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Genetic Factors in Mammalian Prion Diseases

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Abstract

Mammalian prion diseases are a group of neurodegenerative conditions caused by infection of the central nervous system with proteinaceous agents called prions, including sporadic, variant, and iatrogenic Creutzfeldt-Jakob disease; kuru; inherited prion disease; sheep scrapie; bovine spongiform encephalopathy; and chronic wasting disease. Prions are composed of misfolded and multimeric forms of the normal cellular prion protein (PrP). Prion diseases require host expression of the prion protein gene (*PRNP*) and a range of other cellular functions to support their propagation and toxicity. Inherited forms of prion disease are caused by mutation of *PRNP*, whereas acquired and sporadically occurring mammalian prion diseases are controlled by powerful genetic risk and modifying factors. Whereas some PrP amino acid variants cause the disease, others confer protection, dramatically altered incubation times, or changes in the clinical phenotype. Multiple mechanisms, including interference with homotypic protein interactions and the selection of the permissible prion strains in a host, play a role. Several non-*PRNP* factors have now been uncovered that provide insights into pathways of disease susceptibility or neurotoxicity.

INTRODUCTION AND OVERVIEW

Mammalian prions are proteinaceous infectious agents that cause fatal neurodegenerative conditions. Prions share remarkably similar biological phenomena with infectious organisms despite the absence of nucleic acids as essential components. For example, analogous to strains of influenza, prion strains with distinct clinical and neuropathological features may be isolated and serially propagated in mammalian species. Griffith (71) originally proposed three potential mechanisms by which proteins might self-replicate and cause sheep scrapie (the protein-only hypothesis), and the purification of abnormal disease-causing forms of mammalian prion protein (PrP) (the prion concept) was subsequently achieved by Prusiner (162). The protein-only hypothesis and the prion concept are now accepted as correct for conditions associated with abnormal PrP. Some researchers have argued that it is difficult to accommodate strains of agent within a protein-only mechanism, but it is now widely agreed that these strains are likely to be encoded by diversity in the structure of prions. Since the original proposals by Griffith and Prusiner, other transmissible phenotypes have been associated with the prion mechanism, particularly in fungi (213). Researchers have highlighted similarities between prion diseases and many common neurodegenerative disorders of aging associated with abnormal protein propagation and aggregation, which are commonly referred to as prion-like in their disease mechanisms (44, 92).

All mammalian prion diseases are associated with the accumulation of abnormal isoforms of PrP, a host-encoded cellular glycoprotein. Disease-related isoforms, which when protease resistant are termed PrP^{Sc}, are derived from the host-encoded precursor, PrP^{C}, by a posttranslational process that involves conformational change and aggregation. PrP^{C} is largely α-helical in structure while PrP^{Sc} is composed predominantly of β-sheets. Recent cryo-electron microscopy imaging of prions suggests they comprise rods, 20 nm wide, that contain two fibers of PrP, each with a double helical repeating substructure, separated by a central gap 8–10 nm wide that contains heterogeneous material (193).

Molecular genetic approaches are fundamental to understanding the etiology of prion diseases that may occur sporadically, be acquired by dietary or iatrogenic exposure to prions, or be inherited as germline disorders. The discovery that human prion protein gene (*PRNP*) mutations were causal of transmissible diseases was a landmark in medicine, providing key evidence in support of the prion hypothesis (82, 152), and the advent of neurodegenerative disease diagnosis by gene test (46). The clinical diversity of inherited prion disease (IPD) continues to expand and includes nonneurological presentations (123). Similarly, recognition of the central importance of *PRNP* variation in disease susceptibility, in phenotypic modification, and in the selection of strains are key observations.

Human prion disease clinical syndromes are classified as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler syndrome (GSS), fatal familial insomnia (FFI), or kuru. All these diseases share common histopathological features; the classical triad of spongiform vacuolation (affecting any part of the cerebral gray matter), astrocytic proliferation, and neuronal loss is accompanied by abnormal PrP deposition, sometimes including PrP-amyloid plaques (14). Kuru reached epidemic proportions among the Fore population in the Eastern Highlands of Papua New Guinea and was transmitted by ritual cannibalism (4). A new epidemic of human prion disease known as variant CJD (vCJD) appeared in the United Kingdom beginning in 1995 and is caused by the human transmission of epizootic bovine spongiform encephalopathy (BSE) (29, 41, 49, 79, 214). Human prion infection is associated with long, clinically silent and, in part, genetically determined incubation periods that may span over half a century (50), and while the numbers of recognized cases of vCJD have thankfully been relatively small, uncertainty remains about the number of subclinically infected individuals in the United Kingdom and other BSE-affected countries and the eventual

epidemic size of BSE-related human prion disease and the risks of its secondary transmission from such asymptomatic carriers via medical and surgical procedures (65).

Sheep scrapie, a naturally occurring prion disease of sheep and goats, has been recognized in Europe for several centuries (119). Scrapie was demonstrated to be transmissible between sheep by inoculation of brain homogenate in 1936 (53). The realization that kuru, and then CJD, resembled scrapie in its histopathological appearances led to the proposal that these diseases may also be transmissible (74), which was followed by transmissions of kuru in 1966, CJD in 1968, GSS in 1981 (to chimpanzee), and FFI in 1994 (to mouse) (48, 62, 64, 117). Recent evidence points to the potential for classical sheep scrapie as an epizootic (38).

In this article we review the role of genetic factors in susceptibility to, and modification of, prion disease in humans, laboratory animal models, and wild animals.

STRUCTURE AND FUNCTION OF THE MAMMALIAN PRION PROTEIN GENE

Gene Structure and Expression Studies

The prion protein gene (human, *PRNP*; mouse, *Prnp*) is found on chromosome 2 in mice and chromosome 20 in humans. The human gene comprises two exons (three in mouse) with the open reading frame, encoding the 253–amino acid human protein (PrP) entirely within the larger second exon (146). PrP undergoes posttranslational maturation, during which a signal peptide is cleaved from the N terminus and a C-terminal peptide is cleaved upon attachment of the protein to its glycosylinositolphosphatidyl anchor (**Figure 1**). The mature protein found on the cell surface has an unstructured N-terminal domain containing an octapeptide repeat region and a predominantly α-helical C-terminal domain with two N-glycosylation sites.

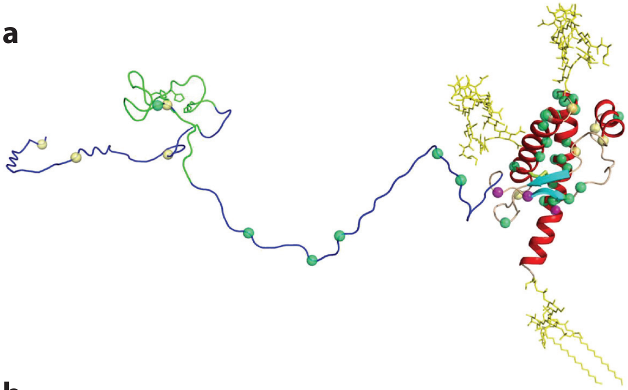
PrP Gene Family

The *PRNP* gene is one member of a small gene family that includes *SPRN* (shadoo, or shadow of prion protein), expressed mainly in brain, and *PRND* (doppel protein), expressed in testis. PrP is expressed during early embryogenesis and is found in most tissues in adults (115). The highest levels of expressed protein are found in the central nervous system (CNS), in particular in association with synaptic membranes (100, 187), but also microglia (27). PrP is also widely expressed in cells of the immune system: B and T cells, dendritic cells, macrophages, and other cells of the hematopoietic system (57). PrP has been identified in marsupials and birds, and the gene is likely to be present in all vertebrates. Like many genes, interspecies studies suggest that *PRNP* evolution is largely driven by the conservation of amino acids through negative selection (199), although in some specific circumstances disease-related evolutionary mechanisms are active (see the section titled Balancing Selection in Human Populations). The prion gene family is thought to have evolved from an ancestral gene coding for a member of the ZIP family of metal ion transporters (179).

