

IN RECENT YEARS, a number of diseases caused by proteinaceous infectious agents called prions have been described (Prusiner 1995) and collectively referred to as the 'transmissible spongiform encephalopathies' (TSE). Prions, which are composed mainly of protein, differ significantly in behaviour from either bacteria or viruses. Prion diseases include

'scrapie' in sheep, 'bovine spongiform encephalopathy' (BSE) in cattle, and Creutzfeldt-Jakob disease (CJD) in humans.

These diseases are important because they can be transmitted between members of the same species and can also cross the species barrier and adapt to new hosts. Of particular concern, is whether a new form of CJD in humans termed variant CJD (vCJD) can be acquired by eating beef

contaminated with prions. This article describes the nature of prions, how prions may cause disease, and the symptoms and pathology associated with the TSE.

Prion protein

Prions lack either DNA or RNA and are composed almost entirely of different forms of protein called prion protein (PrP). PrP is a protein that is made normally in the brain and is coded by a gene, which

Richard Armstrong untangles the prions responsible for transmissible spongiform encephalopathies

Getting your proteins in a twist: disease caused by prions

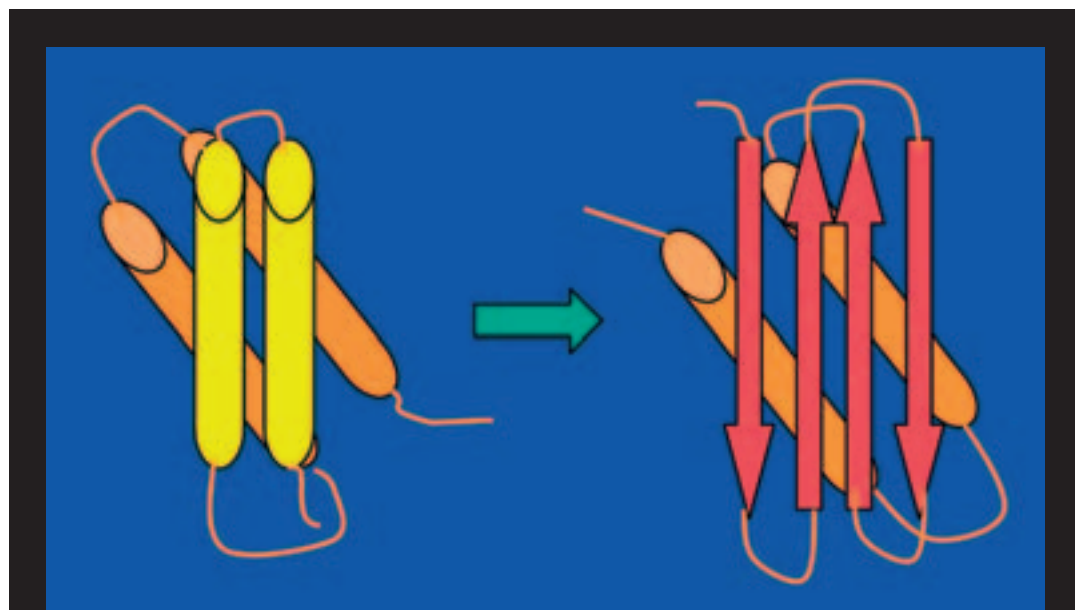


Fig 1. Conversion of normal prion protein (PrP_c) to the scrapie form of PrP (PrP_{sc}) in the presence of PrP_{sc}. PrP_c is believed to comprise four α -helices with virtually no β -sheets. PrP_{sc} displays an identical primary structure to PrP_c but differs in secondary and tertiary structure. PrP_{sc} displays a four-stranded β -sheet configuration (vertical arrows) covered on one face by two α -helices.

