Prion Diseases

47

Ermias D. Belay and Jason C. Bartz

1 Introduction

Prion diseases, also known as transmissible spongiform encephalopathies, are a group of invariably fatal neurodegenerative diseases that affect both humans and animals. They are caused by an unconventional agent termed a prion. Strong evidence indicates that their etiology and pathogenesis involve modification of a host-encoded normal cellular protein known as the prion protein (PrP^C) [1, 2]. Unusual characteristics of prion diseases include their occurrence sporadically without any apparent environmental source of infection in some species, genetically in association with specific prion protein gene mutations in humans, and their transmissibility either within or across different species [2]. In most prion diseases, characteristic neuropathologic features include widespread neuronal loss, spongiform lesions, and astrogliosis, corresponding with accumulation of the agent in different parts of the brain. The presence of the abnormal prions can be demonstrated in the brain and often in other tissues of humans and animals affected by prion diseases [3–6]. Several laboratory tests such as immunohistochemistry and biochemical tests are used to determine the presence of infecting prions in tissue specimens [4, 6, 7]. Acquired forms of prion diseases have very long incubation periods often lasting for years and sometimes even decades [8–10].

Prion diseases of humans include kuru, Creutzfeldt-Jakob disease (CJD), variant CJD, Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI) (Table 47.1) [2, 11].

Kuru was first described in the 1950s as a fatal ataxic neurologic disease among the Fore tribe of the highlands of Papua New Guinea [9, 12–15]. In 1959, Hadlow made the crucial observation that the neuropathology of kuru was similar to scrapie, raising the possibility that, similar to scrapie, kuru might also be transmissible [16, 17]. However, transmissibility was not confirmed until the 1960s when Gajdusek and colleagues successfully transmitted kuru by intracerebral inoculation of brain tissue from deceased patients into chimpanzees [18–23]. This was the first instance of human prion disease to be successfully transmitted to experimental animals.

Kuru is the first epidemic human prion disease to be thoroughly investigated. Since the 1950s, over 2,700 kuru cases have been documented [9, 24]. Strong epidemiologic evidence suggests that the disease spread among the Fore people by ritualistic cannibalism. In ancient Fore culture, giving respect for the dead involved a mourning ritual with plastering of brain tissue all over the body, including mucous membranes and consumption of the decedent's body parts. Relatives who died of kuru were honored with this practice exposing surviving family members to infectious brain tissue which might have amplified the kuru epidemic [25, 26]. After the ritualistic practice ended in the late 1950s, the number of new cases dramatically declined and no persons born after 1959 developed the disease. Likely incubation periods of the most recent seven male cases reported by Collinge et al. ranged from 39 to 56 years, although the longest incubation period may have been up to 7 years longer [9].

Prion diseases of animals include scrapie in sheep, goats, and mouflon; bovine spongiform encephalopathy (BSE) in cattle; feline spongiform encephalopathy in domestic and zoo cats; ungulate spongiform encephalopathy in exotic zoo ruminants; chronic wasting disease (CWD) in deer, elk, and

†Author was deceased at the time of publication.

E.D. Belay, MD (✉)
Division of High-Consequence Pathogens and Pathology,
NCEZID, Centers for Disease Control and Prevention,
1600 Clifton Road, Atlanta, GA 30333, USA
e-mail: ebelay@cdc.gov

J.C. Bartz, PhD
Department of Medical Microbiology and Immunology,
Creighton University, 2500 California Plaza,
Omaha, NE 68178, USA
e-mail: jbartz@creighton.edu

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency.

R.A. Kaslow et al. (eds.), *Viral Infections of Humans*,
DOI 10.1007/978-1-4899-7448-8_47, © Springer Science+Business Media New York 2014

Table 47.1 Animal and human prion diseases

Type of prion disease	Affected host	Year first described or identified	Mode of disease transmission/occurrence
Animal prion diseases			
Scrapie	Sheep and goats	1730s	Contaminated environment, possibly direct contact, oral
Bovine spongiform encephalopathy	Cattle	1986	Contaminated feed, oral
Chronic wasting disease	Deer, elk, and moose	1967	Contaminated environment, direct animal contact, oral
Transmissible mink encephalopathy[a]	Mink	1947	Contaminated feed, oral
Feline spongiform encephalopathy[b]	Domestic and wild cats	1990	BSE-contaminated feed, oral
Ungulate spongiform encepahlopathy[b]	Exotic ruminants (e.g. kudu, nyala)	1986	BSE-contaminated feed, oral
Human prion diseases			
Kuru		1950s	Ritualistic cannibalism involving brain tissue ingestion, oral
Sporadic CJD		1920s	Unknown
Iatrogenic CJD		1974[c]	Via pituitary hormones, dura and cornea grafts, neurosurgical equipment
Variant CJD		1996	Consumption of BSE-contaminated cattle products, human blood products
Familial CJD		1924	Prion protein gene germline mutations, inherited
Gerstmann-Sträussler-Scheinker syndrome		1936	Prion protein gene germline mutations, inherited
Fatal familial insomnia		1986	Prion protein gene germline mutations, inherited

CJD Creutzfeldt-Jakob disease
[a]The last known outbreak of transmissible mink encephalopathy occurred in 1981 in Wisconsin
[b]The known feline and ungulate spongiform encephalopathies are believed to have resulted from BSE transmission
[c]The first report of iatrogenic CJD was in 1974 in a recipient of cornea obtained from a CJD decedent; human pituitary growth hormone-associated CJD was first reported in 1985 and dura mater graft-associated CJD in 1987

moose; and transmissible mink encephalopathy (TME) in farmed mink (Table 47.1) [27].

Epidemiologic evidence indicates that feline and ungulate spongiform encephalopathies were caused by transmission of the BSE agent via consumption of BSE-contaminated feed [28]. Although strong evidence is lacking, speculations persist that scrapie in sheep was the original source of prion diseases in other animals, such as BSE and CWD.

TME occurred in outbreaks among farmed mink primarily in the United States but also in Canada, Finland, Germany, and Russia [28]. TME outbreak investigations suggested that the disease was causally linked with consumption of scrapie-contaminated meat [28]. The last known outbreak of TME occurred in 1985 in Wisconsin. An investigation of this outbreak indicated that the mink on that particular farm were fed only downer cattle, igniting much speculation about the existence of a spontaneous prion disease in cattle even before BSE was identified in the United Kingdom. Non-epizootic cases of TME have not been reported.

2 Etiologic Agent of Prion Diseases

Before the 1980s, prion diseases were widely believed to be caused by "slow viruses" despite the fact that no viral particles or disease-specific nucleic acids were identified in association with scrapie transmission in laboratory animals [29, 30]. The scrapie agent, which was most widely studied at the time, could not be grown in cell culture. Its successful transmission to laboratory mice in 1961 greatly facilitated research efforts to understand the nature of the agent [1]. Because of the long incubation period associated with scrapie, transmission to wild-type mice was tedious and in many instances required a year to complete. Development of transgenic mice greatly facilitated prion disease research by allowing closer modeling of the diseases and reducing the incubation period in the experimental models [31, 32].

Two distinctive properties of the scrapie agent led to the suspicion that the agent was devoid of nucleic acids and, thus, may not be a virus but primarily composed of a protein. These properties included: (1) resistance of the scrapie agent to procedures, such as treatment with ultraviolet light and ionizing radiation, that normally inactivate other microorganisms, including viruses, and (2) the reduction of scrapie infectivity by procedures that denature or degrade proteins [1]. The concept that the scrapie agent might replicate in the absence of nucleic acids or might just be a protein was postulated as early as the 1960s by Alper and colleagues, Pattison and Jones, and Griffith [30, 33, 34]. In 1982, Prusiner and colleagues described the successful enrichment of a hydrophobic protein, the presence of which was required for scrapie transmission in laboratory animals [1, 35, 36]. Prusiner

introduced the term "prion" to describe this protein by borrowing and mixing the first few letters from the descriptive phrase "*pro*teinaceous *in*fectious" particle [1]. Since then, additional evidence has accumulated indicating that prions may be acting alone in causing prion diseases. However, the critical steps in the production, propagation, and pathogenesis of this infectious protein remain unclear. As a result, the study of prions has become an important, relatively new area of biomedical research. Some critics of the prion hypothesis still believe that nucleic acids undetected by current methods may play a crucial role in the pathogenesis of prion diseases [37–40]. Prions seem to be almost entirely composed of proteins with a glucose moiety attached to them. Creation of prion infectivity by modifying the conformation of synthetic or recombinant polypeptides has led credence to the view that prions may entirely consist of proteins with no nucleic acid genome [41, 42]. How a protein-only agent would confer strain specificity to prions causing different diseases in different species has been a topic of debate for many years. Studies have suggested that strain specificity may be enciphered in the different physical properties of prions and their varying protein conformations.

2.1 Cellular Prion Protein

Soon after the nature of infecting prions was described, researchers discovered the similarity of the abnormal prions with that of a normal protein found as a structural component of cell membranes [43–45]. This discovery marked a turning point in our understanding of prion diseases and partially explained the absence of inflammatory infiltrates in pathologic specimens of infected tissues and the lack of humoral response in several prion diseases. PrP^{C} is usually found as a monomeric GPI-linked glycoprotein on the cell membrane and is soluble in mild detergents. In humans, PrP^{C} is encoded by the prion protein gene located on the short arm of chromosome 20 [43, 44]. Similar to other proteins, PrP^{C} is produced in the endoplasmic reticulum and transits through the Golgi apparatus to the cell surface.

PrP^{C} is expressed in large quantities in neurons, but it is also expressed in lymphoid tissues and other organs and tissues in relatively smaller quantities. Several possible functions have been proposed for PrP^{C} [46]. Its location in parts of the cell membrane specializing in signaling was believed to indicate that it may be involved in signal transduction. Other studies have hypothesized that PrP^{C} interacts with other membrane proteins to provide neuroprotective functions, although more direct evidence is lacking. Another perhaps more widely studied proposed function of PrP^{C} is binding copper and involvement in its metabolism [46]. PrP^{C} readily binds copper in physiologic concentrations, and its overexpression has been shown to promote cellular uptake of copper, indicating that it may play a significant role in copper homeostasis [46].

Prions appear to be composed largely or entirely of the abnormal protein designated as PrP^{Sc}. The underlying pathophysiologic mechanism in the occurrence of prion diseases involves the biochemical conversion of PrP^{C} into the pathogenic PrP^{Sc}. This conversion occurs by a poorly defined post-translational autocatalytic process, possibly requiring the aid of cofactors such as proteins or nucleic acids. It appears that PrP^{Sc} reproduces itself by recruiting PrP^{C} and stimulating its conversion into the disease-causing isoform [47–51]. This conformational change confers protease-resistant properties to PrP^{C} and a three-dimensional structure distinguishable from the infecting prions [49, 52]. The initial instigating PrP^{Sc} molecule may originate from exogenous sources or within the brain from somatic or germ line prion protein gene mutations. Knockout mice devoid of the prion protein gene are resistant to scrapie infection, indicating that the production of PrP^{C} is required for the generation and propagation of PrP^{Sc} [53]. During its conversion, PrP^{Sc} acquires more beta-sheet structure that renders it resistant to proteolytic enzymes, conventional disinfectants, and standard sterilization methods. A higher proportion of the tertiary structure of PrP^{C}, on the other hand, is composed of alpha helices which make it more sensitive to denaturation by proteinase-K treatment [54, 55]. Removal of the neuroprotective functions of PrP^{C} as more of it becomes converted to the pathogenic PrP^{Sc} and accumulation of PrP^{Sc} in neurons have been suggested as major contributory factors in the underlying pathogenesis of prion diseases and widespread neuronal death.

3 Creutzfeldt-Jakob Disease

CJD is the most common form of prion disease in humans and has been reported in all continents of the world. It was first recognized in Europe in the early 1920s and bears the name of two German neurologists, Hans Gerhard Creutzfeldt and Alfons Maria Jakob, who separately reported patients with rapidly progressive neurodegenerative diseases [56]. At least two of the patients initially reported by Jakob had the typical neuropathologic features that have since been recognized as the hallmark of CJD.

The clinical manifestation of CJD is usually characterized by the onset of dementia, ataxia, or behavioral abnormalities. As the disease progresses, patients commonly develop dysarthria, movement disorders such as gait abnormalities, myoclonus, and tremors. These neurologic deficits are rapidly progressive and patients often develop akinetic mutism towards the terminal stages of the illness, usually over a period of weeks to several months [57–62]. CJD is invariably fatal with median illness duration of about 4 months. Over 50 % of patients die within 6 months and about 80 % within 1 year of disease onset [57]. The presence of a characteristic electroencephalogram (EEG) finding of triphasic, periodic sharp wave complexes can be demonstrated with multiple

testing in approximately 75 % of patients [63–65]. Elevated levels of 14-3-3 proteins in the cerebrospinal fluid (CSF) can also be found in most CJD patients [66–71]. Elevated CSF 14-3-3 is a marker for rapid neuronal death and, in the appropriate clinical context, can often help in making a premortem diagnosis of CJD. This test is nonspecific, however, and may be elevated in other neurologic conditions that result in rapid neuronal death. A similar CSF test that detects tau proteins can also aid in the premortem diagnosis of CJD. Elevated tau protein levels in combination with a positive 14-3-3 test may increase the sensitivity and specificity of a CJD diagnosis [71]. Magnetic resonance imaging (MRI) findings showing high intensity in the basal ganglia and cortical regions of the brain have been correlated with a CJD diagnosis [64, 72–79]. The characteristic EEG and MRI findings and elevated levels of 14-3-3 in the appropriate clinical context are used as diagnostic criteria for CJD [78].

In the United States, the median age of CJD patients at the time of death is 68 years with approximately 70 % of cases occurring between 55 and 75 years of age (Fig. 47.1) [80–82]. Overall, CJD has been reported in many countries with an annual incidence ranging from one to two cases per million populations, including in the United States (Fig. 47.2). However, the incidence increases with age and approaches five cases per million populations for those ≥65 years of age. A decline in CJD incidence in persons ≥80 years of age has been consistently reported in many developed countries which may be due to ascertainment bias in this older age group. In the United States, epidemiologic studies have consistently shown that the incidence of CJD is at least 2.5 times lower in blacks than whites [81–83]. The precise reason for this wide discrepancy is not well understood, but under diagnosis among black populations is unlikely to entirely explain the striking difference in CJD incidence. The age-adjusted incidence for males in the United States is slightly higher than that for females, particularly among those ≥60 years of age [83, 84]. CJD has been shown to occur in three different forms: sporadic, iatrogenic, and familial forms.

A definitive diagnosis of CJD can only be made by histopathologic or immunodiagnostic testing of brain tissue obtained at autopsy or biopsy. Histopathologic examination of brain tissue demonstrates the hallmark triad of spongiform lesions, neuronal loss, and astrogliosis. Since more specific diagnostic tests have been developed, histopathology alone is rarely used to confirm the diagnosis of CJD. Immunodiagnostic assays, such as immunohistochemistry and Western blot testing that show the presence of PrP^{Sc}, are widely used to confirm the CJD diagnosis. Recently, a new test termed real-time quaking-induced conversion (RTQuIC) was developed to detect prions in peripheral tissues or fluids such as the CSF, blood, or urine [85]. The detection method used in this test is based on that used in protein misfolding cyclic amplification. RTQuIC can detect minute amounts of prions and has been shown to be 80% sensitive and over 90% specific in confirming a CJD diagnosis from CSF specimens. In addition, the presence of infecting prions has been demonstrated in the olfactory epithelium of CJD patients [86]. Because these tissues are accessible from the nasal cavity, specimen collection by deep nasal swab and subsequent testing by RTQuIC has shown promising results as a non-invasive, rapid, antemortem, diagnostic assay for CJD.

Currently, no effective treatment exists for CJD or any other prion disease. Clinical management of patients is primarily supportive to ameliorate some of the aggravating symptoms of the disease [87]. Many compounds and drugs (e.g., pentosan polysulfate, quinacrine, and doxycycline) have been investigated as possible therapeutic modalities in CJD patients and in animal models [88–97]. Although some compounds have been shown to be effective antiprion agents in in vitro experiments, they were only successful in prolonging the incubation period and survival times in animal models. Prolongation of survival or no effect has been reported in human patients treated with some of the drugs that have already been licensed for indications other than CJD treatment. Limited randomized trials are underway to evaluate the usefulness of some of these drugs. Development of disease-specific treatment regimens is complicated by the fact that widespread neuronal damage has already occurred by the time signs and symptoms appear. Therefore, the ideal antiprion treatment should not only inhibit the propagation of infecting prions but also reverse neuronal damage or degeneration that may have already resulted in cognitive impairment and physical disability. Such a drug or a compound should also be able to readily pass the blood-brain barrier. Lack of a reliable antemortem test poses a challenge in diagnosing patients as early as possible so that they could be enrolled in clinical trials to initiate investigational treatment in time to alter the course of the disease.