Function of PrP

Studies of PrP function highlight some intriguing potential roles, but there is yet no consensus and there is no agreed unitary function. Importantly, *Prnp* knockout mice ($Prnp^{0/0}$) have no (or only relatively subtle) phenotypes and a normal fertility and life span. The most significant and consistent characteristic of PrP^{C} knockout mice is their complete resistance to prion disease when inoculated with prions, indicating that PrP expression is an obligate requirement for prion propagation and prion-induced neurotoxicity (30, 31). Constitutive knockout of PrP^{C} or knockout of

a



b

Human	1	--MANLGCWMLVLFVATWSDLGLCKKRPKPG-GWNTGGSRYPGQGSPGGNRYPPQ	52
Sheep	1	MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQ	55
Bovine	1	MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQ	55
Elk	1	MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQ	55
White-tailed deer	1	MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQ	55
Mule deer	1	MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQ	55
Mouse	1	--MANLGYWLLALFVTMWTDVGLCKKRPKPG-GWNTGGSRYPGQGSPGGNRYPPQ	52
Bank vole	1	--MANLSYWLLAFFVTTWTDVGLCKKRPKPG-GWNTGGSRYPGQGSPGGNRYPPQ	52

Human	53	GGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQG--------GGTHSQW	99
Sheep	56	GGGGWGQPHGGGWGQPHG--------GGWGQPHGGGWGQPHGGGGWGQGGSHSQW	102
Bovine	56	GGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGTHGQW	110
Elk	56	GGGGWGQPHGGGWGQPHG--------GGWGQPHGGGWGQPHGGGGWGQGGTHSQW	102
White-tailed deer	56	GGGGWGQPHGGGWGQPHG--------GGWGQPHGGGWGQPHGGGGWGQGGTHSQW	102
Mule deer	56	GGGGWGQPHGGGWGQPHG--------GGWGQPHGGGWGQPHGGGGWGQGGTHSQW	102
Mouse	53	G-GTWGQPHGGGWGQPHGGSWGQPHGGSWGQPHGGGWGQG--------GGTHNQW	98
Bank vole	53	GGGTWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQG--------GGTHNQW	99

Beta strand 1 | Alpha helix 1

Human	100	NKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHFGSDYEDRYYRENM	154
Sheep	103	NKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYYRENM	157
Bovine	111	NKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYYRENM	165
Elk	103	NKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYYRENM	157
White-tailed deer	103	NKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYYRENM	157
Mule deer	103	NKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMNRPLIHFGNDYEDRYYRENM	157
Mouse	99	NKPSKPKTNLKHVAGAAAAGAVVGGLGGYMLGSAMSRPMIHFGNDWEDRYYRENM	153
Bank vole	100	NKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPMIHFGNDWEDRYYRENM	154

Beta strand 2 | Alpha helix 2 | Alpha helix 3

Human	155	HRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERV	209
Sheep	158	YRYPNQVYYRPVDRYSNQNNFVHDCVNITVKQHTVTTTTKGENFTETDIKIMERV	212
Bovine	166	HRYPNQVYYRPVDQYSNQNNFVHDCVNITVKEHTVTTTTKGENFTETDIKMMERV	220
Elk	158	YRYPNQVYYRPVDQYNNQNTFVHDCVNITVKQHTVTTTTKGENFTETDIKMMERV	212
White-tailed deer	158	YRYPNQVYYRPVDQYNNQNTFVHDCVNITVKQHTVTTTTKGENFTETDIKMMERV	212
Mule deer	158	YRYPNQVYYRPVDQYNNQNTFVHDCVNITVKQHTVTTTTKGENFTETDIKMMERV	212
Mouse	154	YRYPNQVYYRPVDQYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERV	208
Bank vole	155	NRYPNQVYYRPVDQYNNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERV	209

Human	210	VEQMCITQYERESQAYYQR--GSSMVLFSSPPVILLISFLIFLIVG	253
Sheep	213	VEQMCITQYQRESQAYYQR--GASVILFSSPPVILLISFLIFLIVG	256
Bovine	221	VEQMCITQYQRESQAYYQR--GASVILFSSPPVILLISFLIFLIVG	264
Elk	213	VEQMCITQYQRESEAYYQR--GASVILFSSPPVILLISFLIFLIVG	256
White-tailed deer	213	VEQMCITQYQRESQAYYQR--GASVILFSSPPVILLISFLIFLIVG	256
Mule deer	213	VEQMCITQYQRESQAYYQR--GASVILFSSPPVILLISFLIFLIVG	256
Mouse	209	VEQMCVTQYQKESQAYYDGRRSSSTVLFSSPPVILLISFLIFLIVG	254
Bank vole	210	VEQMCVTQYQKESQAYYEGRSSRAVLLFSSPPVILLISF-------	248

(Caption appears on following page)

Figure 1 (*Figure appears on preceding page*)

(*a*) Prion protein (PrP) and prion protein gene (*PRNP*) structure. A model structure of human PrP (81), including the unstructured region (*purple*), the octapeptide repeat region (*green*), α-helix (*red ribbon*), β-strand (*light blue*), glycans, and glycosylphosphatidylinositol anchor (*yellow*), is shown. Spheres show the location of amino acid polymorphisms, with common benign missense changes (*yellow*), definite or probable mutations (*green*), and protective factors (*purple*). Alleles of each category are widely distributed across structural features of the protein. (*b*) Alignment of eight species of PrP, with structural features (β-strands, *light blue*; α-helices, *red*) and species-specific disease-modifying polymorphisms highlighted (*light purple*).

neuronal PrP expression in adult mouse brain does not cause neurodegeneration and indeed has no overt phenotypic effect that influences life span or fertility (113). These findings demonstrate that acute loss of PrP^C in neurons in adulthood is well tolerated and that loss of PrP^C function is not a sufficient cause of prion neurodegeneration.

Several abnormalities in *Prnp*$^{0/0}$ mice may highlight PrP functions; examples include electrophysiological abnormalities (51), susceptibility to seizures (208), abnormality of the sleep–wake cycle (196), demyelinating peripheral neuropathy (25), abnormal olfactory physiology (104), and protection from acute brain injury (121). Expression of N-terminal PrP deletion constructs and CNS expression of the homologous Doppel protein encoded by another *PRNP* gene family member result in Purkinje cell loss and cerebellar ataxia. These outcomes can be rescued by overexpression of wild-type PrP, suggesting that the normal function of PrP may involve a hypothetical ligand (termed π) that interacts with PrP to provide neurotrophic support (59, 182). π has not been identified; therefore, such a role for PrP remains hypothetical at present. Recently, the flexible tail of PrP has been proposed to act through a G protein–coupled receptor, Gpr126, to regulate myelin homeostasis (101).

Newly synthesized PrP^C is transported to the cell surface and is recycled via either clathrin-coated vesicles or caveolae-dependent endocytic pathways. A range of PrP^C-interacting molecules have been identified, the most consistent being NCAM1 and the 37-kDa/67-kDa laminin receptor; however, the in vivo relevance of such interactions has yet to be convincingly demonstrated (63, 177). PrP^C exhibits a high-affinity interaction with oligomeric assemblies of amyloid-beta protein, which may be important in the neurotoxic mechanisms of Alzheimer disease (61, 103, 201).

INHERITED PRION DISEASES

Mutation of *PRNP* was first discovered in 1989 with segregation in pedigrees showing autosomal dominant inheritance of human prion disease (82, 152). Now over 60 variants of *PRNP* are known and approximately 10–15% of the annual incidence of prion disease is caused by mutation of *PRNP* (**Figure 2**). Inherited prion disease, rather than eponymous syndromes, is a preferred term for all diseases caused by mutation of *PRNP* because the clinical phenotype is variable both in and between families. The proportion of prion disease caused by mutation varies widely among countries because certain mutations are particularly prevalent in specific ethnic groups and regions, presumably due to founder effects. For example, the E200K mutation is particularly prevalent in Slovakia, where it is responsible for more than 65% of the annual incidence of prion disease and in one study was found to be carried by between 0.2% and 0.6% of the populations examined (136).

Core Clinical Phenotypes of CJD, FFI, GSS, OPRI, and PrP Systemic Amyloidosis

The inherited forms of human prion disease can present in a way similar to the rapidly progressive dementia, myoclonus, and ataxia typical of sporadic CJD or indeed any other neurodegenerative

