

OBSERVATION

Creutzfeldt-Jakob Disease, Prion Protein Gene Codon 129VV, and a Novel PrP^{Sc} 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 (PrP^{Sc} 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 PrP^{Sc} (the scrapie isoform of PrP) from cerebellar tissue demonstrated a novel PrP^{Sc} type similar to that seen in vCJD (PrP^{Sc} 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 PrP^{Sc}.

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THE ORIGINAL RECOGNITION of variant Creutzfeldt-Jakob disease (vCJD) was based on a case series of young patients with rapidly progressive dementia, a geographic and temporal association with bovine spongiform encephalopathy (BSE), and novel neuropathological findings consisting of abundant florid prion protein (PrP) plaques.¹ Molecular strain typing allowed identification of a unique type of PrP^{Sc} (the scrapie isoform of PrP) (type 4) in the brain that was distinct from those seen in classic (sporadic or iatrogenic) CJD and similar to that seen in BSE prion infection of cattle and other species.² Subsequent biological strain typing in both conventional and transgenic mice confirmed that vCJD and BSE were caused by the same prion strain.^{3,4}

Variant CJD also differs markedly from classic CJD in having prominent and consistent involvement of lymphoreticular tissue, allowing its diagnosis by tonsil biopsy findings.⁵⁻⁷ To date, more than 160 individuals have died of vCJD in the United Kingdom; the number infected by BSE prions and who may develop prion disease in the years ahead is unknown because human prion incubation periods may exceed 50 years.⁸

All clinical cases of vCJD studied have had a methionine-homozygous (MM) genotype at polymorphic codon 129 of the prion protein gene (PRNP).⁹ The extension of BSE prion-related disease to individuals with valine-homozygous (VV) or heterozygous (MV) genotypes at PRNP codon 129 has been predicted by comparison with other acquired human prion diseases^{10,11} and by transgenic mouse

models.¹²⁻¹⁴ 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, PrP^{Sc} 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 (50 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 impassive 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.

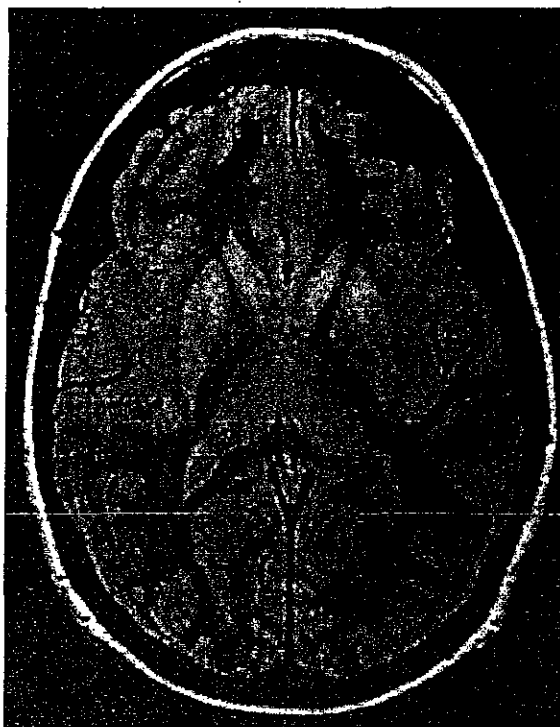


Figure 1. T2-weighted axial magnetic resonance image showing diffuse increased signal within both caudate nuclei and each putamen.