3.1 Sporadic CJD

Sporadic CJD accounts for approximately 85 % of cases and occurs in the absence of outbreaks with no known environmental source of infection. Decades of research has not identified a specific source of infection for sporadic CJD patients. Spontaneous generation of the pathogenic prions was hypothesized as a cause for sporadic CJD, possibly resulting from age-related random somatic mutations or errors during prion protein gene expression. The surprisingly stable and uniform incidence of sporadic CJD in time and space and the absence of recognizable transmission patterns to account for a substantial number of the cases were the strongest arguments favoring the spontaneous occurrence of sporadic CJD. Not all researchers are convinced about the spontaneous occurrence of sporadic CJD, and many studies have been

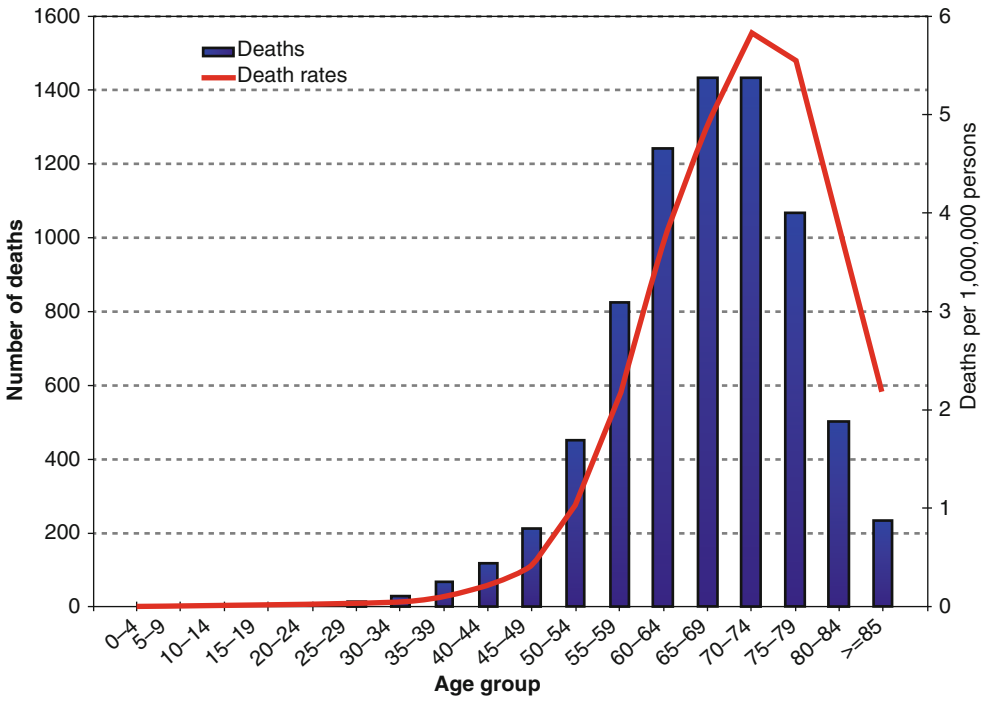

Fig. 47.1 Creutzfeldt-Jakob disease deaths and death rates by age group, the United States, 1979–2008. Deaths obtained from the multiple cause-of-death data for 1979–1998 are based on ICD-9 codes, and those beginning in 1999 are based on ICD-10 codes with available computerized literal death certificate data. Death information was also obtained from other surveillance mechanisms; includes familial prion disease

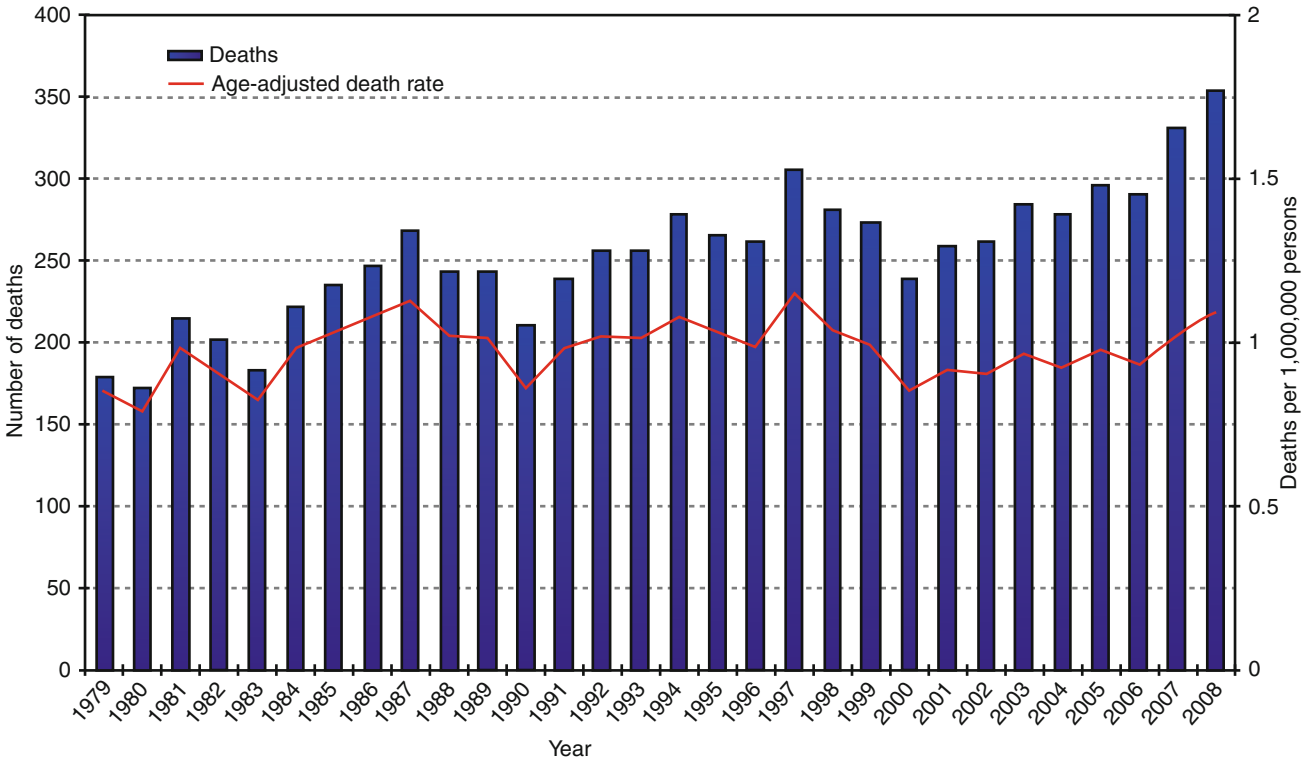

Fig. 47.2 Creutzfeldt-Jakob disease deaths and age-adjusted death rates, the United States, 1979–2008. Deaths obtained from the multiple cause-of-death data for 1979–1998 are based on ICD-9 codes, and those beginning in 1999 are based on ICD-10 codes with available computerized literal death certificate data. Death information was also obtained from other surveillance mechanisms; includes familial prion disease. Rates are adjusted to the US standard 2000 projected population

Table 47.2 Characteristics of subtypes of sporadic Creutzfeldt-Jakob disease (CJD) and sporadic fatal insomnia (sFI)

Subtype	Number of patients ($n=609$)	Percent	Clinicopathologic features
MM1/MV1	352	57.8	Typical CJD clinical and neuropathologic manifestations, typical EEG, rapidly progressive disease.
VV2	90	14.8	Commonly ataxia at onset and late dementia, typical EEG rare, short duration, subcortical pathology, plaque-like deposits
MV2	83	13.6	Similar to VV2 but long duration and presence of kuru-type amyloid plaques in cerebellum
MM2	52	8.5	Progressive dementia, typical EEG rare, long duration, cortical pathology, coarse spongiosis
VV1	25	4.1	Usually young age at onset, typical EEG rare, severe pathology in cerebral cortex with relative sparing of cerebellum, faint synaptic prion staining
MM2 (sFI)	7	1.1	Similar to FFI but without prion protein gene mutations

Data used in the table were obtained from the National Prion Disease Pathology Surveillance Center, Cleveland, OH
M Methionine, *V* Valine

conducted to search for possible sources of infection and risk factors for the disease [98–102].

Possible environmental sources of infection for sporadic CJD have been explored using multiple case-control studies [100–109]. Consumption of animal products, including the brain and other organs; receipt of blood and blood products; occupational exposures; and exposures via surgical procedures were evaluated in these studies. Because of the long incubation period of CJD, obtaining a reliable history of such exposures many years in the past may not be easy. In addition, exposure histories are usually obtained from next-of-kin who may not be familiar with the timing and extent of the exposures. Types of controls used (community or hospitalized) and the timing of selection and interview of the controls could also bias findings of case-control studies [109]. In a study that combined data from Denmark and Sweden, researchers attempted to minimize these biases specifically for evaluating surgical exposures by obtaining information about surgical procedures for both cases and controls from existing hospital records [102]. The study indicated that any major surgery conducted ≥20 years before CJD onset was significantly more common in cases than both matched and unmatched controls. A much larger European study using community controls showed a similar association of history of surgery with the risk of sporadic CJD, albeit using different data collection methodologies [101]. A similar study in Australia also showed that surgical procedures were significantly associated with the development of sporadic CJD [110]. No significant difference in the frequency of surgical procedures was identified among sporadic CJD cases compared with controls in a study performed in Japan [111]. These studies raised the possibility that a certain proportion of sporadic CJD cases may result from exposure to prion-contaminated surgical instruments. Because of the rarity of CJD, confirming or refuting such an association in a small proportion of patients is extremely difficult.

A possible risk of occupational transmission of CJD to health professionals has been raised repeatedly. This risk has recently been reviewed in a study published in Eurosurveillance [112]. The authors used various data sources, including published case reports, case-control studies, and surveillance data from 21 countries contributing to the EuroCJD program. A wide variety of health professionals had been reported with CJD, but the study findings did not suggest increased risk of CJD among health professionals [112].

Sporadic CJD is a heterogeneous disorder which can be further subdivided into five different subtypes based on the Western blot characteristics of protease-resistant fragment of PrP^{Sc} and the polymorphism at codon 129 of the host prion protein gene. These different subtypes, first proposed by Parchi et al., correspond with characteristic clinical and neuropathologic phenotypes (Table 47.2) [113–118]. The most common subtype is associated with a 21 kDa PrP^{Sc} fragment, designated type 1, and the presence of methionine at the polymorphic codon 129 of the prion protein gene. The phenotypic expression does not necessarily neatly fit into these various categories in some patients. In fact, both type 1 and type 2 prion fragments have been reported to coexist in up to 25 % of sporadic CJD patients [113, 119].

A cluster of sporadic prion disease cases termed variably protease-sensitive prionopathy (VPSPr) with a phenotype distinct from other known subtypes of sporadic CJD was recently reported in the United States [120]. The major distinguishing characteristics of VPSPr include sensitivity of the agent to proteinase-K digestion and clinicopathologic manifestations different from sporadic CJD patients. On Western blot analysis, the electrophoretic profile of proteinase-K-resistant fragments from VPSPr patients shows a ladder-like pattern with five major bands corresponding to fragments with different molecular weights. This pattern is clearly different from that seen in sporadic CJD patients but shares some similarities with that observed in some GSS patients. However, no prion protein gene mutations have been identified in association with VPSPr [120]. All the three codon 129 polymorphisms have been identified in patients with VPSPr with slightly variable clinical and histopathologic phenotypes, indicating that the polymorphism at codon 129 modifies the phenotype as it has been shown to do in sporadic CJD patients and several genetic prion diseases.

3.2 Iatrogenic CJD

Iatrogenic CJD, which accounts for <1 % of CJD cases, is associated with transmission of the CJD agent via medical interventions such as administration of contaminated human pituitary hormones and the use of contaminated dura mater and corneal grafts and neurosurgical equipment [121]. Iatrogenic transmission of CJD was first identified by Duffy et al. in 1974 in a patient who, 18 months before CJD onset, received a corneal graft obtained from a donor who died of confirmed CJD [122]. Since then, 10 cornea-associated CJD cases have been reported worldwide, but definitive evidence of CJD in the donors was available for only two of the cases [121, 123, 124]. In the remainder, information on the donors was unavailable or they died of conditions other than a prion disease [124]. Sporadic CJD unrelated to transplanted corneas is expected to occur among elderly patients with a history of cornea transplantations because of a large number of such patients alive in the United States (over 30,000 corneal transplantations are performed annually). Using statistical analysis, Maddox et al. have suggested that one coincidental sporadic CJD case among patients with a history of cornea transplantation is expected to occur every 1.5 years in the United States [124].

In 1977, CJD transmission was reported in two unusually young patients who underwent EEG procedures with implantation of depth electrodes 16–20 months before CJD onset; several months earlier, the electrodes were implanted in a patient who subsequently died of confirmed CJD [125]. Experimental implantation of the EEG electrodes into a chimpanzee 18 months after their original use transmitted CJD, demonstrating that the electrodes were indeed contaminated with the CJD agent [126].

Almost a decade after iatrogenic transmission of CJD was first reported in the corneal recipient and via EEG electrodes, other modes of CJD transmissions were identified, including the use of contaminated cadaveric human growth hormone in 1985 and dura mater grafts in 1987 [59, 127–132]. Reports of these initial iatrogenic CJD cases in the United States were followed by identification of similar cases in other countries [121, 128, 133–151]. Human growth hormone-associated CJD cases were primarily reported in France, the United Kingdom, and the United States. In these countries, large cohorts of young individuals were given human growth hormone (hGH) injections as a treatment for stunted growth resulting from growth hormone deficiency. Extraction of hGH by batch processing of pituitaries from multiple cadaver donors may have led to contamination of an entire batch if one of the donors was in the preclinical phase of disease or had died of CJD. In the United States, after identification of the first three hGH-associated CJD cases, a cohort of >6,000 recipients of hGH sponsored by the National Hormone and Pituitary Program were enrolled in a follow-up study. Among this cohort, as of April 2012, a total of 29 hGH recipients have developed CJD [121, 152]. All of these patients began their treatment prior to the introduction of a size exclusion chromatography purifying step in 1977. To date, no patient who began treatment with hGH purified using this method has developed CJD. A recent analysis of the US data indicated that the absence of cases among patients treated after 1977 may represent a real difference in risk between hGH produced before and after that year [152]. Worldwide, a total of 226 hGH-associated CJD patients have been identified, including the 29 US cases, 119 cases from France, and 65 cases from the United Kingdom [121]. The attack rate ranged from 0.4 % in the United States to 6.3 % in France. Incubation periods ranged from 5 to 42 years with a mean of 17 years [121].

Beginning in the mid-1980s, a parallel iatrogenic CJD outbreak was occurring among patients who received dura mater grafts. The initial case was identified by an astute physician in the United States in a patient who received Lyodura, a brand of dura mater graft processed by a German company [131, 133]. Subsequent to this report, several cases of dura mater graft-associated CJD cases were identified, including 142 from Japan, 14 from Spain, 13 from France, 10 from Germany, 9 from Italy, 8 from the United Kingdom, 5 each from Australia and the Netherlands, 4 each from Canada and the United States, and a smattering of cases from other countries [121]. Over 60 % of the worldwide 228 dura mater graft-associated CJD cases were reported from Japan, where Lyodura was used in much higher quantities than elsewhere [121, 153, 154]. In addition, isolated cases of CJD associated with Tutoplast, another brand of dura mater graft produced by a different German company, have been reported, including in the United States and Japan [137, 153, 154]. The higher number of iatrogenic CJD transmissions associated with Lyodura is believed to be due to the sourcing and processing practices prevalent at the company in the 1980s before revisions were made in response to the occurrence of the initial few Lyodura-associated CJD cases. Almost all Lyodura-associated CJD cases received products processed before these revisions were made. The mean incubation period for the cases identified worldwide was 12 years with a range of 1.3–30 years [121].