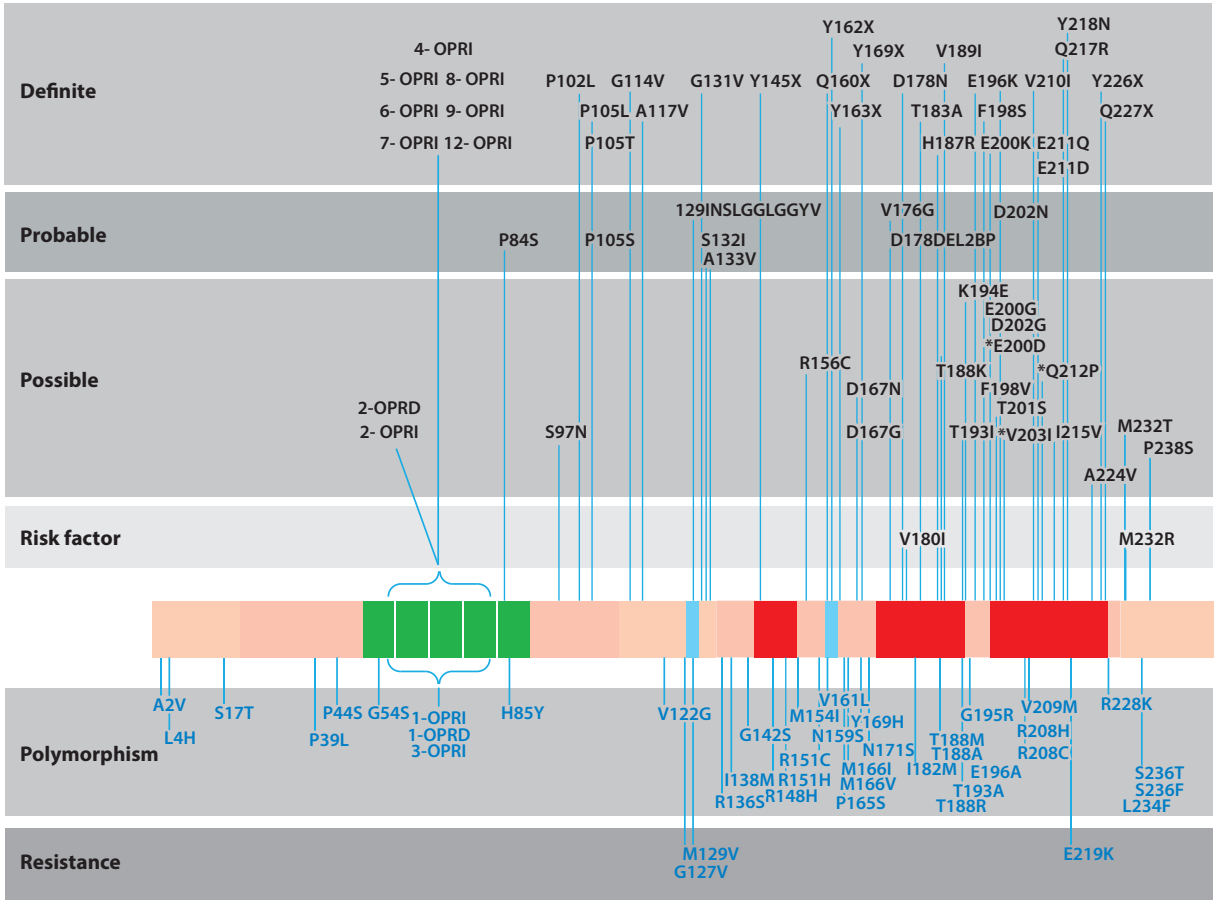


Figure 2

Mutations and polymorphisms of *PRNP*. Sixty-two missense, structural, and termination mutations of *PRNP* are shown together with numerous variants found in healthy populations. The colored bar represents structural features of the protein, including C- and N-signal peptides (*peach*), octapeptide repeat region (*green*), β-strand (*light blue*), and α-helix (*red*). Variants are classified above and below the bar as definite (occurring in multigenerational pedigrees with pathological confirmation), probable (at least one case showing typical pathology associated with IPD at a young age, or at least an atypical PrP pattern for sporadic disease), possible (occurrence in clinically/pathologically diagnosed sporadic CJD with a negative family history), risk factor (occurring in clinically/pathologically diagnosed sporadic CJD and in controls, but statistically significant excess in cases), polymorphism (occurring at similar frequency in cases and controls), or resistance factor (showing statistically significant protective or modifying effect in acquired or sporadic disease). The asterisk denotes homozygous PrP observed in patients. Abbreviations: CJD, Creutzfeldt-Jakob disease; IPD, inherited prion disease; *PRNP*, prion protein gene; PrP, prion protein.

disease syndrome (125). Mistaken diagnoses including Alzheimer disease, frontotemporal dementia variants, multiple system atrophy, corticobasal syndrome, and stroke are well recognized. There are, however, some characteristic features. GSS typically presents with a slowly progressive cerebellar ataxia associated with distal pain and loss of sensation, absent ankle reflexes, and mild abnormalities of frontal lobe function including changes in personality, planning, judgement, and emotional regulation, with a mean disease duration of 4 years (range 0.5–12 years) (210). The progressive loss of thermal sensation, which can be objectively assessed by neurophysiology, associates with abnormal PrP in the dorsal horn of the lumbar spinal cord and typically predates

significant disabilities (173). Less frequently, GSS has a psychiatric presentation often associated with evidence of frontal and parietal lobe dysfunctions. FFI presents with fragmented sleep, a gait disturbance characterized by slow, stiff movements and abnormal postures, and autonomic overactivity and instability; typical disease duration is 1–2 years (99). Octapeptide repeat insertion (OPRI)-related IPD can be indistinguishable from CJD, but larger repeat insertions (five or more extra repeats) typically manifest as a slowly progressive frontal and parietal lobe cognitive syndrome with apraxia and later-onset ataxia. The recently described PrP systemic amyloidosis presents with autonomic and sensory neuropathy, with diarrhea being particularly prominent; disease duration is approximately 30 years (123). Pathological features are illustrated in **Figure 3**.

Genotype–Phenotype Correlations

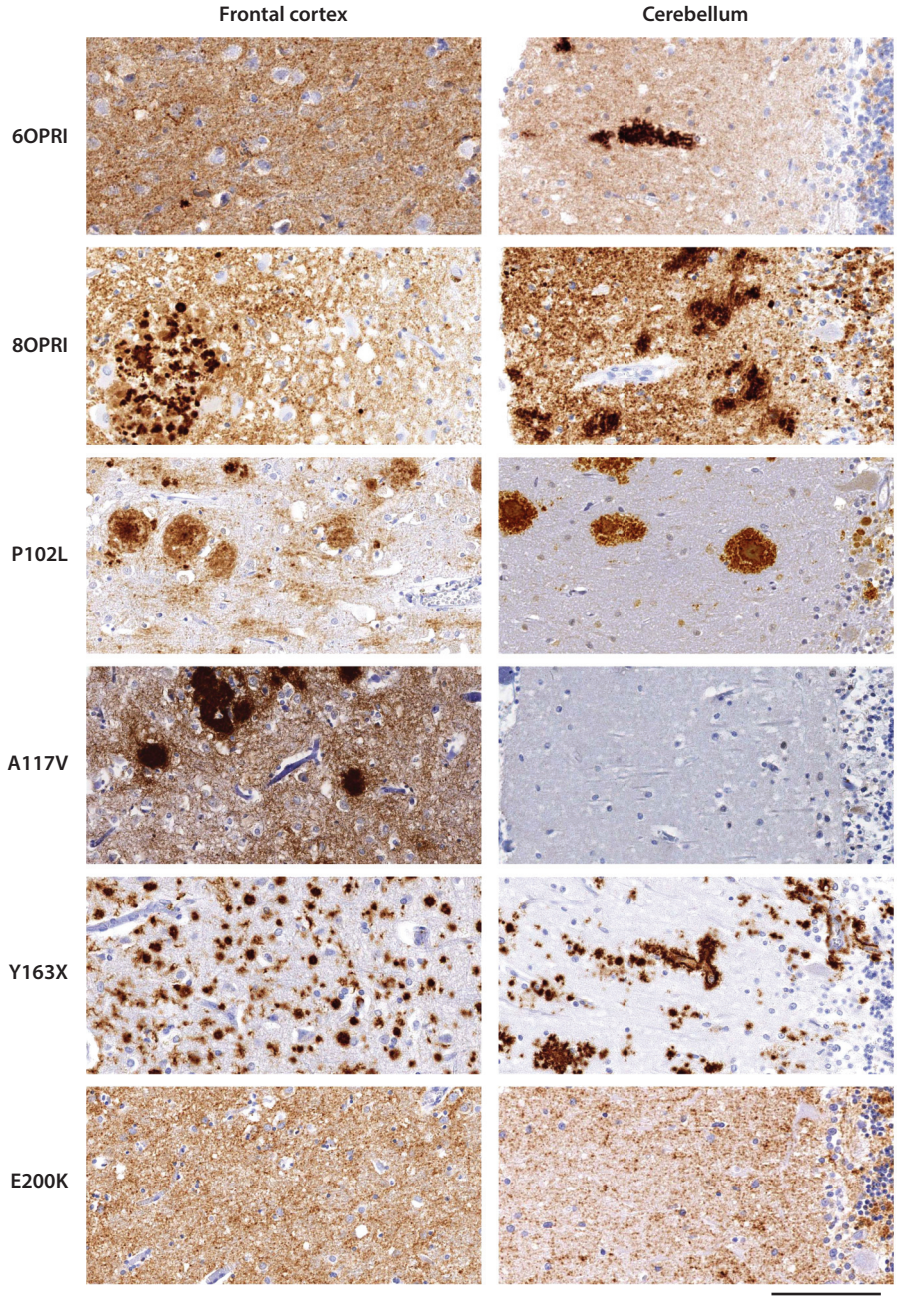
PRNP variants that cause IPD may be divided into several types: missense mutations that alter an amino acid; alterations to the number of octapeptide repeats in the N-terminal region of the gene; premature termination mutations leading to a truncated, anchorless PrP; and rare in-frame or frameshift insertions outside the repeat region (**Figure 2**). Genotype–phenotype correlations can be made, but these are imperfect and considerable intrafamilial diversity is to be expected, some of which may relate to the propagation of alternative prion strains (207).

A spectrum of severity of effect, measured by mean age of clinical onset and penetrance, is well established for *PRNP*. Some *PRNP* mutations are associated with fully penetrant conditions with typical onset in early adulthood, including large OPRI mutations, A117V, and G114V, among others (125). Many variants associated with familial CJD or FFI with onset in late middle-old age are not fully penetrant before other causes of death become common. Family history may therefore be negative, and diagnoses may be missed unless *PRNP* analysis is routinely offered in apparently sporadic cases. Other variants, for example, V180I and M232R, are uncommon population polymorphisms in the Japanese and other East Asian populations and enriched in studies of sporadic CJD in these populations (15, 145, 164). These variants might be better classified as risk factors rather than IPDs because their penetrance appears to be low even in old age (133).

