Journal of NeuroVirology, 9: 183–193, 2003
 ISSN 1355-0284/03 $12.00+.00
DOI: 10.1080/13550280390194082

Taylor & Francis
Taylor & Francis Group

Prion diseases

Edward McKintosh, Sarah J Tabrizi, and John Collinge

Department of Neurodegenerative Disease/MRC Prion Unit, Institute of Neurology, University College London, London, United Kingdom

Prion diseases are incurable neurodegenerative conditions affecting both animals and humans. They may be sporadic, infectious, or inherited in origin. Human prion diseases include Creutzfeldt–Jakob desease (CJD), Gerstmann–Straussler–Scheinker disease, kuru, and fatal familial insomnia. The appearance of variant CJD, and the demonstration that is caused by strains indistinguishable from bovine spongiform encephalopathy (BSE) in cattle, has led to the threat of a major epidemic of human prion disease in the UK and other countries where widespread dietary exposure to bovine prions has occurred. This article reviews the history and epidemiology of these diseases, and then focuses on important areas of current research in human prion disorders. *Journal of NeuroVirology* (2003) **9,** 183–193.

Keywords: bovine spongiform encephalopathy; Creutzfeldt–Jakob disease; fatal familial insomnia; Gerstmann–Straussler–Scheinker syndrome; kuru; prion; scrapie; spongiform encephalopathy

Background

In the 18th century, sheep diseases characterized by trembling were described in French sheep (la tremblante) and as *itching disease* (Gnubberkrankheit) or *trotting disease* (Traberkrankheit) in Germany (McGowan, 1922). This disease is now generally known by the Scottish term "scrapie," a term that describes the tendency for affected animals to relieve the presumed pruritis by scraping their fleeces against hard objects. The economic effects of this disease on the British wool industry were serious enough for the British Government to include funds for scrapie research in its first grant to the Royal Veterinary College in London in 1910. In the 1940s and 1950s, it also led to many countries banning imports of British sheep until proved scrapie-free.

In 1922, Spielmeyer introduced the term "Creutzfeldt–Jakob disease" (CJD) to describe a human neurological disease that was characterized by rapidly progressive myoclonus, ataxia, and dementia, drawing from earlier case reports by Creutzfeldt (1920) and Jakob (1921). This term was initially used to describe a wide range of conditions, which would not necessarily have met modern diagnostic criteria for CJD.

In the 1950s, Australia started to explore the highlands of Papua New Guinea, which it had previously been given responsibility for by the United Nations. Travel into this isolated area led to the discovery of a new disease, kuru. Doctors who investigated this novel disease found that it was characterized by truncal ataxia and tremor, with relentless and inevitable progression to dementia and death. Initial investigation of its cause focused on an environmental or genetic etiology. It was eventually found, however, that the disease was transmissible and shared the same spongiform features on histology as scrapie and CJD. The pivotal finding that these "transmissible spongiform encephalopathies" (TSEs) had an infectious etiology led to the award of the Nobel Prize for medicine in 1970 to Dr Carleton Gajdusek (Gajdusek *et al*, 1966).

It was the introduction of this concept of transmissibility that allowed development of the diagnostic criteria for CJD, atypical cases being classified according to their transmissibility or not. The histological triad of spongiform vacuolation (in any part of cerebral grey matter), neuronal loss, and astroglial proliferation, with or without amyloid plaques, were

Address correspondence to Dr. John Collinge, Imperial College of Medicine at St. Mary's, MRC Prion Unit, Norfold Place, London, W2 1PG, USA.

The authors would like to thank Jonathan Wadsworth for critically reviewing this paper, and Ray Young for his help in designing the illustrations.

Received 17 January 2003; accepted 24 January 2003.

proposed by Beck and Daniel in 1987 and recognized to be a uniform finding across human and animal prion diseases.

When the infectious nature of the disease was discovered, the constitution of the agent was still unclear. It was thought to be a virus due to its ability to pass through filters with a small pore size. The incubation period of these diseases was found, however, to be much longer than those of other infectious diseases (up to 50 years for kuru), leading to the term "slow virus." Further investigation of the nature of the infectious agent led to rejection of the idea that it contained DNA. This heretical suggestion arose because infectivity was not affected by treatments that would usually inactivate nucleic acids (such as ultraviolet [UV] light and nucleases) (Alper *et al*, 1966, 1967). Also, unlike viral infections, these diseases fail to elicit an immune response; and importantly, no virus has ever been consistently demonstrated in association with the disease.

In 1967, Griffith suggested that the infectious agent might be a protein, and a protease-resistant sialoglycoprotein was isolated by Bolton *et al* in 1982, using progressive enrichment of brain homogenates for infectivity. This protein was shown to accumulate in affected brains, and to be the major constituent of infective brain fractions (Griffith, 1967; Bolton *et al*, 1982; Prusiner *et al*, 1982). In 1982, Prusiner coined the term prion (from proteinaceous infectious particles) to distinguish these infectious particles from viruses or viroids. The protein component of these particles was then designated the prion protein (PrP) (Prusiner, 1982) (work for which he was later awarded the Nobel Prize). More recent x-ray crystallography studies on prions suggest that even if nucleic acids are present in association with prion protein, this would be at a size too small to encode phenotypic diversity (Alper, 1993), further strengthening the protein-only 'prion hypothesis.'

Epidemiology

Human TSEs can be divided up by etiology into infectious (5%), sporadic (80%), and inherited (15%). Although study of infectious prion diseases such as kuru, and more recently new variant CJD (vCJD), has lead to large advances in the study of these diseases, the majority of human sufferers are affected by sporadic CJD.

Sporadic CJD occurs at a rate of 0.5 to 1 per million population per year; this incidence is seen worldwide and until recently has remained constant. It is thought to arise as a result of a conformational change in a single prion protein as a rare stochastic event in an otherwise healthy human. An alternate possibility is that the initial misfolded prion molecules are produced following a somatic mutation in the prion gene in a single neuronal cell.

About 15% of human TSEs occur due to inherited mutations in the prion gene, located on chromosome 20p. Over 30 pathogenic mutations have now been described, all causing autosomal dominantly inherited diseases. These different mutations exhibit wide phenotypic variability, even within affected families carrying the same mutation.

The most common familial TSE is Gerstmann-Straussler-Scheinker syndrome (GSS). This usually occurs in the third or fourth decade of life and generally presents as a chronic cerebellar ataxia with pyramidal features. Dementia arises much later than in CJD, with death usually occurring 5 years after diagnosis. The mutation causing GSS was first identified by Hsiao in 1989 as being P102L, although several other mutations in the prion protein gene have since been shown to cause the disease (Hsiao *et al*, 1989). Other familial TSEs include cases that present with symptoms 'classical' of CJD and GSS, but also includes cases that lack either the usual symptoms or the usual histological features of TSEs, although immunohistochemistry for abnormal PrP is usually positive (Collinge *et al*, 1992). A varying mix of progressive dementia, cerebellar ataxia, pyramidal signs, extrapyramidal features, pseudobulbar signs, myoclonus, chorea, seizures, and amyotrophic features are seen. The diagnosis is confirmed by sequencing of the prion gene.

