Variant Creutzfeldt-Jakob disease: pathology, epidemiology, and public health implications

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ABSTRACT Prion diseases, or transmissible spongiform encephalopathies, include Creutzfeldt-Jakob disease (CJD) in humans and scrapie and bovine spongiform encephalopathy (BSE) in animals. These neurodegenerative diseases are invariably fatal and can be transmitted by inoculation or dietary exposure. They are associated with the accumulation of an altered, disease-associated form of the normal prion protein. Pathologically, prion diseases result in neuronal cell death and a characteristic spongiform appearance of the brain tissue. The emergence of a variant form of CJD (vCJD) in the United Kingdom in 1996 has been causally and experimentally linked to the UK BSE epidemic in the 1980s and early 1990s. The finding that BSE is transmissible to different animal species, unlike previously characterized prion diseases such as sheep scrapie, has raised enormous public health concerns worldwide. Although it is not yet possible to gauge the size of a potential vCJD epidemic, preliminary data indicate a significant dietary exposure to BSE-infected material in Britain and wider implications of the transmissibility of prion diseases. The threat to public health has intensified research efforts to understand the molecular basis of prion diseases, understand their transmission between species, improve methods of diagnosis, and develop therapeutic strategies for treatment and prevention of disease. In this review, we summarize current data on the pathology of BSE and vCJD and the epidemiology of vCJD, and we outline public health implications based on these data, emphasizing preventative measures and areas of research for screening and diagnosis.

INTRODUCTION

The transmissible spongiform encephalopathies (TSEs) are a family of neurodegenerative diseases affecting both humans and animals (Tables 1 and 2). They are also known as prion diseases (1) and are associated with abnormal forms of a protein known as the prion protein. The first documented TSE was scrapie, a naturally occurring disease of sheep and goats that has been recognized in Europe for over 200 y (2) and is endemic in many countries around the world. Other occurrences of TSEs in animals include outbreaks of transmissible mink encephalopathy in US mink farms (3), chronic wasting disease of US mule deer and elk populations (4), and most notably, bovine spongiform encephalopathy (BSE), which was first recognized in the United Kingdom in 1986 (5). BSE rapidly evolved into a major epidemic in the United Kingdom (6, 7) and has been causally linked to the occurrence of novel TSEs in a variety of cats and exotic zoo animals and to a new variant of the human prion disease Creutzfeldt-Jakob disease in the UK population (8–10); this variant disease has significantly increased in incidence since its recognition (11). In this review, we summarize current data on the pathology of BSE and variant CJD (vCJD) and the epidemiology of vCJD, and we outline the public health implications of the current data on these diseases.

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

Features of prion diseases

Prion diseases are incurable and fatal and are unique among diseases in that the disease etiology can be sporadic, acquired, or hereditary, as indicated by the variety of human TSEs listed in Table 2. TSEs generally have long incubation times and are transmissible within and between species by inoculation with or dietary exposure to prions from diseased brain tissue—a mechanism that gives rise to the acquired prion diseases and that has been experimentally demonstrated.

The characteristic neuropathology of prion diseases features spongiform vacuolation and neuronal cell loss in the central nervous system accompanied by proliferation of astrocytes and in some cases deposition of amyloid plaques. Prion diseases are also associated with the accumulation in the brain of the host-encoded prion protein PrP in an abnormally folded form that is designated PrPSc (for the scrapie isoform). Although the nature of the transmissible prion has not been unequivocally demonstrated, the major component of the prion is PrPSc (12).

Prion protein in prion disease and the protein-only hypothesis

The normal form of the prion protein, PrPC (for cellular isoform), is a cell-surface glycoprotein of unknown function with a predominantly...
α-helical structure (13–15) that is highly conserved in both sequence and structure between species. The disease-associated PrPSc is derived from the cellular PrPC by posttranslational conformational change (16, 17) but without alteration of the primary sequence of the protein (18). PrPSc is extracted from prion-diseased brain tissue as aggregated material that can be distinguished from PrPC by its partial resistance to proteases and by its detergent insolubility. In contrast to PrPC, PrPSc has a β-sheet-rich structure with little α-helix (19).