Large-scale human sequencing studies have also revealed rare adult carriers of heterozygous loss-of-function mutations (133), which suggests that at least for truncation mutations in or near the N-terminal (residues 1–144) there is no highly penetrant early-onset clinical syndrome related to loss of function of *PRNP*. Several variants have been observed on only a small number of occasions in apparently sporadic CJD, and it remains unclear whether these are simply chance findings of rare genetic variants, risk factors, or partially penetrant mutations (**Figure 2**). Large-scale sequencing studies of multiple populations have helped clarify this issue by revealing that several *PRNP* variants previously seen in only sporadic CJD are in fact uncommon but benign population polymorphisms, an issue of importance for genetic counseling (18, 133).

The youngest patient with IPD presented at age 13 with P105T IPD (170), and the oldest died at age 94 with 4-OPRI IPD (94). The more commonly occurring mutations have liability curves that show a wide range of age at clinical onset (**Figure 4**) (134). For 4-OPRI, 5-OPRI, 6-OPRI, A117V, and F198S mutations there appear to be strong modifying effects of polymorphic codon 129 either in *cis* or in *trans*, with individuals heterozygous for methionine and valine at this position showing a delayed age of clinical onset (66, 94, 125, 129, 161, 210, 211). Reports of genetic anticipation (the earlier presentation of disease in subsequent generations) have been contested on the basis of potentially biased ascertainment of children with clinical onset close to parental onset in calendar year (135, 158, 171).

Several IPD mutations have been modeled in transgenic mice on a mouse or human PrP background and with varying expression levels (7, 8, 39, 40, 86, 87, 116, 189). Broadly, this work has shown that IPD mutations can lead to spontaneous neurodegenerative diseases associated with abnormal forms of PrP, the generation of prions, and, to some extent, phenotypes comparable to those of the relevant human disease.



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Typical histopathological appearance of prion protein (PrP) deposits in various inherited prion diseases. The octapeptide repeat inserts (OPRIs) show a characteristic cerebellar striping pattern that is perpendicular to the cerebellar pial surface, while the neocortex shows mostly a synaptic pattern, with occasional granular or miniplaques. The patterns associated with the P102L mutation show a conspicuous deposition of large plaques in the frontal cortex and cerebellum. Brains with the A117V mutation can show a heavy PrP load in the cortex, with formation of plaques, but usually have only little cerebellar pathology. The truncation mutation Y163X causes a widespread prion amyloid deposition, as shown here in the neocortex and cerebellum, notably with prion amyloid angiopathy (shown here in the cerebellum) (123). The E200K mutation is histologically indistinguishable from some forms of sporadic Creutzfeldt-Jakob disease. All images show identical magnification (scale bar: 100 μm). Images of the neocortex and cerebellum are oriented with the surface to the left. Sections were stained with the antibody ICSM35 and provided by Sebastian Brandner, Division of Neuropathology, UCLH NHS Foundation Trust.

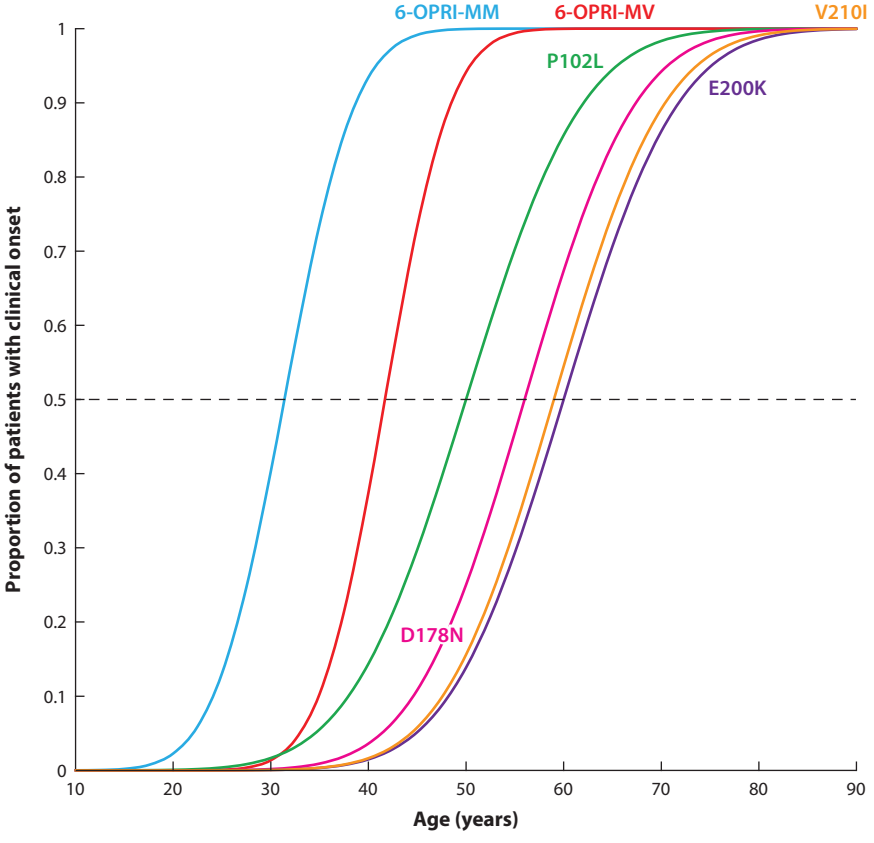


Figure 4

Liability curves of the more common IPDs. Modeled cumulative age at clinical onset of most frequently occurring IPDs are shown. Different IPD mutations can have markedly different ages at clinical onset (e.g., 6-OPRI versus E200K). In some cases onset can be strongly modified by the genotype at codon 129 (compare *red* and *light blue curves*). All IPDs are highly variable in their age at clinical onset, and the determinants of this are not well understood (data derived from the unpublished experience of the National Prion Clinic, UK; see Reference 134 for a global multicenter perspective). Abbreviation: IPD, inherited prion disease.

Predictive Genetic Testing

Predictive genetic testing at *PRNP* has been possible since the early 1990s. In the United Kingdom, where this has been offered for nearly 30 years, approximately 25% of eligible first-degree relatives have chosen to undergo testing (153). Some of the determinants of this decision include a recent diagnosis in a relative, a concern about nonspecific symptoms, changes in personal circumstances, and a wish to be closely involved in research projects. Prenatal genetic diagnosis for the 6-OPRI, F198S, and E200K mutations has also been done (200).

Individuals at risk of IPD based on a predictive genetic diagnosis or diagnosis in a blood relative may have prions in their tissues and body fluids. Few studies have examined when and where prions appear in the preclinical state; therefore, on the basis of a precautionary principle, at-risk individuals should not be blood or organ donors and should advise surgeons of their situation prior to invasive procedures. Precautions to be taken will vary between different organizations and procedures. Active research projects in the United Kingdom as part of the National Prion Monitoring Cohort study (195), as well as other studies in the United States and Europe, are working with these individuals to develop biomarkers that might predict disease onset. An increasing community of individuals who know they carry *PRNP* gene mutations are important advocates of work toward preventive human prion disease therapeutics and early diagnostics.

PRION PROTEIN GENE POLYMORPHISMS AND SUSCEPTIBILITY TO PRION DISEASE

Natural Hosts

Prion diseases occur in humans, in wild and farmed animals, and in laboratory settings by inoculation.

Sheep. Clinical signs of sheep scrapie have been recognized for centuries, and although there is no epidemiological evidence that it is transmissible to humans, occasional transmission cannot be excluded and the disease still continues to be of significance to farmers in terms of animal welfare and economics. Ovine PrP is highly polymorphic, with more than 40 alleles already described across a variety of breeds (67). While no evidence of genetic prion disease has been detected in sheep, the role of PrP variation in susceptibility to classical scrapie has long been established and is associated primarily with variation at three codons, A136V/T, R154H/L, and Q171R/H/K (13); however, not all of these variants are associated with scrapie. An accurate reflection of allele frequencies is difficult because these vary with breed and can quickly change within a flock due to selective breeding. Based on the three-codon haplotype, only five alleles are seen at significant frequency in the United Kingdom, ARQ (56%), VRQ (5%), AHQ (6%), ARR (30%), and ARH (3%), and combinations of these alleles produce 15 genotypes (54). On the basis of susceptibility to scrapie and potential genotype of offspring, researchers have placed these 15 genotypes into five risk groups, R1–R5, where R5 is the highest risk and R1 is the lowest (**Table 1**). The most susceptible allele is VRQ and this is reflected in its inclusion in R5 and R4. Homozygous VRQ/VRQ is the most susceptible genotype, with VRQ/ARQ and VRQ/ARH showing similar susceptibilities. VRQ/AHQ exhibits a significantly lower risk of infection, suggesting that the presence of the AHQ allele is partially protective; however, the VRQ/AHQ genotype is included in the R5 group because highly susceptible VRQ/VRQ offspring may be produced. Similarly, the ARR allele significantly reduces the risk of infection for VRQ/ARR sheep. All the genotypes in R3 are considered of average risk; however, as seen in R5, the presence of the AHQ allele gives a further threefold reduction in risk. R2 genotypes are considered quite resistant but their offspring could be R3. ARR/ARR sheep are considered resistant to classical scrapie (R1).

