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 predominately 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.

Arch Neurol. 2007;64(12):1780-1784

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. Molecular strain typing allowed identification of a unique type of PrPSc (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. Subsequent biological strain typing in both conventional and transgenic mice confirmed that vCJD and BSE were caused by the same prion strain.

Variant CJD also differs markedly from classic CJD in having prominent and consistent involvement of lymphoreticular tissue, allowing its diagnosis by tonsil biopsy findings. 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.

All clinical cases of vCJD studied have had a methionine-homozygous (MM) genotype at polymorphic codon 129 of the prion protein gene (PRNP). 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 and by transgenic mouse...
Our patient was able to visually fixate and follow movements but also had abnormal, spontaneous horizontal roving eye movements with a supranuclear vertical gaze palsy. Her face was impassive with occasional horizontal roving eye movements. Although walking was still possible, and the memory loss became more profound. The patient then developed para-noid ideation, aggression, restless nocturnal behavior, anorexia, and mood disturbance. By 5½ months after onset, the patient 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.

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.
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 astrogliosis 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, astrogliosis, and microglial activation. The thalamus, midbrain, and substantia nigra showed mild to moderate spongiform change, neuronal loss, and astrogliosis 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 astrogliosis. 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-
is as yet no internationally agreed-on classification of PrPSc associated with sporadic and iatrogenic CJD (types 1-3) and variant CJD. However, Hill et al \(^2\) described 3 PrPSc types associated with sporadic CJD, 19-21 this is not observed in mice expressing human PrP 129V inoculated with vCJD. \(^3\) Hill et al \(^2\) recently described a novel PrPSc type 6 in sporadic CJD.

The PrPSc type from our case has features similar to PrPSc type 4 (vCJD) in the predominance of the diglycosylated band; however, it is distinct from PrPSc type 4 in the dependence of the protease cleavage pattern of PrPSc on metal ions, suggesting a distinct PrPSc conformation. Unfortunately, only cerebellum was available for Western blotting in this case, although in vCJD cases from which whole brain was available we have not found evidence of any regional variation in PrPSc type. Others have reported coexistence of Gambetti PrPSc type 1 in the brain from patients with vCJD as a minority component. \(^2\) It would also have been interesting to look for peripheral lymphoreticular PrP deposition because this is prominent in vCJD, but that tissue was not available for analysis. Transmission of BSE isolates to transgenic mice expressing human PrP 129V valine results in clinical prion disease with undetectable PrPSc; however, transmission of vCJD isolates to the same mice produces PrPSc type 5 that shares the same predominance of diglycosylated PrPSc to that of PrPSc type 4, and these data suggest that the molecular signature of BSE may be preserved after BSE transmission to PRNP codon 129 VV humans. \(^1\) Transmission studies of the current case in transgenic mice are now being undertaken to investigate transmission characteristics.

We have described a novel PrPSc type that would be designated type 7 by our classification. A firm conformation between novel PrPSc types and BSE cannot be made on the basis of a single case, and it will be important to see whether other similar cases occur in the United Kingdom and other BSE-exposed countries but not elsewhere and to perform detailed transmission studies of prions from this patient into transgenic and conventional mice to compare with BSE-derived isolates from...
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.

Accepted for Publication: February 22, 2006.

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Financial Disclosure: None reported.

Funding/Support: This work was funded by the MRC and undertaken at the University College London Hospitals and University College London, who received a proportion of funding from the Department of Health’s National Institute for Health Research Biomedical Research Centres funding scheme.

Additional Contributions: Ray Young assisted with figure design. The MRC London Neurodegenerative Diseases Brain Bank (Institute of Psychiatry) provided pathological material. We also acknowledge the many clinicians involved in the care of this patient.

REFERENCES