It was suggested initially that prion diseases were caused by slow viruses because of their transmissibility with long incubation times, but neither a disease-associated virus (1) nor any immune response has been demonstrated. Current evidence favors the protein-only hypothesis (20), which states that PrPSc is the essential element of the transmissible prion and that it serves as a template that promotes the conversion of endogenous PrPC to PrPSc. The autosomal dominant inherited forms of prion diseases are associated with pathogenic mutations of the prion protein gene (31) and can be diagnosed by genotyping. More than 20 pathogenic mutations that may predispose the protein to formation of the pathogenic isoform, thus causing disease, have been documented (31).

The first evidence of the transmissibility of human prion diseases came from kuru, a disease discovered in a remote population in Papua New Guinea in the 1950s that has been linked to ritualistic cannibalism (32). Further transmission of human prion disease was demonstrated following accidental exposure to prions through medical procedures, resulting in around 200 cases of iatrogenic CJD worldwide (33). Most recently, a new variant of CJD (now called simply vCJD) has been observed in the United Kingdom (34), and evidence that it has arisen as a result of transmission of BSE prions will be given below.

In addition to the pathogenic mutations of the prion protein gene linked to familial forms of disease, there are also polymorphisms that affect incubation times and disease susceptibility regardless of disease etiology (22, 35, 36). The polymorphism at residue 129, which can be either methionine or valine, is a major determinant of genetic susceptibility to prion diseases; acquired and sporadic diseases occur mostly in individuals homozygous at codon 129, heterozygous individuals have later onset and longer disease course in some inherited forms of disease (21), that replication efficiency is highest with proteins of the same primary sequence or species (22, 23), and that PrPC is required for prion disease susceptibility (24).

The conformational change from PrPC to PrPSc is the key feature of prion diseases, but the mechanism for the conversion remains unknown. To date, in vitro efforts to produce transmissible material, which would prove the protein-only hypothesis, have not been successful (25–27), suggesting that the cellular context of the process (or processes) is significant.

The novel pathogenic mechanisms of protein-based replication first described in prion diseases are now found to have wider relevance since demonstration in other diseases (28) and in yeast and fungi (29). It has recently been shown that transmissible prions from a filamentous fungus can be generated in vitro (30), demonstrating the validity of the protein-only hypothesis.

### HUMAN PRION DISEASES AND THE LINK BETWEEN BSE AND vCJD

Table 2 shows the range and different origins of the various human prion diseases. Sporadic, or classical, CJD (sCJD) was first described in the 1920s by Creutzfeldt and Jakob. It is a late-onset neurodegenerative dementia with an aggressive clinical course; around 70% of those affected die within 6 mo. The origin of sCJD is unknown, but the disease may be triggered by either a somatic mutation of the prion gene or a spontaneous conformational change of endogenous PrPC to PrPSc. The autosomal dominant inherited forms of prion diseases are associated with pathogenic mutations of the prion protein gene (31) and can be diagnosed by genotyping. More than 20 pathogenic mutations that may predispose the protein to formation of the pathogenic isoform, thus causing disease, have been documented (31).

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vCJD is differentiated from sCJD by various criteria (39, 40), including the following: 1) vCJD shows a much younger age at onset of disease (the mean age of onset is 29 y), a more insidious onset of disease, and a longer clinical course (9–35 mo); 2) vCJD shares the common TSE neuropathological features but with abundant amyloid plaques in the brain tissue (34) and with a much broader tissue distribution of PrPSc (and thus transmissible material), notably including lymphoreticular tissue as well as the central nervous system (41); and 3) sCJD has a uniform worldwide distribution and incidence that are not linked to scrapie incidence (42), whereas vCJD has occurred only in countries that have reported BSE-infected cattle.

Epidemiologic and biochemical evidence that vCJD has arisen as a result of transmission of bovine prions from BSE-infected cattle is discussed below.
Epidemiologic evidence for the BSE-vCJD link

The UK epidemic of BSE has been attributed to the practice of feeding cattle with rendered animal products in the form of meat and bone meal (MBM) (43). Initially, it was assumed that BSE was caused by contamination with sheep scrapie, but because characterization of prion strains has differentiated BSE prions from scrapie prions (44), it is perhaps more probable that BSE has arisen by the recycling in MBM of a rare sporadic case of BSE (45). The emergence of novel TSEs since the onset of the BSE epidemic that have been shown to be caused by a BSE-like strain (see below) confirms that BSE, whatever its origin, is distinct from classical sheep scrapie.