4 Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease

4.1 Bovine Spongiform Encephalopathy

Prion diseases attracted unprecedented scientific and public attention after a large outbreak of BSE in cattle emerged in the United Kingdom in the mid-1980s and spread to other countries [155–157]. This attention increased dramatically when strong scientific evidence indicated in 1996 that the

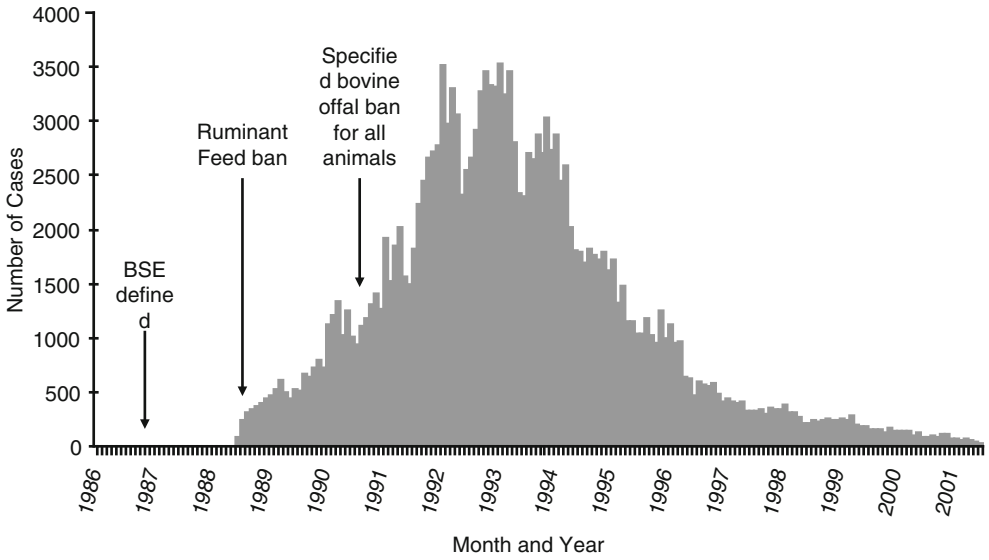

Fig. 47.3 Epidemic curve of bovine spongiform encephalopathy outbreak, the United Kingdom

BSE agent has been transmitted to humans causing a new form of CJD, later termed variant CJD [158]. The implication that prion diseases could be transmitted via contaminated food or bovine-derived products potentially exposing a large number of consumers sent shockwaves through the cattle industry and international beef trade. By far the largest number of BSE cases was reported from the United Kingdom, followed by several other European countries, including Ireland and Portugal [27, 133].

Although BSE was first recognized in the United Kingdom in 1986, undetected cases probably occurred since the early 1980s [159]. The number of UK BSE cases increased rapidly in the second half of the 1980s and early 1990s, peaked in 1992 with 37,280 confirmed cases, and has markedly declined since then (Fig. 47.3). As more stringent control measures were implemented to prevent cattle exposure to meat and bone meal, the number of confirmed UK BSE cases continued its dramatic decline throughout the late 1990s and 2000s consistent with the hypothesis that BSE was orally transmitted via contaminated meat and bone meal [27, 157, 160].

Clinically, the signs of BSE include neurologic dysfunction, including altered behavior, unsteady gait with falling, and abnormal responses to touch and sound [161]. In some animals, the onset of BSE can be insidious and subtle and may be difficult to recognize. During the early phase of the UK BSE outbreak, the public media introduced the popular term "mad cow" disease to describe the strange disease causing fearful and aggressive behavior in some of the cattle infected with BSE.

Although the original source of the BSE outbreak is unknown, the two most accepted hypotheses are cross-species transmission of scrapie from sheep to cattle and the transmission of a spontaneously occurring BSE within the cattle population [157, 160, 162, 163]. The latter hypothesis is predicated on the occurrence of spontaneous BSE, and more convincing data about its occurrence may become available with increased detection and monitoring of rates of atypical as well as classic cases of BSE [164, 165]. Strong epidemiologic evidence indicates that the practice of feeding cattle protein derived from rendered animal carcasses presumably contaminated with the scrapie or spontaneous BSE agent may have triggered the cattle epidemic [157]. In the past, cattle feed rendering in the United Kingdom involved several treatment steps, including exposure of the feed to prolonged heating in the presence of a hydrocarbon solvent. Some researchers have suggested that omission of these steps in the late 1970s and early 1980s in the United Kingdom contributed to the emergence of BSE by allowing scrapie or spontaneous BSE infectivity to survive the rendering process [157, 162, 166, 167]. Regardless of the origin of BSE, the epidemiologic evidence indicates that feeding cattle rendered BSE-infected carcasses greatly amplified the BSE outbreak. Several other factors may have contributed to the emergence of BSE in the United Kingdom, including a relatively high rate of endemic scrapie, a high population ratio of sheep to cattle, and the inclusion of rendered meat and bone meal at high rates in cattle feed.

Since the BSE outbreak was first detected, an estimated >2 million cattle have been infected with BSE in the United Kingdom [133, 167]. Approximately half of these BSE-infected cattle would have been slaughtered for human consumption, potentially exposing millions of UK residents [133, 168, 169]. Beginning in 1988, UK animal and public health authorities implemented several protective measures to prevent further exposure of animals and humans to BSE-infected cattle products. The implementation of these measures, particularly animal feed bans, led to a dramatic decline in the UK BSE outbreak (Fig. 47.3).

Because cattle carcasses were included in the production of animal feed, potential transmission of BSE to other animals was considered during the early phase of the BSE outbreak in the United Kingdom. BSE-like dis-

eases were identified in zoo animals (ungulate spongiform encephalopathy) beginning in the late 1980s and in domestic cats (feline spongiform encephalopathy) beginning in 1990, indicating the potential for the BSE agent to cross the species barrier and infect other animals [28, 170]. This development increased the concern about the possible transmission of BSE to humans and led to the establishment of enhanced CJD surveillance in the United Kingdom.

BSE was reported for the first time outside the United Kingdom in Ireland in 1989 and in Portugal and Switzerland in 1990. By August 2006, the number of countries that reported one or more BSE cases in native cattle increased to 25, including 21 countries in Europe. The four countries outside Europe that reported BSE cases are Canada, Israel, Japan, and the United States. The BSE outbreak appears to be declining in most European countries, although small numbers of cases continue to occur.

In North America, BSE was first detected in 1993 in a cow that had been imported into Canada from the United Kingdom. Rendered remains of imported cohorts of this cow may have been responsible for the BSE cases subsequently identified during 2003–2011 among cattle born in Canada. One of these cases was identified in Washington State but was later traced to a farm in Canada [171]. As of May 2012, a total of 19 BSE cases have been identified in Canada, and at least 13 of these cases were born after the 1997 ruminant feed ban which was implemented to prevent BSE transmission among cattle [172]. Because of the continued occurrence of new BSE infections after the 1997 ruminant feed ban, US and Canadian authorities tightened the specified risk material ban in 2007 by excluding potentially infectious nervous tissues from all animal feed. In 2005 and 2006, respectively, nonclassic forms of BSE termed atypical BSE were confirmed in an approximately 12-year-old cow born and raised in Texas and a 10-year-old cow from Alabama [173, 174]. The source of BSE infection for these two cows remains unknown. In 2012, as part of USDA's ongoing surveillance, a third BSE case was identified in a dairy cow aged over 10 years in California. Similar to the previous 2 BSE cases reported in the United States, the third case was reported to have atypical BSE. The initial two cases were reported with H–type and the third case with L-type BSE. The occurrence of these cases renewed speculations that atypical BSE may in fact constitute a prion disease that arises spontaneously among older potentially predisposed cattle.

4.2 Variant Creutzfeldt-Jakob Disease

In 1996, the identification of a cluster of young patients (median age, 28 years) with a prion disease was reported in the United Kingdom as part of the CJD surveillance system that was established in response to concerns about the potential spread of BSE to humans [158]. Because of the patients' unusually young age and the distinct clinical and neuropathologic findings, which were different from patients with the classic form of CJD, the occurrence of the cluster was believed to signify the transmission of BSE to humans. Since 1996, variant CJD cases increased in number and geographic distribution, and strong scientific evidence supported initial suspicions that variant CJD was indeed BSE in humans [175, 176]. As of June 2014, a total of 229 variant CJD patients were reported worldwide, including 177 patients from the United Kingdom; 27 from France; 5 from Spain; 4 from the United States; 3 from the Netherlands; 2 each from Canada, Italy, and Portugal; and 1 each from Japan, Saudi Arabia, and Taiwan [177]. Seven of the non-UK variant CJD patients (2 each from the United States and Ireland and 1 each from Canada, France, and Japan) were believed to have acquired variant CJD during their past residence or visit in the United Kingdom. The third US and second Canadian vCJD patients were believed to have acquired the disease during their residence in Saudi Arabia.

Variant CJD can be distinguished from the more common classic CJD by the clinical and laboratory findings (Table 47.3) [133, 175, 178]. The median age at death of variant CJD patients is 40 years younger than that of sporadic CJD patients (28 and 68 years, respectively); the median illness duration for vCJD is longer (14 months) than that of sporadic CJD (<6 months). The distinguishing clinical features of variant CJD include a predominantly psychiatric manifestation at onset with delayed appearance of frank neurologic signs and the appearance of sensory abnormalities with dysesthesia and paresthesia [175]. On the MRI, a typical "pulvinar sign" is often demonstrated in a majority of vCJD patients consisting of a hockey-stick-like, symmetrical hyperintensity in the pulvinar region relative to the intensity in other structures [179]. The diagnostic EEG finding that is common in classic CJD patients is very rare in patients with variant CJD. All variant CJD patients tested to date had methionine homozygosity at the polymorphic codon 129 of the prion protein gene [177, 180]. This homozygosity is present in approximately 35–40 % of the general UK population. A definitive diagnosis of variant CJD requires laboratory testing of brain tissues. In addition to the spongiform lesion, neuronal loss, and astrogliosis typical of most prion diseases, the neuropathology in variant CJD is characterized by the presence of numerous "florid plaques," consisting of amyloid deposits surrounded by a halo of spongiform lesions [177, 181] (See Fig. 47.4).

Studies in the United Kingdom have indicated the probable secondary person-to-person transmission of the variant CJD agent in three patients by blood (non-leukodepleted red blood cells) collected 17–40 months before variant CJD onset in the donors [177, 182–187]. The incubation period in

Table 47.3 Clinical and pathologic characteristics distinguishing variant Creutzfeldt-Jakob disease (variant CJD) from classic CJD

Characteristic	Variant CJD	Classic CJD
Median age (range) at death (years)	28 (14–74)	68 (23–97)[a]
Median duration of illness (months)	13–14	4–5
Clinical presentation	Prominent psychiatric/behavioral symptoms, painful sensory symptoms, delayed neurologic signs	Dementia, early neurologic signs
Periodic sharp waves on electroencephalogram	Almost always absent	Often present
"Pulvinar sign" on magnetic resonance imaging[b]	Present in >75 % of cases	Very rare or absent
Presence of "florid plaques" on neuropathologic sample	Present in great numbers	Rare or absent
Immunohistochemical analysis of brain tissue	Marked accumulation of PrP-res[c]	Variable accumulation
Presence of agent in lymphoid tissue	Readily detected	Not readily detected
Increased glycoform ratio on Western blot analysis of PrP-res	Present	Not present
Genotype at codon 129 of prion protein	Methionine/methionine[d]	Polymorphic

[a]U.S. CJD surveillance data 1979–2001
[b]Symmetrical high signal in the posterior thalamus relative to that of other deep and cortical gray matter
[c]Protease-resistant prion protein
[d]A patient with preclinical vCJD related to bloodborne transmission was heterozygous for methionine and valine

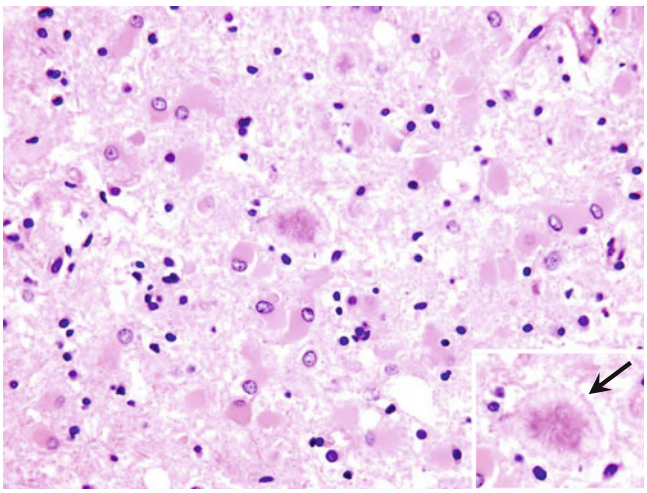

Fig. 47.4 Variant CJD neuropathology. Cerebral cortex shows marked astroglial reaction and the occasional presence of relatively large florid plaques surrounded by vacuoles (*arrow in insert*). Frontal cortex, hematoxylin and eosin stain

these patients ranged from 6.5 to 8.5 years, and all three had methionine homozygosity at the polymorphic codon 129 of the prion protein gene. A fourth patient who was heterozygous at codon 129 had laboratory evidence of vCJD but died of a non-neurologic condition >5 years after receipt of red blood cells from a donor diagnosed with vCJD 18 months after donation [182]. Because a large proportion of the UK population has potentially been exposed to the BSE agent, concerns still exist about additional secondary spread of the agent via blood products and possibly via contaminated surgical instruments. Testing of retrospectively collected appendectomy samples from 12,674 UK residents identified three positive samples, indicating an estimated prevalence of 237 vCJD infections per million populations [188]. Two of the three positive samples with prion protein gene analysis were homozygous for valine at codon 129.

4.3 Atypical Bovine Spongiform Encephalopathy

Since 2003, BSE cases with histopathologic features and Western blot characteristics of the infecting prions distinct from that of the classic form of BSE were increasingly identified from several European countries, Canada, Japan, and the United States [173, 189–193]. Based primarily on the molecular weight of the unglycosylated, proteinase-K-resistant fragment of the PrP^{Sc}, atypical BSE cases were classified into two groups. The fragment with a higher molecular weight than the classic type (C-type) was designated the H-type, and the fragment with a lower molecular weight than the C-type was designated the L-type [194, 195]. It is widely believed that these different properties of the infecting prions may represent different strains of the BSE agent. Initially, the L-type BSE was called bovine amyloid spongiform encephalopathy or BASE because of the presence of amyloid plaques in histopathologic preparations of brain tissues from infected cattle [189]. Because the H- and L-types of BSE seem to be rare and because they tend to occur in older cattle, some researchers have suggested that these atypical BSE cases arise spontaneously as a result of sporadic, random mutations of the prion protein gene [195]. However, the possibility that they may also be strain variations of the large C-type BSE outbreak should not be excluded. Regardless of the origin of atypical BSE, its continued occurrence could still pose a risk of contamination of the animal feed and human food supplies. Spontaneous occurrence of BSE may actually be an ominous sign because feed control measures may not completely eliminate its occurrence and herald the need for continuing surveillance and maintaining effective feed bans even after the C-type BSE outbreak is under control.

Whether or not atypical BSE can be transmitted to humans and the possible phenotype it may represent remain unknown. The possible transmission of L- and

H-type BSE to humans has been assessed using transgenic mouse models expressing the human prion protein homozygous for methionine at codon 129. L-type BSE transmitted to the transgenic mice with no apparent barrier, whereas C-type BSE had a substantial transmission barrier; no transmission of the H-type was reported [196, 197]. Transmission of L-type BSE to lemurs has also been reported via intracerebral and oral challenges and to cynomolgus macaques by intracerebral inoculation [191, 198]. A comparison between the pathogenesis of classic and atypical BSE is currently an area of active research because this could have potential implications for possible exposure to humans and animals [199–201].

5 Chronic Wasting Disease and Interspecies Transmission

CWD was first identified in the late 1960s as a fatal wasting syndrome of captive mule deer in research facilities in Colorado and subsequently in a similar facility in Wyoming. It was not recognized as a spongiform encephalopathy until 1977 [202, 203]. CWD was recognized as a disease of free-ranging animals in the early 1980s, and by the mid-1990s, its endemic occurrence was reported in deer and elk in a contiguous area in northeastern Colorado and southeastern Wyoming. Mule deer, white-tailed deer, and Rocky Mountain elk are the major known natural hosts for CWD [204]. In 2005, a hunter-killed moose was confirmed with CWD in Colorado, suggesting that this member of the deer family is also a natural host. As of December 2013, CWD has been identified in free-ranging cervids in 18 US states (Fig. 47.5) and 2 Canadian provinces and in greater than 100 captive herds in 15 states and provinces in North America.