in humans, is located on chromosome 20. The natural function of PrP is unknown but it is found in synaptic nerve endings suggesting that it may function in neural transmission. There are two forms of PrP, viz., normal cellular PrP (PrP^c), which does not cause disease and the 'scrapie' form of PrP (PrP^{sc}), the presence of which can induce the disease 'scrapie' (DeArmond and Prusiner 1994) (Fig 1). These two forms of PrP are identical in amino acid composition but they differ in conformation or shape of the molecule. The two forms also differ in glycoform, i.e., in the composition of attached chains of carbohydrates. Hence, the pathogenic form of PrP (PrP^{sc}) is a stereoisomer of PrP^c the tertiary structure of which is made up of β -pleated sheets resistant to proteases. The key to understanding prion disease is the fact that 'benign' PrP^c can be converted into 'malignant' PrP^{sc} in the presence of PrP^{sc} (Fig 1). Prusiner (1993) found

that transgenic mice reared without PrP^c could not be infected with scrapie suggesting that PrP^c was required for the disease process to take place. This process is auto-catalytic and once started proceeds at an exponential rate within the brain. PrP^{sc}, due to the presence of β sheets, has a greater tendency than PrP^c to form insoluble aggregates in brain tissue. Hence, the deposition of PrP^{sc} in association with neurons presumably causes their death leading to the appearance of vacuoles in the tissue (Armstrong et al., 2002). Within the brain, PrP^{sc} appears to target specific nerve cell populations and may spread through the brain via cerebral spinal fluid or by anatomical connections (Prusiner and DeArmond 1994; Armstrong et al., 2000). The appearance of PrP^{sc} causes little or no immune response in the patient since the host immune system is presumably tolerant of the host protein.

Each species has its own PrP with a specific amino acid composition. In theory, it is difficult to transmit prion diseases across the species barrier, e.g., to transmit 'scrapie' from sheep to rodents or BSE from cows to humans. However, the more closely the amino acid sequence of an infective PrP^{sc} is to that of the host PrP^c, the greater the chance of initiating the conversion of PrP^c to PrP^{sc}. Sheep and cow PrP are very similar, supporting the contention that the agent may have crossed this species barrier. By contrast, there is much less similarity between cow and human PrP presumably making it more difficult for this species barrier to be crossed.

Prion diseases (TSE)

The fact that prions could cause a transmissible disease was first demonstrated when the 'scrapie' agent derived from sheep was injected into the brains of healthy mice. Mice acquired 'scrapie' like symptoms and the brain

pathology associated with this disease.

This occurred even when the agent was exposed to UV light that would destroy any nucleic acid present. Several TSE's are known and continue to be identified especially in animals (Table 1).

Animal TSE

The best known of the animal TSEs is the 'trembling disease of sheep' called 'scrapie' first described in 1772 (Scott, 1993). Animals with this disease lose coordination and balance but the name 'scrapie' is taken from the tendency of the animals to scrape off their wool. The brains of affected animals exhibit the typical signs of prion disease including the development of vacuolation, neuron loss, and astrocytosis (a proliferation of astrocytic glial cells). BSE is a relatively new form of prion disease first described in cattle in 1986. By 1989, 50,000 cattle in the UK were infected. Affected cows become apprehensive and uncoordinated and develop similar brain pathology to that seen in 'scrapie'. Two theories have been proposed to account for the BSE epidemic. First, that TSE infected animal feed supplement containing the scrapie agent was given to cattle. Second, that the disease has its origin in the very uncommon hereditary form of the disease and that the remains of these animals may have entered the processing of animal feed. Prion diseases have also been described in cats, deer, elk and mink.