The spectrum of human TSEs was further broadened by description of fatal familial insomnia (FFI) in 1986, which presents with progressive untreatable insomnia and autonomic dysfunction (Lugaresi *et al*, 1986). Although FFI patients show moderate cortical astrocytosis, the most consistent finding is atrophy of the anterior-ventral and medial-dorsal thalamic nuclei. Two patients with FFI have been described to have widespread spongiosis and a periodic electroencephalogram (EEG); these patients were subsequently found to have mutations at codon 178 in the prion gene (Medori *et al*, 1992a, 1992b; Gambetti *et al*, 1993), a mutation that had previously been described in patients with CJD. FFI has since been transmitted to laboratory animals (Collinge *et al*, 1995; Tateishi *et al*, 1995). The finding that even when spongiform encephalopathies occur as a result of mutations in the prion gene (Medori *et al*, 1992; Kretzschmar *et al*, 1995; Mastrianni *et al*, 1996) the prions formed are still transmissible strongly supports the case in favor of the prion protein as a sole constituent of the disease. This finding also makes TSEs unique among diseases as they may occur spontaneously, due to genetic mutations, or due to infectious passage.

Currently, the lowest annual incidence of TSEs are those acquired by either iatrogenic or dietary exposure. There are many documented examples of iatrogenic prion disease, occurring as a result of cross contamination via surgical instruments (Gibbs *et al*, 1994), injection of hormones prepared from pooled cadaveric pituitary glands (Rappaport and Graham, 1987), corneal transplantation (Kennedy *et al*, 2001;

Gottesdiener, 1989), and implantation of cadaveric dura mater grafts (Clavel and Clavel, 1996). Greater awareness of the risks of transmission of TSEs via these routes has led to a change in practices, with pituitary hormones now manufactured by recombinant techniques and greater screening of cadaveric donors for transplanted organs and tissues. This screening is done by seeking a history of familial TSEs, or of potential exposure to TSE transmission by previous use of cadaveric growth hormone or dura mater implantation. Because there is still no rapid diagnostic test, this screening process, although helpful, is not infallible.

The occurrence of bovine spongiform encephalopathy (BSE) in the UK cow herds in the 1990s led to transmission to humans (Hill *et al*, 1997a; Collinge *et al*, 1996). The origin of BSE will probably always remain unclear but it is assumed that a case of "sporadic BSE" arose in a single cow, by conformational change in a single prion protein molecule or by mutation of a single prion gene, in much the same way that sporadic CJD is thought to arise in humans. This process may have occurred as a rare event for hundreds of years. In the 1980s, however, practices in animal husbandry changed, resulting in parts of cow carcases reentering the bovine food chain. This allowed for the infection of other cows with BSE, and over time, produced an epidemic of BSE in cows, which then entered the human food chain on a large scale.

The transmission of BSE to humans as vCJD (Hill *et al*, 1997a) gave rise to a vast public problem, with the death of 119 people from vCJD so far (National CJD Surveillance Unit, 2002). This represents a small fraction of the annual incidence of sporadic and familial TSEs, but the number of people in the UK currently incubating the disease is still unclear (Ghani *et al*, 1999; Hilton *et al*, 2000). It is known from the study of kuru that the mean incubation period of these diseases is in the order of decades and the fact that vCJD involves bovine prions crossing a "transmission barrier" will further prolong this incubation period (see below). This has implications for epidemiological models, in which mean incubation period is related to eventual epidemic size, therefore these models need to be interpreted with caution. A worrying finding concerns an increase in the incidence of sporadic CJD in the UK over the past 20 years. This was thought to represent better case ascertainment, but recent research has raised the possibility that these cases of sporadic CJD may include individuals who have actually developed the disease as a result of eating BSE-infected beef, but presenting with a different phenotype to that usually seen with vCJD (Asante *et al*, 2002).

The long incubation period of vCJD means that there is a considerable risk that asymptomatic carriers will pass the disease on via surgical instruments, donated blood, or cadaveric tissue donation, significantly increasing in the size of any UK epidemic. Although mathematical models suggest that it is unlikely that vCJD will become endemic in the UK population (*Risk assessment for transmission of vCJD via surgical instruments: a modelling approach and numerical scenarios*. UK Department of Health, 2001), it still remains a remote possibility. The reality of these risks is demonstrated by previous infections from surgical instruments (Gibbs *et al*, 1994), but their magnitude will remain impossible to quantify until better data on the number of asymptomatic carriers are available (Hilton *et al*, 2002; Ghani, 2002).

Structure of the prion protein

The unifying hallmark of the prion diseases is the aberrant metabolism of the prion protein (PrP), which exists in at least two conformational states with different physicochemical properties. The normal form of the protein, referred to as PrP^{C}, is a highly conserved cell-surface protein attached via a glycophosphatidyl inositol anchor. It is expressed in a wide range of cell types, and particularly in neuronal cells. PrP^{C} is a sialoglycoprotein of molecular weight 33 to 35 kDa, with a high content of α-helical secondary structure that is sensitive to protease treatment and soluble in detergents. The disease-associated isoform, referred to as PrP^{Sc}, is found only in infected brains as aggregated material, is partially resistant to protease treatment and insoluble in detergents, and has a high content of β-sheet secondary structure.

As discussed above, prions do not appear to contain significant amounts of nucleic acids, and this is backed by the experiments of Kellings *et al*, who demonstrated that no group of similar DNA fragments consistently copurified with PrP^{Sc} (Kellings *et al*, 1994). This has necessitated a new approach to describe how prions replicate. The protein-only hypothesis proposed by Griffith in 1967 was modified by Prusiner, who proposed a model of infection that relied on a misfolded "scrapie" form of the normal prion protein molecule (PrP^{Sc}) being able to induce the refolding of the host's constitutive cellular prion protein (PrP^{c}) to mimic its aberrant conformation [7, 10] (Figure 1). This proteinaceous infectious particle (prion) would encode differences in disease

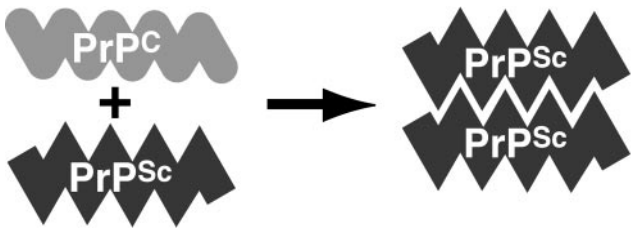


Figure 1 The "Prion Hypothesis" suggests that an abnormal conformer (PrP^{Sc}) of the cellular prion protein (PrP^{c}) is capable of inducing PrP^{c} to undergo a change of conformation into PrP^{Sc}.

