Published in final edited form as:

Methods Mol Biol. 2017 ; 1658: 219–252. doi:10.1007/978-1-4939-7244-9_16.

Insights into mechanisms of transmission and pathogenesis from transgenic mouse models of prion diseases

Julie A. Moreno, PhD and Glenn C. Telling, MA (Oxon), PhD

Prion Research Center, Department of Microbiology, Immunology and Pathology, Cell and Molecular Biology Graduate Program, Molecular, Cellular & Integrative Neuroscience Graduate Program

Abstract

Prions represent a new paradigm of protein-mediated information transfer. In the case of mammals, prions are the cause of fatal, transmissible neurodegenerative diseases, sometimes referred to as transmissible spongiform encephalopathies (TSE's), which frequently occur as epidemics. An increasing body of evidence indicates that the canonical mechanism of conformational corruption of cellular prion protein (PrP^C) by the pathogenic isoform (PrP^{Sc}) that is the basis of prion formation in TSE's, is common to a spectrum of proteins associated with various additional human neurodegenerative disorders, including the more common Alzheimer's and Parkinson's diseases. The peerless infectious properties of TSE prions, and the unparalleled tools for their study, therefore enable elucidation of mechanisms of template-mediated conformational propagation that are generally applicable to these related disease states. Many unresolved issues remain including the exact molecular nature of the prion, the detailed cellular and molecular mechanisms of prion propagation, and the means by which prion diseases can be both genetic and infectious. In addition, we know little about the mechanism by which neurons degenerate during prion diseases. Tied to this, the physiological role of the normal form of the prion protein remains unclear and it is uncertain whether or not loss of this function contributes to prion pathogenesis. The factors governing the transmission of prions between species remain unclear, in particular the means by which prion strains and PrP primary structure interact to affect inter-species prion transmission. Despite all these unknowns, advances in our understanding of prions have occurred because of their transmissibility to experimental animals and the development of transgenic (Tg) mouse models has done much to further our understanding about various aspects of prion biology. In this review we will focus on advances in our understanding of prion biology that occurred in the past eight years since our last review of this topic.

Keywords

Prions; transmissible spongiform encephalopathies (TSEs); scrapie; chronic wasting disease (CWD); Creutzfeldt Jakob disease (CJD); bovine spongiform encephalopathy (BSE); transgenic mice

1. Introduction

Prions challenge fundamental concepts of inheritance and infection. Prion-mediated phenotypes, in organisms such as yeast and *A. plasia*, as well as a variety of mammalian neurodegenerative diseases result from the ability of the prion conformation to interact with and induce further structural conversion of counterpart endogenous proteins in their normal states. The prototype prion diseases are the transmissible spongiform encephalopathies (TSEs) of animals and humans, which include scrapie in sheep, bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) of deer and elk, transmissible mink encephalopathy (TME), and various human disorders, the most common being Creutzfeldt Jakob disease (CJD). They share a number of common features, the most consistent being that disease is generally naturally and/or experimentally transmissible; the extraordinary physical properties of the infectious agent that account for its extreme resistance to conventional disinfection procedures; and the neuropathologic changes that accompany disease in the central nervous system (CNS). These features typically consist of neuronal vacuolation and degeneration, a reactive proliferation of astroglia, and, in some conditions such as the human prion diseases kuru, Gerstmann Straussler Scheinker (GSS) syndrome, and variant CJD (vCJD), the deposition of amyloid plaques.

Central to all prion states are the protean conformational properties of particular host encoded proteins. In the case of TSE's, this is the prion protein (PrP). The normal form, referred to as PrP^C, is a sialoglycoprotein of molecular weight ~ 33 kDa that is attached to the surface of neurons and other cell types by means of a glycosylphosphatidylinositol (GPI) anchor. Experimentally, PrP^C is sensitive to protease treatment, can be released from cell surfaces by treatment with phosphoinositide phospholipase C (PIPLC), and is soluble in detergents as a monomer. During disease, a conformational variant of PrP^C referred to as PrP^{Sc}, accumulates in infected brains, and, in most cases, tissues of the lymphoreticular system. PrP^{Sc} is partially resistant to protease treatment, is resistant to PIPLC treatment, insoluble in detergents, and is prone to aggregate. In most examples of prion disease, experimental protease cleavage of the amino-terminal 66, or so, amino acids of PrP^{Sc} gives rise to a protease-resistant core, originally referred to as PrP27-30. Considerable evidence now supports the once unorthodox hypothesis that prions lack nucleic acid, are composed largely, if not entirely, of PrP^{Sc}, and that replication involves corruption of the benign cellular form of the prion protein by its abnormally conformed infective counterpart, which results in the exponential accumulation of prions, and inevitable demise of the infected host as a result of profound CNS neurodegeneration [1].

2. Prion transmission barriers

A crucial aspect of prion disorders is their transmissibility. Inoculation of prion diseased brain material into individuals of the same species will typically reproduce disease with a remarkably synchronous time to disease onset. Although transmission efficacy between species is, by comparison, generally significantly less than when prions propagate within their natural hosts, cross-species infection is nevertheless known to have featured in the etiologies of several epidemics, in particular BSE, vCJD, and most likely prions disease in mink, referred to as transmissible mink encephalopathy (TME).

2.1 PrP primary structure, prion strains and the species barrier

Although the elements controlling interspecies prion transmission are not completely understood, seminal studies in Tg mice [2, 3] and cell-free systems [4] suggested that minimal amino acid divergences may have a major impact on the transmission efficiency, and that barriers to transmission between species resulted from primary structural incompatibilities between PrP^{Sc} constituting the prion, and substrate PrP^C expressed in the newly infected host.

Experimentally, the barrier to prion transmission between species may be absolute, in which case no transmission is recorded, or, more commonly, primary interspecies transmissions are characterized by variable rates of infection, and protracted, inconstant intervals to disease onset. Mechanistically, this is thought to reflect a two-step process in which initial stochastic conversion of host PrP^C by structurally mismatched PrP^{Sc} is followed by effective PrP^C conversion by the resulting structurally compatible PrP^{Sc}, leading to efficient replication of adapted prions, neurodegeneration and inevitable death of the host. Consistent with this notion, serial passage of such adapted prions to additional animals expressing the same PrP^C results in a relatively short, synchronous time to disease in all inoculated recipients.

Transgenic approaches have also been used to address the notion that PrP primary structures expressed in particular species are inherently resistant to infectious conversion. Vidal and co-workers generated Tg mice overexpressing rabbit PrP (TgRab) under the control of the mouse prion protein gene promoter [5]. Their rationale for this approach was that rabbits have generally been considered to be resistant to prion infection. This group showed, however, that PMCA-derived rabbit PrP^{Sc} caused a prion disease in rabbits [6]. In subsequent studies, the susceptibility of TgRab to different prions was assessed. Susceptible prions included BSE, sheep BSE, L-type BSE, *in vitro* generated rabbit prions, and the same passaged through rabbits, ME7, and RML. SSBP-1, CWD and atypical scrapie did not cause disease.

The influence of intra-species PrP polymorphisms on prion disease susceptibility in mice [7], sheep [8] and humans [9–11] supported the concept that the primary structure of PrP was a crucially important determinant of prion species barriers. Nonetheless, while the foregoing mechanism is in accordance with the outcomes of the majority of prion interspecies transmission barriers, other circumstances indicate that our knowledge of the parameters controlling interspecies prion transmission remains incomplete. For example, the criteria governing PrP primary structural control over transmission appear to be moot in the case of the bank voles, which are susceptible to prions from a number of mammals with divergent PrP primary structures [12].

Mammalian prion strains are an equally important component affecting prion transmission. Seminal studies indicated that distinct heritable prion strain phenotypes, including the incubation time to disease, and the profile of CNS lesions, are enciphered within the conformation of PrP^{Sc} [13, 14]. This provided investigators with the ability to use the biochemical properties of PrP^{Sc} as a means of tracking, and to some extent identifying strains by assessing of PrP^{Sc} conformation, and/or the extent of PrP^{Sc} glycosylation [15–18]. Since a change in PrP^{Sc} conformation accompanies the emergence of newly adapted prions

following passage across species barriers [16], the concept of a strain barrier was introduced to account for the influence of distinct conformations of PrP^{Sc} molecules [19]. The unexpectedly wide host range of BSE prions, exemplified by transmission to humans as variant CJD (vCJD), as well as the restricted transmission of vCJD prions in human PrP Tg mice [20, 21] compared to bovine PrP Tg mice [22] exemplify the capacity of particular prion strains to overcome the influence of PrP primary structure.

While mutational events in agent-associated nucleic acid were originally cited as the cause of strain instability [23], more recently, changes in the conformation of PrP^{Sc} have been shown to be associated with the acquisition of new strain properties [16]. To account for the phenomena of prion transmission barriers, strain instability, heterogeneity, and adaptation in the context of PrP^{Sc} conformation, the conformational selection model [24, 25] was proposed to reconcile how PrP primary structure and prion strain conformations interact to control transmission barriers to prion propagation. This model postulates that only a subset of PrP^{Sc} conformations is compatible with each individual PrP primary structure [24, 26]. By extension, this leads to the notion that prions exist as an array of quasispecies conformations, a model first applied to populations of a virus within its host [27]. There is a mounting body of experimental evidence for this concept [28–30]. Quasispecies acquire fitness when populations of prion conformers are subjected to selective pressure, for example during propagation in a host expressing a different PrP primary structure following interspecies transmission. From this perspective, PrP^C primary structure influences the portfolio of thermodynamically preferred PrP^{Sc} conformations that are kinetically selected during propagation, such that only a subset of such PrP^{Sc} conformers are optimized for fitness in a particular host [31, 32].