Human TSE

Creutzfeldt-Jakob disease is one of a group of human diseases caused by prions (Table 1). In addition to CJD, three other human prion diseases have been identified. Firstly, 'kuru' is a disease found in the 'Fore' ▶

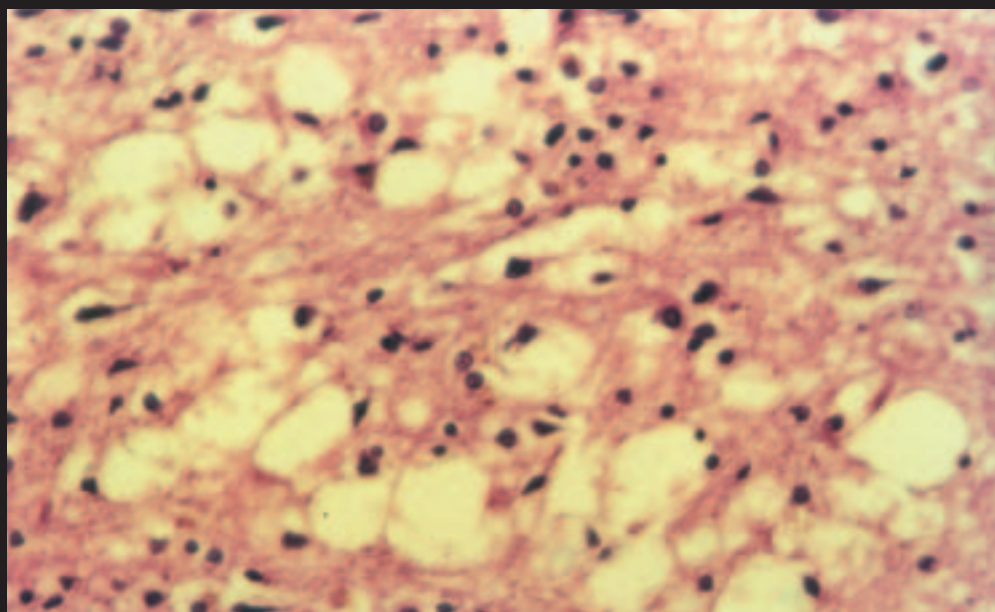


Fig 2. Vacuolation ('spongiform change') in the cerebral cortex of a patient with sporadic Creutzfeldt-Jakob disease (sCJD). Section stained with H/E. Vacuoles appear as distinct holes in the section while the dark structures are neuronal cell bodies or glial cell nuclei.

highlanders', natives of Papua and New Guinea, and probably acquired as a result of ritual cannibalism. Secondly, 'fatal familial insomnia' (FFI) is a recently described genetic disorder that is characterised by sleep disturbance followed by insomnia and agitation and later, by the development of hallucinations, stupor, and coma. Thirdly, 'Gerstman-Straussler-Scheinker' (GSS) disease is a rare genetic disorder first described in 1936. It is characterised by ataxia (muscular incoordination), dementia (loss of short-term memory, judgement, and emotional disturbance), limb weakness, and speech problems. It can

sometimes resemble types of multiple sclerosis and therefore, can be difficult to diagnose in individual patients.

CJD was first described by Creutzfeldt and Jakob in the 1920s and is the most important human form of prion disease. Patients develop dementia, which is sometimes preceded by ataxia, and loss of coordination, with death resulting in about 3-12 months after the onset of symptoms. The characteristic pathological signs of prion disease are observed in the brain including vacuolation (Fig 2), neuronal loss, the proliferation of astrocytes and the deposition of PrPsc in the