The occurrence of CWD in free-ranging animals is spreading to wider geographic areas, and prevalence is increasing in many CWD endemic locations. Parts of Wyoming now have CWD prevalence rates approaching 50 % in mule deer, and prevalence in areas of Colorado and Wisconsin is less than 15 % in deer. The prevalence of CWD in elk is generally lower than in deer but can reach 10 % in parts of Wyoming. Known risk factors for CWD include sex and age of the animals, with adult male deer having the highest disease prevalence [203, 205]. Polymorphisms in the prion protein gene correlate with the incidence of CWD in deer and elk but remain less understood than the strong genetic influence described for scrapie in sheep [206–209]. The long-term effects of CWD infection on the dynamics of cervid populations are unclear. Epidemiologic modeling studies have predicted that CWD infection could have negative effects on the density of free-ranging cervid populations. These long-term effects may be influenced by variations in hunting management practices and persistence of the CWD agent in the environment [210, 211]. CWD infection in free-

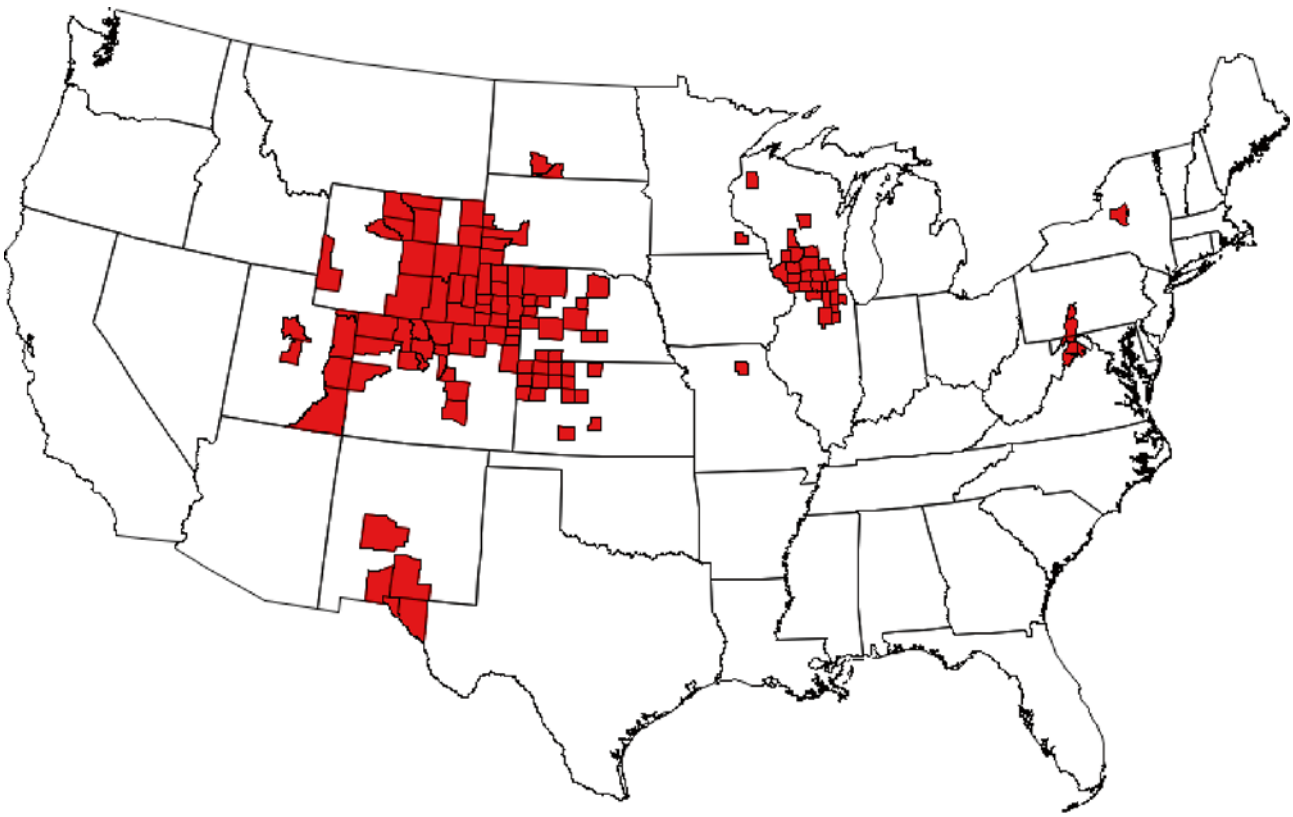

Fig. 47.5 Chronic wasting disease among free-ranging cervids by county, the United States, July 2012

ranging mule deer has been associated with large decreases in cervid populations in Boulder, Colorado, an area with a high rate of CWD infection [205]. Consistent with the clinical manifestations of the disease, deer with CWD are weaker and are preyed upon by mountain lions more readily than healthy deer [205]. They are also more likely to be involved in collisions with vehicles, further contributing to thinning of cervid populations [212].

5.1 CWD Transmission in the Natural Host

Horizontal transmission of the CWD agent is a major mechanism of disease spread in the wild. CWD prions can enter the environment through shedding from diseased animals and from decomposing carcasses. The CWD agent is shed from infected deer in urine, feces, saliva, blood, and antler velvet, and shedding can occur during the preclinical phase of CWD or from clinically affected animals [213, 214]. CWD prions are present in many organs and tissues of an infected cervid, including skeletal muscle, cardiac muscle, fat, lymphoid tissues, and peripheral and central nervous system tissues [209, 215, 216]. Ingestion is an effective route of CWD agent transmission among cervids and lesions in the oral cavity can facilitate entry of the agent enhancing the transmission of CWD [217, 218]. Prions bind to a range of soils and soil minerals and retain the ability to replicate [219–223]. Ingestion and inhalation of soil by cervids has been hypothesized to play an important role in CWD transmission [224, 225]. Nasal inoculation has been shown to be an efficient route of prion transmission [226, 227]. Consistent with the role of the environment in CWD transmission, exposure of CWD-naïve deer to drinking water, feed buckets, and bedding used by CWD-infected deer resulted in the naïve deer developing CWD [228].

5.2 Interspecies CWD Transmission

Human prion disease with evidence of a link with CWD has not been identified despite several epidemiologic investigations of suspected cases [202, 229–232]. Consistent with this observation, in vitro prion conversion assays indicate that the efficiency of human PrP^C conversion by CWD prions is low. In addition, transgenic mice expressing human PrP^C are not susceptible to CWD infection [233–235]. However, the CWD agent has been transmitted to squirrel monkeys by intracerebral and oral routes of inoculation, while cynomolgus macaques, which are genetically closer to humans, are resistant to CWD infection [236]. Transmission of CWD to non-cervid species has not been observed under natural conditions. Raccoons, opossums, and coyotes who may scavenge CWD-infected carcasses have not been shown to be infected with CWD in Wisconsin [237]. Transmission of CWD to cattle has not been observed in experimentally controlled environmental exposure studies [209]. However, CWD has been transmitted to cattle, sheep, goats, mink, ferrets, voles, and mice by intracerebral inoculation [209, 238–240]. Limitations of the aforementioned negative transmission studies include the small number of animals inoculated, which would be unlikely to identify low transmission rates, and the fact that strain variations have not been fully accounted for. Compelling evidence suggests the existence of distinct CWD strains which may influence host range, pathogenicity, and the zoonotic potential of CWD [241–243]. Currently, knowledge about the natural distribution and prevalence of CWD strains in free-ranging cervids is very limited. Interactions of the CWD agent with the environment particularly soil may alter CWD strain properties or exert selective pressure on different strains, further complicating the interpretation of CWD transmission studies [219]. Although the negative transmission studies provide reassurance indicating the existence of a substantial species barrier protecting humans from CWD transmission, the animal model studies should be interpreted with caution. Epidemiologic surveillance is ongoing to monitor the zoonotic transmission of CWD to individuals who hunted in areas where the disease has been endemic for decades. Although these studies have not detected any evidence of CWD transmission to humans, long-term follow up is necessary because of the long incubation period associated with any potential zoonotic transmission.

6 Prion Diseases of Humans Associated with Genetic Mutations

One of the intriguing properties of prion diseases in humans is the fact that they can be both infectious and heritable. The inherited or genetic forms of prion diseases are associated with insertion, deletion, or point mutations of the open reading frame of the prion protein gene [113, 244]. At least 24 different point mutations of the prion protein gene have been described in association with human prion diseases [113, 245]. These genetic prion diseases have widely varying clinical and neuropathologic manifestations and account for 10–15% of prion diseases in humans. Historically, genetic forms of prion diseases, in part based on their phenotypical expression, are classified as familial CJD, GSS, and FFI. They mostly follow an autosomal dominant inheritance pattern and have high penetrance. Beginning in 1989, many types of insertion mutations associated with markedly heterogeneous phenotypes have been reported in familial clusters.

6.1 Familial CJD

Patients with familial CJD generally have clinicopathologic phenotype similar to sporadic CJD. The disease has a

dominant inheritance pattern, and over half of affected family members carrying the mutation eventually die of CJD [2]. Familial CJD has been reported among family clusters in many countries, including from Canada, Europe, Japan, Israel, the United States, and several Latin American countries [2, 113, 244, 246–250]. The largest familial cluster was reported among Jews of Libyan and Tunisian origin, in rural Chile, and in Slovakia. It is most frequently associated with a mutation substituting glutamic acid with lysine at codon 200 of the prion protein gene. Arguably, familial CJD associated with codon 200 mutation is the most common heritable form of prion disease in humans. Perhaps the next relatively common form of familial CJD is associated with a mutation at codon 178 substituting aspartic acid with asparagine. This mutation has been reported in families originating from England, Finland, France, Hungary, and the Netherlands [245, 251]. A pedigree analysis of the original Finish family indicated that codon 178 mutation could have a disease penetration rate of 100 % [252]. Familial CJD with codon 178 mutation occurs when the mutant allele coding for asparagine at codon 178 also codes for valine at codon 129 [2, 253]. Compared with sporadic CJD patients, familial CJD patients with codon 178 mutation tend to have illness onset at an earlier age (mean, 46 years). About 12 other less frequent mutations with phenotypical expressions resembling familial CJD have been reported from many countries.

6.2 Gerstmann-Sträussler-Scheinker Syndrome

GSS bears the names of the three physicians who first described the disease in 1936 [56]. The original Austrian family spanning many generations reported by these physicians was later shown to carry the codon 102 mutation, which we now know is the most common form of GSS. Since its first report, the term GSS is used to describe a heterogeneous group of inherited human prion diseases that are characterized by a long duration of illness (median: ~5 years but sometimes exceeding 20 years) and the presence of numerous PrP-amyloid plaques, primarily in the cerebellum.

At least 13 different types of prion protein gene mutations or a combination of mutations in at least 56 kindred or families have been reported in association with the GSS phenotype. Familial clusters with the GSS phenotype have been reported from Canada, Europe, Japan, Israel, Mexico, and the United States [2, 245]. Many of the GSS mutations are associated with a greater degree of variability in the disease phenotype than other inherited forms of prion diseases. The most frequent GSS mutation results in a substitution of leucine for proline at codon 102 of the prion protein gene and is coupled with methionine at the polymorphic codon 129 of the mutant allele [254]. Patients with this mutation commonly manifest with cerebellar dysfunction, including ataxia and dysarthria, movement disorders, and possibly dementia and akinetic mutism. In some patients with the GSS 102 mutation, the illness can last for up to 6 years.

GSS with codon 105 mutations substituting proline for leucine or serine has been reported in several Japanese families. Patients predominantly have spastic paraparesis associated with cerebellar dysfunction and dementia and a neuropathologic picture of numerous amyloid plaques with neurofibrillary tangles and absence of spongiform changes. A large US family of German descent followed up for decades with an illness resembling GSS has been shown to have a mutation at codon 117 substituting alanine for valine [255]. Patients in this family cluster predominantly presented with dementia and pyramidal and extrapyramidal signs with minimal signs of cerebellar dysfunction. Arguably the largest and perhaps most studied family cluster with GSS was identified in the US state of Indiana with over 70 affected family members identified over 6 generations, and a total of over 1,000 family members involved in the investigation [256–263]. Many patients in this cluster had a mutation at codon 198 substituting phenylalanine for serine. At least six additional mutations of the prion protein gene have been reported in patients manifesting with a phenotype resembling GSS.

6.3 Fatal Familial Insomnia

The name FFI was first used in 1986 to describe a patient who predominantly presented with insomnia and autonomic dysfunction and had a history of other family members in several generations who had died of a similar illness [264–266]. Neuropathologic studies in FFI predominantly show marked involvement of the thalamus, resulting in a clinical phenotype characterized often by intractable insomnia and autonomic nervous system dysfunction, including abnormalities in temperature regulation, increased heart rate, hypertension, and sexual and urinary dysfunction [266–272]. The neuropathologic lesions are more severe in the thalamus than other regions of the brain. FFI is primarily associated with a mutation at codon 178 of the prion protein gene resulting in a substitution of aspartic acid with asparagine in combination with methionine at the polymorphic codon 129 of the mutant allele. The mutation seems to follow an autosomal dominant inheritance pattern. FFI has been identified in Australia, Canada, Japan, the United States, and several European countries. Recently, patients with no prion protein gene mutations but having clinical and pathologic manifestations indistinguishable from that seen in FFI patients have been reported. These seemingly sporadic cases with no family history of a similar disease are now recognized as sporadic fatal insomnia (sFI) and are classified as one of the subtypes of sporadic CJD [273, 274].

6.4 Codon 129 Polymorphism

The prion protein gene in humans exhibits a polymorphism at codon 129 which codes either for methionine or valine. Approximately 40 % of predominantly Caucasian populations are homozygous for methionine, 50% heterozygous with methionine and valine, and 10 % homozygous for valine. Methionine and valine homozygosity seem to be overrepresented among sporadic CJD (84 %) and hGH-associated CJD (67 %) patients, indicating that it may influence disease susceptibility in some individuals [121]. To date, all variant CJD patients who have been tested are homozygous for methionine at codon 129 [180, 181]. Heterogeneity at codon 129 seems to be protective against CJD, but when disease occurs the incubation period is usually prolonged in some iatrogenic CJD patients. The polymorphism also markedly influences the clinicopathologic phenotype of sporadic CJD and several inherited prion diseases. The most striking example of this influence is the phenotype associated with codon 178 mutation that substitutes aspartic acid with asparagine. Patients who have this mutation in combination with methionine on the mutant allele at codon 129 present with the FFI phenotype, whereas patients who have valine at codon 129 of the mutant allele present with the familial CJD phenotype [253]. The codon 129 polymorphism may also influence the age at onset and duration of illness in some prion diseases.

7 Scrapie

Scrapie was first reported in the 1730s in England but has been identified in many countries since then. Although it was recognized as a distinct clinical entity of sheep over 250 years ago, many aspects of the disease including its natural origin in flocks and the precise means by which it usually spreads remain uncertain. Experimentally, the disease was first transmitted by intraocular inoculation of scrapie brain extracts.

Scrapie transmission may occur by different postulated mechanisms. A commonly cited source of transmission, for example, is the placenta and amniotic fluid of scrapie-infected ewes. These tissues are known to harbor the infectious agent and can cause scrapie when fed to other sheep. They may contaminate pastures and barns that, in turn, may remain potentially infectious for years. Another possible source of spread is feces because prion replication occurs in gut lymphoid tissues after oral inoculation in sheep and goats. The importance of oral transmission is supported by experimental studies that detected prions in sheep tonsils examined early during the incubation period. Other poorly defined scrapie transmission mechanisms include: (a) the vertical transfer of the scrapie agent and (b) the possible chance occurrence of scrapie caused by hypothesized rare, spontaneous changes in the animal's cellular prion protein.

Scrapie occurs endemically in many countries, including in Europe and North America. Australia and New Zealand have sizable sheep populations but are generally recognized as free of the disease. To protect their "scrapie-free" status, these countries have established extensive safeguards to prevent the introduction of scrapie into their herds from imported animals.