Figure 1 shows the superimposed prion disease epidemic curves for BSE (46) and vCJD (39) in the United Kingdom since 1988, when BSE became a notifiable disease. Since the emergence of vCJD there have been increased awareness and improved surveillance of prion diseases, which is believed to account for the slight increase in reported incidence of vCJD during that period; however, of all the human prion diseases, only vCJD has significantly increased in incidence (increase of around 23% per year) since its recognition in 1996 (11). As of July 2002, there had been 115 deaths from (probable and definite) vCJD, with a further 9 cases still alive (44), in the United Kingdom. A further 4 cases had been reported in France and 1 in the Republic of Ireland; both countries had also reported significant BSE cases per head of cattle (47).

There have been 180,000 confirmed cases of BSE in the United Kingdom, but the actual numbers affected are more likely to be around 1 million, with perhaps 750,000 BSE-infected cattle entering the human food chain in the 1980s and early to mid-1990s (7). There has therefore been considerable and widespread exposure of the UK population to contaminated bovine material, despite government measures to protect human health, which included a ban on the use of specified risk material (SRM; largely nervous tissue and offal) for human consumption in 1989 and a subsequent ban on the consumption of cattle over 30 mo of age (48). Measures taken to protect animal health included a ban on mammalian products for use in ruminant feed (1989), followed in 1996 by a ban on the use of such material for any farmed animals, which prevented the possibility of cross-contamination of feedstuffs (48).

Biochemical evidence for the BSE-vCJD link

Biochemical evidence indicates that BSE and vCJD are caused by the same prion strain. Distinct prion disease strains can be distinguished by their biological properties, including incubation periods and patterns of neuropathology on transmission to mice (known as the lesion profile), PrPSc from brain tissue of different prion disease strains also shows different molecular weights and glycosylation patterns on Western blotting following partial protease digestion (8, 49)—characteristic profiles that are maintained on passage to mice (8). Of the 4 PrPSc types associated with human prion diseases, type 4 is associated only with vCJD; in all vCJD cases subjected to this analysis, the type 4 pattern has been seen, and no samples from other prion diseases have shown a type 4 profile (8). Samples of BSE-infected bovine brain show a profile similar to that seen in vCJD (8). Transmission of vCJD and BSE to a mouse host and analysis of the mouse-derived PrPSc showed the same PrPSc profile, as well as similar lesion profiles distinct from those caused by other prion diseases, indicating that BSE and vCJD are indistinguishable in the same host (8–10). Recent cases of feline spongiform encephalopathy and related diseases in zoo animals have also shown the BSE PrPSc profile, confirming BSE as a common causative agent for the novel TSEs of the 1990s (8, 44). The PrPSc type is used for unequivocal diagnostic differentiation between vCJD and other prion diseases.

EPIDEMIOLOGY OF vCJD

Dietary transmission of prions

The major route of transmission of BSE to humans, causing vCJD, is likely to be dietary consumption of BSE-infected beef. Meat products containing central nervous system tissues (processed meats such as sausages, ground meat, meat pies, and pastries) are more likely to contain a high titer of the BSE agent than other meat products and therefore represent a greater transmission risk. The question of whether consumption of specific types and cuts of meat is linked to vCJD incidence has been addressed by spatial epidemiology studies that relate clusters of vCJD cases to regional trends in dietary consumption (50). Spatial epidemiology of vCJD in Great Britain revealed a 2-fold increase in vCJD incidence in the north of the country compared with the south (rate ratio = 1.94, 95% CI: 1.12, 3.36 north compared with south). Regional incidence of vCJD was related to regional dietary intake of meat products likely to contain high-titer BSE material as assessed by 2 national surveys: 1) the Household Food Consumption and Expenditure Report and 2) the Dietary and Nutritional Survey of British Adults. A positive correlation between vCJD incidence and consumption of such meat products was found using the data from the first survey (r = 0.72, P = 0.03) but not the second (50).