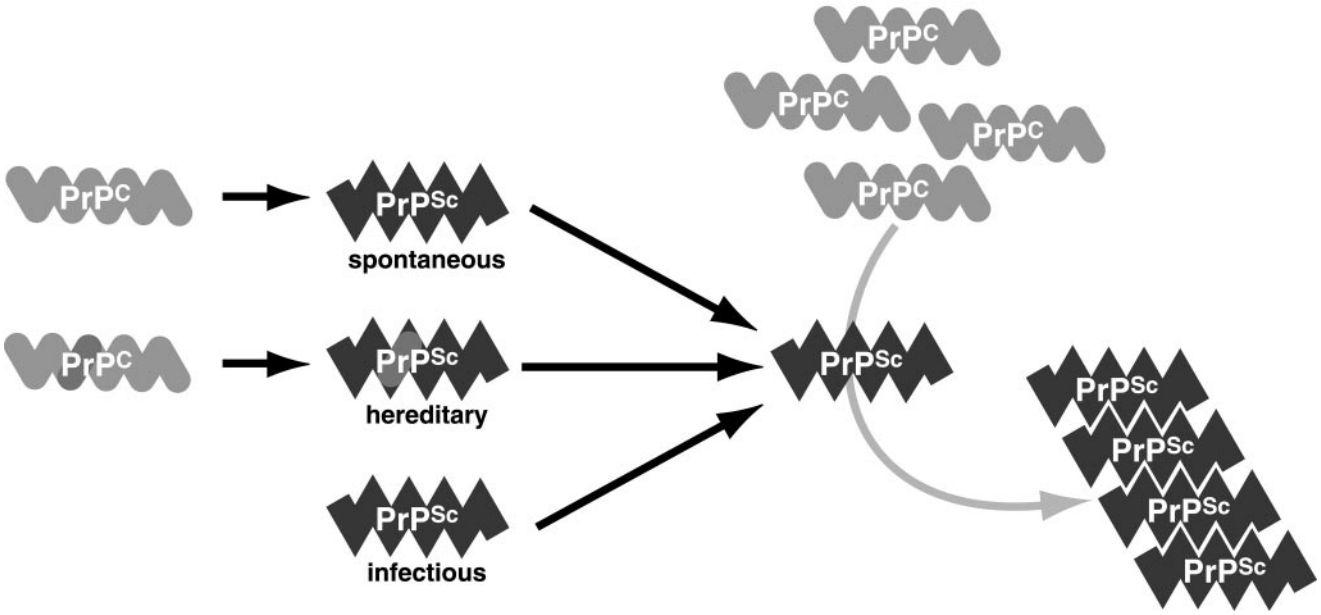


Figure 2 The abnormally folded PrP^{Sc} forms of the prion protein may arise either spontaneously, or in patients carrying a mutation that makes misfolding more likely, or from an exogenous infective source. Once a misfolded form has arisen, other PrP^{c} molecules are converted, propagating the disease in a form of a chain reaction.

pathogenesis by differences in its conformation, allowing for the transmission of varying "phenotypes" in the absence of nucleic acid (Prusiner, 1982).

One of the strengths of this model is that it provides a mechanism by which TSEs can arise through infectious, hereditary, or spontaneous mechanisms (Figure 2).

The greatest objection to the prion hypothesis, however, is the idea that an infectious agent could be capable of producing diseases with different phenotypes without the presence of DNA (or RNA) to encode this information. Many experiments demonstrate that different forms of scrapie do result in different phenotypes, and that these phenotypes are maintained even when the infectious agent is passaged through different species, demonstrating that they are not encoded by the primary structure of a species' prion protein (Bruce *et al*, 1994; Bessen and Marsh, 1992, 1994).

An alternative hypothesis was proposed by Weissmann in 1991 (Weissmann, 1991). This hypothesis suggests that the protein component of a prion (designated the apoprion) can cause disease by itself but that a small host-derived nucleic acid (designated the coprion) can associate with it. The combination of both (the holoprion) can then give rise to a host-modified strain of disease. From this model, it can be predicted that removal of the coprion would result in a loss of strain-specific properties, and substitution of coprions from another strain would lead to a corresponding change in strain properties. Additionally, infectivity would be resistant to treatments that inactivate nucleic acids, but strain-specific properties would not. Experiments testing these hypotheses have not yet been published, but demonstration that PrP^{Sc} is able to acquire strain-specific properties *in vitro* argues against it, and favors prion protein conformation as the mediator of strain diversity (Bessen *et al*, 1995).

Strain typing

PrP produces three distinct bands on Western blot, due to the presence of one, two, or no sugar molecules at the two potential glycosylation sites on the prion protein (Figure 3). Treatment of infected brain homogenates with the protease proteinase K under defined conditions degrades PrP^{c} completely, leaving PrP^{Sc} substantially intact (Figure 3). Proteinase K treatment of PrP^{Sc} does cleave approximately 70 amino acids from the N terminus of the molecule, making it appear to have a lower molecular mass (Figure 3).

Different strains produce different migration patterns on polyacrylamide gels after this limited proteolysis, suggesting that they have different conformations [36]. In addition, the ratio of unglycosylated to mono- and diglycosylated forms also varies. This has allowed the ratio of percentages of the protein in different forms to be used as marker of strain "type," with the finding that different strain types of prion produce sporadic or variant CJD in humans (Figure 4**A**, **B**) (and discussed below) (Wadsworth *et al*, 1999).

If they are to be responsible for encoding diversity in strain type, these biochemical properties must be

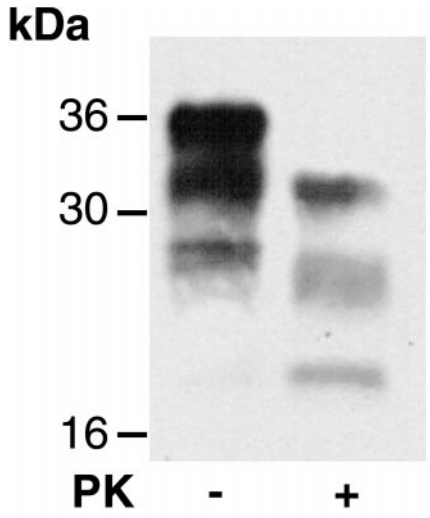


Figure 3 PrP^{C} is seen to result in three bands, with masses between 16 and 36 kDa, on Western blot. Treatment with proteinase K (PK) under defined conditions degrades the more numerous PrP^{C} molecules, revealing PrP^{Sc}. Proteinase K cleaves some residues from PrP^{Sc}, causing an apparent decrease in its molecular mass (figure courtesy of Dr Andy Hill).

retained after transmission to experimental animals of both the same and different species. Studies with CJD isolates have demonstrated that this is so, with both PrP^{Sc} fragment size and glycoform ratio maintained on passage in transgenic mice expressing human PrP (Collinge *et al*, 1996). Transmission of human prions and bovine prions to wild-type mice also results in murine PrP^{Sc} with fragment sizes and glycoform ratios that correspond to the original inoculum (Collinge *et al*, 1996). vCJD is associated with PrP^{Sc} glycoform ratios that are distinct from those seen in classical CJD but similar to those seen in BSE, both in cows and when transmitted to several other species (Collinge *et al*, 1996; Hill *et al*, 1997b). These data strongly support the "protein only" hypothesis of infectivity by suggesting that strain variation is encoded by a combination of PrP conformation and glycosylation. As PrP glycosylation occurs before conversion to PrP^{Sc}, different glycoform ratios may represent selection of a particular PrP^{C} glycoform by PrP^{Sc} of different conformations. According to this hypothesis, PrP conformation would be the primary determinant of strain type, with glycosylation being involved as a secondary process. Because it is known that different cell types glycosylate proteins differently, PrP^{Sc} glycosylation patterns may explain the different neuropathological phenotypes that distinguish different prion strains (Collinge *et al*, 1996). Particular PrP^{Sc} glycoforms may replicate most favorably in neuronal populations with a similar PrP glycoform expressed on the cell surface. Such targeting could also explain the different incubation periods seen in different strains, with targeting of more critical brain regions, or regions with higher levels of PrP expression, producing shorter incubation periods.

