Prion diseases: **Transmission from mad cows?**

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Prion diseases in humans show considerable clinical and pathological heterogeneity. The identification of a new variant of Creutzfeldt–Jakob disease, and its interpretation as evidence of transmission of mad cow disease to man, rely critically on our understanding of the epidemiology of prion diseases.

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Prion diseases have been much in the news following the description [1] of what has been claimed to be a new variant of Creutzfeldt–Jakob disease (CJD), possibly linked to the recent UK epidemic of 'mad cow disease', or bovine spongiform encephalopathy (BSE). Prion diseases show a number of well-recognized characteristics, such as rapidly progressing dementia, myoclonus (seizure involving muscle spasms) and widespread spongiform degeneration in the brain. But they also show considerable variation in their clinical presentation, disease duration and the type and distribution of brain lesions [2]. About 85 % of human prion diseases occur sporadically, but there are also inherited familial forms, such as Gerstmann–Sträussler–Scheinker (GSS) syndrome, familial CJD and fatal familial insomnia (FFI), which are genetic diseases associated with mutations in the prion protein gene. Kuru, which occurs among certain tribes in New Guinea, is acquired only by transmission and is believed to be caused by the consumption of infected human material. Given the known variability of human prion diseases, how compelling is the evidence that the recent CJD cases really do reflect a new form of the disease transmitted from cattle with BSE?

The pathology of prion diseases is characterized by spongiform degeneration of regions of the brain, with gliosis and variable deposition of a protease-resistant isoform of a normal cellular protein — the prion protein (PrP). The term 'prion', derived from 'protein infectious agent', was coined to reflect the belief that the protein itself, in an abnormal form, is the transmissible, disease-causing agent [3]. This belief was based on many studies, primarily with the sheep prion disease scrapie, which failed to identify any nucleic acid associated with the infectious agent. The prion protein was originally identified in the disease-associated form (PrP^{Sc}), but was subsequently shown to be a normal cellular protein (PrP^{C}). The most popular current view is that PrP^{Sc} is infectious because small amounts of the protein in some way induce a conformational change in PrP^{C}, which thus switches to the pathological isoform PrP^{Sc}. The switch is therefore an autocatalytic process, and this is thought to precede spongiform degeneration and be a critical event in disease pathogenesis [3,4]. Indeed, it has been speculated that heritable disease variability, associated with different 'strains' of the infectious agent, are caused by the self-propagation of protease-resistant PrP^{Sc} polymers with distinct conformations (see [5] for a recent discussion of the nucleated-polymerization model of PrP^{Sc} replication).

Although the physiological function of PrP^{C} is uncertain, its conservation among mammals implies it does have an important role. The available evidence suggests that PrP^{C} is essential for the maintenance of neuronal integrity in the brain. The normal function of PrP has been addressed by creating PrP^{C} 'knockout' mice, in which the *PrP* gene has been inactivated by homologous recombination. Although *PrP* is highly conserved and widely expressed during early embryogenesis, mice homozygous for a disrupted *PrP* gene initially seemed developmentally and behaviourally normal. Significantly, they were resistant to prion disease, fulfilling an important prediction of the hypothesis that the transmission of prion diseases involves an interaction between an abnormal, infectious protein and the normal cellular protein [3].

More recently, however, it has been reported that, as the *PrP* mutant mice age, they do show symptoms of neuronal disease. In particular, they appear to lose cerebellar Purkinje neurons and exhibit weakened γ-amino-butyric acid (GABA) receptor-mediated fast neuronal inhibition and impaired long-term potentiation [6]. These observations suggest that PrP is necessary for normal synaptic function and the long-term survival of Purkinje cells. Furthermore, these mutant mice show behavioural abnormalities resembling those associated with the human prion disease FFI — they have altered circadian rhythms and abnormally fragmented sleep, in addition to being especially susceptible to sleep deprivation [7].

Although their phenotype mirrors aspects of human prion diseases, the *PrP* mutant mice are, as mentioned above, resistant to infection by the prion disease agent, and they do not display pathological hallmarks of the disease, such as spongiform changes or gliosis. Expression of PrP^{C} by the host is thus necessary for prion disease-induced neurotoxicity. This conclusion is further supported by the observation that, when wild-type tissue was grafted into the brains of *PrP* mutant mice, only the graft developed pathological

changes following inoculation with the prion disease agent [8]. Furthermore, although PrP^{Sc} migrated from the graft into the host brain, it was insufficient to cause any spongiform changes or gliosis in the mutant tissue.

All cases of inherited prion disease analysed have been found to be linked to a point mutation or extra copies of a repeat sequence within the protein-coding region of the *PrP* gene [2,3]. Although the molecular basis of the phenotypic variability exhibited by sporadic CJD is largely unknown, polymorphism at residue 129 — which is either methionine or valine — of the PrP protein may affect pathological features of familial prion diseases as well as susceptibility to sporadic CJD [9].