The breed of sheep and polymorphisms of the prion protein gene can greatly influence susceptibility to scrapie [275–277]. Experimental transmissions with scrapie-infected tissues, for example, have confirmed the differing susceptibility to scrapie of different breeds of sheep. Other studies of Suffolk sheep in the United States indicated that susceptibility to scrapie was highly correlated with a polymorphism in the prion protein gene at codon 171 (glycine or arginine); the presence of arginine confers resistance to the disease [278, 279].

7.1 Atypical Scrapie

A new prion disease of sheep was identified in Norway in 1998 (Nor98) and has since been identified in several countries worldwide [280–287]. This new form, commonly referred to as atypical scrapie, differs from the classic form of scrapie in several important ways. The electrophoretic migration of Nor98/atypical scrapie PrP^{Sc} is characterized by lower molecular weight PrP^{Sc} species than that of the classic form [288, 289]. The distribution of PrP^{Sc} in sheep infected with Nor98/atypical scrapie is largely localized to the CNS in contrast to classic forms of scrapie that have a more widespread distribution. Interestingly, Nor98/atypical scrapie is mainly found in sheep with prion protein genotypes that correspond with relative resistance to the classic strains of scrapie [290–293]. Atypical scrapie occurs in older animals compared to classic forms of scrapie, and the incidence of disease is similar in infected flocks compared to the general population, suggesting that animal-to-animal transmission is not a prominent feature of Nor98/atypical scrapie [281, 294–296].

The etiology of Nor98/atypical scrapie is unclear. The predominance of PrP^{Sc} distribution in the CNS, the occurrence of disease in older animals, and the lack of clear evidence of animal-to-animal transmission have led to the hypothesis that Nor98/atypical scrapie may be a spontaneous prion disease with an etiology similar to sporadic CJD [281]. In addition, transmission of Nor98/atypical scrapie to transgenic mice expressing ovine prion protein produces a disease phenotype that is distinct from BSE-infected mice suggesting that Nor98/atypical scrapie is not due to transmission of BSE to sheep [285, 297, 298]. However, recent data indicate that Nor98/atypical scrapie is experimentally transmissible via oral inoculation and that extraneural tissues that are PrP^{Sc} negative as assessed by immunodetection techniques contain significant amounts of prion infectivity when tested using animal bioassays [299]. The implications of these findings are twofold. First, the conditions exist for transmission of Nor98/atypical

scrapie between individual animals (peripheral distribution of agent and oral susceptibility) suggesting that a spontaneous etiology is not the only mechanism of disease occurrence. Second, disease surveillance is based on immunodetection of PrP^{Sc}; therefore, the incidence of disease may be underreported. Clearly much work is needed to resolve the etiology and transmission history of this newly emerging prion disease of sheep.

8 Diagnostic Tests for Prion Diseases

In general, infecting prions can be detected in high concentrations in central nervous system tissues, particularly the brain. Their presence outside of the brain in peripheral tissues varies by the host species and infecting prion strains. In many prion diseases, confirmatory diagnosis requires testing of brain tissues by one of the widely used diagnostic methods, including histopathology using H&E staining, immunohistochemistry after labeling with antibodies directed to certain epitopes of the prion molecule, Western blot techniques, or rapid diagnostic tests developed for BSE screening such as prionics and biorad. Most of these diagnostic methods require protease digestion of biological samples to degrade native PrP^{C} that could interfere with the test results. They rely on the resistance of PrP^{Sc} to protease digestion to detect its presence. In contrast, confirmation-dependent immunoassay (CDI), a test developed by researchers at University of California, San Francisco, does not rely on the protease-resistant nature of PrP^{Sc} to be used as a diagnostic tool [300, 301]. The high-affinity antibodies used in CDI recognize confirmation-dependent epitopes of PrP^{Sc} to distinguish it from that of PrP^{C}.

8.1 Prion Amplification in In Vitro Systems

Current prion diagnostic tests, such as immunohistochemistry and Western blot, mainly rely on the immunodetection of endogenous PrP^{Sc} that is present in the host. They are very specific in the diagnosis of prion diseases but have limited sensitivity of detection of low amounts of PrP^{Sc}. The ability to detect low amounts of PrP^{Sc} would allow for an earlier diagnosis of prion diseases and potentially from easily accessible tissues (e.g., blood) that may harbor low levels of PrP^{Sc} compared to the brain. Recent promising studies have developed in vitro means of prion conversion that allow for the amplification of low levels of PrP^{Sc} to an abundance that is in the range of detection of current immunodetection technologies. In these in vitro systems, the natural properties of prions to recruit and convert PrP^{C} are exploited by mimicking the in vivo prion replication process to amplify and detect even minute amounts of prions in biological samples [302]. Byron Caughey and colleagues first demonstrated in a cell-free system that PrP^{C} could be converted to a PK-resistant form in the presence of PrP^{Sc} [303, 304]. This cell-free conversion assay was useful for understanding the nature of the prion agent, prion strain properties, and the species barrier effect [303]. Cell-free conversion reactions were not very efficient; therefore, such assays had limited ability to be used as diagnostic tools for prion diseases. Claudio Soto and colleagues subsequently developed a system they termed protein misfolding cyclic amplification (PMCA) to increase the efficiency of in vitro conversion assays [305–308]. PMCA utilizes incubation of uninfected brain homogenate that contains PrP^{C} with a biological sample that contains PrP^{Sc} resulting in the conversion of PrP^{C} to PrP^{Sc}. The PrP^{Sc} acts like a seed and elongates into an oligomer by attracting PrP^{C} molecules and incorporating them into the growing oligomer. The rate of conversion is limited by the availability of seeds actively recruiting PrP^{C}. To accelerate the process, a sonication step was introduced to fragment the growing oligomer into smaller units that would serve as multiple seeds to recruit even more PrP^{C}, thereby amplifying the conversion reactions. Serial rounds of incubation and sonication result in a highly efficient in vitro conversion process [307]. PMCA has been shown to amplify PrP^{Sc} from samples containing a single infectious dose to levels that are easily detectible by Western blot analysis. Real-time quacking-induced conversion (RTQuIC) is a modification of PMCA methodology and utilizes recombinant PrP^{C} that has single PrP^{Sc} molecule sensitivity and detects PrP^{Sc} using thioflavin T fluorescence resulting in real-time detection of PrP^{Sc} formation allowing for a more rapid assay [303, 304, 309, 85]. Other promising in vitro technologies include the amyloid seeding assay and the surround optical fiber immunoassay. As with all other highly sensitive diagnostic tests (e.g., PCR), care must be taken to minimize contamination, and in an aspect that is unique to prion diseases, the spontaneous conversion of PrP^{C} to PrP^{Sc} must be controlled for. With these controls in place, PMCA, RTQuIC, and other tests developed using similar methodologies have the potential to be highly sensitive and accurate prion diagnostic tests.

References

1. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science. 1982;216:136–44.
2. Belay ED. Transmissible spongiform encephalopathies in humans. Annu Rev Microbiol. 1999;53:283–314.
3. Bell JE, Gentleman SM, Ironside JW, et al. Prion protein immunocytochemistry–UK five centre consensus report. Neuropathol Appl Neurobiol. 1997;23:26–35.
4. Kovacs GG, Head MW, Hegyi I, et al. Immunohistochemistry for the prion protein: comparison of different monoclonal antibodies in human prion disease subtypes. Brain Pathol. 2002;12:1–11.
5. Piccardo P, Safar J, Ceroni M, Gajdusek DC, Gibbs Jr CJ. Immunohistochemical localization of prion protein in spongiform encephalopathies and normal brain tissue. Neurology. 1990;40:518–22.
6. Privat N, Sazdovitch V, Seilhean D, LaPlanche JL, Hauw JJ. PrP immunohistochemistry: different protocols, including a procedure for long formalin fixation, and a proposed schematic classification

for deposits in sporadic Creutzfeldt-Jakob disease. Microsc Res Tech. 2000;50:26–31.
7. Zaborowski A, Kordek R, Botts GT, Liberski PP. Immunohistochemical investigations of the prion protein accumulation in human spongiform encephalopathies. Special report II. Pol J Pathol. 2003;54:39–47.
8. Boelle PY, Valleron AJ. Incubation period of human prion disease. Lancet. 2006;368:914.
9. Collinge J, Whitfield J, McKintosh E, et al. Kuru in the 21st century–an acquired human prion disease with very long incubation periods. Lancet. 2006;367:2068–74.
10. Klitzman RL. Incubation period of human prion disease. Lancet. 2006;368:913.
11. Johnson RT. Prion diseases. Lancet Neurol. 2005;4:635–42.
12. Gajdusek DC, Zigas V. Degenerative disease of the central nervous system in New Guinea; the endemic occurrence of kuru in the native population. N Engl J Med. 1957;257:974–8.
13. Gajdusek DC. Kuru. Trans R Soc Trop Med Hyg. 1963;57:151–69.
14. Zigas V, Gajdusek DC. Kuru: clinical study of a new syndrome resembling paralysis agitans in natives of the Eastern Highlands of Australian New Guinea. Med J Aust. 1957;44:745–54.
15. Gajdusek DC, Zigas V. Kuru; clinical, pathological and epidemiological study of an acute progressive degenerative disease of the central nervous system among natives of the Eastern Highlands of New Guinea. Am J Med. 1959;26:442–69.
16. Hadlow WJ. Neuropathology and the scrapie-kuru connection. Brain Pathol. 1995;5:27–31.
17. Hadlow WJ. Scrapie and kuru. Lancet. 1959;2:289–90.
18. Gajdusek DC, Gibbs CJ. Attempts to demonstrate a transmissible agent in kuru, amyotrophic lateral sclerosis, and other sub-acute and chronic nervous system degenerations of man. Nature. 1964; 204:257–9.
19. Gajdusek DC, Rogers NG, Basnight M, Gibbs Jr CJ, Alpers M. Transmission experiments with kuru in chimpanzees and the isolation of latent viruses from the explanted tissues of affected animals. Ann N Y Acad Sci. 1969;162:529–50.
20. Gajdusek DC, Gibbs Jr CJ, Asher DM, David E. Transmission of experimental kuru to the spider monkey (Ateles geoffreyi). Science. 1968;162:693–4.
21. Gajdusek DC, Gibbs Jr CJ, Alpers M. Transmission and passage of experimental "kuru" to chimpanzees. Science. 1967;155: 212–4.
22. Beck E, Daniel PM, Alpers M, Gajdusek DC, Gibbs Jr CJ. Experimental "kuru" in chimpanzees. A pathological report. Lancet. 1966;2:1056–9.
23. Gajdusek DC, Gibbs CJ, Alpers M. Experimental transmission of a Kuru-like syndrome to chimpanzees. Nature. 1966;209:794–6.
24. Liberski PP. Kuru and D. Carleton Gajdusek: a close encounter. Folia Neuropathol. 2009;47:114–37.
25. Lindenbaum S. Cannibalism, kuru and anthropology. Folia Neuropathol. 2009;47:138–44.
26. Liberski PP, Sikorska B, Lindenbaum S, et al. Kuru: genes, cannibals and neuropathology. J Neuropathol Exp Neurol. 2012;71: 92–103.
27. Sejvar JJ, Schonberger LB, Belay ED. Transmissible spongiform encephalopathies. J Am Vet Med Assoc. 2008;233:1705–12.
28. Sigurdson CJ, Miller MW. Other animal prion diseases. Br Med Bull. 2003;66:199–212.
29. Gajdusek DC. Unconventional viruses and the origin and disappearance of kuru. Science. 1977;197:943–60.
30. Alper T, Haig DA, Clarke MC. The exceptionally small size of the scrapie agent. Biochem Biophys Res Commun. 1966;22:278–84.
31. Scott MR, Supattapone S, Nguyen HOB, DeArmond SJ, Prusiner SB. Transgenic models of prion disease. Arch Virol. 2000; 16(Suppl):113–24.
32. Weissmann C, Flechsig E. PrP knock-out and PrP transgenic mice in prion research. Br Med Bull. 2003;66:43–60.
33. Pattison IH, Jones KM. The possible nature of the transmissible agent of scrapie. Vet Rec. 1967;80:2–9.
34. Griffith JS. Self-replication and scrapie. Nature. 1967;215: 1043–4.
35. Prusiner SB, Bolton DC, Groth DF, Bowman KA, Cochran SP, McKinley MP. Further purification and characterization of scrapie prions. Biochemistry. 1982;21:6942–50.
36. Bolton DC, McKinley MP, Prusiner SB. Identification of a protein that purifies with the scrapie prion. Science. 1982;218: 1309–11.
37. Manuelidis L. A 25 nm virion is the likely cause of transmissible spongiform encephalopathies. J Cell Biochem. 2007;100: 897–915.
38. Manuelidis L, Liu Y, Mullins B. Strain-specific viral properties of variant Creutzfeldt-Jakob disease (vCJD) are encoded by the agent and not by host prion protein. J Cell Biochem. 2009;106:220–31.
39. Miyazawa K, Emmerling K, Manuelidis L. High CJD infectivity remains after prion protein is destroyed. J Cell Biochem. 2011;112:3630–7.
40. Miyazawa K, Emmerling K, Manuelidis L. Replication and spread of CJD, kuru and scrapie agents in vivo and in cell culture. Virulence. 2011;2:188–99.
41. Eiden M, Buschmann A, Kupfer L, Groschup MH. Synthetic prions. J Vet Med B Infect Dis Vet Public Health. 2006;53:251–6.
42. Legname G, Nguyen HOB, Baskakov IV, Cohen FE, DeArmond SJ, Prusiner SB. Strain-specified characteristics of mouse synthetic prions. Proc Natl Acad Sci U S A. 2005;102:2168–73.
43. Oesch B, Westaway D, Walchli M, et al. A cellular gene encodes scrapie PrP 27–30 protein. Cell. 1985;40:735–46.
44. Basler K, Oesch B, Scott M, et al. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell. 1986;46: 417–28.
45. Pan KM, Stahl N, Prusiner SB. Purification and properties of the cellular prion protein from syrian-hamster brain. Protein Sci. 1992;1:1343–52.
46. Viles JH, Cohen FE, Prusiner SB, Goodin DB, Wright PE, Dyson HJ. Copper binding to the prion protein: structural implications of four identical cooperative binding sites. Proc Natl Acad Sci U S A. 1999;96:2042–7.
47. Collinge J, Clarke AR. A general model of prion strains and their pathogenicity. Science. 2007;318:930–6.
48. DeArmond SJ, Prusiner SB. Perspectives on prion biology, prion disease pathogenesis, and pharmacologic approaches to treatment. Clin Lab Med. 2003;23:1–41.
49. Jansen K, Schafer O, Birkmann E, et al. Structural intermediates in the putative pathway from the cellular prion protein to the pathogenic form. Biol Chem. 2001;382:683–91.
50. Milhavet O, Casanova D, Chevallier N, McKay RD, Lehmann S. Neural stem cell model for prion propagation. Stem Cells. 2006;24:2284–91.
51. Wickner RB, Edskes HK, Shewmaker F, Kryndushkin D, Nemecek J. Prion variants, species barriers, generation and propagation. J Biol. 2009;8:47.
52. Prusiner SB. Prions. Proc Natl Acad Sci U S A. 1998;95: 13363–83.
53. Bueler H, Aguzzi A, Sailer A, et al. Mice devoid of prp are resistant to scrapie. Cell. 1993;73:1339–47.
54. Baskakov IV, Legname G, Prusiner SB, Cohen FE. Folding of prion protein to its native alpha-helical conformation is under kinetic control. J Biol Chem. 2001;276:19687–90.
55. Principe S, Maras B, Schinina ME, Pocchiari M, Cardone F. Unraveling the details of prion (con)formation(s): recent advances by mass spectrometry. Curr Opin Drug Discov Devel. 2008;11: 697–707.
56. Richardson EP, Masters CL. The nosology of Creutzfeldt-Jakob disease and conditions related to the accumulation of PrPCJD in the nervous system. Brain Pathol. 1995;5:33–41.