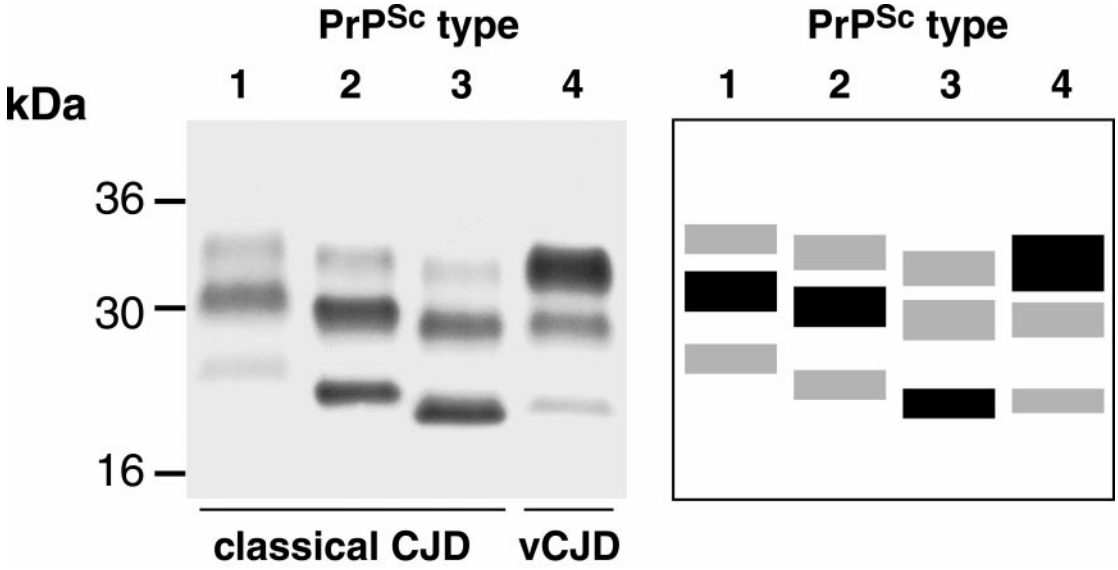
Host genotype

As mentioned above, TSEs are unusual in having familial as well as infectious etiologies. Since its original discovery, over 30 disease-causing mutations have been described in the prion gene (Figure 5).

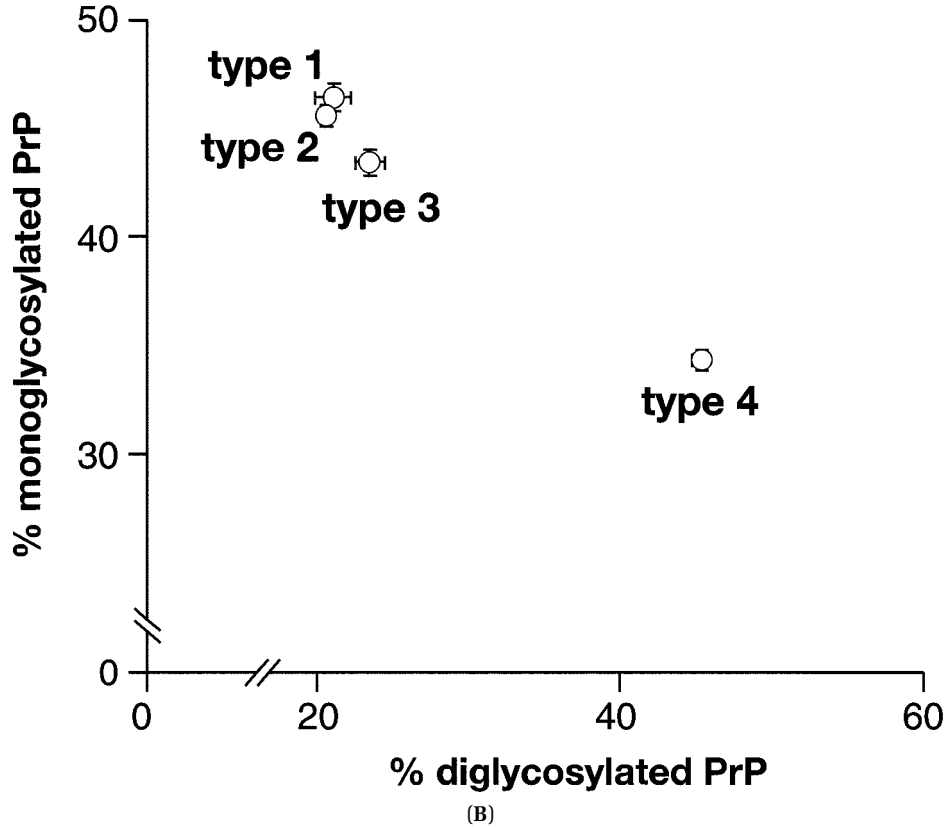
Nonpathogenic polymorphims are also described (Figure 5), and the most important of these is the methionine/valine polymorphism at position 129 (38% MM, 51% MV, 11% VV in the UK population). A study of 22 sporadic CJD cases found that all but one was homozygous for either methionine or valine at codon 129 (Palmer *et al*, 1991). This finding has subsequently been repeated in larger studies, both in the UK (Windl *et al*, in press) and abroad (Laplanche *et al*, 1994; Salvatore *et al*, 1994). Genotyping of seven CJD patients who had been treated with human pituitary hormones also showed a significant excess of valine homozygotes at codon 129 (Collinge *et al*, 1991), and this finding has subsequently been replicated in the USA (Brown *et al*, 1994) and France (Laplanche *et al*, 1994). Furthermore, all cases of vCJD to date have occurred in patients who are homozygous for methionine at this position (Ironside *et al*, 2000). These findings suggest that the presence of heterozygosity at codon 129 provides protection from prion diseases presumably by reducing the prion protein's ability to refold into the conformation necessary to propagate CJD prions. It is, however, not yet known whether this protection is partial or total, because infection with acquired prion disease may still occur in codon 129 heterozygotes, but with longer incubation periods. This has major implications for estimation of the eventual size of the vCJD epidemic.

The "species barrier"

It has been known for many years that transmission of prion strains between species is restricted by a "species barrier" (Pattison, 1965). This is demonstrated by an increase in incubation period, and a decrease in the percentage of animals succumbing to disease, when prions from one species are inoculated into another ("first passage"). This contrasts with the situation when prions are inoculated into animals of the same species, when these animals are seen to all become sick, with remarkably consistent incubation periods. If after inoculation into a different species infectious tissue is taken from the animals that do become sick and transmitted to further animals ("second passage"), the pattern of infectivity seen resembles that of the initial species, with most if not all animals becoming sick after relatively short and consistent incubation periods (Figure 6). This transmission barrier between species can be quantified by the difference in incubation period and rate of infection between first and second passages. It can also be quantified more rigorously by inoculating 10-fold serial dilutions and determining the infectious



(A)



(B)

Figure 4 (**A**) Different strains of CJD are seen on Western blot to have differences in both molecular mass and the percentage of un-, mono-, and diglycosylated forms. These features can be used as a biochemical indicator of "Strain Type." (For further details see AF Hill *et al*, Molecular classification of sporadic CJD, *Brain*, in press.) (**B**) Using the ratio of the percentage of PrP^{Sc} in mono- or diglycosylated forms demonstrates the clear difference between sporadic and variant strains of CJD (figure courtesy of Dr Jonathan Wadsworth).

dilution at which 50% of animals succumb, the ID_{50}.