The issue of strain effects on the efficacy of cross-species transmission was elegantly addressed in transgenic experiments by Béringue and colleagues who compared the ability of brain and lymphoid tissues from ovine and human PrP Tg mice to replicate foreign, inefficiently transmitted prions [33]. They observed that lymphoid tissue was consistently more permissive than the brain for CWD and BSE prions. Furthermore, when the transmission barrier was overcome through strain shifting in the brain, a distinct agent propagated in the spleen, which retained the ability to infect the original host.

An additional factor affecting the efficiency of interspecies transmissions was revealed by recent experiments using Tg mice expressing PrP^C lacking the GPI moiety that tethers the protein to the cell surface. Previous *in vitro* studies showed that an artificially mutant version of PrP that lacks the terminal sequence for addition of the GPI lipid anchor could acquire resistance to protease digestion and therefore resembled PrP^{Sc} [4, 34]. To determine whether this mutant PrP can support prion propagation and PrP^{Sc} production *in vivo*, Chesebro and colleagues produced Tg mice expressing “anchorless” PrP [35]. As expected, anchorless PrP was not expressed on the surface of cells derived from Tg mice; instead it was secreted. When inoculated with prions, the mice accumulated mutant PrP in the form of abundant amyloid plaques throughout the brain as early as 70 days after inoculation. But despite their accumulation of amyloid-forming protease-resistant PrP (in many cases at levels higher than PrP^{Sc} found in the brains of clinically sick wild-type mice), these Tg mice failed to develop neurologic signs of prion disease up to 600 days following prion inoculation—long after

inoculated control mice succumbed to disease. To test the effect of lack of GPI anchoring on a species barrier model anchorless 22L mouse prions derived from TgGPI⁻ mice were more infectious than wild type 22L prions in tg66 mice expressing HuPrP at levels 8- to 16-fold above normal, but not in tgRM transgenic mice, which expressed human PrP at 2- to 4-fold above normal.

2.2 Variable effects of endogenous mouse PrP expression in transgenic mouse models

The availability of *Prnp*^{0/0} knockout mice, and the characterization of increasing numbers of Tg mice expressing different PrP alleles revealed interesting protective functions of wild type PrP on various PrP-related pathologies. In some cases disease can be partially or fully suppressed by co-expression of wild type PrP. This effect was first observed during the characterization of Tg mice expressing human (Hu) PrP, referred to as Tg(HuPrP) mice, which only became susceptible to CJD prions when endogenous wild type mouse PrP was eliminated by crossing the HuPrP transgene array to *Prnp*^{0/0} knockout mice [36, 37]. Because of this dominant negative effect, subsequent Tg mouse models expressing foreign PrP coding sequences have generally been either produced in, or ultimately crossed with *Prnp*^{0/0} knockout mice. Coincidentally, these observations also formed the partial basis for the model of prion replication involving an auxiliary factor, referred to as protein X, which was proposed to bind to PrP^C and facilitate conversion to PrP^{Sc} [21, 37, 38]. While subsequent experimental evidence questioned the requirement for protein X [39], this model remains a guiding principle that has informed structural analyses of PrP^C [40, 41].

Interactions between wild-type and mutant prion proteins have also been shown to modulate neurodegeneration in Tg mouse models expressing mutated PrP, the seminal observations being made in Tg mouse models of the inherited human prion disease called Gerstmann Strausler Sheinker (GSS) Syndrome, caused by mutation of codon 102 which results in substitution of proline for leucine [42]. In contrast, neurodegeneration induced by expression of a nine octapeptide insertion associated with familial Creutzfeldt Jakob disease (CJD), designated Tg(PG14), is unaffected by wild type mouse PrP expression [43]. These studies are also consistent with a model in which mutated and wild type PrP compete for a hypothetical binding partner that, in this case, serves to transduce neurotoxic signals. Interestingly, while Tg mice expressing PrP tagged at its amino terminus with green fluorescent protein (GFP) supported compromised prion replication, prion propagation was facilitated by co-expression of wild type PrP, suggesting that wild-type PrP rescued an altered amino terminal function in the tagged PrP [44]. In contrast, the effect of tagging PrP at the C-terminus with GFP was that Tg mice expressing this construct were incapable of sustaining prion infection and the PrP-GFP chimera acted as a dominant-negative inhibitor of wild type PrP conversion to PrP^{Sc} [45]. In similar fashion, co-expression of wild type mouse PrP was shown to rescue the neurodegenerative phenotype of mice expressing amino-terminal deletions [46–48].

2.3 Transgenic mouse models of mammalian prion diseases

Seminal experiments by Scott and co-workers abrogated the resistance of mice to hamster prions by transgenic expression of hamster PrP^C in mice [2, 49], and paved the way for the development of a variety of facile Tg mouse models that authentically recapitulate known

transmission barriers [50]. The lack of species barrier during homotypic transmission, i.e. when the host expresses a PrP gene identical to that of the infecting species, led to the development of many different facile mouse models in which to study the biology of mammalian prions, including sheep, bovine, human, cervid and mink, by transgenic expression in mice of PrP coding sequences from these various species. These Tg resources largely circumvented the imprecise approach of studying the biology of various mammalian prion diseases by transmission to generally non-susceptible experimental animals, or, where possible, the natural host, but has also greatly contributed to our understanding of various mechanistic aspects of these extraordinary pathogens [51]. Generally, an inverse correlation exists between the length of prion incubation time in these Tg mouse models, and transgene expression level. Transgenes are usually expressed on a *Prnp* knockout background (*Prnp*^{0/0}) [52] in order to avoid partial or full suppression of disease caused by co-expression of wild type PrP. A variety of different transgenic mice expressing chimeric versions of PrP in which specific regions of mouse PrP primary structure were replaced by the corresponding elements from human, sheep and bovine PrP have also been created [36, 37, 53–55]. Inclusion of certain mouse PrP primary structural elements in such constructs, in particular MHu2MPPrP, also countermanded the inhibitory effect of mouse PrP co-expression [36, 37].

In an alternative approach, expression of foreign PrP genes in mice has been accomplished by gene replacement methods. This approach ensures that the PrP coding sequence is controlled by the same regulatory elements as wild type mouse PrP, in which case gene expression is expected to recapitulate authentic PrP^C expression. In both cases, PrP sequence identity between the transgenic host and donor usually lead to a higher transmission rate as compared to wild-type mice. When considering such transmission models, it is important to consider that outcomes might be limited by the life span of the recipient host, which might be exceeded by the incubation period, although pathological examination, PrP^{Sc} detection and secondary passage may identify infected animals. These problems can be partially overcome by transgenic mice over expressing PrP^C which might be desirable to fully assess the extent of a species barrier, since it results in highly reduced incubation times. For example, while human PrP knock-in mice did not register disease when challenged with prions from cattle affected with BSE [56], infection did occur in Tg mice expressing higher levels of human PrP [20].

Human prion diseases—The initial transmission of human prions to experimental primates has a rich history beginning with William Hadlow's recognition of the similarity between kuru and scrapie, and his prediction that patient brain extracts would cause disease in inoculated non-human primates after a prolonged incubation period [57]. Seven years later, Gajdusek, Gibbs and Alpers demonstrated the transmissibility of kuru to chimpanzees after incubation periods ranging from 18 to 21 months [58], and subsequently showed that CJD was similarly infectious [59]. For many years the transmission of human prion diseases was investigated using this approach [60], but the expense of housing these animals for long time periods, as well as ethical concerns surrounding their use, severely limited these studies. Inoculations of laboratory rodents produced variable results [61–65]. Generally, only ~10% of intracerebrally inoculated mice developed CNS dysfunction with incubation

times of >500 days (d) [36], limiting the ability of this approach to characterize specific agent strains.

While seminal transgenic investigations by Prusiner and colleagues studied the transmission properties of scrapie prions experimentally adapted to hamsters or mice [3, 49], Tg mice expressing human PrP^C, referred to as Tg(HuPrP) mice, were the first to abrogate a species barriers to naturally occurring prions, in this case prions causing human diseases such as human disorders such as sporadic and iatrogenic CJD (sCJD and iCJD) [36, 37]. As previously described for the animal prion diseases, human prion disease susceptibility is strongly influenced by polymorphic variation of *PRNP*. In particular, homozygosity at *PRNP* codon 129, which encodes methionine (M) or valine (V), predisposes to the development of sporadic and acquired CJD [10, 11]. Surprisingly, two lines of Tg(HuPrP) mice expressing HuPrP with V at residue 129 (HuPrP-V129), referred to as Tg(HuPrP)152 and Tg(HuPrP)110, inoculated with CJD prions failed to develop CNS dysfunction more frequently than non-transgenic controls [36]. Subsequently, mice expressing a chimeric human/mouse PrP transgene, designated MHu2M, were constructed, because earlier studies had shown that a chimeric hamster/mouse PrP gene supported transmission of either mouse or hamster prions [3, 66]. These Tg(MHu2M)5378 mice were found to be highly susceptible to human prions suggesting that Tg(HuPrP) mice have considerable difficulty converting HuPrP^C into PrP^{Sc} [36]. However, Tg(HuPrP)152 mice, and another line designated Tg(HuPrP)440, which expresses HuPrP with M at 129, when crossed with *Prnp*^{0/0} [52], were rendered susceptible to human prions [37]. These observations demonstrated that Tg(HuPrP) mice were resistant to human prions because mouse PrP^C inhibited the conversion of HuPrP^C into PrP^{Sc}. In contrast, Tg(MHu2M)5378 mice crossed onto the null background were only slightly more susceptible to human prions compared to Tg(MHu2M)5378 mice that expressed both chimeric and MoPrP^C. Furthermore, Tg(MHu2M) mice inoculated with either Hu or chimeric MHu2M prions exhibited similar incubation times.