Table 1 Common PrP gene disease modifying polymorphisms

Species	Polymorphism	Comment
Natural hosts		
Sheep	A136V, R154H, Q171R,H	Classical scrapie
	VRQ/VRQ	R5: Highest risk of infection
	VRQ/ARQ	
	VRQ/ARH	
	VRQ/AHQ	
	VRQ/ARR	R4: High risk of infection
	ARQ/ARQ	R3: Average risk of infection
	ARQ/AHQ	
	ARQ/ARH	
	AHQ/AHQ	
	AHQ/ARH	
	ARH/ARH	
	ARR/ARQ	R2: Partially resistant
	ARR/ARH	
	AHQ/AHQ	
	ARR/ARR	R1: Fully resistant
Cattle	12-bp/23-bp del (promoter)	Higher BSE risk
Elk	M132L	L: Reduced CWD susceptibility
White-tailed deer	Q95H	H: Reduced CWD susceptibility
	G96S	S: Reduced CWD susceptibility
	A116G	G: Reduced CWD susceptibility
Mule deer	S225F	F: Reduced CWD susceptibility
Experimental models		
Mouse	*Prnp*^{a} 108L, 189T	Short incubation time
	Prnp^{b} 108F, 189V	Long incubation time
	Prnp^{c} 108F, 189T	Long incubation time
Bank vole	M109I	M: Shorter incubation time

PrP codon numbers are species specific. R1–R5 for sheep refer to risk groups for classical scrapie for the National Scrapie Plan of Great Britain. Abbreviations: BSE, bovine spongiform encephalopathy; CWD, chronic wasting disease; del, deletion.

Genotypes may behave differently in the presence of alternative prion strains, and this was confirmed with the identification of so-called atypical scrapie in 1998. Although atypical scrapie has been detected in only hundreds of sheep, compared with the many thousands affected by classical scrapie, it has been seen mostly in genotypes belonging to the R1–R3 groups (19). In particular, the genotypes AHQ/AHQ (R3), AHQ/ARQ (R3), and ARR/ARR (R1) are overrepresented in naturally occurring cases. In addition to codons 136, 154, and 171, susceptibility to atypical scrapie is also linked to codon 141, where $AF_{141}RQ$ is associated with a higher susceptibility than $AL_{141}RQ$ (6, 111, 142, 178).

The classification of classical scrapie risk genotypes is also of little value in predicting susceptibility to BSE, because in experimental transmissions to sheep the ARQ genotype was highly susceptible relative to VRQ and ARR (5, 68). The susceptibility of the ARQ genotype was also confirmed in transgenic mice expressing ovine PrP (ARQ) infected with various sources of BSE including the BSE-L strain (21, 52). As for atypical scrapie, codon 141 also influences

susceptibility to experimental BSE in ARQ sheep in which FF_{141} and LF_{141} had a longer incubation time than the LL_{141} genotype (192).

Goats. As in sheep, scrapie is naturally found in goats. At least 28 PrP amino acid polymorphisms have been described, some of which have been associated with susceptibility to prion disease (67, 202). Goats share the A136, R154H, and Q171 alleles with sheep, although A136 and Q171 are not polymorphic (1, 69, 155). As in sheep, the H_{154} allele is associated with a low risk of classical scrapie but a high risk of atypical scrapie (6, 19). Other alleles associated with scrapie resistance are H143R, N146S/D, R211Q, and Q222K.

PRNP sequencing of healthy Norwegian dairy goats identified a novel null variant created by a naturally occurring nonsense mutation at codon 32 (GGA/TGA; 32Stop) that is predicted to encode only seven amino acids of the mature protein (20). The 32Stop allele was found at a frequency of 11% in Norwegian dairy goats and represented the first naturally occurring *PRNP* null allele. Reduced levels of PrP were detected in heterozygous goats while none was detected in homozygous 32Stop animals. PrP knockout mice are completely resistant to prion infection; therefore, it is predicted that the 32Stop homozygous goats will also be resistant to all strains of prions (30).

Cattle. Unlike sheep, bovine PrP exhibits much less variation, with only eight single amino acid changes identified, along with an octapeptide repeat polymorphism resulting in either five or six repeats (67). The reduced variation compared with that in sheep may reflect the highly inbred nature of European herds. Despite the large number of cattle infected with BSE, no association between susceptibility to BSE and PrP protein polymorphisms has been found (83). This may be due to the inherent lack of genetic variation, the uniformity of the BSE strain, or its more permissive nature. However, a 12-bp deletion in the bovine *PRNP* promoter has been associated with BSE susceptibility and reported to increase risk of BSE in either the homozygous or the heterozygous state (73, 176). The highest risk of BSE is seen when the 12-bp deletion occurs together with an additional 23-bp promoter deletion on the same haplotype. Experimental models have shown that susceptibility is correlated with PrP expression level, so evidence that the promoter polymorphisms affect *PRNP* transcription suggests that genetic variation at the *PRNP* locus does play a role in cattle susceptibility to BSE (30, 163, 175, 216).

Although the origin of BSE is unresolved, the many differences between the BSE strain and known scrapie strains do not support a scrapie origin. However, the possibility cannot be excluded that the novel BSE strain arose from mutation of a scrapie strain (28). Alternative hypotheses include the possibility that it arose spontaneously in a cow that was subsequently recycled into the food chain (42). The discovery of rare alternative strains of BSE (L- and H-type) in asymptomatic older cattle sent for slaughter suggests that this may not be an unrealistic proposal (22, 37). All but one of the atypical BSE cases had no mutations in *PRNP* and in that respect are reminiscent of sporadic CJD (167). In the case of one cow with H-type BSE, a mutation in *PRNP* was discovered (E211K) (143, 167). This is of particular interest because the equivalent human codon carries a mutation (E200K) that is a common cause of inherited CJD. This raises the possibility of both sporadic and genetic prion disease in cattle, which could generate new prion strains in the future.

Cervids. Chronic wasting disease (CWD), the only naturally occurring prion disease recognized in wild animals, is seen in free-ranging and captive deer and elk across a wide geographical area of North America; was exported to South Korea; and has been recently identified in reindeer, moose, and deer in Norway, Sweden, and Finland (205). The horizontal spread and environmental persistence of CWD raise questions about the natural susceptibility of various species and their genetic

variants. Sixteen single amino acid polymorphisms in cervid PrP have been described and some of these have been associated with susceptibility to CWD (67, 169). In Rocky Mountain elk, codon 132 is polymorphic [major allele is methionine and minor allele (37%) is leucine] (147, 157). This codon is of particular interest because it is the cervid amino acid that corresponds to human codon 129. Observations in both wild and farmed CWD-infected animals suggest that the presence of L132 is protective (147, 157). This is supported by experimental CWD prion transmissions of various genotypes to elk and is further corroborated by CWD infection of transgenic mice expressing cervid PrP [Tg(Cer-PrP)mice] bearing the M132 or L132 variants. The Tg(Cer-PrP)MM_{132} mice were highly susceptible to CWD prions from all genotypes, but Tg(Cer-PrP)LL_{132} mice showed no clinical signs of disease (70, 75). The presence of L132 did not afford complete protection, as low levels of PrP^{Sc} were detected in the brains of Tg(Cer-PrP)LL_{132} mice, indicative of subclinical infection. This was subsequently confirmed by second passage into Tg(Cer-PrP)LL_{132} mice resulting in clinical disease. This finding raises the possibility that L132 elk may be healthy carriers of prions, furthering the risk of CWD spread through the environmental dissemination of prions. Furthermore, Tg(Cer-PrP)LL_{132} mice were susceptible to the scrapie strain SSBP/1, reinforcing the view that L132 is only partially protective and that this effect is prion strain specific.

In white-tailed deer, three single amino acid PrP polymorphisms have been associated with CWD susceptibility: Q95H, G96S, and A116G (67, 89, 95, 148, 169, 215). In all cases, the minor allele (95H, 2%; 96S, 26%; and 116G, 13%) was underrepresented in CWD-affected animals. For G96S these observations were confirmed by experimental transmissions of CWD prions to transgenic mice expressing cervid PrP with G96 or S96 (90, 132, 165). Tg(Cer-PrP)GG_{96} mice were susceptible to CWD prions regardless of the codon 96 genotype of the inocula, whereas Tg(Cer-PrP)SS_{96} mice were resistant to all CWD prions.

CWD susceptibility in mule deer is also associated with PrP variation at codon 225, where the most common amino acid is serine and the minor allele (5%) is phenylalanine. As seen for other cervid PrP polymorphisms, the minor allele (F225) is associated with a reduced risk of CWD and is underrepresented in affected animals (77, 88). Because F225 is present at such a low frequency and these data are derived from free-ranging animals, it is possible that these findings do not accurately represent the influence of codon 225 on susceptibility. Experimental transmissions of CWD prions to SS_{225} and SF_{225} deer showed that both genotypes were susceptible, though the accumulation of PrP^{Sc} and the appearance of spongiform change in the CNS were considerably delayed in SF_{225} animals (60). This longer incubation time raises the possibility of an extended period of infectivity dissemination into the environment, thus increasing the risk of infecting other animals.