It was initially demonstrated that this species barrier was due to differences in PrP primary structure between originator species and host. When mice, which are normally resistant to hamster prions, were modified to express the hamster PrP gene, they became highly susceptible to infection with Sc237 hamster prions (Prusiner *et al*, Rubin *et al*, Stanton *et al*, 1990). The finding that sporadic and acquired CJD

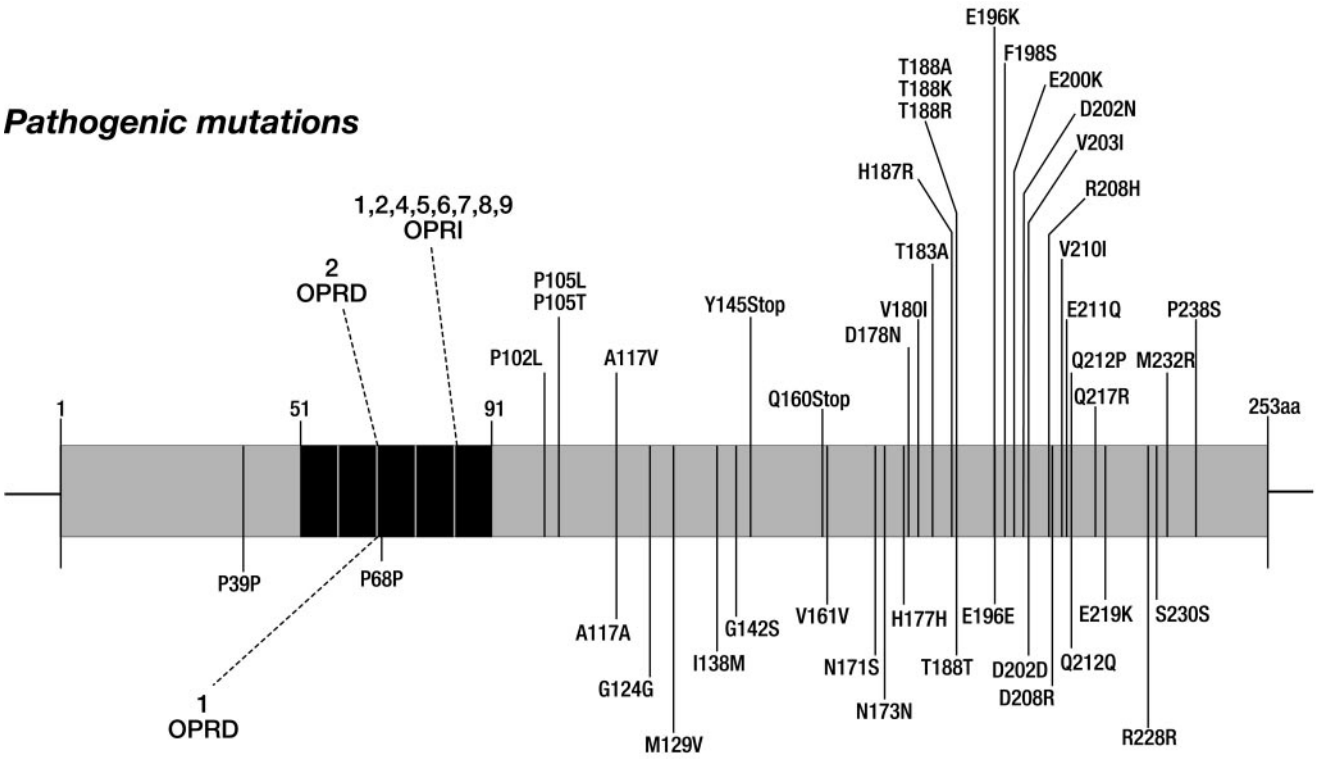


Figure 5 Pathogenic mutations and polymorphisms in the human prion protein. The pathogenic mutations associated with human prion disease are shown above the PrP coding sequence. These consist of 1, 2, or 4–9 octapeptide repeat insertions within the octarepeat region between codons 51 and 91, a deletion of 2 octapeptide repeats, and various point mutations causing missense amino acid substitutions. Point mutations are designated by the wild-type amino acid preceding the codon number, followed by the mutant residue, using single-letter amino acid conventions. Polymorphic variants are shown below the PrP coding sequence. Deletion of one octapeptide repeat is not associated with disease (figure courtesy of Mr Jon Beck).

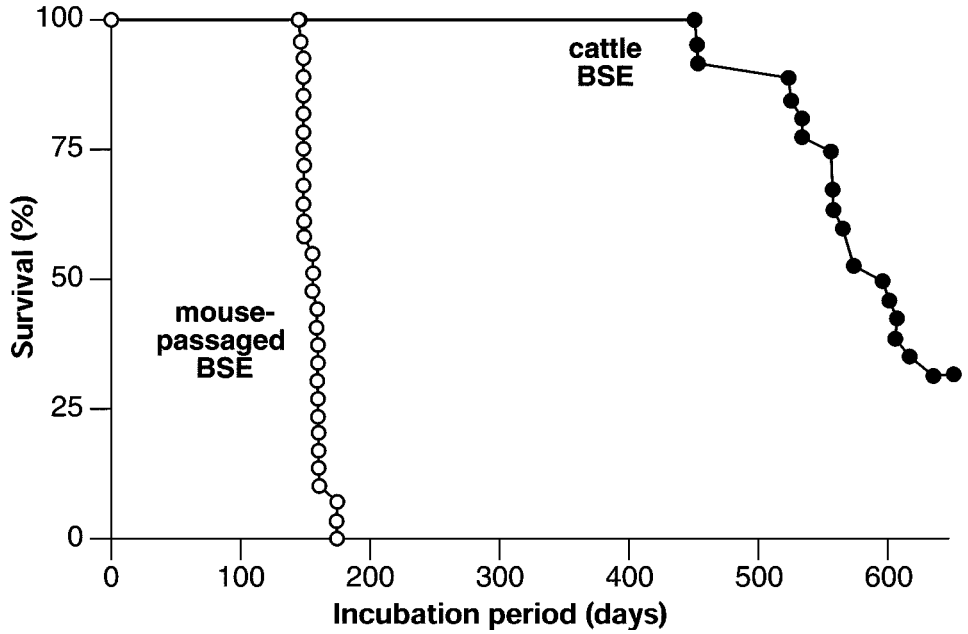


Figure 6 Decrease in incubation period with second passage. Initial inoculation of mice with prions derived from a bovine source (cattle BSE) results in less that 100% of mice succumbing to infection. Additionally, those mice that succumb do so after prolonged and variable incubation periods. If homogenate is prepared from mice that did succumb following initial BSE inoculation, then transmission of this "mouse-passaged" BSE ("second passage") results in a much more uniform and rapid incubation of disease, with approximately 100% clinical infection (figure courtesy of Dr Jonathan Wadsworth).

occurred in patients homozygous for the methionine/valine polymorphism at position 129 on the prion gene also suggested infection occurred most readily when PrP^{c} primary structure was identical with the PrP^{Sc} responsible for prion propagation (Palmer *et al*, 1991; Collinge *et al*, 1991).