The availability of such susceptible Tg mice made possible the rapid and relatively inexpensive transmission of human prion diseases for the first time. Based on these findings, several additional similar Tg mouse models expressing HuPrP have been produced, with identical results [20, 67, 68]. Gene-targeting approaches have also been employed to produce mice expressing human PrP [69]. This approach has the obvious advantage of expressing “normal” levels of transgene-encoded PrP under the control of the *Prnp* transcriptional elements, and, for example, to conveniently model the effects of heterozygosity at codon 129. Transmission of a limited number of sporadic CJD cases in these mice has provided evidence for four distinct prion strains. Evidence of strain variation in sCJD have also come from laboratory studies in bank voles [12].

Early transmission studies to Tg(MHu2M)5378 mice provided evidence that different human prion strains, namely in patients with fatal familial insomnia (FFI), caused by mutation at codon 178 (D178N), and familial CJD, caused by mutation at codon 200 (E200K), are enciphered by different conformational states of PrP^{Sc} [13], a concept first elaborated following transmission of the hamster-adapted strains of TME called hyper (HY) and drowsy (DY) [14]. While the conformational enciphering hypothesis is supported by

considerable additional experimental evidence, defining human prion strain prevalence has been hampered by difficulties in arriving at an internationally accepted classification system for human prion strains [70, 71], and by the observation that multiple PrP^{Sc} subtypes coexist in the same brain [29, 72]. Coincidentally, there transmissions also supported the concept that genetically programmed prion diseases are also transmissible. Other examples of inherited human prion diseases that have been transmitted under similar circumstances include transmission of an inherited form of CJD caused by mutation of codon 210 which changes valine to isoleucine at this residue [73].

Given the importance of infectious transmission in prion diseases, the availability of Tg mice with susceptibility to human prions has increased the number of known sporadic prion diseases. Transmission to Tg(MHu2M) mice of an unusual case of human prion diseases that presented with insomnia but no *PRNP* abnormalities led to the discovery of a novel prion disease referred to as sporadic fatal insomnia [74]. More recently transmission of a new prion disorder, referred to as variably protease-sensitive prionopathy (VPSPr), a seemingly sporadic disease that is distinct from CJD but shares features of GSS, confirmed that VPSPr might be the long-sought sporadic form of GSS [75]. While inefficient transmission of VPSPr was recorded on first passage to Tg mice over expressing HuPrP, infectivity was not serially transmissible. Thus, while VPSPr is an authentic prion disease, Tg(HuPrP) mice do not appear able to sustain replication beyond the first passage. Similar findings using gene-targeted mice expressing HuPrP confirmed the interpretation that VPSPr has limited potential for human-to-human transmission [76].

A significant goal of Tg development was to produce mice in which the incubation time of prions was as rapid as possible using additional chimeric mouse/human PrP transgenes [21]. Korth and co-workers refined the MHu2M PrP approach, and optimized human prion transmission by replacing key human PrP residues with the equivalent residues from mouse. The resulting chimera, referred to as Tg(MHu2M,M165V,E167Q) mice resulted in shortening the incubation time to approximately 110 days for prions from sCJD patients and divergence into two strain types [21]. Even shorter incubation times and CJD strain evolution were also observed in another line, termed Tg1014 in which a single additional residue (M111V) was reverted to mouse [77].

Development of Tg mice with susceptibility to human prions was timely, as it occurred in the context of significant human exposure to BSE prions, at least in the UK, and the consequential occurrence of vCJD in young adults and teens [78]. Tg(HuPrP) created by Prusiner and colleagues, and similar mice subsequently generated by other groups [20] were used to characterize vCJD prions, and to model human susceptibility to BSE [18, 79–81]. As previously described for the animal prion diseases, human prion disease susceptibility is strongly influenced by polymorphic variation of *PRNP*. In particular, homozygosity at *PRNP* codon 129, which encodes M or V, predisposes to the development of sporadic and acquired CJD [10, 11]. Transmission studies of human CJD cases to transgenic mice confirm the influence of this polymorphism. While mice expressing V129 are susceptible to all PrP^{Sc} types and PrP 129 genotypes [18, 21, 37, 79, 80], mice expressing the HuPrP-129M allele are susceptible to prions from M129 homozygous patients, transmissions from patients mismatched at this codon, or heterozygotes are generally more inefficient [20, 21, 37].

Strikingly, all neuropathologically confirmed vCJD cases studied so far have been homozygous for M at codon 129 [82]. Transmission studies of human CJD cases to Tg mice confirm the influence of this polymorphism. While mice expressing V129 are susceptible to all PrP^{Sc} types and PrP 129 genotypes [18, 21, 37, 79, 80], mice expressing the HuPrP-129M allele are susceptible to prions from M129 homozygous patients, transmissions from patients mismatched at this codon, or heterozygotes are generally more inefficient [20, 21, 37]. Although initial BSE transmissions to Tg(HuPrP)152 mice were uniformly negative, suggesting a substantial species barrier in humans to BSE prions [79], subsequent BSE transmission to Tg mice expressing M at human PrP codon 129 revealed inefficient transmission, characterized by low attack rates and long incubation times. Moreover, a strain shift was occasionally observed in these transmissions, producing a sCJD-like phenotype in a proportion of inoculated Tg mice [20, 56]. In contrast, gene-targeted transgenic mice expressing human PrP were not susceptible to BSE prions [56]. However, these mice were susceptible to sheep-adapted BSE prions suggesting increased susceptibility of humans to BSE prions following passage through sheep [83], an effect that is unrelated to increased titer of BSE prions in sheep brain [84]. The effect of codon 129 heterozygosity on human prion susceptibility has been further examined in Tg mice co-expressing transgene arrays expressing HuPrP-M129 and HuPrP-V129 [85].

The emergence of vCJD in the late 20th century renewed interest in kuru, another acquired human prion disease among the Fore peoples of the inner highlands of Papua New Guinea. Kuru exemplifies the epidemic nature of prion diseases. By mid-twentieth century it was the leading cause of death in this region, killing over 3,000 people in the exposed population of 30,000. While its etiology was initially puzzling, studies showing that kuru patient brain extracts produced a progressive neurodegenerative condition in inoculated chimpanzees after a prolonged incubation period [58], supporting the notion that kuru was an infectious disorder. Despite neuropathological similarities, scrapie was clearly not a candidate for the origin of this infectious disorder. Moreover, kuru predated the BSE/vCJD epidemic by several decades. In fact kuru was a devastating consequence of ritualistic endocannibalism, where brains and other body parts of tribal elders were consumed as an act of mourning.

Remarkably, a novel *PRNP* variant was found uniquely among unaffected individuals in the kuru-exposed population, in which V at residue 127 was replaced by glycine (G) [86]. This polymorphism was hypothesized to be an acquired prion disease resistance factor, selected in response to the kuru epidemic. Asante and colleagues modeled this kuru resistance polymorphism, referred to as HuPrP V¹²⁷, in Tg mice [87]. Tg mice having the genotype associated with disease resistance, specifically heterozygosity (G/V) at position 127, and M/M homozygosity at 129, referred to as G¹²⁷M¹²⁹/V¹²⁷M¹²⁹, were completely protected against kuru prions. In contrast all kuru inoculated mice expressing G/G at 127 and either M/M or V/V at 129 (G¹²⁷M¹²⁹/G¹²⁷M¹²⁹ and G¹²⁷V¹²⁹/G¹²⁷V¹²⁹) developed disease after ~ 200 days. Complete recapitulation of genetic susceptibility to kuru therefore validated this transgenic modeling approach. Given the previously recognized similarities between kuru and sCJD, it came as no surprise that G¹²⁷M¹²⁹/V¹²⁷M¹²⁹ were also protected from CJD prions. Interestingly however, these same mice were incompletely protected against vCJD prions. Remarkably, mice homozygous for G¹²⁷ M¹²⁹ were completely protected against all forms of human prion diseases, including vCJD, and failed to manifest either clinical or

subclinical signs of prion disease. As such, they behaved like mice which fail to express PrP as a result of disruption of the PrP gene, and yet still express HuPrP. The availability of Tg mice expressing different levels of HuPrP M¹²⁹ and V¹²⁹ showed that the mechanism underlying the profound inhibitory effects of HuPrP V¹²⁷ occur independently from M129V, which is thought to influence PrP interactions during the replicative process. Moreover, HuPrP V¹²⁷ acts as a dominant negative inhibitor of prion conversion.

Transgenic models of inherited human prion diseases—Approximately 10–20% of human prion diseases exhibit an autosomal dominant mode of inheritance resulting from missense or insertion mutations in the coding sequence of the human PRNP. Several mutations are genetically linked to loci controlling familial CJD, GSS syndrome and FFI. GSS syndrome, which is characterized clinically by ataxia and dementia and neuropathologically by the deposition of PrP amyloid, most commonly results from mutation at codon 102 of PRNP resulting in the substitution of leucine (L) for proline (P) [88]. GSS linked to this mutation is transmissible to non-human primates [60], wild-type mice [63], Tg mice expressing a chimeric mouse-human PrP gene expressing the GSS mutation [37], and *Prnp* gene-targeted mice referred to as 101LL [89].