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 98 ng/mL (reference cutoff, <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. Tonsil biopsy was not possible because of a previous tonsillectomy from which little tissue remained. Genetic testing for mutations associated with spinocerebellar ataxia 1, 2, 3, 6, and 7 and Friedreich ataxia gave negative re-

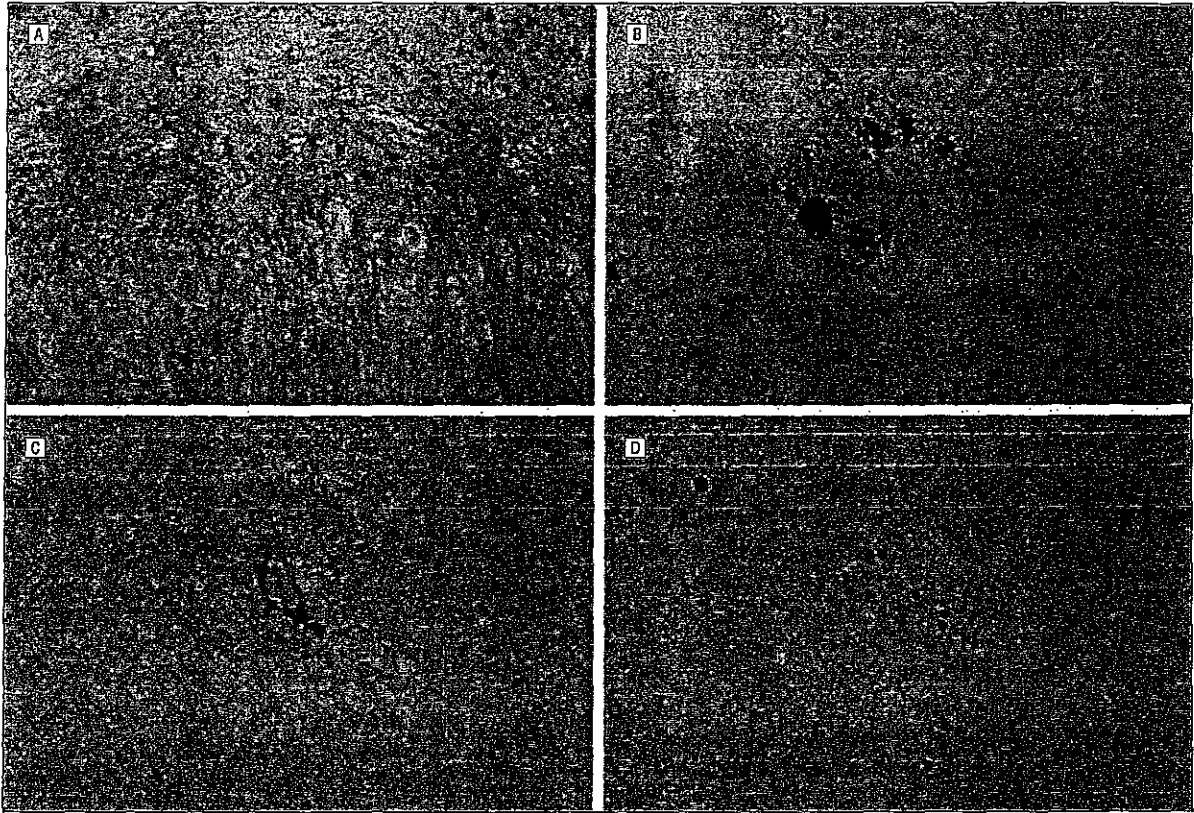


Figure 2. Immunohistochemical analysis of brain sections from the patient. A, Glial fibrillary acidic protein immunohistochemistry of the molecular and granule cell layers of the cerebellum showing neuronal loss and Bergmann astrocytosis (original magnification $\times 20$). B, Granular prion protein staining in the cerebellum (original magnification $\times 40$). C, Perineuronal prion protein staining in the temporal lobe (original magnification $\times 20$). D, Prion protein plaques in the temporal lobe (original magnification $\times 20$).

sults. 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 astrocytosis 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, astrocytosis, and microglial activation. The thalamus, midbrain, and substantia nigra showed mild to moderate spongiform change, neuronal loss, and astrocytosis 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 astrocytosis. 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.

PrP^{Sc} TYPING STUDIES

Western blot analysis was performed on fresh frozen cerebellar tissue from the patient. Identical results were ob-