Human. Strong risk factors for the most common human prion disease, sporadic CJD, are found in *PRNP*. Codon 129, either methionine and valine, is highly polymorphic in European, African, and American populations, with a diminished frequency of valine in East Asia, where it is rare (18, 127, 151). Increased risk of sporadic CJD is associated with both homozygous genotypes, whereas heterozygotes are relatively protected (154). Susceptibility therefore implicates homotypic protein interactions in homozygotes. However, more complex mechanisms also play a role, as codon 129 influences the range of prion strains that may be propagated by conformational selection (41, 45). The clinical phenotype is also influenced. More typical, rapidly progressive cases are seen in 129MM individuals, and atypical cases that might be mistaken for more common causes of dementia are seen in 129MV individuals, with implications for ascertainment (43, 159). In East and South Asian populations, the presence of lysine at codon 219 in the heterozygous state with glutamic acid is a powerful resistance factor (181). Intriguingly, the lysine allele has been found in two vCJD patients, implying again that risk factors in *PRNP* may be specific to particular prion strains (112).

Uncommon polymorphisms observed by the Japanese CJD surveillance system at positions 180 and 232 (in the C-terminal signal peptide) may also be viewed as risk factors for sporadic CJD (15, 145, 164). Several other variants are benign (e.g., a deletion polymorphism of octapeptide repeat region) or are of unclear pathogenicity (e.g., G142S and N171S found in African populations) (**Figure 2**).

Acquired prion diseases also show strong susceptibility at *PRNP*. Pathologically proven vCJD had been seen only in 129MM individuals until 2016 (126), although 129MV was identified in a subclinically infected individual (23), a suspected case in whom autopsy was not possible (93), and a single case recently studied postmortem (137). Kuru occurs in all three genotypes at codon 129; however, the incubation time differs, and in heterozygous individuals this can be remarkably long (50). A G127V polymorphism localized to the center of the kuru-affected region in the Eastern Highlands of Papua New Guinea seems to confer complete resistance to kuru (131). Indeed, this variant appears to have been increased in frequency through Darwinian evolution because of the selection pressure imposed by the kuru epidemic (9). In iatrogenic CJD caused by the use of cadaver-derived pituitary growth hormone, codon 129 influences both susceptibility and incubation time (47, 172, 190).

Polymorphisms near *PRNP* may affect its expression and could confer additional risk of disease, as appears to be the case for cattle BSE. Small studies initially provided evidence in support of this hypothesis (118, 124, 206); however, the largest and most recent genome-wide association study (GWAS) did not provide confirmation (128) (see the section titled Human Genome-Wide Association Study).

Experimental Models

Although major advances in prion genetics have come from the study of natural diseases and populations, for more detailed hypothesis-driven studies an experimental model is required. Experimental transmissions with sheep, cattle, and cervids are routinely undertaken; however, these are costly, necessitating small studies, and they require specialized facilities not easily accessible to most researchers. For the study of both human and animal prion diseases the mouse has become the model of choice.

Mouse. Wild-type mice are naturally susceptible to prion diseases; therefore, unlike with many other neurodegenerative diseases they faithfully reproduce all the characteristics of the disease, including prolonged incubation times; prion strain properties; and pathological features such as PrP^{Sc} deposition, spongiosis, and gliosis. The ability to generate transgenic, knock-in, and knockout mice expressing various alleles of human, sheep, cattle, and cervid *PRNP* has facilitated detailed transmission studies that have not only delineated the key PrP residues but also made major contributions to our understanding of prion diseases and their associated risks.

Though wild-type mice are susceptible to prion diseases, there is considerable variation in attack rate and incubation period depending on the experimental paradigm. For example, the primary transmission of sheep scrapie and cattle BSE is inefficient; however, on subsequent passage there is evidence of adaptation to the new host, resulting in 100% susceptibility and shorter incubation times (42), termed a transmission barrier. Other parameters such as prion strain, infectious dose, and route of inoculation also affect incubation time; nevertheless, when these variables are kept constant, the main factor influencing incubation time is genetic background.

Historically it was observed that for a given inbred line of mice and mouse-adapted prion strains the incubation time was remarkably constant and that broadly mice fall into two groups, one in which the incubation time is short (100–200 days) and the other in which the incubation

time is long (greater than 255 days) (33, 55, 56, 97, 212). This has been explained by the presence of two *Prnp* alleles named $Prnp^a$ (108L, 189T) and $Prnp^b$ (108F, 189V) in mice with short and long incubation times, respectively (138, 212). Detailed targeting experiments suggest that codons 108 and 189 modify incubation time independently, whereby codon 189 is the major determinant and regulates the initial interaction and binding to PrP^{Sc} and codon 108 influences the rate of PrP^{c}-to-PrP^{Sc} conversion (10).

$Prnp^b$ was first identified in the VM/Dk mouse strain, and subsequently the $Prnp^b$ allele has been identified in 8 different mouse strains (33, 105). The extent of inbreeding during the establishment of different laboratory mouse lines suggests that the $Prnp^b$ allele arose only once and that all $Prnp^b$ mice are related (17). This contrasts with $Prnp^a$, which has been identified in over 60 strains. Sequencing *Prnp* in a more diverse panel of mice identified a new allele, $Prnp^c$ (108P, 189T) (107). To date, $Prnp^c$ has been seen only in the MAI/Pas mouse strain, which was inbred from Austrian wild-trapped mice. In terms of incubation time, MAI/Pas mice more closely resemble $Prnp^b$ mice, with a long incubation time of 360 ± 11 days with Chandler/RML prions, thus reflecting the influence of codon 189.

Bank vole. Bank voles (*Myodes glareolus*) are small rodents that have also been used as experimental models for laboratory prion transmissions. Bank voles lack the genetic and technological resources available for mouse, but their popularity stems from their apparent permissiveness to different prion strains, including natural scrapie and sporadic CJD, from a variety of species (2, 78, 144). Following additional passages and strain adaptation, short incubation times of less than 100 days have been recorded, which are usually only seen in transgenic mice greatly overexpressing PrP. Paradoxically, bank voles are more resistant than wild-type mice to BSE, which is generally considered a highly promiscuous prion strain (2). However, bank voles are able to propagate BSE prions, as subsequent passage produces a 100% attack rate and an incubation time of approximately 200 days. The difference in prion strain propagation properties despite the similarity in PrP sequence between mouse and bank vole (eight amino acid differences in the mature protein) reinforces the hypothesis that strain structural properties as well as primary PrP sequence are key to determining the transmission barrier (41, 45, 209).

Bank voles have a naturally occurring PrP polymorphism at codon 109 that may be a methionine or isoleucine (36). This corresponds to mouse PrP codon 108, which is also polymorphic (L108F) and is associated with determining incubation time. Experimental challenge of 109MM and 109MI bank voles with different scrapie isolates showed a significant reduction in incubation time for the homozygous voles (202 ± 28 days MM; 298 ± 51 days MI) (36). The influence of the M109L polymorphism was also seen where spontaneous prions were produced in transgenic mice overexpressing bank vole PrP 109M but not 109I (209).

PRION PROTEIN POLYMORPHISM AND EVIDENCE OF EVOLUTIONARY SELECTION

Balancing Selection in Sheep

Despite the high degree of PrP sequence conservation across species, sheep exhibit a surprising number of amino acid variants (67). The maintenance of such a large number of ovine *PRNP* alleles, several of which are associated with susceptibility to the naturally occurring disease scrapie, is suggestive of balancing selection within the population. Indeed, a study of the sequence evolution of *PRNP* in ruminants confirmed the influence of balancing selection (183). Although selective breeding in domesticated animals distorts the allele frequencies, of particular note is the maintenance of the VRQ allele despite the high risk imposed by the VRQ/VRQ homozygous genotype.

In this instance, the relatively low risk of VRQ/AHQ and VRQ/ARR heterozygotes may provide some protection against scrapie while maintaining the VRQ allele. In sheep, as in humans and other animals, heterozygosity appears to afford a degree of protection against prion diseases, thereby favoring the maintenance of a diverse allele pool. Equally, as demonstrated by classical and atypical scrapie, the degree of selective advantage offered by a given genotype is entirely dependent on the prion strain encountered and the prevalence of prion disease in the population. The existence and maintenance of multiple alleles may therefore reflect the exposure to different prion strains during evolution. Similarly, the genetic diversity of sheep may explain the multiple prion strains in sheep, as the number of amino acid polymorphisms increases the variety of strain conformations able to be populated by ovine PrP.

Selection in Cervids

The presence of resistance alleles to CWD in the cervid population has the potential to confer selective advantage to less susceptible genotypes and through positive selection alter the allele frequencies in the population (169). In white-tailed deer the 96G allele is four times more susceptible to CWD than the 96S allele; further, 96S allele deer survive approximately eight months longer once infected (168). A study of relative fitness in a prion disease–endemic region showed that 96S-resistant deer achieve greater population growth and have a long-term fitness advantage, suggesting that over time the 96S allele will become the major allele (168). This potential for positive selection in cervids contrasts with the balancing selection demonstrated for sheep. This difference may be reflected in the greater variation seen in the *PRNP* sequence for sheep than for cervids. It may also reflect the fact that scrapie has been recognized for much longer than CWD; therefore, multiple scrapie prion strains and their respective susceptibility alleles have coexisted for longer.