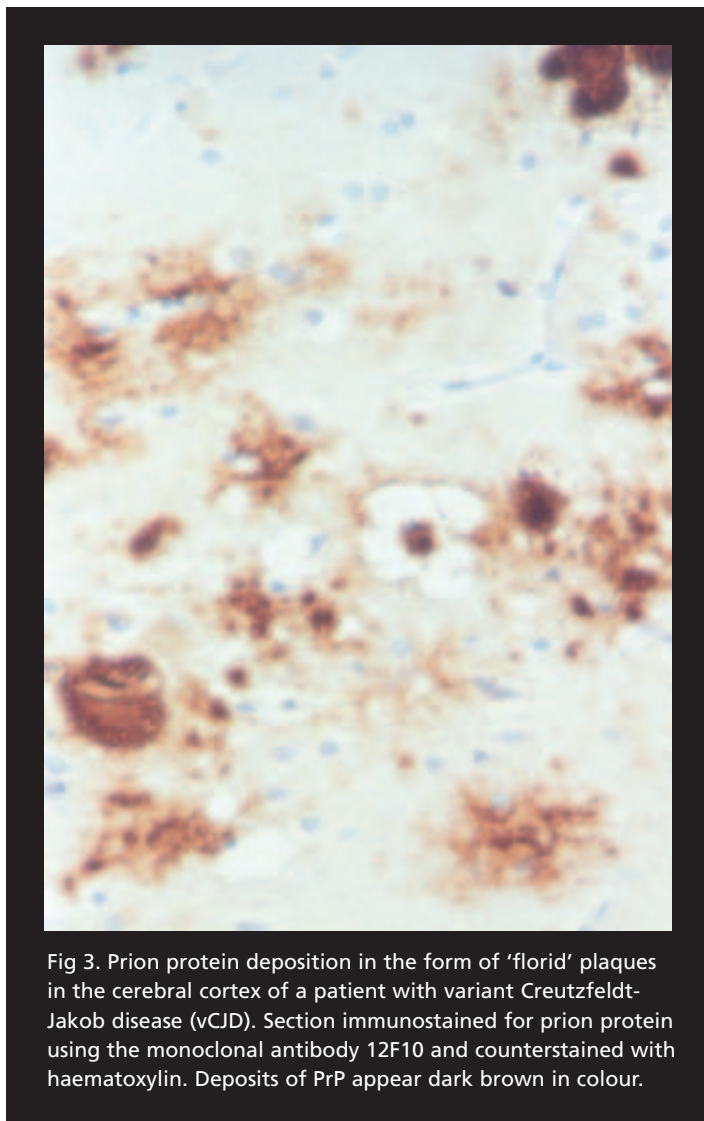


Fig 3. Prion protein deposition in the form of 'florid' plaques in the cerebral cortex of a patient with variant Creutzfeldt-Jakob disease (vCJD). Section immunostained for prion protein using the monoclonal antibody 12F10 and counterstained with haematoxylin. Deposits of PrP appear dark brown in colour.

Table 1.
Human and animal transmissible spongiform encephalopathies (TSE)

Disease	Abbreviation	Host
Creutzfeldt-Jacob disease	CJD	Human
Kuru	-	Human
Fatal familial insomnia	FFI	Human
Gerstmann-Straussler Scheinker disease	GSS	Human
Scrapie	-	Sheep/mouse
Bovine spongiform encephalopathy	BSE	Cattle
Feline spongiform encephalopathy	-	Cat
Chronic wasting disease	-	Deer/elk
Transmissible mink encephalopathy	-	Mink

form of discreet 'plaques' (Fig 3). CJD exists in several forms including those associated with genetic factors (familial or fCJD), those resulting from transmission of PrPsc (iatrogenic or iCJD) and those that occur sporadically within the population (sCJD). About 10-15% of cases of CJD are genetic and this condition is inherited as an autosomal dominant gene. These cases appear to be caused by point mutations (a change in one codon of the DNA resulting in the substitution of one amino acid for another) of the PrP gene. A small number of cases can be traced to a transmissible cause (see Table 2). Possible sources of transmission include corneal transplant, the use of electrodes implanted in the brain, neurosurgery, dura matter grafts, and the use of growth hormone. The latter was acquired as a result of the use of growth hormone extracted from human

pituitary glands. This cause of CJD has now been largely eliminated as a result of the use of genetically engineered growth hormone. About 85% of recorded cases of CJD are sporadic. The mean age of onset of sCJD is 60 years of age and its incidence is approximately 1/1,000,000. Two theories have been proposed to explain cases of sporadic CJD. Firstly, a mutation of the PrP gene could result in the formation of PrPsc rather than PrPc or secondly, PrPc could be converted spontaneously to PrPsc. The chance of both processes occurring could increase with age.