It has, however, also been demonstrated that this is not always the case. BSE prions transmit efficiently to a wide range of species but maintain their transmission characteristics even when transmitted through intermediate species with different PrP primary structures (Bruce *et al*, 1994). Sporadic CJD prions do not readily transmit to wild-type mice but transmit readily to mice transgenic for the human prion gene, with consistent short incubation periods that are unaltered by second passage and therefore consistent with a lack of species barrier (Hill *et al*, 1997a). vCJD prions (which are also by definition of the same primary protein structure), on the other hand, transmit more readily to wild-type mice but less efficiently to transgenic mice (Hill *et al*, 1997a). These findings suggest that primary structure is not the sole determinant of the species barrier and that conformation of the protein is also important. As a result, the term "transmission barrier" has been suggested to be a more suitable term (Collinge, 1999). Originally, assessment of transmission barriers relied on animals developing clinical disease. Using this method of assessment, there are highly efficient barriers limiting transmission of hamster prions to mice (Kimberlin and Walker, 1979; Scott *et al*, 1989). Although mice inoculated with hamster Sc237 prions live as long as mock-inoculated mice and don't show any signs of clinical disease, examination of their brains at long time points after inoculation reveals high levels of PrP^{Sc} and/or infectivity (Hill *et al*, 2000). This finding suggests that a transmission barrier occurs not just to the replication of infectious prions, but also to subsequent neurodegeneration caused by accumulation of these prions.

Function of PrP^{C}

A major goal of research into TSEs is an understanding of the role of the normal prion protein, and the mechanisms by which PrP^{Sc} accumulation is linked with neurodegeneration. The essential role of host PrP^{C} for prion propagation and pathogenesis is demonstrated by the fact that mice in which the PrP gene has been disrupted (referred to as $Prnp^{0/0}$) are resistant to scrapie infection (Bueler *et al*, 1993; Manson *et al*, 1994), and that reintroduction of the murine PrP^{C} transgene restores susceptibility to infection (Fischer *et al*, 1996).

$Prnp^{0/0}$ mice have also been studied to probe the normal function of PrP^{C}. Two independently generated lines of gene-targeted $Prnp^{0/0}$ mice developed normally and appeared to suffer no gross phenotypic abnormalities (Manson *et al*, 1994; Bueler *et al*, 1992). The relative normality of these PrP-null mice was thought to result from effective adaptive changes during development. However, data from *Prnp* conditional knockout mice suggest this is not the case (Mallucci *et al*, 2002); these mice undergo ablation of neuronal PrP expression at 9 weeks of age. The mice remain healthy without evidence of neurodegeneration or an overt clinical phenotype, demonstrating that acute loss of neuronal PrP in adulthood is tolerated, and that the pathophysiology of prion diseases is unlikely to be due to loss of normal PrP function (Mallucci *et al*, 2002).

The prion protein is expressed in most adult tissues (Manson *et al*, 1992), but is found at the highest levels in the central nervous system (CNS) and immune systems (Dodelet and Cashman, 1998). It is a cell-surface sialoglycoprotein with high affinity for copper (Hornshaw *et al*, 1995; Brown *et al*, 1997; Stöckel *et al*, 1998) and possesses superoxide dismutase activity *in vitro* when refolded in the presence of high concentrations of copper chloride (Brown *et al*, 1999). Newly synthesised PrP^{c} is transported to the cell-surface and then cycles rapidly via a clathrin-mediated mechanism, with a transit time of approximately 1 hour between the surface and early endosomes (Shyng *et al*, 1994). This process is seen with cell-surface receptors, suggesting a similar role for PrP^{c}; its femtomolar affinity for copper suggests that copper metabolism or transport may be in fact be its true function (Jackson *et al*, 2001).

Antiprion therapeutics

Various compounds are known to interact with PrP^{Sc}, these include anthracycline (Tagliavani *et al*, 1997), Congo red (Ingrosso *et al*, 1995), dextran sulfphate, pentosan polysulphate, and other polyanions (Ehlers and Diringer, 1984; Farquhar and Dickinson, 1986; Kimberlin and Walker, 1986), and β-sheet breaker peptides (Soto *et al*, 2000). Unfortunately, most of these compounds are only effective if administered well before the onset of clinical disease, and frequently also show either high levels of toxicity, low levels of bioavailability, or both. Although the ability to bind PrP^{Sc}, and prevent further conversion of PrP^{c}, provides a logical approach to slowing or preventing disease progression, it is unlikely to lead to a cure for prion diseases. The development of therapies that provide a cure will need a clearer understanding of the role of PrP^{c}, as well as advances in early diagnosis.

Currently clinical trials using quinacrine and chlorpromazine treatment in CJD and vCJD patients are underway in both the UK and USA, but results are not yet published. However, there is no evidence that these drugs are useful against prion disease *in vivo*, and recently quinacrine treatment in a rodent

model of CJD demonstrated no efficacy (Collins *et al*, 2002). This has highlighted the difficulty of transferring *in vitro* experiments to the clinical setting. Yet public demand for any treatment that will slow or abolish the relentless progression of these extremely distressing diseases is understandably high. A recent legal challenge has obliged a UK health authority to offer intraventricular pentosan polysulphate to two patients (*Independent Newspaper*, 24th December, 2002), that this highly experimental treatment will go ahead underlines the drive to find a viable antiprion therapeutic.

Diagnostic testing

The key to therapy for prion diseases is early diagnosis before significant neurological impairment has occurred. Diagnosis of TSEs was initially made by the finding of spongiform change and amyloid-plaque deposition on histology. The presence of infectious prions could only be demonstrated by passaging brain homogenate to indicator animals, a costly and lengthy process. Spongiform change and gliosis on histology are still used in diagnosis, whether from experimental animals, livestock, or humans. The production of PrP-specific antibodies has allowed immunohistochemistry to be added to the histologist's arsenal, improving the specificity of the diagnosis, especially in cases where plaques are not prolific or there is doubt about a nonprion cause for spongiform change. This, in addition to highly sensitive Western blot analysis, has increased the sensitivity and specificity of diagnosis (Wadsworth *et al*, 2001). Although the production of PrP-specific antibodies has led to advances in histology, brain tissue is still required for the diagnosis of most TSEs, thus necessitating a brain biopsy.

However, vCJD is unusual, in that lymphotropism appears to be a major feature of the disease. Spongiform change and amyloid plaques are not seen in lymphoid tissue but histological demonstration of the presence of prion protein is possible with PrP-specific antibodies. Histological sections are first treated with formic acid, which disinfects the tissue, removes PrP^{c}, and denatures the prion protein to permit antibody binding to PrP^{Sc}. This allows vCJD to be diagnosed reliably from tonsil biopsy, avoiding the need for a more invasive brain biopsy in these patients (Hill *et al*, 1997b; Wadsworth *et al*, 2001; Hill *et al*, 1999). This combination of immunohistochemistry and Western blotting analysis of tonsil biopsies from suspected vCJD patients has been shown to provide a diagnostic test with 100% specificity and sensitivity when studied in a recent case series of 43 cases (24 positive and 19 negative) (A Kennedy, paper in preparation).

Conclusion

Recent research has suggested that epidemics of human TSEs may have occurred for thousands of years (S Mead, paper submitted); and the occurrence of TSEs in UK animal herds has had economic consequences for many decades. Despite these statistics, it is the potential threat of an epidemic of vCJD in the UK population that has provided an increased impetus to try to understand theses novel diseases. Animal TSEs are now recognized to occur across all countries (with the exception of Australia and New Zealand), and to occur not only as scrapie in sheep, but as transmissible mink encephalopathy, BSE in cows, domestic cats, and zoo animals, and as chronic wasting disease in American deer and elk herds. Infectious diseases are not known for their tendency to respect national borders; BSE has already spread through many European countries and vCJD may therefore still do so.