57. Brown P, Cathala F, Castaigne P, Gajdusek DC. Creutzfeldt-Jakob disease – clinical analysis of a consecutive series of 230 neuropathologically verified cases. Ann Neurol. 1986;20:597–602.
58. Brown P, Cathala F, Sadowsky D, Gajdusek DC. Creutzfeldt-Jakob disease in France: II. Clinical characteristics of 124 consecutive verified cases during the decade 1968–1977. Ann Neurol. 1979;6:430–7.
59. Gibbs Jr CJ, Joy A, Heffner R, et al. Clinical and pathological features and laboratory confirmation of Creutzfeldt-Jakob disease in a recipient of pituitary-derived human growth hormone. N Engl J Med. 1985;313:734–8.
60. Brown P, Cathala F, Gajdusek DC. Creutzfeldt-Jakob disease in France: III. Epidemiological study of 170 patients dying during the decade 1968–1977. Ann Neurol. 1979;6:438–46.
61. Zerr I, Poser S. Clinical diagnosis and differential diagnosis of CJD and vCJD with special emphasis on laboratory tests. APMIS. 2002;110:88–98.
62. Krasnianski A, Meissner B, Heinemann U, Zerr I. Clinical findings and diagnostic tests in Creutzfeldt-Jakob disease and variant Creutzfeldt-Jakob disease. Folia Neuropathol. 2004;42(Suppl B):24–38.
63. Brown P. EEG findings in Creutzfeldt-Jakob disease. JAMA. 1993;269:3168.
64. Cambier DM, Kantarci K, Worrell GA, Westmoreland BF, Aksamit AJ. Lateralized and focal clinical, EEG, and FLAIR MRI abnormalities in Creutzfeldt-Jakob disease. Clin Neurophysiol. 2003;114:1724–8.
65. Sakuishi K, Hanajima R, Kanazawa I, Ugawa Y. Periodic motor cortical excitability changes associated with PSDs of EEG in Creutzfeldt-Jakob disease (CJD). Clin Neurophysiol. 2005;116:1222–6.
66. Cuadrado-Corrales N, Jimenez-Huete A, Albo C, et al. Impact of the clinical context on the 14-3-3 test for the diagnosis of sporadic CJD. BMC Neurol. 2006;6:25.
67. Huang N, Marie SK, Livramento JA, Chammas R, Nitrini R. 14-3-3 protein in the CSF of patients with rapidly progressive dementia. Neurology. 2003;61:354–7.
68. Peoc'h K, Delasnerie-Laupretre N, Beaudry P, Laplanche JL. Diagnostic value of CSF 14-3-3 detection in sporadic CJD diagnosis according to the age of the patient. Eur J Neurol. 2006;13:427–8.
69. Saiz A, Nos C, Yague J, Dominguez A, Graus F, Munoz P. The impact of the introduction of the 14-3-3 protein assay in the surveillance of sporadic Creutzfeldt-Jakob disease in Catalonia. J Neurol. 2001;248:592–4.
70. Shiga Y, Wakabayashi H, Miyazawa K, Kido H, Itoyama Y. 14-3-3 protein levels and isoform patterns in the cerebrospinal fluid of Creutzfeldt-Jakob disease patients in the progressive and terminal stages. J Clin Neurosci. 2006;13:661–5.
71. Sanchez-Juan P, Green A, Ladogana A, et al. CSF tests in the differential diagnosis of Creutzfeldt-Jakob disease. Neurology. 2006;67:637–43.
72. Cohen OS, Hoffmann C, Lee H, Chapman J, Fulbright RK, Prohovnik I. MRI detection of the cerebellar syndrome in Creutzfeldt-Jakob disease. Cerebellum. 2009;8:373–81.
73. Geschwind MD, Potter CA, Sattavat M, et al. Correlating DWI MRI with pathologic and other features of Jakob-Creutzfeldt disease. Alzheimer Dis Assoc Disord. 2009;23:82–7.
74. Kong A, Kleinig T, Van der Vliet A, et al. MRI of sporadic Creutzfeldt-Jakob disease. J Med Imaging Radiat Oncol. 2008;52:318–24.
75. Lou X, Ma L, An NY, Cai YQ, Liang Y, Guo XG. Diffusion-weighted magnetic resonance imaging in diagnosis of Creutzfeldt-Jakob disease. Chin Med J (Engl). 2006;119:1242–7.
76. Poon MA, Stuckey S, Storey E. MRI evidence of cerebellar and hippocampal involvement in Creutzfeldt-Jakob disease. Neuroradiology. 2001;43:746–9.
77. Zanusso G. Should MRI, signs be included in the diagnostic criteria for sporadic Creutzfeldt-Jakob disease? Nat Clin Pract Neurol. 2006;2:68–9.
78. Zerr I, Kallenberg K, Summers DM, et al. Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Brain. 2009;132:2659–68.
79. Meissner B, Kallenberg K, Sanchez-Juan P, et al. MRI lesion profiles in sporadic Creutzfeldt-Jakob disease. Neurology. 2009;72:1994–2001.
80. Belay ED, Holman RC, Schonberger LB. Creutzfeldt-Jakob disease surveillance and diagnosis. Clin Infect Dis. 2005;41:834–6.
81. Gibbons RV, Holman RC, Belay ED, Schonberger LB. Creutzfeldt-Jakob disease in the United States: 1979–1998. JAMA. 2000;284:2322–3.
82. Holman RC, Belay ED, Christensen KY, et al. Human prion diseases in the United States. PLoS One. 2010;5:e8521.
83. Holman RC, Khan AS, Belay ED, Schonberger LB. Creutzfeldt-Jakob disease in the United States, 1979–1994: using national mortality data to assess the possible occurrence of variant cases. Emerg Infect Dis. 1996;2:333–7.
84. Holman RC, Khan AS, Kent J, Strine TW, Schonberger LB. Epidemiology of Creutzfeldt-Jakob disease in the United States, 1979–1990: analysis of national mortality data. Neuroepidemiology. 1995;14:174–81.
85. Peden AH, McGuire LI, Appleford NEJ, et al. Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion. J Gen Virol. 2012;93:438–49.
86. Zanusso G. Detection of the pathological prion protein in the olfactory epithelium of Subjects with Sporadic Creutzfeldt-Jakob Disease. Chemical Senses 2008;33:S12. Fifteenth International Symposium on Olfaction and Taste, July 21–26, 2008, San Francisco, California.
87. Smith-Bathgate B. Creutzfeldt-Jakob disease: diagnosis and nursing care issues. Nurs Times. 2005;101:52–3.
88. Parry A, Baker I, Stacey R, Wimalaratna S. Long term survival in a patient with variant Creutzfeldt-Jakob disease treated with intraventricular pentosan polysulphate. J Neurol Neurosurg Psychiatry. 2007;78:733–4.
89. Spilman P, Lessard P, Sattavat M, et al. A gamma-secretase inhibitor and quinacrine reduce prions and prevent dendritic degeneration in murine brains. Proc Natl Acad Sci U S A. 2008;105:10595–600.
90. Collinge J, Gorham M, Hudson F, et al. Safety and efficacy of quinacrine in human prion disease (PRION-1 study): a patient-preference trial. Lancet Neurol. 2009;8:334–44.
91. Iuvone T, Esposito G, De Filippis D, Scuderi C, Steardo L. Cannabidiol: a promising drug for neurodegenerative disorders? CNS Neurosci Ther. 2009;15:65–75.
92. Ludewigs H, Zuber C, Vana K, Nikles D, Zerr I, Weiss S. Therapeutic approaches for prion disorders. Expert Rev Anti Infect Ther. 2007;5:613–30.
93. Rainov NG, Tsuboi Y, Krolak-Salmon P, Vighetto A, Doh-Ura K. Experimental treatments for human transmissible spongiform encephalopathies: is there a role for pentosan polysulfate? Expert Opin Biol Ther. 2007;7:713–26.
94. Stewart LA, Rydzewska LH, Keogh GF, Knight RS. Systematic review of therapeutic interventions in human prion disease. Neurology. 2008;70:1272–81.
95. Tremblay P, Meiner Z, Galou M, et al. Doxycycline control of prion protein transgene expression modulates prion disease in mice. Proc Natl Acad Sci U S A. 1998;95:12580–5.
96. Gallardo-Godoy A, Gever J, Fife KL, Silber BM, Prusiner SB, Renslo AR. 2-Aminothiazoles as therapeutic leads for prion diseases. J Med Chem. 2011;54:1010–21.
97. Mallucci G, Collinge J. Rational targeting for prion therapeutics. Nat Rev Neurosci. 2005;6:23–34.
98. Ward HJ, Knight RS. Surgery and risk of sporadic Creutzfeldt-Jakob disease. Neuroepidemiology. 2008;31:241–2.
99. Ruegger J, Stoeck K, Amsler L, et al. A case-control study of sporadic Creutzfeldt-Jakob disease in Switzerland: analysis of

potential risk factors with regard to an increased CJD incidence in the years 2001–2004. BMC Public Health. 2009;9:18.
100. van Duijn CM, Delasnerie-Laupretre N, Masullo C, et al. Case-control study of risk factors of Creutzfeldt-Jakob disease in Europe during 1993–95. European Union (EU) Collaborative Study Group of Creutzfeldt-Jakob disease (CJD). Lancet. 1998;351:1081–5.
101. Ward HJ, Everington D, Croes EA, et al. Sporadic Creutzfeldt-Jakob disease and surgery: a case-control study using community controls. Neurology. 2002;59:543–8.
102. Mahillo-Fernandez I, de Pedro-Cuesta J, Bleda MJ, et al. Surgery and risk of sporadic Creutzfeldt-Jakob disease in Denmark and Sweden: registry-based case-control studies. Neuroepidemiology. 2008;31:229–40.
103. Bobowick AR, Brody JA, Matthews MR, Roos R, Gajdusek DC. Creutzfeldt-Jakob disease: a case-control study. Am J Epidemiol. 1973;98:381–94.
104. Davanipour Z, Alter M, Sobel E, Asher D, Gajdusek D. A case-control study of Creutzfeldt-Jakob disease. Am J Epidemiol. 1984;120:472.
105. Davanipour Z, Alter M, Sobel E, Asher D, Gajdusek DC. Creutzfeldt-Jakob disease – possible medical risk-factors. Neurology. 1985;35:1483–6.
106. Davanipour Z, Alter M, Sobel E, Asher DM, Gajdusek DC. A case-control study of Creutzfeldt-Jakob disease – dietary risk-factors. Am J Epidemiol. 1985;122:443–51.
107. Wientjens D, Davanipour Z, Hofman A, et al. Risk factors for Creutzfeldt-Jakob disease: a reanalysis of case-control studies. Neurology. 1996;46:1287–91.
108. Kondo K, Kuroiwa Y. A case control study of Creutzfeldt-Jakob disease – association with physical injuries. Ann Neurol. 1982; 11:377–81.
109. de Pedro CJ, Ruiz Tovar M, Ward H, et al. Sensitivity to biases of case-control studies on medical procedures, particularly surgery and blood transfusion, and risk of Creutzfeldt-Jakob disease. Neuroepidemiology. 2012;39:1–18.
110. Collins S, Law MG, Fletcher A, Boyd A, Kaldor J, Masters CL. Surgical treatment and risk of sporadic Creutzfeldt-Jakob disease: a case-control study. Lancet. 1999;353:693–7.
111. Hamaguchi T, Noguchi-Shinohara M, Nozaki I, et al. Medical procedures and risk for sporadic Creutzfeldt-Jakob disease, Japan, 1999–2008. Emerg Infect Dis. 2009;15:265–71.
112. Alcalde-Cabero E, Almazan-Isla J, Brandel JP, et al. Health professions and risk of sporadic Creutzfeldt-Jakob disease, 1965 to 2010. Euro Surveill. 2012;17:13–22.
113. Gambetti P, Kong QZ, Zou WQ, Parchi P, Chen SG. Sporadic and familial CJD: classification and characterisation. Br Med Bull. 2003;66:213–39.
114. Parchi P, Giese A, Capellari S, et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol. 1999;46:224–33.
115. Parchi P, Saverioni D. Molecular pathology, classification, and diagnosis of sporadic human prion disease variants. Folia Neuropathol. 2012;50:20–45.
116. Parchi P, Strammiello R, Giese A, Kretzschmar H. Phenotypic variability of sporadic human prion disease and its molecular basis: past, present, and future. Acta Neuropathol. 2011;121:91–112.
117. Parchi P, Strammiello R, Notari S, et al. Incidence and spectrum of sporadic Creutzfeldt-Jakob disease variants with mixed phenotype and co-occurrence of PrPSc types: an updated classification. Acta Neuropathol. 2009;118:659–71.
118. Parchi P, Castellani R, Capellari S, et al. Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. Ann Neurol. 1996;39:767–78.
119. Cali I, Castellani R, Alshekhlee A, et al. Co-existence of scrapie prion protein types 1 and 2 in sporadic Creutzfeldt-Jakob disease: its effect on the phenotype and prion-type characteristics. Brain. 2009;132:2643–58.
120. Zou W-Q, Puoti G, Xiao X, et al. Variably protease-sensitive prionopathy: a new sporadic disease of the prion protein. Ann Neurol. 2010;68:162–72.
121. Brown P, Brandel J-P, Sato T, et al. Latrogenic Creutzfeldt-Jakob disease, final assessment. Emerg Infect Dis. 2012;18:901–7.
122. Duffy P, Wolf J, Collins G, DeVoe AG, Streeten B, Cowen D. Letter: possible person-to-person transmission of Creutzfeldt-Jakob disease. N Engl J Med. 1974;290:692–3.
123. Hogan RN, Brown P, Heck E, Cavanagh HD. Risk of prion disease transmission from ocular donor tissue transplantation. Cornea. 1999;18:2–11.
124. Maddox RA, Belay ED, Curns AT, et al. Creutzfeldt-Jakob disease in recipients of corneal transplants. Cornea. 2008;27:851–4.
125. Bernoulli C, Siegfried J, Baumgartner G, et al. Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. Lancet. 1977;1:478–9.
126. Gibbs CJ, Asher DM, Kobrine A, Amyx HL, Sulima MP, Gajdusek DC. Transmission of Creutzfeldt-Jakob-disease to a chimpanzee by electrodes contaminated during neurosurgery. J Neurol Neurosurg Psychiatry. 1994;57:757–8.
127. Koch TK, Berg BO, De Armond SJ, Gravina RF. Creutzfeldt-Jakob disease in a young adult with idiopathic hypopituitarism. Possible relation to the administration of cadaveric human growth hormone. N Engl J Med. 1985;313:731–3.
128. Preece MA. Creutzfeldt-Jakob disease: implications for growth hormone deficient children. Neuropathol Appl Neurobiol. 1986; 12:509–15.
129. Centers for Disease Control. Fatal degenerative neurologic disease in patients who received pituitary-derived human growth hormone. Morb Mortal Wkly Rep. 1985;34:359.
130. Centers for Disease Control and Prevention. Update: Creutzfeldt-Jakob disease in a patient receiving a cadaveric dura mater graft. Morb Mortal Wkly Rep. 1987;36:324–5.
131. Centers for Disease Control (CDC). Rapidly progressive dementia in a patient who received a cadaveric dura mater graft. Morb Mortal Wkly Rep. 1987;36:49–50, 5.
132. Thadani V, Penar PL, Partington J, et al. Creutzfeldt-Jakob disease probably acquired from a cadaveric dura mater graft – case-report. J Neurosurg. 1988;69:766–9.
133. Belay ED, Schonberger LB. The public health impact of prion diseases. Annu Rev Public Health. 2005;26:191–212.
134. Blossom DB, Maddox RA, Beavers SF, et al. A case of Creutzfeldt-Jakob disease associated with a dura mater graft in the United States. Infect Control Hosp Epidemiol. 2007;28:1396–7.
135. Centers for Disease Control and Prevention. Creutzfeldt-Jakob disease in patients who received a cadaveric dura mater graft – Spain, 1985–1992. Morb Mortal Wkly Rep. 1993;42:560–3.
136. Dobbins JG, Belay ED, Malecki J, et al. Creutzfeldt-Jakob disease in a recipient of a dura mater graft processed in the US: cause or coincidence? Neuroepidemiology. 2000;19:62–6.
137. Hannah EL, Belay ED, Gambetti P, et al. Creutzfeldt-Jakob disease after receipt of a previously unimplicated brand of dura mater graft. Neurology. 2001;56:1080–3.
138. Centers for Disease Control (CDC). Update: Creutzfeldt-Jakob disease in a second patient who received a cadaveric dura mater graft. Morb Mortal Wkly Rep. 1989;38:37–8, 43.
139. Centers for Disease Control and Prevention (CDC). Creutzfeldt-Jakob disease in patients who received a cadaveric dura mater graft–Spain, 1985–1992. Morb Mortal Wkly Rep. 1993;42: 560–3.
140. Centers for Disease Control and Prevention (CDC). Creutzfeldt-Jakob disease associated with cadaveric dura mater grafts – Japan, January 1979-May 1996. Morb Mortal Wkly Rep. 1997;46:1066–9.
141. Centers for Disease Control and Prevention (CDC). Update: Creutzfeldt-Jakob disease associated with cadaveric dura mater grafts – Japan, 1979–2003. Morb Mortal Wkly Rep. 2003; 52:1179–81.