The possible development of CJD as a result of eating infected beef is of great current concern. The risk of transmission of BSE to humans is believed to be low because of the differences in structure between the 'scrapie' agent and human PrP. However, the recent

Table 2.
Methods of transmission of
Creutzfeld-Jacob disease

Mode of transmission	'Incubation period'
Corneal transplant	>2 years
Depth electrodes	>2years
Neurosurgery	>2years
Dura matter grafts	>2years
Growth hormone extracts	4 - 30 years, average 12 yrs
Eating infected beef	?

appearance of vCJD, which may be related to BSE, is causing considerable concern. The BSE agent is virtually identical to that causing vCJD supporting the hypothesis of a direct link. The first cases of vCJD were recorded in 1995 and by 2003, the total number of deaths ascribed to this disease was 125. This new variant differs significantly from previously described forms of CJD being characterised by an earlier age of onset (mean 28 years) and a prolonged duration of illness (up to 2 years). Presentation of the disease is largely psychiatric with patients exhibiting anxiety, depression, and behavioural changes. After a period of weeks or months, a cerebellar syndrome develops with problems in walking and movement. Memory problems develop late in the clinical course and the patient ultimately becomes mute and unable to move. Myoclonus (limb jerking) occurs usually at some stage in the disease in the majority of patients. In addition, the pathology of vCJD differs significantly from other forms of CJD with widespread concentrated deposits of PrP^{Sc} throughout the brain referred to as 'florid plaques' (Armstrong *et al.*, 2002b) (Fig 2). Whether these cases

represent a small cluster of cases linked to BSE or the beginning of a larger BSE epidemic of vCJD is controversial and remains to be established.

Conclusions

Although TSE have been known for many years, the knowledge that these diseases are caused by prions is relatively recent and extends the classification of human infectious disorders. Prions exhibit little of the behaviour characteristic of viruses or bacteria and the principles of infectious disease derived from these entities may be of little help in understanding prion disease. This lack of basic knowledge was a major problem in the 1980s and 90s when the BSE epidemic was at its height and government policy was formulated often in a climate of scientific ignorance.

A major concern with reference to the TSE is that disease can be transmitted both within and between species and therefore, can be passed to humans. Despite the occurrence of some 'clustering' of cases of CJD, it seems very unlikely that the disease can be transmitted by close human contact. There is no evidence, for example, of increased incidence of CJD in

neurosurgeons or pathologists who are likely to have increased exposure to PrP. In some rare instances, CJD does appear to have been transmitted to humans via corneal grafts (Hogan and Cavanagh, 1995). In experiments using the 'scrapie' agent, mice infected through the retina exhibited neuronal losses in the lateral geniculate body coincident with the onset of vacuolation. This suggests that the disease could spread into the brain via the visual pathway. However, the risk of CJD transmission by this method would seem to be remote. It has been calculated that between 0.5-4 CJD infected organ donors would be expected in a single year in the USA. The current 'Eye Bank Association of America' criteria for exclusion of donor corneas based on suspicious history are usually considered adequate to protect against accidental transmission.

There remains considerable anxiety in the UK about the

likely development of new cases of vCJD that have been incubating since the 1980s although the most recent data do suggest a decrease in the rate of increase of new cases. It has been known for some time, however, that the phenotypic features of CJD are influenced by a polymorphism of the PrP gene at codon 129, *viz.*, the presence of either methionine or valine at this codon. In the TSE kuru, methionine homozygosity at codon 129 results in cases with earlier onset and shorter duration, a similar phenotype to vCJD. All vCJD cases described to date have been methionine homozygotes. Hence, there could be larger numbers of future vCJD cases, heterozygous at codon 129, and which have much longer incubation periods. □

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