Balancing Selection in Human Populations

The only example of a human prion disease of sufficient severity and in individuals of reproductive age who will contribute to evidence of selection pressure is kuru. This epidemic devastated the Fore and neighboring populations in the mid-twentieth century and was documented as the leading cause of death in women (4). Several thousand survivors of the kuru epidemic and their neighboring unexposed populations have been analyzed (131). These groups show marked Hardy–Weinberg disequilibrium at codon 129, which becomes most extreme in those who, by virtue of age, gender, and location, had the highest potential for exposure at mortuary feasts and which is absent from unexposed groups and the modern young population (130). The selection event in the Fore was not only intense but also complex, with resistance conferred by the heterozygous state at codon 129 and the proximal V allele at codon 127, which appears to be under positive selection. The relative excess of nonsynonymous:synonymous *PRNP* polymorphism in world populations, and the allele frequency distribution, led to the suggestion that balancing selection occurred more widely than the kuru region (127, 185, 186), with the potential implication that the kuru epidemic in the Fore region was not unique and prehistoric kuru-like epidemics may have occurred in other populations.

NON-PRION PROTEIN GENE GENETIC RISK FACTORS

QTL Mapping Studies of Mouse

Genetic analyses in natural hosts and experimental models demonstrate that coding polymorphisms within the prion gene itself are the major genetic determinants of susceptibility or incubation time. It is also clear, particularly from mouse studies, that genetic background is also

Table 2 Summary of prion disease QTL mapping

Mouse cross or species	Prion strain (route)	Chromosome (peak location cM)
CAST × NZW F_2 intercross	Chandler/RML(ic)	*Mmu2* (61.2) *Mmu11* (43.7) *Mmu12* (47.0)
CAST × SJL F_2 intercross	Chandler/RML(ic)	*Mmu9* (17.0) *Mmu11* (43.0)
CAST × NZW F_2 intercross	Mouse passaged BSE(ic)	*Mmu2* (61.2) *Mmu11* (43.7)
F_1 × C57 backcross F_1 × RIII backcross	BSE(ic)	*Mmu2* (34.0) *Mmu8* (43.0) *Mmu4* (42.5) *Mmu15* (49.6)
RIII × C57 F_2 intercross	ME7(ic)	*Mmu5* (68.0) Mmu7 (6.0)
C57BL/6 × DBA RI and F_2 intercross Meta-analysis	ME7(ic) BSE(ip)	*Mmu4* (61.9) *Mmu11* (52.0) *Mmu18* (4.0)
Cow: four half-sibling families (Holstein sire)	Natural BSE	*BTA17* (144.0) *X/*Y_{ps} (58.0)
Sheep (Romanov flock)	Natural scrapie	*OAR18* (100.0)

Abbreviations: BSE, bovine spongiform encephalopathy; ic, intracerebral; ip, intraperitoneal; QTL, quantitative trait locus.

important. For example, within *Prnp*^{a} mice, where there are no PrP coding differences, there is a difference of over 100 days in incubation time, with Chandler/RML prions between the mouse strains exhibiting the shortest (105 ± 4 days, SJL/J) and longest (221 ± 5, PWK/Pas) incubation times (34, 35, 105, 166, 212).

Investigators have carried out several mapping studies to identify non-*Prnp*-modifying genes (85, 106, 108, 114, 139, 188). Although different in the details, all studies were broadly similar, relying on classical crosses between two inbred mouse lines (*Prnp*^{a}) with different incubation times to generate recombinants and segregate the parental alleles within the resulting population. The offspring were challenged with prions and their disease incubation times were recorded. Microsatellite markers were used to genotype the mice and statistical analyses were used to measure the linkage between genotype and phenotype. A variety of long incubation time mouse strains (CAST/Ei, C57/FaDk, C57BL6/J) and short incubation time mouse strains (SJL/J, NZW/OlaHsd, RIII/FaDk, DBA/J) in F_2 intercrosses, backcrosses, and recombinant inbred lines have been used. A variety of prion strains (Chandler/RML, ME7, mouse-adapted BSE, primary passage BSE) and both intracerebral and intraperitoneal routes of inoculation have been used. Individually, each of these studies successfully identified multiple regions of significant linkage; however, given the variation in study design, only a few loci have been replicated across studies (**Table 2**).

Replication of a quantitative trait locus (QTL) provides confidence in the data and suggests that a QTL may be of general importance rather than a route of inoculation or prion strain specific. Multiple studies have identified loci on mouse Chromosomes 2, 4, and 11 (85, 106, 108, 188). Although the peak of linkage varies, the regions are broad, containing hundreds of genes, but considerable overlap in the confidence intervals suggests that these studies have identified a common locus. Of note, the *Mmu2* locus includes *Prnp*. However, as there are many other genes in this region and no PrP coding differences between the mice, it remains possible that differential regulation of *Prnp* may underlie this QTL.

QTL Mapping in Cattle and Sheep

QTL mapping in experimental mouse crosses has been successful owing to the availability of mouse genetic resources and multiple inbred lines of mice, the capacity to generate large numbers of animals for statistically powerful studies, and the ability to control the experimental conditions. Despite the advantages, mouse crosses do not represent natural infections with naturally occurring strains of prions; therefore, QTL mapping studies have used cattle and sheep. In both cattle and sheep, studies have compared natural infections in infected (susceptible) and noninfected (resistant) animals. Although natural infection reflects the epidemiology, its disadvantage is that unlike in experimental infection it is impossible to control for exposure of infectivity, which may reduce the power of the study (140, 219) (**Table 2**).

IDENTIFICATION OF CANDIDATE GENES

Use of Heterogeneous Stock Mice

Mouse QTL studies confirmed the importance of non-*Prnp* genetic modifiers in prion disease incubation time and successfully identified multiple loci across the genome. However, the broad confidence intervals hindered progress toward identifying the underlying candidate genes. One approach to fine mapping has been to use heterogeneous stock mice, which originated from eight parental strains and were bred semirandomly over several generations. This complex cross results in greater allele diversity and multiple recombination events per chromosome and, when combined with high-density genotyping, can achieve a mapping resolution of 1–3 cM (80, 203). Using 1,000 mice at generation 37 of the Northport heterogeneous stock inoculated with the Chandler/RML prion strain, researchers have successfully resolved QTLs on *Mmu15* and *Mmu19* (109, 110).

***Cpne8*.** Sequence analysis of all the genes in the region in the heterogeneous stock parental strains subsequently identified the copine family gene *Cpne8* as the most likely candidate for the *Mmu15* QTL (**Table 3**). The precise role of *Cpne8* in incubation time has not been resolved. Copine family members encode Ca^{2+}-dependent phospholipid-binding proteins, so *Cpne8* may be involved in PrP trafficking to and from the cell membrane (197).

***Hectd2*.** The same approach was used to narrow the region of interest on *Mmu19* to 2.9 Mb and identified *Hectd2* as the most promising candidate gene (110). Additional *HECTD2* human single nucleotide polymorphism case-control association studies were conducted in patients with vCJD, sporadic CJD, and kuru (110). These data suggest that although there is some evidence that *HECTD2* is associated with acquired human prion diseases, the molecular mechanism may vary in different populations or in response to different prion strains.

Little is known of the normal function of Hectd2 and how this impacts disease susceptibility and incubation time; however, allele-specific differential expression of messenger RNA (mRNA) occurs, with a five-fold increase in expression by the end stage of disease (110). On the basis of sequence homology, Hectd2 appears to be a member of the E3 ubiquitin ligase family, whose role is to ubiquitinate proteins targeted for degradation by the proteasome and autophagy. It is expected that the identification of Hectd2 substrates will provide insight into the pathways regulated by Hectd2 and their role in prion disease.

Expression Profiling

The availability of high-density microarrays for human, mouse, cow, and sheep has facilitated genome-wide analysis of differential mRNA expression in affected versus unaffected animals and

Table 3 Genetic modifiers of prion disease

Gene or locus	Method	Evidence
PRNP	Human GWAS and mouse QTL	All human prion diseases and mouse transmission studies
Non-*PRNP* genes		
RARB	Human GWAS	vCJD and iCJD, SNP association (HS mice)
STMN2		vCJD and kuru, SNP association (HS mice)
STX6, *GAL3ST1*, *PDIA4*		sCJD, top-ranked hits
MTMR7		vCJD
NPAS2		vCJD
Hectd2	Mouse QTL	QTL (HS mice), SNP association (vCJD and kuru)
Cpne8		QTL (HS mice)
Hspa13 (*Stch*)	Expression profiling	Differential expression and Tg mouse transmissions
Candidate genes		
SPRN	Neurodegeneration	vCJD rare mutations
SOD1		Human Tg and knockout mouse transmissions, SNP association (HS mice)
APP		Knockout mouse transmissions
CLU		Knockout mouse transmissions
BACE1		sCJD candidate gene association study
CTSD		vCJD candidate gene association study
IL-1r	Immune response	Knockout mouse transmissions, SNP association (HS mice)
IL-10		Knockout mouse transmissions
Mcp-1 (*Ccl2*)		Knockout mouse transmissions

Abbreviations: GWAS, genome-wide association study; HS, heterogeneous stock; iCJD, inherited Creutzfeldt-Jakob disease; QTL, quantitative trait locus; sCJD, sporadic Creutzfeldt-Jakob disease; SNP, single nucleotide polymorphism; Tg, transgenic; vCJD, variant Creutzfeldt-Jakob disease.

also at different stages during disease progression in experimental infections (12, 84, 218). These studies have produced a wealth of data highlighting the gene networks and pathways associated with disease-specific changes, although it is difficult to differentiate between causative changes and those that are secondary effects reflecting the ongoing cell stress and death, particularly at the terminal stages.

