal on 2 separate occasions. A polymerase chain reaction performed with primers designed to amplify the octapeptide repeat region of PRNP did not demonstrate an insertion mutation. The codon 129 polymorphism was homozygous for valine.

Fourteen months after onset, the patient died and an autopsy was performed.

**AUTOPSY FINDINGS**

Histopathologic examination was limited to the brain and spinal cord (Figure 2). The findings were atypical of sporadic CJD in the severity of white matter degeneration and the extensive nature of PrP deposition in the cortex and white matter. The frontal cortex showed extremely severe neuronal loss with striking astrocystosis and prominent spongiform vacuolation. There was severe overall loss of white matter, in places reminiscent of infarction. Deposition of PrP was extensive throughout the cortex and white matter. In places this was a diffuse punctate deposition similar to the recognized synaptic pattern. Occasionally, individual cells, mainly pyramidal neurons, were outlined by PrP deposition and had a fine granular intracellular deposition. More dense deposits, similar to plaques, were seen in the cortex. Also in the white matter, PrP deposits were seen ranging from a couple of micrometers to much larger plaquelike deposits, although these were not florid.

Temporal, parietal, and occipital lobes showed histologic features similar to those described in the frontal lobe, the occipital lobe being most severe. The hippocampus was relatively well preserved. In the caudate, putamen, and amygdala there was neuronal loss, astrocystosis, and microglial activation. The thalamus, midbrain, and substantia nigra showed mild to moderate spongiform change, neuronal loss, and astrocystosis with intraneuronal and extracellular punctate deposits. The pons and medulla were less severely affected than the midbrain with punctate PrP deposits. The cerebral peduncles were severely affected, with nearly complete loss of myelin. The cerebellum was very severely affected, with a dramatic loss of Purkinje and granule cells accompanied by vacuolation and astrocystosis. The cerebellar white matter showed severe white matter loss similar to incipient infarcts. Deposition of PrP in the cerebellum was marked with accumulation of punctate deposits resembling plaques, most commonly in the granule cell layer. In the white matter the deposits were denser still, occasionally plaquelike or forming irregular linear deposits.

**PrPsc TYPING STUDIES**

Western blot analysis was performed on fresh frozen cerebellar tissue from the patient. Identical results were ob-
Does the PrPSc typing suggest a BSE-related cause, or can our findings be accommodated by the spectrum seen in sporadic CJD cases worldwide? The molecular strain typing of the patient's brain material demonstrated a novel PrPSc type when compared with our archived cases. There is as yet no internationally agreed-on classification of PrPSc type. Parchi and colleagues identified 2 PrPSc types in sporadic CJD. However, Hill et al. recently described a novel PrPSc type 6 in sporadic CJD.

**Figure 3.** Immunoblotting of 10% brain homogenate after limited proteinase K digestion using anti–prion protein (PrP) monoclonal antibody 3F4. Lanes 1, 2, and 3 show 3 types of PrPSc: (the scrapie isoform of PrP) seen in sporadic and iatrogenic cases of Creutzfeldt-Jakob disease; lane 4 shows PrPSc type 4, which is uniquely seen in brain tissue from patients with variant Creutzfeldt-Jakob disease. Lane 5 shows PrPSc from the cerebellum of our patient demonstrating the same predominance of the high-molecular-mass diglycosylated PrP glycoform and a molecular mass of all PrP fragments similar to those of PrPSc type 4.

**Figure 4.** Immunoblotting of 10% brain homogenate after limited proteinase K (PK) digestion using anti–PrP monoclonal antibody 3F4. Lanes 1, 2, and 3 show 3 types of PrPSc: (the scrapie isoform of PrP) with an identical pattern of PrP fragments observed after proteolysis in the presence of 25mM EDTA. B, Cerebellum from our patient demonstrating a doublet of low-molecular-mass nonglycosylated PrPSc bands. All bands migrate with lower apparent molecular mass following proteolysis in the presence of 25mM EDTA. C, Aliquots of cerebellum homogenate from our patient digested directly with proteinase K or after treatment with 25mM EDTA and sequential washing of insoluble pellets with N-ethyl morpholine buffer either lacking (+) or containing (−) 20µM zinc chloride (ZnCl2).

**Figure 4.** Immunoblotting of 10% brain homogenate after limited proteinase K digestion using anti–prion protein (PrP) monoclonal antibody 3F4. A, Cerebellum from a patient with variant Creutzfeldt-Jakob disease demonstrating a doublet of low-molecular-mass nonglycosylated bands of PrPSc (the scrapie isoform of PrP) with an identical pattern of PrP fragments observed after proteolysis in the presence of 25mM EDTA. B, Cerebellum from our patient demonstrating a doublet of low-molecular-mass nonglycosylated PrPSc bands. All bands migrate with lower apparent molecular mass following proteolysis in the presence of 25mM EDTA. C, Aliquots of cerebellum homogenate from our patient digested directly with proteinase K or after treatment with 25mM EDTA and sequential washing of insoluble pellets with N-ethyl morpholine buffer either lacking (+) or containing (−) 20µM zinc chloride (ZnCl2).
cattle and other species. Two other cases of prion disease with valine homozygosity and atypical features have been reported in the United Kingdom and the Netherlands. One of these cases was atypical because of very young onset and a protracted psychiatric history; the other was notable because certain clinical and molecular features of the case overlapped with those of vCJD, including Western blot analysis of autopsied brain showing a predominance of a diglycosylated PrPSc isoform.

We recommend keeping an open mind about the etiology of such cases during the ensuing years. These cases emphasize the importance both of continued surveillance of prion disease and the further development and refinement of molecular classification of prion diseases of humans and animals. It will also be important to assess lymphoreticular involvement in subsequent cases either at diagnostic tonsil biopsy or at autopsy.

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