142. Centers for Disease Control and Prevention (CDC). Update: Creutzfeldt-Jakob disease associated with cadaveric dura mater grafts – Japan, 1978–2008. Morb Mortal Wkly Rep. 2008;57:1152–4.
143. Brown P, Preece M, Brandel JP, et al. Iatrogenic Creutzfeldt-Jakob disease at the millennium. Neurology. 2000;55:1075–81.
144. Esmonde T, Lueck CJ, Symon L, Duchen LW, Will RG. Creutzfeldt-Jakob-disease and lyophilized dura-mater grafts – report of 2 cases. J Neurol Neurosurg Psychiatry. 1993;56:999–1000.
145. Iwasaki Y, Mimuro M, Yoshida M, Hashizume Y, Kitamoto T, Sobue G. Clinicopathologic characteristics of five autopsied cases of dura mater-associated Creutzfeldt-Jakob disease. Neuropathology. 2008;28:51–61.
146. Kim HL, Do JY, Cho HJ, et al. Dura mater graft-associated creutzfeldt-jakob disease: the first case in Korea. J Korean Med Sci. 2011;26:1515–7.
147. Toovey S, Britz M, Hewlett RH. A case of dura mater graft-associated Creutzfeldt-Jakob disease in South Africa. S Afr Med J. 2006;96:592–3.
148. Brown P, Gajdusek DC, Gibbs Jr CJ, Asher DM. Potential epidemic of Creutzfeldt-Jakob disease from human growth hormone therapy. N Engl J Med. 1985;313:728–31.
149. Dixit K, Kreitschmann-Andermahr I, Basu A, et al. Human growth hormone-related iatrogenic Creutzfeldt-Jakob disease–being aware of diagnostic features 25 years later. J Clin Endocrinol Metab. 2009;94:2684–5.
150. Furtner M, Gelpi E, Kiechl S, et al. Iatrogenic Creutzfeldt-Jakob disease 22 years after human growth hormone therapy: clinical and radiological features. J Neurol Neurosurg Psychiatry. 2008;79:229–31.
151. Rappaport EB, Graham DJ. Pituitary growth hormone from human cadavers: neurologic disease in ten recipients. Neurology. 1987;37:1211–3.
152. Abrams JY, Schonberger LB, Belay ED, et al. Lower risk of Creutzfeldt-Jakob disease in pituitary growth hormone recipients initiating treatment after 1977. J Clin Endocrinol Metab. 2011;96:E1666–9.
153. Centers for Disease Control and Prevention. Update: Creutzfeldt-Jakob disease associated with cadaveric dura mater grafts – Japan, 1979–2003. Morb Mortal Wkly Rep. 2003;52:1179–81.
154. Centers for Disease Control and Prevention. Update: Creutzfeldt-Jakob disease associated with cadaveric dura mater grafts – Japan, 1978–2008. Morb Mortal Wkly Rep. 2008;57:1152–4.
155. Wells GAH, Scott AC, Johnson CT, et al. A novel progressive spongiform encephalopathy in cattle. Vet Rec. 1987;121:419–20.
156. Collee JG. Bovine spongiform encephalopathy. Lancet. 1990;336:1300–3.
157. Collee JG, Bradley R. BSE: a decade on–part I. Lancet. 1997;349:636–41.
158. Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet. 1996;347:921–5.
159. Cohen CH, Valleron AJ. When did bovine spongiform encephalopathy (BSE) start? Implications on the prediction of a new variant of Creutzfeldt-Jakob disease (nvCJD) epidemic. Int J Epidemiol. 1999;28:526–31.
160. Belay ED, Schonberger LB. Variant Creutzfeldt-Jakob disease and bovine spongiform encephalopathy. Clin Lab Med. 2002;22:849–62, v–vi.
161. Haessig M, Haessig BU, Knubben-Schweizer G. Objectivity of BSE symptoms using Bayes theorem. Schweiz Arch Tierheilkd. 2011;153:565–7.
162. Brown P. Bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease. Br Med J. 2001;322:841–4.
163. Capobianco R, Casalone C, Suardi S, et al. Conversion of the BASE prion strain into the BSE strain: the origin of BSE? Plos Pathog. 2007;3:e31.
164. Baron T, Biacabe AG. Origin of bovine spongiform encephalopathy. Lancet. 2006;367:297–8.
165. Torres J-M, Andreoletti O, Lacroux C, et al. Classical bovine spongiform encephalopathy by transmission of H-type prion in homologous prion protein context. Emerg Infect Dis. 2011;17:1636–44.
166. Brown P. Afterthoughts about bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease. Emerg Infect Dis. 2001;7:598–600.
167. Smith PG, Bradley R. Bovine spongiform encephalopathy (BSE) and its epidemiology. Br Med Bull. 2003;66:185–98.
168. Ghani AC, Ferguson NM, Donnelly CA, Anderson RM. Predicted vCJD mortality in Great Britain – Modelling the latest data puts a ceiling on the likely number of vCJD cases. Nature. 2000;406:583–4.
169. Ghani AC, Ferguson NM, Donnelly CA, Anderson RM. Factors determining the pattern of the variant Creutzfeldt-Jakob disease (vCJD) epidemic in the UK. Proc Biol Sci. 2003;270:689–98.
170. Kelly DF, Wells GA, Haritani M, Higgins RJ, Jeffrey M. Neuropathological findings in cats with clinically suspect but histologically unconfirmed feline spongiform encephalopathy. Vet Rec. 2005;156:472–7.
171. Centers for Disease Control and Prevention (CDC). Bovine spongiform encephalopathy in a dairy cow – Washington state, 2003. Morb Mortal Wkly Rep. 2004;52:1280–5.
172. Centers for Disease Control and Prevention (CDC). Overview of BSE in North America. (Accessed at http://www.cdc.gov/ncidod/dvrd/bse/index.htm).
173. Richt JA, Kunkle RA, Alt D, et al. Identification and characterization of two bovine spongiform encephalopathy cases diagnosed in the United States. J Vet Diagn Invest. 2007;19:142–54.
174. Richt JA, Hall SM. BSE case associated with prion protein gene mutation. PLoS Pathog. 2008;4:e1000156.
175. Zeidler M, Stewart GE, Barraclough CR, et al. New variant Creutzfeldt-Jakob disease: neurological features and diagnostic tests. Lancet. 1997;350:903–7.
176. Bruce ME, Will RG, Ironside JW, et al. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature. 1997;389:498–501.
177. Ironside JW. Variant Creutzfeldt-Jakob disease: an update. Folia Neuropathol. 2012;50:50–6.
178. Will RG, Zeidler M, Stewart GE, et al. Diagnosis of new variant Creutzfeldt-Jakob disease. Ann Neurol. 2000;47:575–82.
179. Collie DA, Summers DM, Sellar RJ, et al. Diagnosing variant Creutzfeldt-Jakob disease with the pulvinar sign: MR imaging findings in 86 neuropathologically confirmed cases. AJNR Am J Neuroradiol. 2003;24:1560–9.
180. Zeidler M, Stewart G, Cousens SN, Estibeiro K, Will RG. Codon 129 genotype and new variant CJD. Lancet. 1997;350:668.
181. Ironside JW, Head MW, Bell JE, McCardle L, Will RG. Laboratory diagnosis of variant Creutzfeldt-Jakob disease. Histopathology. 2000;37:1–9.
182. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet. 2004;364:527–9.
183. Peden AH, Ritchie DL, Ironside JW. Risks of transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Folia Neuropathol. 2005;43:271–8.
184. Zou S, Fang CT, Schonberger LB. Transfusion transmission of human prion diseases. Transfus Med Rev. 2008;22:58–69.
185. Llewelyn CA, Hewitt PE, Knight RS, et al. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet. 2004;363:417–21.
186. Wilson K, Ricketts MN. A third episode of transfusion-derived vCJD. Lancet. 2006;368:2037–9.
187. Hewitt PE, Llewelyn CA, Mackenzie J, Will RG. Three reported cases of variant Creutzfeldt-Jakob disease transmission following transfusion of labile blood components. Vox Sang. 2006;91:348.

188. Hilton DA, Ghani AC, Conyers L, et al. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. J Pathol. 2004;203:733–9.
189. Casalone C, Zanusso G, Acutis P, et al. Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. Proc Natl Acad Sci U S A. 2004;101:3065–70.
190. Biacabe AG, Laplanche JL, Ryder S, Baron T. Distinct molecular phenotypes in bovine prion diseases. EMBO Rep. 2004;5: 110–5.
191. Ono F, Tase N, Kurosawa A, et al. Atypical L-type bovine spongiform encephalopathy (L-BSE) transmission to cynomolgus macaques, a non-human primate. Jpn J Infect Dis. 2011;64:81–4.
192. Brown P, McShane LM, Zanusso G, Detwile L. On the question of sporadic or atypical bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. Emerg Infect Dis. 2006;12:1816–21.
193. Gavier-Widen D, Noremark M, Langeveld JP, et al. Bovine spongiform encephalopathy in Sweden: an H-type variant. J Vet Diagn Invest. 2008;20:2–10.
194. Buschmann A, Gretzschel A, Biacabe AG, et al. Atypical BSE in Germany–proof of transmissibility and biochemical characterization. Vet Microbiol. 2006;117:103–16.
195. Biacabe AG, Morignat E, Vulin J, Calavas D, Baron TG. Atypical bovine spongiform encephalopathies, France, 2001–2007. Emerg Infect Dis. 2008;14:298–300.
196. Beringue V, Herzog L, Reine F, et al. Transmission of atypical bovine prions to mice transgenic for human prion protein. Emerg Infect Dis. 2008;14:1898–901.
197. Kong Q, Zheng M, Casalone C, et al. Evaluation of the human transmission risk of an atypical bovine spongiform encephalopathy prion strain. J Virol. 2008;82:3697–701.
198. Mestre-Frances N, Nicot S, Rouland S, et al. Oral transmission of L-type bovine spongiform encephalopathy in primate model. Emerg Infect Dis. 2012;18:142–5.
199. Balkema-Buschmann A, Fast C, Kaatz M, et al. Pathogenesis of classical and atypical BSE in cattle. Prev Vet Med. 2011;102: 112–7.
200. Hoffmann C, Eiden M, Kaatz M, et al. BSE infectivity in jejunum, ileum and ileocaecal junction of incubating cattle. Vet Res. 2011; 42:21.
201. Suardi S, Vimercati C, Casalone C, et al. Infectivity in skeletal muscle of cattle with atypical bovine spongiform encephalopathy. PLoS One. 2012;7:e31449.
202. Belay ED, Maddox RA, Williams ES, Miller MW, Gambetti P, Schonberger LB. Chronic wasting disease and potential transmission to humans. Emerg Infect Dis. 2004;10:977–84.
203. Miller MW, Williams ES. Chronic wasting disease of cervids. Curr Top Microbiol Immunol. 2004;284:193–214.
204. Saunders SE, Bartelt-Hunt SL, Bartz JC. Occurrence, transmission, and zoonotic potential of chronic wasting disease. Emerg Infect Dis. 2012;18:369–76.
205. Miller MW, Swanson HM, Wolfe LL, et al. Lions and prions and deer demise. PLoS One. 2008;3:e4019.
206. White SN, Spraker TR, Reynolds JO, O'Rourke KI. Association analysis of PRNP gene region with chronic wasting disease in Rocky Mountain elk. BMC Res Notes. 2010;3:314.
207. Wilson GA, Nakada SM, Bollinger TK, Pybus MJ, Merrill EH, Coltman DW. Polymorphisms at the PRNP gene influence susceptibility to chronic wasting disease in two species of deer (Odocoileus Spp.) in Western Canada. J Toxicol Environ Health A. 2009;72:1025–9.
208. Wilson R, Plinston C, Hunter N, et al. Chronic wasting disease and atypical forms of bovine spongiform encephalopathy and scrapie are not transmissible to mice expressing wild-type levels of human prion protein. J Gen Virol. 2012;93:1624–9.
209. Sigurdson CJ. A prion disease of cervids: chronic wasting disease. Vet Res. 2008;39:41.
210. Almberg ES, Cross PC, Johnson CJ, Heisey DM, Richards BJ. Modeling routes of chronic wasting disease transmission: environmental prion persistence promotes deer population decline and extinction. PLoS One. 2011;6:e19896.
211. Wasserberg G, Osnas EE, Rolley RE, Samuel MD. Host culling as an adaptive management tool for chronic wasting disease in white-tailed deer: a modelling study. J Appl Ecol. 2009;46:457–66.
212. Krumm CE, Conner MM, Miller MW. Relative vulnerability of chronic wasting disease infected mule deer to vehicle collisions. J Wildl Dis. 2005;41:503–11.
213. Gough KC, Maddison BC. Prion transmission, prion excretion and occurrence in the environment. Prion. 2010;4:275–82.
214. Maddison BC, Baker CA, Terry LA, et al. Environmental sources of scrapie prions. J Virol. 2010;84:11560–2.
215. Spraker TR, Zink RR, Cummings BA, Sigurdson CJ, Miller MW, O'Rourke KI. Distribution of protease-resistant prion protein and spongiform encephalopathy in free-ranging mule deer (*Odocoileus hemionus*) with chronic wasting disease. Vet Pathol. 2002;39: 546–56.
216. Race B, Meade-White K, Race R, Chesebro B. Prion infectivity in fat of deer with chronic wasting disease. J Virol. 2009;83: 9608–10.
217. Denkers ND, Telling GC, Hoover EA. Minor oral lesions facilitate transmission of chronic wasting disease. J Virol. 2011;85:1396–9.
218. Williams ES. Chronic wasting disease. Vet Pathol. 2005;42: 530–49.
219. Saunders SE, Bartz JC, Bartelt-Hunt SL. Influence of prion strain on prion protein adsorption to soil in a competitive matrix. Environ Sci Technol. 2009;43:5242–8.
220. Saunders SE, Bartz JC, Vercauteren KC, Bartelt-Hunt SL. Enzymatic digestion of chronic wasting disease prions bound to soil. Prion. 2010;4:209.
221. Saunders SE, Bartz JC, Vercauteren KC, Bartelt-Hunt SL. An enzymatic treatment of soil-bound prions effectively inhibits replication. Appl Environ Microbiol. 2011;77:4313–7.
222. Saunders SE, Shikiya RA, Langenfeld K, Bartelt-Hunt SL, Bartz JC. Replication efficiency of soil-bound prions varies with soil type. J Virol. 2011;85:5476–82.
223. Saunders SE, Yuan Q, Bartz JC, Bartelt-Hunt S. Effects of solution chemistry and aging time on prion protein adsorption and replication of soil-bound prions. PLoS One. 2011;6:e18752.
224. Schramm PT, Johnson CJ, Mathews NE, McKenzie D, Aiken JM, Pedersen JA. Potential role of soil in the transmission of prion disease. Med Miner Geochem. 2006;64:135–52.
225. Smith CB, Booth CJ, Pedersen JA. Fate of prions in soil: a review. J Environ Qual. 2011;40:449–61.
226. Denkers ND, Seelig DM, Telling GC, Hoover EA. Aerosol and nasal transmission of chronic wasting disease in cervidized mice. J Gen Virol. 2010;91:1651–8.
227. Kincaid AE, Bartz JC. The nasal cavity is a route for prion infection in hamsters. J Virol. 2007;81:4482–91.
228. Mathiason CK, Hays SA, Powers J, et al. Infectious prions in preclinical deer and transmission of chronic wasting disease solely by environmental exposure. PLoS One. 2009;4:e5916.
229. Belay ED, Gambetti P, Schonberger LB, et al. Creutzfeldt-Jakob disease in unusually young patients who consumed venison. Arch Neurol. 2001;58:1673–8.
230. Anderson CA, Bosque P, Filley CM, et al. Colorado surveillance program for chronic wasting disease transmission to humans: lessons from 2 highly suspicious but negative cases. Arch Neurol. 2007;64:439–41.
231. Mawhinney S, Pape WJ, Forster JE, Anderson CA, Bosque P, Miller MW. Human prion disease and relative risk associated with chronic wasting disease. Emerg Infect Dis. 2006;12:1527–35.
232. Centers for Disease Control and Prevention (CDC). Fatal degenerative neurologic illnesses in men who participated in wild game feasts – Wisconsin, 2002. Morb Mortal Wkly Rep. 2003;52:125–7.