***Hspa13* (*Stch*).** An alternative approach to identifying differentially expressed genes that underlie susceptibility is to look for a correlation between mRNA expression in uninfected mice from lines with different disease incubation times. In this way, five candidate genes, including *Hspa13*, were identified (72). Manipulation of *Hspa13* expression levels in vitro and in vivo validated the microarray findings. Decreased expression in cells induced a reduced susceptibility to prions, and overexpression in transgenic mice showed significantly reduced incubation times with three different prion strains (26, 72). Hspa13 is a member of the Hsp70 ATPase heat shock protein family, and although it lacks a peptide-binding domain, it is localized within the endoplasmic reticulum and is induced by Ca^{2+} release, suggesting a role in endoplasmic reticulum stress and the unfolded protein response (150). Overexpression of *Hspa13* has also been associated with TRAIL-induced apoptosis (217).

Human Genome-Wide Association Study

The appeal of a GWAS is that it is unbiased, the technologies are cost-effective and reliable, and the precedents for reproducibility are good, as long as strict statistical thresholds are applied.

GWAS investigations have been used for kuru, vCJD, and sporadic CJD; however, the sample sizes achieved are smaller than those that have typically been required for success in most complex disease traits. All studies have identified *PRNP* as the dominant risk locus (126, 128, 174). In vCJD, polymorphisms upstream of the *RARB* and *STMN2* genes were flagged by the first study (126), although neither of these variants achieved genome-wide levels of significance. A second study using vCJD samples that were largely but not completely overlapping with those of the first study identified single nucleotide polymorphisms near the *MTMR7* and *NPAS2* genes that exhibit genome-wide significance in a pooled data set (174). In sporadic CJD, an international study that pooled samples between Germany and the United Kingdom did not find genome-wide significant hits aside from *PRNP* (128). None of the polymorphisms identified in vCJD GWAS investigations are risk factors for the sporadic disease. A new GWAS (published in abstract form only) identified only *STX6*, *GAL3ST1*, and *PDIA4* as risk genes for sporadic CJD, implicating intracellular vesicle trafficking, sphingolipid metabolism, and the unfolded protein response in susceptibility (122).

Candidate Genes

Many genes from a variety of sources have been suggested as candidate susceptibility or incubation time genes. These include genes that are already associated with other neurodegenerative diseases and immune response genes such as cytokines and chemokines. In most cases, knockout or transgenic mouse models have been used to test individual hypotheses; in other cases, human association studies have focused on a single gene.

SOD1. Mutation in the human superoxide dismutase 1 (*SOD1*) gene causes familial amyotrophic lateral sclerosis, a form of motor neuron disease, through a toxic gain-of-function (91). To test the hypothesis that *Sod1* is a susceptibility gene for prion disease, Tamguney et al. (191) challenged a transgenic mouse overexpressing human *SOD1* with both RML and 301V mouse–adapted prion strains. With RML the incubation time was extended by 19%; however, no change was detected for 301V prions (191). Using heterogeneous stock mice, Akhtar et al. (3) detected an association between the *Sod1* locus and prion disease incubation time. Further mouse studies using *Sod1* knockout mice showed a reduction in incubation time of 20%, 13%, and 24% with RML, ME7, and mouse-passaged BSE (MRC2) prion strains, respectively (3). Taken together, these data support a protective role for Sod1 in prion disease.

APP. PrP has been linked with the posttranslational processing of APP through *BACE1* and has been implicated as an amyloid-beta receptor in some models of Alzheimer disease (11, 61, 103, 156). To assess the role of App in prion disease, Tamguney et al. (191) challenged *App* knockout mice with the RML mouse–adapted scrapie strain of prions and demonstrated a 13% increase in incubation time (191).

CLU. Two large independent GWAS investigations have shown a genetic association between the clusterin (Apolipoprotein J) gene (*CLU*) and Alzheimer disease (76, 102). Although no genetic association between *CLU* and CJD or in mouse crosses has been seen, clusterin is upregulated and colocalizes with PrP^{Sc} in the brains of BSE-infected mice (120). To evaluate the role of *Clu* in prion disease, Kempster et al. (96) challenged *Clu* knockout mice with BSE prions and showed a 40-day increase in incubation time relative to controls. Consistent with a role in aggregate formation, less PrP^{Sc} deposition but more astrocytosis was observed in *Clu*-deficient mice.

IL-1r1. Both IL-1β and its receptor interleukin-1 receptor type 1 (IL-1r1) are upregulated in scrapie-infected mice, with increased IL-1β also detected in cerebrospinal fluid from patients with CJD (180, 198, 204). IL-1r1 in the CNS is expressed primarily on astrocytes and oligodendrocytes and mice deficient in IL-1r1 show a reduced inflammatory response. Two independent studies have found that *IL-1r1* knockout mice challenged with prions show a delayed onset of clinical signs and an overall increase in survival time (180, 191). This is associated with a delay in gliosis, suggesting that the production of IL-1 in prion disease and the resulting gliosis damage rather than protect the neurons.

IL-10. Studies using interleukin-10 (*IL-10*) knockout mice showed a dramatic decrease in incubation time to 58 ± 14 days compared with wild-type controls, 140 ± 2 (129S1/SvIm/J) (194). Despite the short incubation time, the knockout mice developed the same disease phenotype and neuropathology and the same levels of infectivity. However, the reported ~60% reduction in incubation time may not be a general finding, as an independent study showed only a 19% reduction on a C57Bl/6J background and no difference on the 129S1/SvIm/J background (191).

Mcp-1. Monocyte chemoattractant protein 1 (Mcp1) [a.k.a. chemokine C-C motif ligand 2 (Ccl2)] drives the activation of microglia and shows an increase in expression throughout the course of disease. *Mcp1* knockout mice inoculated with the ME7 mouse–adapted scrapie prion strain showed a 4-week delay in the onset of late clinical signs and an overall increase in survival of 2–3 weeks relative to controls (58). This result may be prion strain specific because inoculation of $Mcp1^{-/-}$ mice and mice deficient in its receptor, *Ccr2*, with the RML prion strain showed no differences in incubation time (149, 191).

Other human genes. A human candidate gene association study has found in *BACE1* evidence for an association between a common polymorphism (rs638405) and sporadic CJD (32). For *CTSD* there is evidence for an association between a common polymorphism (C224T) and vCJD (24). There is no evidence of association with other forms of human prion disease (98). The *C9orf72* hexanucleotide repeat expansion mutation that causes frontotemporal dementia and amyotrophic lateral sclerosis, and the *TREM2* gene, known to be a strong risk factor in Alzheimer disease, are not risk factors for CJD (16, 184). Recently, variants near the *CYP4X1* gene were proposed as disease modifiers in IPD caused by the E200K mutation of *PRNP*, with similar effects in sporadic CJD (160).

CONCLUSIONS AND FUTURE DIRECTIONS

We now have an advanced understanding of the role of PrP and its amino acid variants in susceptibility to mammalian prion diseases. Possible roles for a large number of non-*PRNP* genes have been proposed on the basis of mapping, candidate, or genome-wide studies of mouse and human. Robust genome-wide significance in human studies of sporadic CJD, for which independent replication can be achieved, together with concordant evidence from animal models, is the main goal but has, until recently, been frustratingly elusive. The lack of power to detect modest-effect risk alleles is likely due to sample size. Human genome-wide association arrays have focused on common variants. Attention will increasingly move to next-generation sequencing technologies to detect variants of all frequencies, which in some cases cause complete loss of function of genes and therefore might be more likely to have strong effects. An additional area for attention is the use of *PRNP* variants in prediction of phenotype and in stratified medicine or preventive approaches to therapeutic trials.

Manipulation of non-*Prnp* genes in experimental models, particularly the risk genes discovered by GWASs, will be a crucial activity in future research. The development of a human astrocytic model and other cellular models of prion infection, and of human cerebral organoids that can be infected with prions and develop neurodegeneration, will be attractive choices to complement the study of genetically modified mice.

A relatively unexplored area of research is the role of epigenetics in prion disease susceptibility. Why is age such a strong risk factor for human prion disease but complex in that risk appears to decline at the extremes of old age? What is the role of gene expression and noncoding RNAs in prion infection of tissues and selective vulnerability? Does altered expression of genes confer susceptibility or are changes observed in the disease state simply the consequence of infection and toxicity?

Recent findings using transgenic mouse models of prion infection to explore factors involved in neurotoxicity and synapse regeneration implicate the unfolded protein and cold shock responses (141). These pathways might be further explored with the use of genetically modified models.

A further unexplored and challenging area is the somatic mutation hypothesis of sporadic CJD. Does the absence of strong human genetic risk factors beyond *PRNP*, taken together with the seemly random occurrence of disease in all countries surveyed, imply that stochastic factors are crucial? Aside from the stochastic generation of a prion seed, sporadic CJD may be caused by somatic mutation in a cluster or even a single cell, which may be extremely challenging to show experimentally.

DISCLOSURE STATEMENT

J.C. is a director and shareholder of D-Gen Ltd., an academic spin-out company working in the field of prion disease diagnosis, decontamination, and therapeutics.

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