Initial studies in Tg(GSS) mice which attempted to understand how an inherited disease could also be infectious suggested that prions in the brains of spontaneously sick Tg(GSS) mice could be transmitted to Tg mice expressing lower levels of mutant protein, referred to as Tg196 mice [42, 90]. Disease was also induced in Tg196 mice by a mutant synthetic peptide comprising MoPrP residues 89–103 refolded into a beta-sheet conformation [91] and this disease was subsequently propagated to additional Tg196 mice [92]. While these studies lent support to the prion hypothesis, since they suggested that pathogenic PrP gene mutations resulted in the spontaneous formation of PrP^{Sc} and de novo production of prions [93], this explanation was controversial for several reasons. Although protease-sensitive forms of PrP^{Sc} have been identified using biochemical and immunological methods [15, 94], the lack of protease-resistant PrP^{Sc} in the brains of spontaneously sick or recipient mice eliminated a property that, to some, was synonymous with prion infectivity. Moreover, *Prnp* gene-targeted 101LL mice expressing MoPrP-P101L failed to spontaneously develop neurodegenerative disease [89]. Finally, disease transmission from spontaneously sick mice to wild type mice did not occur and spontaneous disease was eventually registered in aged Tg196 mice [91, 92] complicating the interpretation of the original transmission experiments.

To address the apparent dissociation of prion infectivity and PrP^{Sc} in this well-established Tg model, subsequent studies attempted to use means other than differential resistance to proteinase K treatment to detect disease-associated forms of PrP in spontaneously sick Tg mice expressing MoPrP-P101L. Using the prototype PrP^{Sc}-specific monoclonal antibody (Mab) reagent referred to as 15B3 [95], Nazor and co-workers showed that disease in Tg mice over expressing MoPrP-P101L results from the spontaneous conversion of mutant PrP^C to protease-sensitive MoPrP^{Sc}-P101L, defined by its reactivity with 15B3, that accumulates as aggregates in the brains of sick Tg mice [96]. To understand the influence of mutant PrP expression levels on the transmissibility of spontaneously-generated pathogenic MoPrP-P101L, they produced mice in which transgene copy numbers and levels of MoPrP-P101L

expression were carefully defined. While inoculation of disease-associated MoPrP-P101L accelerated disease in Tg mice expressing MoPrP-P101L from multiple transgenes, disease transmission neither occurred to wild type nor Tg mice expressing MoPrP-P101L from two transgene copies that did not develop disease spontaneously in their natural life span.

Since disease transmission from spontaneously sick Tg(GSS) mice depended on recipient mice expressing MoPrP-P101L at levels greater than that produced by two transgene copies and, since such levels of over expression ultimately resulted in spontaneous disease in older uninoculated recipients, these results suggest that the phenomenon of disease transmission from spontaneously sick Tg(GSS) mice might be more appropriately viewed as disease acceleration whereby inoculation of disease-associated MoPrP-P101L promotes the aggregation of precursors of pathological MoPrP-P101L that result from transgene overexpression. Such a scheme is consistent with a nucleated polymerization mechanism of prion replication originally postulated from cell-free conversion systems [4] and subsequently demonstrated to be the basis of prion propagation in lower eukaryotes [97]. According to this model, PrP^C is in equilibrium with PrP^{Sc}, or its precursor, and the equilibrium normally favors PrP^C. Also, PrP^{Sc} is stable only in its aggregated form that can “seed” polymerization of additional PrP^C, thus converting it into additional PrP^{Sc}. Further, more definitive work combining studies in Tg(GSS) and *Prnp* gene-targeted 101LL mice indicated that clinical or profound neuropathological changes were absent in gene targeted mice inoculated with brain extracts of spontaneously sick Tg(GSS) mice, indicating that *de novo* formation of abnormally aggregated PrP in the host does not always result in a transmissible prion disease [98]. In a related issue, the role of PrP overexpression in the production and transmission of synthetic mammalian prions (SMP) [99] originating from *E. coli*-derived recombinant MoPrP remains to be determined. While the transmission properties and protease-resistance of MoPrP (89–230) SMPs are clearly different from disease-associated MoPrP-P101L, it may be significant that the Tg mice in which these SMPs were initially derived expressed MoPrP(89–231) at levels 16 times higher than normal.

Interestingly, unlike mice expressing the GSS P102L mutation in the context of the mouse PrP primary structure, Tg mice expressing human PrP with the P102L mutation failed to develop disease spontaneously with increasing age. However, these mice were susceptible to infection from patients with the homotypic pathogenic mutation, as well as CJD, producing distinct prion strains with transmission properties distinct from sporadic and acquired human prion disease [100]. In contrast to reports in the gene targeted mouse model, GSS-102L prions produced in this study were incapable of transmitting disease to wild type mice [89].

Transgenic expression of other disease-associated mutations in the context of mouse or human PrP has been met with varying success. While Tg mice expressing mouse PrP containing the most common E200K fCJD mutation (E199K in mouse PrP) did not develop disease spontaneously [42], a Tg mouse expressing chimeric MHu2M PrP [37] containing the E199K mutation PrP developed neurological signs at 5–6 months of age and deteriorated to death several months thereafter [101]. Inoculation of brain extracts from diseased Tg(MHu2M-E199K) mice induced a distinct fatal prion disease in wildtype mice. Mice expressing a mouse PrP version of a nine octapeptide insertion associated with familial CJD,

designated Tg(PG14), exhibited a slowly progressive neurological disorder characterized by apoptotic loss of cerebellar granule cells, gliosis but no spongiosis [102]. Whether the brains of sick Tg(PG14) mice, like sick Tg(GSS) mice, contain 15B3-immunoprecipitable PrP has not yet been reported; however, in both models, mutated PrP adopts different pathologic conformations either spontaneously or following inoculation with authentic prions [96, 103]. Like Tg(GSS) mice, brain homogenates from spontaneously sick Tg(PG14) mice failed to transmit disease to Tg mice that express low levels of mutated PrP that do not become sick spontaneously. Whether differences in the state of aggregation of PG14spon compared to MoPrP-P101L will affect its ability to accelerate disease progression in over-expressor Tg(PG14) mice remains to be determined.

Chiesa's group also described a Tg mouse model of inherited CJD expressing the mouse homolog of the D178N in combination with the V129 polymorphism. These Tg(CJD) mice had EEG and sleep abnormalities, memory impairment, motor dysfunction, and striking morphological alterations of the neuronal endoplasmic reticulum (ER) associated with ER retention of mutant PrP [104]. This study was followed by reports of the properties of Tg mice expressing the mouse PrP homolog of the same D178N mutation in cis with M129, which is associated with FFI [105]. Spontaneous disease in these so-called Tg(FFI) mice was different from Tg(CJD) mice. Tg(FFI) synthesize misfolded mutant PrP in their brains and, like FFI, illness is associated with sleep disruption. However, unlike this form of fCJD and FFI, bioassay and protein misfolding cyclic amplification (PMCA) showed that prions were not produced in Tg(FFI) and Tg(CJD) brains, suggesting to the authors that the disease-encoding properties of mutant PrP do not depend on its ability to propagate its misfolded conformation. Tg(FFI) mice complement a gene targeted model of this disease that provided a different outcome. Knock-in mice carrying the FFI mutation [106] developed biochemical, physiological, behavioral, and neuropathological abnormalities similar to FFI, and this spontaneous disease could be transmitted, and serially passaged, to mice expressing physiological amounts of PrP without the mutation. Likewise, the same investigators produced knock-in mouse models of CJD caused by the N178/V129 variants. These mice differed phenotypically from FFI knock-in mice, producing a spontaneous, transmissible CJD-like disease [107]. The reasons for the discrepant properties of Tg and knock-in models with respect to spontaneous prion formation are unclear.

Transgenic mice expressing a mutation at codon 117 associated with a telencephalic form of GSS [108], also spontaneously developed neurodegenerative disease and accumulated an aberrant, neurotoxic form of PrP termed CtmPrP, which appears to be distinct from conventional protease-resistant PrP^{Sc} [109]. Matrianni and colleagues also constructed Tg mice that express PrP carrying the mouse homolog of this GSS mutation. These Tg(A116V) mice express approximately six times the endogenous levels of PrP, and recapitulate many clinicopathologic features of GSS(A117V) that are distinct from CJD [110]. More recently, Asante and co-workers produced Tg mice expressing the A117V mutation in the context of HuPrP. Unlike previous attempts at transmission, prions from human patients with GSS A117V transmitted to these Tg mice, producing appropriate neuropathology, and accumulation of PrP^{Sc} [111].

Bovine prion disease—Several lines of Tg mice expressing bovine PrP, referred to as Tg(BoPrP) mice, have been independently produced [55, 112, 113]. All Tg(BoPrP) mice are susceptible to BSE prions, and their availability during the peak years of the BSE and vCJD epidemics were invaluable for ascertaining the pathogenesis of BSE and the properties of these and other bovine prion diseases.

While BSE initially appeared to be a homogeneous disease, the large-scale testing of livestock nervous tissues for the presence of PrP^{Sc} led to the recognition, in Europe, Japan and the USA, of two additional bovine PrP^{Sc} variants termed H- and L-types [114]. The molecular signature of bovine PrP^{Sc} from animals with the bovine amyloidotic spongiform encephalopathy (BASE) variant corresponds to L-type, and appears similar to a distinct subtype of sporadic CJD [115]. L-type has a tendency to form amyloid plaques in cattle brain and has a distribution of brain pathology distinct from BSE [115]. These ‘atypical BSE’ cases have been detected in aged asymptomatic cattle during systematic testing at slaughterhouse. The etiology of these atypical forms remains unexplained but could involve either (i) a change in the biological properties of the BSE agent, (ii) infection of cattle with prions from another source, such as scrapie or CWD, or (iii) previously unrecognized sporadic forms of prion disease in cattle. A case of BSE in the US with an H-type PrP^{Sc} signature in an approximately 10-year old cow from Alabama was also associated with mutation of glutamate (E) to lysine (K) at 211, referred to as E211K [116]. Of particular significance, the identical substitution at the equivalent codon 200 in human *PRNP* is linked to the most frequent form of familial CJD with clusters described in Chileans, Oravian Slovaks, Libyan Jews, Britons and Japanese.