The past few years have seen major increases in our understanding of the etiology and pathology of prion diseases but large research challenges still remain, if a major epidemic of vCJD is to be avoided and suitable treatments are to be found. Not least among these are proof of the exact nature of this novel infectious agent, further elaboration of the role of PrP^{c}, and the development of reliable therapeutics.

References

Alper T (1993). The scrapie enigma: insights from radiation experiments. *Radiat Res* **135:** 283–292.

Alper T, Cramp WA, Haig DA, Clarke MC (1967). Does the agent of scrapie replicate without nucleic acid? *Nature* **214:** 764–766.

Alper T, Haig DA, Clarke MC (1966). The exceptionally small size of the scrapie agent. *Biochem Biophys Res Commun* **22:** 278–284.

Asante EA, Linehan JM, Desbruslais M, Joiner S, Gowland I, Wood A, Welch J, Hill AF, Lloyd SE, Wadsworth JDF, Collinge J (2002). BSE prions propagae as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. *EMBO J*, **21:** 6358–6366.

Bessen RA, Kocisko DA, Raymond GJ, Nandan S, Lansbury PT, Coughey B (1995). Non-genetic propagation of strain-specific properties of scrapie prion protein. *Nature* **375:** 698–700.

Bessen RA, Marsh RF (1992). Biochemical and physical properties of the prion protein from two strains of the transmissible mink encephalopathy agent. *J Virol* **66:** 2096–2101.

Bessen RA, Marsh RF (1994). Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. *J Virol* **68:** 7859–7868.

Bolton DC, McKinley MP, Prusiner SB (1982). Identification of a protein that purifies with the scrapie prion. *Science* **218:** 1309–1311.

Brown DR, Qin K, Herms JW, Madlung A, Manson J, Strome R, *et al* (1997). The cellular prion protein binds copper in vivo. *Nature* **390:** 684–687.

Brown DR, Wong BS, Hafiz F, Clive C, Haswell SJ, Jones IM (1999). Normal prion protein has an activity like that of superoxide dismutase. *Biochemical J* **344:** 1–5.

Brown P, Cervenakova L, Goldfarb MD, McCombie WR, Rubenstein R, Will RG, *et al* (1994). Iatrogenic Creutzfeldt-Jakob disease: an example of the interplay between ancient genes and modern medicine. *Neurology* **44:** 291–293.

Bruce M, Chree A, McConnell I, Foster J, Pearson G, Fraser H (1994). Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. *Philos Trans R Soc Lond [Biol]* **343:** 405–411.

Bueler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M, *et al* (1993). Mice devoid of PrP are resistant to scrapie. *Cell* **73:** 1339–1347.

Bueler H, Fischer M, Lang Y, Bluethmann H, Lipp H-P, DeArmond SJ, *et al* (1992). Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature*, **356:** 577–582.

Clavel M, Clavel P (1996). Creutzfeldt-Jakob disease transmitted by dura mater graft. *Eur Neurol* **36:** 239–240.

Collinge J (1999). Variant Creutzfeldt-Jakob disease. *Lancet* **354:** 317–323.

Collinge J, Brown J, Hardy J, Mullan M, Rossor MN, Baker H, *et al* (1992). Inherited prion disease with 144 base pair gene insertion: II: Clinical and pathological features. *Brain* **115:** 687–710.

Collins SJ, Lewis V, Brazier M, Hill AF, Fletcher A, Masters CL (2002). Quinacrine does not prolong survival in a murine Creutzfeldt-Jakob disease model. *Ann Neurol* **52:** 503–506.

Collinge J, Palmer MS, Dryden AJ (1991). Genetic predisposition to Iatrogenic Creutzfeldt-Jakob disease. *Lancet* **337:** 1441–1442.

Collinge J, Palmer MS, Sidle KCL, Gowland I, Medori R, Ironside J, *et al* (1995). Transmission of fatal familial insomnia to laboratory animals. *Lancet* **346:** 569–570.

Collinge J, Sidle KCL, Meads J, Ironside J, Hill AF (1996). Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. *Nature* **383:** 685–690.

Creutzfeldt HG (1920). Uber eine eigenartige herdfoermige Erkrankung des Zentralnervensystems. *Z Gesamte Neurol Psychiatry* **57:** 1–18.

Dodelet VC, Cashman NR (1998). Prion protein expression in human leukocyte differentiation. *Blood* **91:** 1556–1561.

Ehlers B, Diringer H (1984). Dextran sulphate 500 delays and prevents mouse scrapie by impairment of agent replication in spleen. *J Gen Virol* **65:** 1325–1330.

Farquhar CF, Dickinson AG (1986). Prolongation of scrapie incubation period by an injection of dextran sulphate 500 within the month before or after infection. *J Gen Virol* **67:** 463–473.

Fischer M, Rulicke T, Raber A, Sailer A, Oesch B, Brandner S, *et al* (1996). Prion protein (PrP) with amino terminal deletions restoring susceptibility of PrP knockout mice to scrapie. *EMBO J* **15:** 1255–1264.

Gajdusek DC, Gibbs CJ Jr, Alpers MP (1966). Experimental transmission of a kuru-like syndrome to chimpanzees. *Nature* **209:** 794–796.

Gambetti P, Petersen R, Monari L, Tabaton M, Autilio-Gambetti L, Cortelli P, *et al* (1993). Fatal familial insomnia and the widening spectrum of prion diseases. *Br Med Bull* **49:** 980–994.

Ghani AC (2002). The epidemiology of variant Creutzfeldt-Jakob disease in Europe. *Microb Infect* **4:** 385–393.

Ghani AC, Ferguson NM, Donnelly CA, Hagenaars TJ, Anderson RM (1999). Epidemiological determinants of the pattern and magnitude of the vCJD epidemic in Great Britain. *Proc R Soc Lond B* **265:** 2443–2452.

Gibbs CJ Jr, Asher DM, Kobrine A, Amyx HL, Sulima MP, Gajdusek DC (1994). Transmission of Creutzfeldt-Jakob disease to a chimpanzee by electrodes contaminated during neurosurgery. *J Neurol Neurosurg Psychiatry* **57:** 757–758.

Gottesdiener KM (1989). Transplanted infections: donor-to-host transmission with the allograft. *Ann Intern Med* **110:** 1001–1016.

Griffith JS (1967). Self Replication and scrapie. *Nature* **215:** 1043–1044.

Hill AF, Butterworth RJ, Joiner S, Jackson G, Rossor MN, Thomas DJ, *et al* (1999). Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. *Lancet* **353:** 183–189.

Hill AF, Desbruslais M, Joiner S, Sidle KCL, Gowland I, Collinge J (1997a). The same prion strain causes vCJD and BSE. *Nature* **389:** 448–450.

Hill AF, Joiner S, Linehan J, Desbruslais M, Lantos PL, Collinge J (2000). Species barrier independent prion replication in apparently resistant species. *Proc Natl Acad Sci USA* **97:** 10248–10253.

Hill AF, Zeidler M, Ironside J, Collinge J (1997b). Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. *Lancet* **349:** 99–100.

Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, Penney M, *et al* (2002). Accumulation of prion protein in tonsil and appendix: review of tissue samples. *BMJ* **325:** 633–634.

Hornshaw MP, McDermott Jr, Candy JM, Lakey JH (1995). Copper binding to the N-terminal tandem repeat region of mammalian and avian prion protein: structural studies using synthetic peptides. *Biochem Biophys Res Commun* **214:** 993–999.