Epidemiologic analyses of a specific disease cluster in the United Kingdom suggest that variation in vCJD incidence may be explained by not only dietary intake of certain beef products but also certain methods used in the preparation of beef for human consumption. In Leicestershire (population 870,000) the vCJD incidence was reported to be 5.7/million compared with the national incidence in the United Kingdom of 1.5/million (50, 51). The cluster of 5 cases that produced this excess is highly significant (P = 0.004). The links between these Leicestershire cases...
were found by public health investigations to be local butchers’ shops that processed whole cattle carcasses, thereby contaminating carcass meat with central nervous system tissue (50–53).

**Characterization of the vCJD epidemic and incubation period**

Various predictions of the size of the vCJD epidemic in the United Kingdom have been made (54–56), suggesting epidemic sizes of between a few hundred and more than 1 million cases. In such models, the epidemic size is dependent on both the level of exposure to prions and the disease incubation time, neither of which is quantifiable for vCJD, though it is clear that larger epidemics are mapped by longer and more variable incubation times as well as increased exposure to prions (57). Such predictions are also limited by the assumption of only one susceptible genotype—that is, codon 129 methionine homozygous.

The epidemiology of kuru has important implications for the mapping and prediction of the vCJD epidemic, because there is a common oral route of infection between kuru and vCJD. The gradual decline in kuru cases over the 45 y since the cannibalism ceased (there are still a few cases today) indicates that disease incubation periods can extend to more than 40 y (45, 58). For vCJD we can expect the average incubation period to be greater than the 12-y average for kuru, given that there is a species barrier between cattle and humans (44) that will increase the length and variability of the incubation period. The data from the cluster of 5 cases in Leicestershire allow an estimate of incubation period to be made for some of these early vCJD cases. Some of the Leicestershire cases have been linked to specific butchers who ceased operating in 1982 and 1989 (50, 51), suggesting that infection occurred very early in the BSE epidemic. An estimate of 15–18 y for the incubation period has been made from these data (57).

By definition, the cases to date are those with highest susceptibility and shortest incubation time, suggesting that average incubation periods could be significantly greater than this and that we have to date seen the disease in a highly susceptible subgroup of the population (45). Apart from the prion gene codon 129 status, there are no further genetic similarities at the prion gene locus between the cases of vCJD, not even in the geographical cluster of cases in Leicestershire (59). Other susceptibility loci independent of the prion gene have been identified in mice, and analogy to humans may help in determining genetic susceptibility (60–62).

**PUBLIC HEALTH IMPLICATIONS**

**Preventative measures in the epidemic regions**

The novel human prion disease vCJD was recognized in the United Kingdom in 1996 (34), following the UK BSE epidemic in the late 1980s and early 1990s (6, 7). In addition to epidemiologic evidence, convincing experimental data indicating that vCJD is caused by exposure to BSE-infected material were presented soon after the recognition of the disease (8–10).

UK government measures to protect public health following the emergence of BSE included a ban on the use of SRM for human consumption in 1989 (48). SRM is largely nervous tissue and offal with a high risk of containing BSE prions, and the designation of SRM is under continual review based on developing scientific evidence (48). A subsequent ban on the consumption of cattle over 30 mo of age was put in place in 1996 after the emergence of vCJD, on the rationale that the incubation period of BSE is estimated to be about 5 y. A ban on beef on the bone for human consumption was also in place for the period 1997–1999 and was lifted following the decline of the BSE epidemic. From the early 1990s there have also been controls on the preparation of carcasses to reduce the risk of meat contamination with SRM. All practices implicated in the Leicestershire cases [exposure period mid-to late 1980s (50, 51)] were therefore illegal long before the link was established.

The extreme resistance of prions to standard decontamination methods is a significant factor in the accidental transmission of prions; it has caused both the BSE epidemic and cases of iatrogenic CJD. Iatrogenic spread of variant CJD is an area of considerable concern for human health, particularly given the wide tissue distribution of prions from vCJD patients (41), the potentially large numbers of affected individuals (see above), and the transmissibility of sub- and preclinical prion disease (63). Recent studies have again demonstrated experimental transmission of prion diseases using prion-contaminated metal wires (64), highlighting the risk of transmission via surgical procedures, and there is still uncertainty about the risk of transmission of vCJD via blood products (65). A further route of transmission via the use of biospecimens in drug development has been revealed by a recent recall on batches of a preparation containing albumin from a UK donor who was subsequently diagnosed with vCJD (66); despite the recall, at least 757 patients had already received the drug.