The development of various Tg mouse models expressing bovine PrP were invaluable for characterizing and titrating BSE infectivity in a variety of tissues [55, 113, 117], and provided compelling evidence for a relationship between vCJD and BSE [22]. While these Tg mouse models were characterized by rapid incubation times and 100% attack rates, mice expressing bovine PrP generated by gene replacement of the mouse PrP coding sequence had long incubation times (>500 days) and incomplete attack rates [56]. The experimental transmission of H- and L-type cases to bovine PrP Tg mice unambiguously demonstrated their infectious nature and revealed strain properties distinct from BSE [118–121]. While BSE and BASE transmitted readily to Tgbov XV mice, they produced different clinical, neuropathological, and molecular disease phenotypes [121]. Interestingly, the same study indicated that BASE prions were able to convert into BSE prions upon serial transmission in inbred mice. The relationship of this finding to the apparently protean nature of BSE prions in aforementioned transmission studies [20, 21, 122] remains to be determined.

Strikingly, serial passage of the L-type strain to wild type mice, and mice expressing the VRQ allele of ovine PrP induced a disease phenotype indistinguishable from that of BSE [119, 121], suggesting a possible etiological relationship between atypical and classical BSE. The relevance of these findings to studies in Tg mice, which consistently reveal the existence of more than one molecular type of PrP^{Sc} [20, 21, 122] and suggest that more than one BSE prion strain might infect humans [20], remains to be determined.

Challenge of two lines of Tg mice expressing human PrP with M at codon 129 with L-type isolates produced a molecular phenotype distinct from classical BSE [67, 68]. In one case, L-type transmitted with no transmission barrier [68], and in both cases the L-type PrP^{Sc} biochemical signature was conserved upon transmission. In contrast, the transmission efficiency of classical BSE and H-type isolates to transgenic mice expressing human PrP is relatively low [20, 68, 123]. Increased pathogenicity of sheep-passaged BSE occurred in Tg mice expressing porcine PrP [124] or human PrP [84] raising the possibility that BSE may gain virulence by passage in another species.

Ovine prion disease—Transgenic mice expressing ovine PrP are susceptible to prions from scrapie-affected sheep [125–130]. A clear link to codon 136 genotype and susceptibility/resistance to different sheep scrapie isolates has been described in multiple previous studies. Generally, increased susceptibility to scrapie is associated with expression of sheep PrP with valine (V) at residue 136, referred to as OvPrP-V136 compared to alanine (A) at residue 136, referred to as OvPrP-A136, with A/A136 being the most resistant, and V/V136 the most susceptible genotypes. In the case of SSBP/1 incubation periods are ~170 days in V/V136 sheep, while transmission to A/A136 sheep is relatively inefficient, with no disease recorded after >1000 days [131]. The most widely characterized models include Tg mice expressing OvPrP-V136, including tg338 [129], and Tgov59 mice [132], or Tgov4 [127] mice expressing OvPrP-A136. In the case of tg338 mice, the transgene was comprised of a bacterial artificial chromosome insert of 125 kb of sheep DNA, while in the case of Tgov59 and Tgov4 mice the neuron specific enolase promoter was used to drive OvPrP expression. These lines are maintained on different heterogeneous genetic backgrounds, and CNS expression levels in tg338 mice are ~8- to 10-fold higher than wild type, while Tgov59 and Tgov4 lines each over express OvPrP-ARQ at levels ~2- to 4-fold higher than those found in sheep brain. Spontaneous neurological dysfunction has been reported in Tg lines over expressing OvPrP [125, 129].

Subsequently, two additional Tg models, referred to as Tg(OvPrP-A136)3533^{+/-} and Tg(OvPrP-V136)4166^{+/-} mice were produced and characterized that express either OvPrP-V136 or OvPrP-A136 approximately equivalent to PrP levels normally expressed in the CNS of wild type mice [133]. Importantly, the influence of residue 136 on the transmission of SSBP/1 and CH1641 prions in Tg(OvPrP-A136)3533^{+/-} and Tg(OvPrP-V136)4166^{+/-} mice is in accordance with the properties of these isolates in sheep of various genotypes [134]. While SSBP/1 eventually transmits to Tg(OvPrP-A136)3533^{+/-} mice with incubation times exceeding 400 days, the general effects of the A/V136 dimorphism on SSBP/1 transmission observed in sheep are recapitulated in Tg(OvPrP-A136)3533^{+/-} and Tg(OvPrP-V136)4166^{+/-} mice. Similarly, CH1641, which propagates efficiently in A/A136 sheep [131], preferentially propagates in Tg(OvPrP-A136)3533^{+/-} mice (Table 1). In other studies, CH1641 transmitted to TgOvPrP4 mice with an ~250 d mean incubation time [135].

Tg(OvPrP-A136)3533^{+/-} and Tg(OvPrP-V136)4166^{+/-} lines were produced using the cosSHa.Tet cosmid vector which drives expression from the PrP gene promoter [66], and therefore it was expected that expression of OvPrP^C-A136 and OvPrP^C-V136 occurs in identical neuronal populations. Accordingly, homozygous Tg mice were mated to produce mice, referred to as Tg(OvPrP-A/V) mice, that co-express both alleles [133]. Previous

studies reported on Tg mice expressing OvPrP with V at 136, referred to as Tg(OvPrP)14882^{+/-} mice, that were also produced in a *Prnp*^{0/0}/FVB background using the cosSHa.Tet cosmid vector [130]. However, in that study, comparable Tg mice expressing OvPrP-A136 were not reported. While SSBP/1 incubation times are prolonged in A/V136 compared to V/V 136 sheep [131], incubation times were shorter in Tg(OvPrP-A/V) than in Tg(OvPrP-V136)4166^{+/-} mice.

A novel mAb PRC5, the epitope of which comprises residue 136 [136], was used to monitor conversion of OvPrP^C-A136 in compound heterozygous. Surprisingly, in contrast to its relatively slow conversion when OvPrP^C-A136 is expressed in isolation, co-expression with OvPrP^C-V136 in Tg(OvPrP-A/V136) mice facilitated rapid conversion of OvPrP^C-A136 to OvPrP^{Sc}-A136. The conformation and diffuse CNS distribution of the resulting OvPrP^{Sc}-A136(U) were equivalent to that of OvPrP^{Sc}-V136(U) and not OvPrP^{Sc}-A136(S). These results demonstrate that under conditions of allele co-expression a dominant conformer may alter the conversion potential of an otherwise resistant PrP polymorphic variant to an unfavorable prion strain [133].

Ovine Tg mice have been shown to be a useful tool for discriminating scrapie strains [137] and in particular for differentiating BSE in sheep from natural scrapie isolates [128, 132, 138]. Tg mouse models are also at the forefront of characterizing a relatively newly-emerging 'atypical' scrapie strain [139] related to so-called Nor98 cases first identified in Norwegian sheep. Atypical scrapie was confirmed to be a prion disease following transmission to Tg338 mice expressing OvPrP-V136, and revealed the uniformity of features between atypical scrapie cases [139], confirming limited studies in the natural host [140]. In one study, Tg mice that overexpress human prion protein were found to be susceptible to BSE prions, but not classical or atypical scrapie prions [141]. In contrast, Andrioletti and co-workers show that a panel of classical sheep scrapie prions transmit to several Tg mice expressing human PrP mouse models with an efficiency comparable to that of cattle BSE indicating that classical scrapie prions have zoonotic potential [142].

Cervid prion disease—Some 15 years ago, CWD was perhaps the least understood of all the prion diseases of animals and humans. Known to be highly contagious, its origins and mode of transmission were unclear, and it was not known whether multiple CWD strains exist or whether CWD prions pose a risk to other animals or humans. The main objectives at that time were to develop rapid and sensitive bioassays for CWD prions, and to experimentally address the risks that CWD prions pose to humans and other species. The development of Tg mice that were the first reliable bioassay for rapid and sensitive detection of CWD prions was a significant advance. With these resources in hand, investigators have obtained important information about CWD pathogenesis, and the molecular mechanisms of prion propagation, species barriers and strains. Using CWD-susceptible Tg mice it became possible to bioassay CWD prions in tissues, body fluids and secretions of deer and elk, which provided insights into the mode of transmission of this highly contagious disease. The study of inter-mammalian species barriers in Tg mice allows investigators to model the risks posed to humans and livestock from exposure to CWD prions, and this information helps facilitate management decisions designed to minimize interspecies prion transmission. During this time, the development of more facile, sensitive approaches to amplify prions *in*

vitro, such as PMCA and RT-QuIC, have revolutionized our ability to detect prions, even at extremely low titers. In concert, cell culture models, have provided an alternate means of CWD titration that has largely superseded bioassay in Tg mice, and provided insights into strategies for developing compounds that inhibit CWD propagation. The development of compounds such as IND24 provides significant optimism for treating this currently incurable disease. Finally, studies of CWD using these newly developed tools has provided unexpected mechanistic insights into PrP^C to PrP^{Sc} conversion, particularly the role of the $\beta 2$ - $\alpha 2$ loop/ α -helix 3 epitope, and the proposal that prion strains exist as a continuum of conformational quasi-species.