Two types of PrP^{Sc} have been defined by size and glycosylation differences. When passaged in syngeneic animals, each strain or isolate shows highly preserved characteristics, such as incubation time, the distribution and intensity of spongiform degeneration, and the pattern of intracerebral PrP^{Sc} deposition [3,10]. These strain-specific properties can, however, drastically change if a given PrP^{Sc} strain is passaged in animals with a different *PrP* genotype. Even a single amino-acid change in PrP can cause resistance to infection, or lead to disease with a different incubation time and pathology. Heterogeneity of the infectious PrP^{Sc} agent, either in combination with or independent of the host's *PrP* genotype, may well contribute to the phenotypic variability of sporadic CJD.

Homozygosity for valine at the polymorphic residue 129 mentioned above also enhances susceptibility to iatrogenic CJD *via* peripheral contamination, for example caused by injections of contaminated growth hormone. This form of CJD typically manifests with a cerebellar syndrome similar to that of kuru and often shows larger PrP^{Sc} accumulation in the cerebellum than in the cerebral cortex. Valine 129 homozygotes with sporadic CJD have been found to exhibit similar clinical and pathological features, suggesting that this phenotype is not specific to the iatrogenic form but is associated with either the valine 129 genotype or the selection of a specific prion strain, or both [10].

The description of ten 'atypical' cases of CJD and their presumptive association with exposure to infected material from cows with BSE, has precipitated the recent crisis in public confidence over the safety of British beef [1]. The cases are characterized by the following features: disease onset before the age of 40 years; symptoms of a psychiatric nature, such as depression, aggression and behavioural disturbance; a relatively long time-course of disease (a year or more); and an extensive PrP deposition in plaques throughout the cortex (see Box). All of the cases identified so far are homozygous for *PrP* encoding methionine at residue 129. The atypical nature of these cases is thought to be a new development — a new 'strain' of the disease — and to represent strong evidence that BSE-infected material can cause CJD

WHO case definition of the new CJD variant

- A suspect case shows a psychiatric presentation with anxiety, depression, withdrawal and other behavioural changes with progression to neurological abnormalities; onset of a progressive cerebellar syndrome within weeks or months of presentation; forgetfulness and other memory impairment, with subsequent dementia; and myoclonus in the late stages.

- The electroencephalogram does not show the changes normally observed in classic CJD. Less common features include early onset of dysaesthesia in limbs and face, and chorea, extrapyramidal and pyramidal signs later in the illness.

- Neuropathological diagnosis is mandatory for confirmation of possible cases. Confirmatory examination of the brain should show numerous widespread kuru-type amyloid plaques surrounded by vacuoles; spongiform changes that are mostly evident in the basal ganglia and thalamus; and high-density prion protein accumulation on immunocytochemical analysis, especially in the cerebellum.

One aspect of these cases that is claimed to be of critical epidemiological importance is their recent appearance, in the UK, just a few years after the BSE epidemic — also in the UK — reached its peak. CJD in young people — those under the age of 40 years — is extremely rare [1]. In 14 cases of CJD in those aged less than 30 years previously reported outside the UK, cortical plaques were described in only one, and in this report the possible diagnosis of Gerstmann–Sträussler–Scheinker syndrome was suggested. Only one such case was identified in the UK between 1970 and 1989, and only two patients aged less than 30 years old were identified in France between 1968 and 1982; one was identified in Japan between 1975 and 1977, and none at all in Israel between 1963 and 1987.

The overall incidence of CJD in the UK rose in the 1990s, probably because of improved ascertainment of CJD in the elderly [11], as previously predicted [12]. Might a similar improved ascertainment, due in part at least to the publicity surrounding the BSE epidemic, account for the recently described younger cases of CJD? Scientists at the CJD Surveillance Unit in Edinburgh argue against the view that these cases simply reflect increased ascertainment, as they believe their distinctive neuropathological pattern would have been detected earlier, especially in patients so young. It is noteworthy, however, that three of the ten patients were reported to the CJD Surveillance Unit as suspect CJD cases only after biopsy samples had been examined. So, in the absence of neuropathological examination, these cases might not have come to the attention of the Unit at all.

Detailed clinical documentation of the genetic forms of CJD has revealed a wide variation in clinical presentation

of CJD, including depression and personality disorders and a long time-course of the disease. Sporadic cases with atypical presentations are very likely to escape clinical detection and a recent study has suggested that they may represent a substantial proportion of prion disease patients [13]. This study showed that 40 % of prion disease cases identified neuropathologically had been 'undetected' during life, and most of these were found retrospectively to have had an 'atypical' presentation, including psychiatric symptomatology and a long course of illness, although none had 'kuru plaques'. Two of the cases died aged 40 and 48 years old in 1980 and 1978 [13].

These data have helped fuel the debate over the phenotypic limits of CJD and the reliability of the epidemiological conclusions that can be drawn from studies using a narrow clinicopathological definition of CJD [12,13]. It follows that awareness of the phenotypic variability of CJD, coupled with the enhanced public interest, might have led to the uncovering of cases which do not conform to the 'typical' CJD straitjacket and which would previously have passed unreported [13]. It would seem prudent to reinvestigate, in archival material, the pathological nature of cases of psychiatric/neurological disorders associated with an early age (less than 45 years) of death. Confirmation of the absence of cases sharing the phenomenology of the 'new strain' of CJD prior to 1986 would enhance our confidence in the conclusions drawn from the recent observations.

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