Currently in the United Kingdom, surgical instruments known to have been used on suspected cases of CJD are quarantined until confirmation of diagnosis (31) and reused only if an alternative diagnosis is confirmed. Blood products are now routinely leukodepleted following the identification of transmissible prions in lymphoreticular tissue (38), and plasma products are sourced from outside the United Kingdom (31).

There is also concern in the United Kingdom over the possible transmission of BSE to sheep, the subsequent problems of differentiation between sheep BSE and sheep scrapie, and the attendant problems of further human health risks from infected sheep. A ban on the use of animal products in feed for all farmed animals should have safeguarded sheep populations from contracting BSE, and encouragingly, there has been no observed surge in reported sheep scrapie since the BSE epidemic (67).

**vCJD screening**

Areas of research for public health protection are focused on improvements to screening tests (for both individuals and blood and tissue donations), decontamination procedures for surgical instruments, and therapeutics. The development of preclinical diagnostic and screening tests is imperative to provide the potential to control disease spread and treat disease in affected individuals before the onset of clinical features.

At present, very little can be done following a prion disease diagnosis because progression to the symptomatic stage is marked by irreversible neurodegeneration, and traditionally, diagnosis of nonhereditary prion diseases can be confirmed only at autopsy. There are several criteria now used to determine probable vCJD (40) in living patients, and considerable progress in diagnostics has been made with the development of the diagnostic tonsil biopsy, which exploits the wider tissue distribution of vCJD prions compared with other prion diseases (38). To date, the biopsy has been 100% sensitive and specific for vCJD; it enables a pre-mortem confirmation of the vCJD diagnosis. It also has potential for preclinical diagnosis if the tonsil is shown to give a positive result for presymptomatic individuals.
Identification of a therapeutic target for prion disease is complicated by the many unknowns of prion diseases, but it is possible to identify 3 main areas of investigation: 1) removal of PrP<sub>C</sub>, 2) blockage of the conversion between PrP<sub>C</sub> and PrP<sub>Sc</sub>, and 3) degradation and removal of PrP<sub>Sc</sub>. Degradation and removal of PrP<sub>Sc</sub> may be possible but ineffective because it is not known whether PrP<sub>Sc</sub> itself is the cause of neurodegeneration. Removal of PrP<sub>C</sub> is not applicable to a human population, though it has been shown in experimental animals that the normal function of PrP<sub>C</sub> is dispensable (68, 69). This finding validates any therapeutic agent targeted at blocking the conversion between the 2 prion protein isoforms, making this the most promising therapeutic approach—one that could offer a prophylactic against prion diseases. The absence of a host immune response in prion disease enables immunotherapeutic approaches to be used, and the efficacy of antibody treatment has recently been demonstrated in both cell culture and animal models of prion disease (70–72).

Worldwide BSE incidence and implications for the United States

In recent years, there has been a significant increase in the incidence of BSE in countries around Europe and in Japan not linked to imported cases. Countries with the highest incidence, after the United Kingdom, are Portugal, Ireland, and Switzerland (47).

To date, the United States has observed BSE in only imported stock (73). To protect the US livestock and human populations from the threat of BSE and its transmission, the US Department of Agriculture (USDA) banned imports of all ruminants from the United Kingdom in 1989 and from all European countries in 1997, when BSE cases were reported in other countries across Europe. A ban on feeding ruminants MBM containing mammalian products was put in place in 1997, but such feed is still prepared for pigs and poultry, leaving the potential for contamination of feed (74). There is also concern about the spread of chronic wasting disease (CWD), which has increased within deer and elk populations in the United States and could spread to the human population (75–78). The USDA-commissioned Harvard BSE Risk Assessment Study published in November 2001 (79) states that the risks of a BSE epidemic in the United States are extremely small and that various barriers would have to be simultaneously transgressed in order for the United States to observe an epidemic of BSE originating from imported animals, CWD, or sporadic cattle disease. Since the Harvard BSE Risk Assessment Study, proposals have been made to specify risk materials and ban their use for human consumption and to ban certain stunning devices for cattle slaughter. Restrictions are already in place for blood and tissue donations (73). Three recent cases of CJD in the United States had potential links to consumption of venison; although these were subsequently confirmed as sporadic CJD (76), there is now a worldwide awareness of the potential for transmission of animal TSEs to humans.

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REFERENCES