The expense of housing cervids under prion-free conditions for long periods and the highly communicable nature of CWD present significant challenges for using deer as experimental hosts [143]. As noted above, transmission to other species has yielded mixed results. The resistance of mice [144] and the inefficient transmission of CWD to ferrets [145] are examples of species barriers to CWD prions, albeit of varying extent. The prototype Tg(CerPrP) mice developed signs of neurological dysfunction ~230 days following intracerebral inoculation with four CWD isolates [144]. The brains of sick Tg mice recapitulated the cardinal neuropathological features of CWD. As part of a larger study of CWD pathogenesis, Tg(CerPrP) mice were used as a sensitive means to show that skeletal muscles of CWD-infected deer harbor infectious prions, demonstrating that humans consuming or handling meat from CWD-infected animals are at risk to prion exposure [146]. Similar analyses of skeletal muscle BSE-affected cattle in a larger study of BSE pathogenesis using Tgbov XV mice did not reveal high levels of BSE infectivity [117]. Since the seminal reports of accelerated CWD transmission from deer and elk to Tg(CerPrP) mice [144], several other groups have reported similar results using comparable Tg mouse models [147–150]. CWD has also been transmitted, albeit with less efficiency, to Tg mice expressing mouse [151] or Syrian hamster PrP [152].

The generation of CWD-susceptible Tg mice, in concert with the development of PMCA-based approaches for amplifying CWD infectivity using PrP^C expressed in the CNS of those mice [153, 154], has also provided crucial information about the biology of CWD and cervid prions. Not only was amplification *in vitro* shown to maintain CWD prion strain properties, it also provided a means of generating novel cervid prion strains [153–156]. Transgenic and *in vitro* amplification approaches have also facilitated our understanding of the mechanism of CWD transmission among deer and elk [143, 157–159]. Transmission studies in Tg(CerPrP)1536^{+/-} and similar Tg mice demonstrated that CWD prions were present in urine and feces and saliva [149, 157], and these findings are substantiated by *in vitro* amplification techniques [157, 160, 161]. Tg approaches have been essential for assessing the potential risk of human exposure to CWD prions [146, 162, 163]. The availability of CWD-susceptible transgenic mouse models, for the first time, also provided a means of quantifying CWD infectivity by end-point titration [162].

As demonstrated in other species in which prion diseases occur naturally, susceptibility to CWD is highly dependent on polymorphic variation in deer and elk *PRNP*. Polymorphisms at codons 95 [glutamine (Q) or histidine (H)] [164], 96 [glycine (G) or serine (S)] [164, 165] and 116 [alanine (A) or glycine (G)] [166] in white-tailed deer have been reported. While all

major genotypes were found in deer with CWD, the Q96, G96, A116 allele (QGA) was more frequently found in CWD-affected deer than the QSA allele [164, 167], suggesting a protective effect of the counterpart polymorphisms. The elk *PRNP* coding sequence is polymorphic at codon 132 encoding either methionine (M) or leucine (L) [168, 169]. This position is equivalent to human *PRNP* codon 129. Studies of free-ranging and captive elk with CWD [170], as well as oral transmission experiments [171, 172], indicate that the L132 allele protects against CWD.

Transgenic mouse modeling provided a means of assessing the role of these cervid PrP gene polymorphisms on CWD pathogenesis. In recent work combining studies in Tg mice, the natural host, cell-free prion amplification and molecular modeling approaches, we analyzed the effects of deer polymorphic amino acid variations on CWD propagation and susceptibility to prions from different species [50]. Reflecting the general authenticity of the Tg modeling approach, the properties of CWD prions were faithfully maintained in deer following their passage through Tg mice expressing cognate PrP. Moreover, the protective influences of naturally occurring PrP polymorphisms on CWD susceptibility were accurately reproduced in Tg mice or during cell free amplification. The resistance of Tg mice expressing deer PrP S96 to CWD, referred to as Tg(DeerPrP-S96)7511 mice, is consistent with previously generated tg60 mice expressing serine at residue 96 [147]. In the studies of Angers and colleagues, whereas substitutions at residues 95 and 96 affected CWD propagation, their protective effects were overridden during replication of sheep prions in Tg mice and, in the case of residue 96, deer.

To more fully address the influence of the elk 132 polymorphism, transmissibility of CWD prions was assessed in Tg mice expressing cervid PrP^C with L or M at residue 132 [173]. While Tg mice expressing CerPrP-L132 afforded partial resistance to CWD, SSBP/1 sheep scrapie prions transmitted efficiently to Tg mice expressing CerPrP-L132, suggesting that the elk 132 polymorphism also controls prion susceptibility at the level of prion strain selection. The contrasting ability of CWD and SSBP/1 prions to overcome the inhibitory effects of the CerPrP-L132 allele is reminiscent of studies describing the effects of the human codon 129 methionine (M)/valine (V) polymorphism on vCJD/BSE prion propagation in Tg mice expressing human PrP, which concluded that human PrP V129 severely restricts propagation of the BSE prion strain [122]. It therefore appears that amino acid substitutions in the unstructured region of PrP affect PrP^C-to-PrP^{Sc} conversion in a strain-specific manner.

The susceptibility of Tg(DeerPrP-S96)7511 mice, albeit with incomplete attack rates and long incubation times, are at odds with previous work showing complete resistance of tg60 mice [147, 174]. This apparent discrepancy is most likely related to the low transgene expression in tg60 mice, reported to be 70% the levels found in deer. CWD occurs naturally in deer homozygous for the PrP-S96 allele [175], which is clearly inconsistent with a completely protective effect of this substitution, suggesting that Tg(DeerPrP-S96)7511 mice represent an accurate Tg model in which to assess the effects of the S96 substitution.

In accordance with a role for this region in strain selection, in subsequent studies, Tg mice expressing wild type deer PrP (tg33) or tg60 were challenged with CWD prions from experimentally infected deer with varying polymorphisms at residues 95 and 96 [176].

Passage of deer CWD prions into tg33 mice expressing wild type deer PrP resulted in 100% attack rates, with CWD prions from deer expressing H95 or S96 having significantly longer incubation periods. Remarkably, otherwise resistant tg60 mice [147, 174] developed disease only when inoculated with prions from deer expressing H95/Q95 and H95/S96 PrP genotypes. Serial passage in tg60 mice resulted in propagation of a novel CWD strain, referred to as H95(+), while transmission to tg33 mice produced two disease phenotypes consistent with propagation of two strains.

High resolution structural studies showed the loop region linking the second beta-sheet ($\beta 2$) with the alpha2-helix ($\alpha 2$) of cervid PrP to be extremely well defined compared to most other species, raising the possibility that this structural characteristic correlates with the unusually facile contagious transmission of CWD [177]. Tg mice expressing mouse PrP in which the $\beta 2$ - $\alpha 2$ loop was replaced by the corresponding region from cervid species, spontaneously developed prion disease [178]. Additional studies consistently point to the importance of the $\beta 2$ - $\alpha 2$ loop in regulating transmission barriers, including that of humans to CWD [156, 179–181]. Subsequent work suggested a more complex mechanism in which the $\beta 2$ - $\alpha 2$ loop participates with the distal region of α -helix 3 to form a solvent-accessible contiguous epitope [41]. These and later studies [40] ascribed greater importance to the plasticity of this discontinuous epitope. For example, substitution of D found in horse, which contains a similarly structured loop region, by S at residue 170 of mouse (elk PrP numbering) loop, increased not only the structural order of the loop, but also the long-range interaction with Y228 in α -helix 3. Underscoring the importance of long-range $\beta 2$ - $\alpha 2$ loop/ α -helix 3 interactions, similar structural connections occur between this residue, which is alanine (A) in Tammar wallaby PrP, and residue 169, which is isoleucine (I) in this species (19). Stabilizing long-range interactions between the $\beta 2$ - $\alpha 2$ loop and α -helix 3 also occur in rabbit PrP, a species generally regarded as resistant to prion infection. X-ray crystallographic analyses showed the rabbit $\beta 2$ - $\alpha 2$ loop to be clearly ordered and indicated that hydrophobic interactions between the side chains of V169 and, in this case Y221 (elk PrP numbering) of α -helix 3, contributed to the stability of the $\beta 2$ - $\alpha 2$ loop/ α helix 3 epitope [182].

In mule deer, polymorphism at codon 225 encoding serine (S) or phenylalanine (F) influences CWD susceptibility, the 225F allele being relatively protective. The occurrence of CWD was found to be 30-fold higher in deer homozygous for serine at position 225 (225SS) than in heterozygous (225SF) animals; the frequency of 225SF and 225FF genotypes in CWD-negative deer was 9.3%, but only 0.3% in CWD-positive deer [183]. Recent studies comparing CWD susceptibility in mule deer of the two residue 225 genotypes (225SS, 225FF) showed that 225FF mule deer had differences in clinical disease presentation, as well as more-subtle, atypical traits [184]. Immediately adjacent to the protective mule deer PrP polymorphism at 225, residue 226 encodes the singular primary structural difference between Rocky Mountain elk and deer PrP. Elk PrP contains glutamate (E), and deer PrP Q at this position.