233. Barria MA, Telling GC, Gambetti P, Mastrianni JA, Soto C. Generation of a new form of human PrPSc in vitro by interspecies transmission from cervid prions. J Biol Chem. 2011;286:7490–5.
234. Sandberg MK, Al-Doujaily H, Sigurdson CJ, et al. Chronic wasting disease prions are not transmissible to transgenic mice overexpressing human prion protein. J Gen Virol. 2010;91:2651–7.
235. Tamguney G, Giles K, Bouzamondo-Bernstein E, et al. Transmission of elk and deer prions to transgenic mice. J Virol. 2006;80:9104–14.
236. Race B, Meade-White KD, Miller MW, et al. Susceptibilities of nonhuman primates to chronic wasting disease. Emerg Infect Dis. 2009;15:1366–76.
237. Jennelle CS, Samuel MD, Nolden CA, et al. Surveillance for transmissible spongiform encephalopathy in scavengers of white-tailed deer carcasses in the chronic wasting disease area of Wisconsin. J Toxicol Environ Health A. 2009;72:1018–24.
238. Harrington RD, Baszler TV, O'Rourke KI, et al. A species barrier limits transmission of chronic wasting disease to mink (Mustela vison). J Gen Virol. 2008;89:1086–96.
239. Heisey DM, Mickelsen NA, Schneider JR, et al. Chronic wasting disease (CWD) susceptibility of several North American rodents that are sympatric with cervid CWD epidemics. J Virol. 2010;84:210–5.
240. Bartz JC, McKenzie DI, Bessen RA, Marsh RF, Aiken JM. Transmissible mink encephalopathy species barrier effect between ferret and mink – PrP gene and protein-analysis. J Gen Virol. 1994;75:2947–53.
241. Angers RC, Kang H-E, Napier D, et al. Prion strain mutation determined by prion protein conformational compatibility and primary structure. Science. 2010;328:1154–8.
242. Bessen RA, Marsh RF. Identification of 2 biologically distinct strains of transmissible mink encephalopathy in hamsters. J Gen Virol. 1992;73:329–34.
243. Nicot S, Baron T. Strain-specific barriers against bovine prions in hamsters. J Virol. 2011;85:1906–8.
244. Mastrianni JA. The genetics of prion diseases. Genet Med. 2010;12:187–95.
245. Kovacs GG, Puopolo M, Ladogana A, et al. Genetic prion disease: the EUROCJD experience. Hum Genet. 2005;118:166–74.
246. Brown P, Galvez S, Goldfarb LG, et al. Familial Creutzfeldt-Jakob disease in Chile is associated with the codon 200 mutation of the PRNP amyloid precursor gene on chromosome 20. J Neurol Sci. 1992;112:65–7.
247. Brown P, Goldfarb LG, Gibbs CJ, Gajdusek DC. The phenotypic-expression of different mutations in transmissible familial Creutzfeldt-Jakob disease. Eur J Epidemiol. 1991;7:469–76.
248. Galvez S, Cartier L, Monari M, Araya G. Familial Creutzfeldt-Jakob disease in Chile. J Neurol Sci. 1983;59:139–47.
249. Sanchez-Valle R, Nos C, Yague J, Graus F, Dominguez A, Saiz A. Clinical and genetic features of human prion diseases in Catalonia: 1993–2002. Eur J Neurol. 2004;11:649–55.
250. Lee HS, Sambuughin N, Cervenakova L, et al. Ancestral origins and worldwide distribution of the PRNP 200K mutation causing familial Creutzfeldt-Jakob disease. Am J Hum Genet. 1999;64:1063–70.
251. Goldfarb LG, Brown P, Haltia M, et al. Creutzfeldt-Jakob disease cosegregates with the codon-178ASN PRNP mutation in families of European origin. Ann Neurol. 1992;31:274–81.
252. Goldfarb LG, Brown P, Cervenakova L, Gajdusek DC. Molecular-genetic studies of Creutzfeldt-Jakob-disease. Mol Neurobiol. 1994;8:89–97.
253. Monari L, Chen SG, Brown P, et al. Fatal familial insomnia and familial Creutzfeldt-Jakob-disease – different prion proteins determined by a DNA polymorphism. Proc Natl Acad Sci U S A. 1994;91:2839–42.
254. Dohura K, Tateishi J, Sasaki H, Kitamoto T, Sakaki Y. PRO- LEU change at position-102 of prion protein is the most common but not the sole mutation related to Gerstmann-Straussler syndrome. Biochem Biophys Res Commun. 1989;163:974–9.
255. Tranchant C, Dohura K, Steinmetz G, et al. Mutation of codon 117 of the prion gene in a family of Gerstmann-Straussler-Scheinker disease. Rev Neurol (Paris). 1991;147:274–8.
256. Ghetti B, Dlouhy SR, Giaccone G, et al. Gerstmann-Straussler-Scheinker disease and the Indiana kindred. Brain Pathol. 1995;5:61–75.
257. Giaccone G, Verga L, Bugiani O, et al. Prion protein preamyloid and amyloid deposits in Gerstmann-Straussler-Scheinker disease, Indiana Kindred. Proc Natl Acad Sci U S A. 1992;89:9349–53.
258. Piccardo P, Seiler C, Dlouhy SR, et al. Proteinase-K-resistant prion protein isoforms in Gerstmann-Straussler-Scheinker disease (Indiana kindred). J Neuropathol Exp Neurol. 1996;55:1157–63.
259. Tagliavini F, Prelli F, Porro M, et al. Amyloid fibrils in Gerstmann-Straussler-Scheinker disease (Indiana and Swedish Kindreds) express only prp peptides encoded by the mutant allele. Cell. 1994;79:695–703.
260. Unverzagt FW, Farlow MR, Norton J, Dlouhy SR, Young K, Ghetti B. Neuropsychological function in patients with Gerstmann-Straussler-Scheinker disease from the Indiana kindred (F198S). J Int Neuropsychol Soc. 1997;3:169–78.
261. Dlouhy S, Farlow M, Ghetti B, et al. Preliminary linkage analysis of Gerstmann-Straussler-Scheinker (GSS) disease in the Indiana Kindred. Am J Hum Genet. 1991;49:339.
262. Dlouhy SR, Hsiao K, Farlow MR, et al. Linkage of the Indiana kindred of gerstmann-straussler-Scheinker disease to the prion protein gene. Nat Genet. 1992;1:64–7.
263. Ghetti B, Farlow MR, Frangione B, Giaccone G, Tagliavini F, Bugiani O. Natural-history of Gerstmann-Straussler-Scheinker disease (GSS) in the Indiana Kindred (IK). J Neuropathol Exp Neurol. 1991;50:317.
264. Lugaresi E, Medori R, Montagna P, et al. Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. N Engl J Med. 1986;315:997–1003.
265. Lugaresi E, Medori R, Montagna P, et al. Fatal familial insomnia and dysautonomia – reply. N Engl J Med. 1987;316:1027–8.
266. Medori R, Tritschler HJ, Leblanc A, et al. Fatal familial insomnia, a prion disease with a mutation at codon-178 of the prion protein gene. N Engl J Med. 1992;326:444–9.
267. Gallassi R, Morreale A, Montagna P, Gambetti P, Lugaresi E. Fatal familial insomnia – neuropsychological study of a disease with thalamic degeneration. Cortex. 1992;28:175–87.
268. Manetto V, Medori R, Cortelli P, et al. Fatal familial insomnia – clinical and pathological-study of 5 new cases. Neurology. 1992;42:312–9.
269. Reder AT, Mednick AS, Brown P, et al. Clinical and genetic-studies of fatal familial insomnia. Neurology. 1995;45:1068–75.
270. Sforza E, Montagna P, Tinuper P, et al. Sleep-wake cycle abnormalities in fatal familial insomnia – evidence of the role of the thalamus in sleep regulation. Electroencephalogr Clin Neurophysiol. 1995;94:398–405.
271. Gallassi R, Morreale A, Montagna P, et al. Fatal familial insomnia: behavioral and cognitive features. Neurology. 1996;46:935–9.
272. Krasnianski A, Bartl M, Sanchez Juan PJ, et al. Fatal familial insomnia: clinical features and early identification. Ann Neurol. 2008;63:658–61.
273. Montagna P, Gambetti P, Cortelli P, Lugaresi E. Familial and sporadic fatal insomnia. Lancet Neurol. 2003;2:167–76.
274. Capellari S, Parchi P, Cortelli P, et al. Sporadic fatal insomnia in a fatal familial insomnia pedigree. Neurology. 2008;70:884–5.
275. Groschup MH, Lacroux C, Buschmann A, et al. Classic scrapie in sheep with the ARR/ARR prion genotype in Germany and France. Emerg Infect Dis. 2007;13:1201–7.

276. Fediaevsky A, Tongue SC, Noremark M, Calavas D, Ru G, Hopp P. A descriptive study of the prevalence of atypical and classical scrapie in sheep in 20 European countries. BMC Vet Res. 2008;4:19.
277. Sirakov I, Peshev R, Christova L. Genetic predisposition of some Bulgarian sheep breeds to the scrapie disease. Virus Genes. 2011;43:153–9.
278. Sawalha RM, Villanueva B, Brotherstone S, Rogers PL, Lewis RM. Prediction of prion protein genotype and association of this genotype with lamb performance traits of Suffolk sheep. J Anim Sci. 2010;88:428–34.
279. Hamir AN, Kunkle RA, Richt JA, Greenlee JJ, Miller JM. Serial passage of sheep scrapie inoculum in Suffolk sheep. Vet Pathol. 2009;46:39–44.
280. Benestad SL, Sarradin P, Thu B, Schonheit J, Tranulis MA, Bratberg B. Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. Vet Rec. 2003;153:202–8.
281. Benestad SL, Arsac J-N, Goldmann W, Noremark M. Atypical/Nor98 scrapie: properties of the agent, genetics, and epidemiology. Vet Res. 2008;39:19.
282. De Bosschere H, Roels S, Benestad SL, Vanopdenbosch E. Scrapie case similar to Nor98 diagnosed in Belgium via active surveillance. Vet Rec. 2004;155:707–8.
283. Gavier-Widen D, Noremark M, Benestad S, et al. Recognition of the Nor98 variant of scrapie in the Swedish sheep population. J Vet Diagn Invest. 2004;16:562–7.
284. Onnasch H, Gunn HM, Bradshaw BJ, Benestad SL, Bassett HF. Two Irish cases of scrapie resembling Nor98. Vet Rec. 2004;155:636–7.
285. Arsac J-N, Andreoletti O, Bilheude J-M, Lacroux C, Benestad SL, Baron T. Similar biochemical signatures and prion protein genotypes in atypical scrapie and Nor98 cases, France and Norway. Emerg Infect Dis. 2007;13:58–65.
286. Bruce ME, Nonno R, Foster J, et al. Nor98-like sheep scrapie in the United Kingdom in 1989. Vet Rec. 2007;160:665–6.
287. Seuberlich T, Botteron C, Benestad SL, et al. Atypical scrapie in a Swiss goat and implications for transmissible spongiform encephalopathy surveillance. J Vet Diagn Invest. 2007;19:2–8.
288. Buschmann A, Biacabe AG, Ziegler U, et al. Atypical scrapie cases in Germany and France are identified by discrepant reaction patterns in BSE rapid tests. J Virol Methods. 2004;117:27–36.
289. Gretzschel A, Buschmann A, Langeveld J, Groschup MH. Immunological characterization of abnormal prion protein from atypical scrapie cases in sheep using a panel of monoclonal antibodies. J Gen Virol. 2006;87:3715–22.
290. Moum T, Olsaker I, Hopp P, et al. Polymorphisms at codons 141 and 154 in the ovine prion protein gene are associated with scrapie Nor98 cases. J Gen Virol. 2005;86:231–5.
291. Saunders GC, Cawthraw S, Mountjoy SJ, Hope J, Windl O. PrP genotypes of atypical scrapie cases in Great Britain. J Gen Virol. 2006;87:3141–9.
292. Caspari DK, Balkema-Buschmann A, Brandt HR, Groschup MH, Erhardt G, Luehken G. Polymorphisms in genes CTSB, CTSD, CAPN2, KLK1 and TGFB1 not associated with susceptibility to atypical or classical ovine scrapie. Arch Tierz. 2010;53:457–64.
293. Luehken G, Buschmann A, Brandt H, Eiden M, Groschup MH, Erhardt G. Epidemiological and genetical differences between classical and atypical scrapie cases. Vet Res. 2007;38:65–80.
294. Fediaevsky A, Morignat E, Ducrot C, Calavas D. A case-control study on the origin of atypical scrapie in sheep, France. Emerg Infect Dis. 2009;15:710–8.
295. Fediaevsky A, Gasqui P, Calavas D, Ducrot C. Discrepant epidemiological patterns between classical and atypical scrapie in sheep flocks under French TSE control measures. Vet J. 2010;185:338–40.
296. Fediaevsky A, Maurella C, Noremark M, et al. The prevalence of atypical scrapie in sheep from positive flocks is not higher than in the general sheep population in 11 European countries. BMC Vet Res. 2010;6:9.
297. Arsac J-N, Betemps D, Morignat E, et al. Transmissibility of atypical scrapie in ovine transgenic mice: major effects of host prion protein expression and donor prion genotype. PLoS One. 2009;4:e7300.
298. Griffiths PC, Plater JM, Jayasena D, et al. Transmission of TSEs to transgenic mice overexpressing ovine PrP(ARR). Prion. 2010;4:152.
299. Andreoletti O, Orge L, Benestad SL, et al. Atypical/Nor98 scrapie infectivity in sheep peripheral tissues. PLoS Pathog. 2011;7:e1001285.
300. Safar JG, Prusiner SB. New generation of prion tests: Conformation-dependent immunoassay (CDI) (Abstract). Am Chem Soc. 2003;226:U102–3.
301. Safar JG, Geschwind MD, Deering C, et al. Diagnosis of human prion disease. Proc Natl Acad Sci U S A. 2005;102:3501–6.
302. Castilla J, Morales R, Saa P, Barria M, Gambetti P, Soto C. Cell-free propagation of prion strains. EMBO J. 2008;27:2557–66.
303. Orru CD, Caughey B. Prion seeded conversion and amplification assays. In: Tatzelt J, editor. Prion proteins. Berlin, Germany: Springer; 2011. p. 121–33.
304. Atarashi R, Moore RA, Sim VL, et al. Ultrasensitive detection of scrapie prion protein using seeded conversion of recombinant prion protein. Nat Methods. 2007;4:645–50.
305. Morales R, Duran-Aniotz C, Diaz-Espinoza R, Camacho MV, Soto C. Protein misfolding cyclic amplification of infectious prions. Nat Protoc. 2012;7:1397–409.
306. Castilla J, Saa P, Morales R, Abid K, Maundrell K, Soto C. Protein misfolding cyclic amplification for diagnosis and prion propagation studies. In: Kheterpal IWR, editor. Amyloid, prions, and other protein aggregates, Pt B. San Diego, California: Academic Press; 2006. p. 3–21.
307. Saa P, Castilla J, Soto C. Ultra-efficient replication of infectious prions by automated protein misfolding cyclic amplification. J Biol Chem. 2006;281:35245–52.
308. Soto C, Saborio GP, Anderes L. Cyclic amplification of protein misfolding: application to prion-related disorders and beyond. Trends Neurosci. 2002;25:390–4.
309. Cramm M, Schmitz M, Zerr I. Comparison of two diagnostic approaches-detection of PrPSc via quaking-induced conversion and 14-3-3 in cerebrospinal fluid of Creutzfeldt-Jakob disease patients. Prion. 2012;6:93–4.

Suggested Reading

Collinge J, Palmer MS. Prion diseases. Oxford/New York: Oxford University Press; 1997.
Prusiner SB. Prion biology and diseases. 2nd ed. New York: Cold Spring Harbor Laboratory Press; 2003.
Soto C. Prions: the new biology of proteins. New York: CRC Press, Taylor & Francis Group; 2006.