Hsiao K, Baker HF, Crow TJ, Poulter M, Owen F, Terwilliger JD, *et al* (1989). Linkage of a prion protein missense variant to Gerstmann-Straussler syndrome. *Nature* **338:** 342–345.

Ingrosso L, Ladogana A, Pocchiari M (1995). Congo red prolongs the incubation period in scrapie-infected hamsters. *J Virol* **69:** 506–508.

Ironside JW, Head MW, Bell JE, McCardle L, Will RG (2000). Laboratory diagnosis of variant Creutzfeldt-Jakob disease. *Histopathology* **37:** 1–9.

Jackson GS, Murray I, Hosszu LL, Gibbs N, Waltho JP, Clarke AR, *et al* (2001). Location and properties of metal-binding sites on the human prion protein. *Proc Natl Acad Sci USA* **98:** 8531–8535.

Jakob A (1921). Uber eigenartige Erkrankungen des Zentralnervensystems mit bemerkenswertem anatomischem Befund. *Z Gesamte Neurol Psychiatry* **64:** 147–228.

Kellings K, Prusiner SB, Riesner D (1994). Nucleic acids in prion preparations: unspecific background or essential component. *Philos Trans R Soc Lond [Biol]* **343:** 425–430.

Kennedy RH, Hogan N, Brown P, Holland E, Johnson RT, Stark W, *et al* (2001). Eye banking and screening for Creutzfeldt-Jakob disease. *Arch Ophthalmol* **119:** 721–726.

Kimberlin RH, Walker CA (1979). Pathogenesis of scrapie: agent multiplication in brain at the first and second passage of hamster scrapie in mice. *J Gen Virol* **42:** 107–117.

Kimberlin RH, Walker CA (1986). Suppression of scrapie infection in mice by heteropolyanion 23, dextran sulfate, and some other polyanions. *Antimicrob Agents Chemother* **30:** 409–413.

Kretzschmar HA, Neumann M, Stavrou D (1995). Codon 178 mutation of the human prion protein gene in a German family (Backer family): sequencing data from 72- year-old celloidin-embedded brain tissue. *Acta Neuropathol (Berl)* **89:** 96–98.

Laplanche J-L, Delasnerie-Lauprêtre N, Brandel JP, Chatelain J, Beaudry P, Alpérovitch A, *et al* (1994). Molecular genetics of prion diseases in France. French Research Group on Epidemiology of Human Spongiform Encephalopathies. *Neurology* **44:** 2347–2351.

Lugaresi E, Medori R, Baruzzi PM, Cortelli P, Lugaresi A, Tinupar P, *et al* (1986). Fatal familial insomnia and dysautonomia, with selective degeneration of thalamic nuclei. *N Engl J Med* **315:** 997–1003.

Mallucci GR, Ratté S, Asante EA, Linehan J, Gowland I, Jefferys JGR, *et al* (2002). Post-natal knockout of prion protein alters hippocampal CA1 properties, but does not result in neurodegeneration. *EMBO J* **21:** 202–210.

Manson JC, Clarke AR, Hooper ML, Aitchison L, McConnell I, Hope J (1994). 129/Ola mice carrying a null mutation in PrP that abolishes mRNA production are developmentally normal. *Mol Neurobiol* **8:** 121–127.

Manson J, West JD, Thomson V, McBride P, Kaufman MH, Hope J (1992). The prion protein gene: a role in mouse embryogenesis? *Development* **115:** 117–122.

Mastrianni JA, Iannicola C, Myers RM, DeArmond S, Prusiner SB (1996). Mutation of the prion protein gene at codon 208 in familial Creutzfeldt-Jakob disease. *Neurology* **47:** 1305–1312.

McGowan JP (1922). Scrapie in sheep. *Scott J Agric* **5:** 365–375.

Medori R, Tritschler HJ, LeBlanc A, Villare F, Manetto V, Chen HY, *et al* (1992a). Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene [see comments]. *N Engl J Med* **326:** 444–449.

Medori R, Montagna P, Tritschler HJ, LeBlanc A, Cortelli P, Tinupar P, *et al* (1992b). Fatal familial insomnia: a second kindred with mutation of prion protein gene at codon 178. *Neurology* **42:** 669–670.

Palmer MS, Dryden AJ, Hughes JT, Collinge J (1991). Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. *Nature* **352:** 340–342.

Pattison IH (1965). Experiments with scrapie with special reference to the nature of the agent and the pathology of the disease. In: *Slow latent and temperate virus infections, NINDB Monograph 2*. Gajdusek CJ, Gibbs CJ, Alpers MP (eds). Washington DC: US Government Printing, p. 249–257.

Prusiner SB, Bolton DC, Groth DF, Bowman K, Cochran SP, Mc Kinley MP (1982). Further purification and characterization of scrapie prions. *Biochemistry* **21:** 6942–6950.

Prusiner, SB (1982). Novel proteinaceous infectious particles cause scrapie. *Science* **216:** 136–144.

Prusiner SB *et al*; Rubin E *et al*; Stanton BR *et al* (1990). D 216. Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. D 217. Production of human hemoglobin S antilles in transgenic mice. D 218. Analysis of murine Nmyc function by in situ hydridization and homologous recombination. *J Cellular Biochem* **UCLA Symposium Suppl 14A:** 363.

Rappaport EB, Graham DJ (1987). Pituitary growth hormone from human cadavers: neurologic disease in ten recipients. *Neurology* **37:** 1211–1213.

Salvatore M, Genuardi M, Petraroli R, Masullo C, D'Alessandro M, Pocchiari M (1994). Polymorphisms of the prion protein gene in Italian patients with Creutzfeldt-Jakob disease. *Hum Genet* **94:** 375–379.

Scott M, Foster D, Mirenda C, Serban D, Coufal F, Wälchli M, *et al* (1989). Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell* **59:** 847–857.

Shyng S-L, Heuser JE, Harris DA (1994). A glycolipid-anchored prion protein is endocytosed via clathrin-coated pits. *J Cell Biol* **125:** 1239–1250.

Soto C, Kascsack RJ, Saborío GP, Aucouturier P, Wisniewski T, Prelli F, *et al* (2000). Reversion of prion protein conformational changes by synthetic beta-sheet breaker peptides. *Lancet* **355:** 192–197.

Stöckel J, Safar J, Wallace AC, Cohen FE, Prusiner SB (1998). Prion protein selectively binds copper(II) ions. *Biochemistry* **37**, 7185–7193.

Tagliavini F, McArthur RA, Canciani B, Giaccone G, Porro M, Bugiani M, *et al* (1997). Effectiveness of anthracycline against experimental prion disease in syrian hamsters. *Science* **276:** 1119–1122.

Tateishi J, Brown P, Kitamoto T, Hoque ZM, Roos R, Wollman R, *et al* (1995). First experimental transmission of fatal familial insomnia. *Nature* **376:** 434–435.

Wadsworth JDF, Hill AF, Joiner S, Jackson GS, Clarke AR, Collinge J (1999). Strain-specific prion-protein conformation determined by metal ions. *Nat Cell Biol* **1:** 55–59.

Wadsworth JDF, Joiner S, Hill AF, Campbell TA, Desbruslais M, Luthert PJ, *et al* (2001). Tissue distribution of protease resistant prion protein in variant CJD using a highly sensitive immuno-blotting assay. *Lancet* **358:** 171–180.

Weissmann C (1991). A 'unified theory' of prion propagation. *Nature* **352:** 679–683.