Recent findings show that residues 225 and 226 play a critical role in PrP^C to-PrP^{Sc} conversion and strain propagation, but that their effects are distinct from those produced by the H95Q, G95S, and M132L polymorphisms [50]. Structural analyses confirm that residues 225 and 226 are located in the distal region of α -helix 3 that participates with the $\beta 2$ - $\alpha 2$

loop to form a solvent-accessible contiguous epitope [41]. Consistent with a role for this epitope in PrP conversion, these polymorphisms severely impact replication of both SSBP/1 and, to variable degrees, CWD. In the case of Tg mice expressing deerPrP-F225, referred to as Tg(DeerPrP-F225), SSBP/1 incubation times were prolonged threefold, whereas inoculation with CWD produced incomplete attack rates or prolonged and variable incubation times in small numbers of mice. In those Tg(DeerPrP-F225) mice that did succumb to CWD, PrP^{Sc} distribution patterns were altered compared with Tg(DeerPrP) mice.

To address the effects of substitution of E for Q at residue 226, we assessed whether Tg mice expressing wild type elk or deer PrP differed in their responses to CWD. These studies showed that differences at residue 226 also affected CWD replication, but to a lesser degree than the residue 225 polymorphism, with disease onset prolonged by 20–46% in CWD-inoculated Tg(DeerPrP) compared with Tg(ElkPrP) mice, and PrP^{Sc} distribution and neuropathology varying in each case [162]. In contrast to Tg(DeerPrP) mice which are susceptible to SSBP/1 [173], Tg(ElkPrP) were completely resistant [162], although the resistance of elk PrP^C to propagation of SSBP/1 was overcome following adaptation in deer or Tg(DeerPrP) mice. Passage in Tg mice expressing E226 or Q226 profoundly affected the ability of SSBP/1 to reinfect Tg mice expressing sheep PrP^C. These studies paralleled aspects of our previously published studies indicating that amino acid differences at residue 226 controlled the manifestation of CWD quasi-species or closely related strains [29]. These findings therefore collectively point to an important role for residues 225 and 226 in PrP^C-PrP^{Sc} conversion and the manifestation of prion strain properties, and substantiate the view that long-range interactions between the β 2- α 2 loop and α -helix 3 provide protection against prion infection and suggest a likely mechanism to account for the protective effects of the F225 polymorphism. Molecular dynamics analyses [50] showed that the S225F and E226Q substitutions in deer alter the orientations of D170 in the β 2- α 2 loop and Y228 in α -helix 3. This structural change allows hydrogen bonding between the side chains of these residues, which results in reduced plasticity of the β 2- α 2 loop/ α -helix 3 epitope compared with deer or elk PrP structures. This suggests that the increased stability of this tertiary structural epitope precludes PrP^C-to-PrP^{Sc} conversion of deerPrP-F225.

Although our seminal studies in Tg mice [144], and subsequent work [148] raised the possibility of CWD strain variation, the limited number of isolates and the lack of detailed strain analyses in those studies meant that this hypothesis remained speculative. Subsequent studies supported the feasibility of using Tg(CerPrP)1536^{+/-} mice for characterizing naturally occurring CWD strains, and novel cervid prions generated by PMCA [154]. To address whether different CWD strains occur in various geographic locations or in different cervid species, bioassays in transgenic mice were used to analyze CWD in a large collection of captive and wild mule deer, white-tailed deer and elk from various geographic locations in North America [29]. These findings provided substantial evidence for two prevalent CWD prion strains, referred to as CWD1 and CWD2, with different clinical and neuropathological properties. Remarkably, primary transmissions of CWD prions from elk produced either CWD1 or CWD2 profiles, while transmission of deer inocula favored the production of mixed intra-study incubation times and CWD1 and CWD2 neuropathologies. These findings

indicate that elk may be infected with *either* CWD1 *or* CWD2, while deer brains tend to harbor CWD1/CWD2 strain mixtures.

The different primary structures of deer and elk PrP at residue 226 provides a framework for understanding these differences in strain profiles of deer and elk. Because of the role played by residue 226, the description of a lysine polymorphism at this position in deer [185], and its possible role on strain stability may be significant. It is unknown whether CWD1 and CWD2 interfere or act synergistically, or whether their co-existence contributes to the unparalleled efficiency of CWD transmission. Interestingly, transmission results reported in previous studies suggested that cervid brain inocula might be composed of strain mixtures [149].

Additional studies support the existence of multiple CWD strains. CWD has also been transmitted, albeit with varying efficiency, to transgenic mice expressing mouse PrP [149, 151]. In the former study, a single mule deer isolate produced disease in all inoculated Tga20 mice, which express mouse PrP at high levels. On successive passages, incubation times dropped to ~ 160 d. In the second study, one elk isolate from a total of eight deer and elk CWD isolates induced disease in 75 % of inoculated Tg4053 mice, which also over express mouse PrP. The distribution of lesions in both studies appeared to resemble the CWD1 pattern. Low efficiency CWD prion transmission was also recorded in hamsters and transgenic mice expressing Syrian hamster PrP [152]. In that study, during serial passage of mule deer CWD, fast and slow incubation time strains with different patterns of brain pathology and PrP^{Sc} deposition were also isolated. In yet other studies, serial passages of CWD from white-tailed deer into transgenic mice expressing hamster PrP, and then Syrian golden hamsters, produced a strain, referred to as ‘wasting’ (WST), characterized by a prominent preclinical wasting disease, similar to cachexia, which the authors propose is due to a prion-induced endocrinopathy [186]. These same investigators identified a second strain, defined as ‘cheeky’ (CKY), derived from infection of Tg mice that express hamster PrP [187]. The CKY strain had a shorter incubation period than WST, but after transmission to hamsters, the incubation period of CKY became >150 days longer than WST. In this case, PK digestion revealed strain-specific PrP^{Sc} signatures that were maintained in both hosts, but the solubility and conformational stability of PrP^{Sc} differed for the CWD strains in a host-dependent manner. In addition to supporting the view that there are multiple CWD strains, these findings suggest the importance of host-specific pathways, independent of PrP, that participate in the selection and propagation of distinct strains.

Studies in cell culture also support the existence of CWD strains. Quinacrine altered the transmission properties of CWD prions, as well as the biochemical characteristics of the constitutive PrP^{Sc} [30]. Despite accumulating significantly higher prion titers, quinacrine treated (Q-CWD) prions from Elk21⁺ cells produced prolonged incubation times in Tg(DeerPrP) and Tg(ElkPrP) mice compared to CWD from untreated Elk21⁺ cells. Since kinetics of disease onset in prion-infected animals is inversely related to the titer of a given prion strain, this unusual outcome is consistent with quinacrine affecting the intrinsic properties of the CWD prion. In accordance with this notion, while the deposition patterns of PrP^{Sc} in the brains of diseased Tg(DeerPrP) and Tg(ElkPrP) mice receiving prions from Elk21⁺ are concordant with our previously published descriptions following transmissions of

naturally-occurring or PMCA-generated CWD prions [154], these distinctive patterns were not recapitulated in either line of Tg mice receiving Q-CWD prions. Prion incubation times and neuronal targeting are the biological criteria by which prion strains are defined. Previous studies showed that strain properties are enciphered within the conformation of PrP^{Sc} [13, 154], and that cervid prion incubation times positively correlate with PrP^{Sc} conformational stability [154]. It is therefore significant that the longer incubation times of Q-CWD prions and altered patterns of PrP^{Sc} deposition in both Tg models were associated with an increase in the relative stability of PrP^{Sc} conformers constituting Q-CWD prions.

The properties of Q-CWD prions provided convincing evidence for conformational mutation that were suggested, but could not be observed, in previous studies of mouse-adapted scrapie prions in cell cultures treated with swainsonine, an inhibitor of Golgi α -mannosidase II [28]. Since the swainsonine-induced properties of cell-derived mouse prions reverted to their original characteristics when propagated *in vivo*, and there were no detectable differences in the conformations of PrP^{Sc} generated under conditions of swainsonine treatment compared to untreated cells, claims of strain mutation were indirectly based on the differing properties of prions from swainsonine treated and untreated cells using a cell panel assay. In contrast, CWD and Q-CWD prions are comprised of distinct PrP^{Sc} conformers that produce discernable phenotypes in two lines of Tg mice.

In accordance with Western blotting-based analyses showing higher conformational stabilities of PrP^{Sc} produced in the brains of CWD-infected Tg(DeerPrP) mice compared with Tg(ElkPrP) mice [29], C-SPA confirmed that the conformation of deer PrP^{Sc} in RK13 cells expressing deer PrP (RKD⁺) was more stable than elk PrP^{Sc} produced during infection of Elk21⁺. Because Elk21⁺ and RKD⁺ propagated CWD prions from the same source differences in conformational stability result from the effects of residue 226, the sole primary structural difference between elk and deer PrP.

The identification and characterization of distinct CWD strains, and the influence of PrP primary structure on their stabilities, is of importance when considering the potential for inter-species transmission. The appearance of vCJD following human exposure to BSE [18, 188], place the human species barrier to other animal prion diseases, particularly CWD, at the forefront of public health concerns. Since North American hunters harvest thousands of deer and elk each year, and it is not currently mandatory to have these animals tested, the demonstration of CWD prions in skeletal muscle and fat of deer [146, 163], make it is likely that humans consume CWD prions. The substantial market for elk antler velvet in traditional Asian medicine also warrants concern [162].

Estimates of the zoonotic potential of CWD are currently mixed. Surveillance currently shows no evidence of transmission to humans [189, 190]. While initial cell-free conversion studies suggested that the ability of CWD prions to transform human PrP^C into protease resistant PrP was low [165], subsequent results showed that cervid PrP^{Sc} induced the conversion of human PrP^C by protein misfolding cyclic amplification (PMCA), following CWD prion strain stabilization by successive passages *in vitro* or *in vivo* [191]. These results have implications for the human species barrier to CWD, and underscore the role of strain adaptation on interspecies transmission barriers. Additional studies using transgenic mice

expressing human PrP^C showed that CWD failed to induce disease following intracerebral CWD infection [149, 150, 192]. However, CWD transmission was reported to nonhuman primates through the intracerebral inoculation of squirrel monkeys (*Saimiri sciureus*) [193, 194]. Moreover, using RT-QuIC to model the transmission barrier of CWD to human rPrP, the work of Davenport and co-workers results suggest that, at the level of protein-protein interactions, CWD adapts to a new species more readily than does BSE, and that the barrier preventing transmission of CWD to humans may be less robust than estimated [195].

References

1. Prusiner, SB. Prions (Les Prix Nobel Lecture). In: Frängsmyr, T., editor. Les Prix Nobel. Stockholm, Sweden: Almqvist & Wiksell International; 1998. p. 268-323.
2. Prusiner SB, Scott M, Foster D, Pan K-M, Groth D, Mirenda C, Torchia M, Yang S-L, Serban D, Carlson GA, et al. Transgenic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell*. 1990; 63:673–686. [PubMed: 1977523]
3. Scott M, Groth D, Foster D, Torchia M, Yang S-L, DeArmond SJ, Prusiner SB. Propagation of prions with artificial properties in transgenic mice expressing chimeric PrP genes. *Cell*. 1993; 73:979–988. [PubMed: 8098995]
4. Kocisko DA, Come JH, Priola SA, Chesebro B, Raymond GJ, Lansbury PT Jr, Caughey B. Cell-free formation of protease-resistant prion protein. *Nature*. 1994; 370:471–474. [PubMed: 7913989]
5. Vidal E, Fernandez-Borges N, Pintado B, Erana H, Ordonez M, Marquez M, Chianini F, Fondevila D, Sanchez-Martin MA, Andreoletti O, et al. Transgenic Mouse Bioassay: Evidence That Rabbits Are Susceptible to a Variety of Prion Isolates. *PLoS Pathog*. 2015; 11(8):e1004977. [PubMed: 26247589]
6. Chianini F, Fernandez-Borges N, Vidal E, Gibbard L, Pintado B, de Castro J, Priola SA, Hamilton S, Eaton SL, Finlayson J, et al. Rabbits are not resistant to prion infection. *Proc Natl Acad Sci U S A*. 2012; 109(13):5080–5085. [PubMed: 22416127]
7. Westaway D, Goodman PA, Mirenda CA, McKinley MP, Carlson GA, Prusiner SB. Distinct prion proteins in short and long scrapie incubation period mice. *Cell*. 1987; 51:651–662. [PubMed: 2890436]
8. Hunter N, Goldmann W, Marshall E, O'Neill G. Sheep and goats: natural and experimental TSEs and factors influencing incidence of disease. *Arch Virol Suppl*. 2000; (16):181–188. [PubMed: 11214921]
9. Baker HF, Poulter M, Crow TJ, Frith CD, Lofthouse R, Ridley RM. Amino acid polymorphism in human prion protein and age at death in inherited prion disease. *Lancet*. 1991; 337:1286.
10. Collinge J, Palmer MS, Dryden AJ. Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. *Lancet*. 1991; 337:1441–1442. [PubMed: 1675319]
11. Palmer MS, Dryden AJ, Hughes JT, Collinge J. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. *Nature*. 1991; 352:340–342. [PubMed: 1677164]
12. Nonno R, Di Bari MA, Cardone F, Vaccari G, Fazzi P, Dell'Omo G, Cartoni C, Ingrosso L, Boyle A, Galeno R, et al. Efficient transmission and characterization of Creutzfeldt-Jakob disease strains in bank voles. *PLoS Pathog*. 2006; 2(2):e12. [PubMed: 16518470]
13. Telling GC, Parchi P, DeArmond SJ, Cortelli P, Montagna P, Gabizon R, Mastrianni J, Lugaresi E, Gambetti P, Prusiner SB. Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science*. 1996; 274:2079–2082. [PubMed: 8953038]
14. Bessen RA, Marsh RF. Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. *J Virol*. 1994; 68:7859–7868. [PubMed: 7966576]
15. Safar J, Wille H, Itri V, Groth D, Serban H, Torchia M, Cohen FE, Prusiner SB. Eight prion strains have PrP^{Sc} molecules with different conformations. *Nat Med*. 1998; 4(10):1157–1165. [PubMed: 9771749]

16. Peretz D, Williamson RA, Legname G, Matsunaga Y, Vergara J, Burton DR, DeArmond SJ, Prusiner SB, Scott MR. A change in the conformation of prions accompanies the emergence of a new prion strain. *Neuron*. 2002; 34(6):921–932. [PubMed: 12086640]
17. Collinge J, Sidle KCL, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of “new variant” CJD. *Nature*. 1996; 383:685–690. [PubMed: 8878476]
18. Hill AF, Desbruslais M, Joiner S, Sidle KCL, Gowland I, Collinge J, Doey LJ, Lantos P. The same prion strain causes vCJD and BSE. *Nature*. 1997; 389:448–450. [PubMed: 9333232]
19. Scott MR, Peretz D, Nguyen HO, Dearmond SJ, Prusiner SB. Transmission barriers for bovine, ovine, and human prions in transgenic mice. *Journal of virology*. 2005; 79(9):5259–5271. [PubMed: 15827140]
20. Asante EA, Linehan JM, Desbruslais M, Joiner S, Gowland I, Wood AL, Welch J, Hill AF, Lloyd SE, Wadsworth JD, et al. BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. *Embo J*. 2002; 21(23):6358–6366. [PubMed: 12456643]
21. Korth C, Kaneko K, Groth D, Heye N, Telling G, Mastrianni J, Parchi P, Gambetti P, Will R, Ironside J, et al. Abbreviated incubation times for human prions in mice expressing a chimeric mouse-human prion protein transgene. *Proc Natl Acad Sci U S A*. 2003; 100(8):4784–4789. [PubMed: 12684540]
22. Scott MR, Will R, Ironside J, Nguyen H-OB, Tremblay P, DeArmond SJ, Prusiner SB. Compelling transgenic evidence for transmission of bovine spongiform encephalopathy prions to humans. *Proc Natl Acad Sci USA*. 1999:26.
23. Bruce ME, Dickinson AG. Biological evidence that the scrapie agent has an independent genome. *J Gen Virol*. 1987; 68:79–89. [PubMed: 3100717]
24. Collinge J, Clarke AR. A general model of prion strains and their pathogenicity. *Science*. 2007; 318(5852):930–936. [PubMed: 17991853]
25. Haldiman T, Kim C, Cohen Y, Chen W, Blevins J, Qing L, Cohen ML, Langeveld J, Telling GC, Kong Q, et al. Co-existence of distinct prion types enables conformational evolution of human PrP^{Sc} by competitive selection. *J Biol Chem*. 2013; 288(41):29846–29861. [PubMed: 23974118]
26. Collinge J. Variant Creutzfeldt-Jakob disease. *Lancet*. 1999; 354(9175):317–323. [PubMed: 10440324]
27. Eigen M. On the nature of virus quasispecies. *Trends Microbiol*. 1996; 4(6):216–218. [PubMed: 8795155]
28. Li J, Browning S, Mahal SP, Oelschlegel AM, Weissmann C. Darwinian evolution of prions in cell culture. *Science*. 2010; 327(5967):869–872. [PubMed: 20044542]
29. Angers RC, Kang HE, Napier D, Browning S, Seward T, Mathiason C, Balachandran A, McKenzie D, Castilla J, Soto C, et al. Prion strain mutation determined by prion protein conformational compatibility and primary structure. *Science*. 2010; 328(5982):1154–1158. [PubMed: 20466881]
30. Bian J, Kang HE, Telling GC. Quinacrine promotes replication and conformational mutation of chronic wasting disease prions. *Proc Natl Acad Sci U S A*. 2014; 111(16):6028–6033. [PubMed: 24711410]
31. Makarava N, Baskakov IV. The same primary structure of the prion protein yields two distinct self-propagating states. *The Journal of biological chemistry*. 2008; 283(23):15988–15996. [PubMed: 18400757]
32. Rezaei H, Choiset Y, Eghiaian F, Treguer E, Mentre P, Debey P, Grosclaude J, Haertle T. Amyloidogenic unfolding intermediates differentiate sheep prion protein variants. *J Mol Biol*. 2002; 322(4):799–814. [PubMed: 12270715]
33. Beringue V, Herzog L, Jaumain E, Reine F, Sibille P, Le Dur A, Vilotte JL, Laude H. Facilitated cross-species transmission of prions in extraneural tissue. *Science*. 2012; 335(6067):472–475. [PubMed: 22282814]
34. Rogers M, Yehiely F, Scott M, Prusiner SB. Conversion of truncated and elongated prion proteins into the scrapie isoform in cultured cells. *Proc Natl Acad Sci USA*. 1993; 90:3182–3186. [PubMed: 8475059]

35. Chesebro B, Trifilo M, Race R, Meade-White K, Teng C, LaCasse R, Raymond L, Favara C, Baron G, Priola S, et al. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. *Science*. 2005; 308(5727):1435–1439. [PubMed: 15933194]
36. Telling GC, Scott M, Hsiao KK, Foster D, Yang SL, Torchia M, Sidle KC, Collinge J, DeArmond SJ, Prusiner SB. Transmission of Creutzfeldt-Jakob disease from humans to transgenic mice expressing chimeric human-mouse prion protein. *Proc Natl Acad Sci U S A*. 1994; 91(21):9936–9940. [PubMed: 7937921]
37. Telling GC, Scott M, Mastrianni J, Gabizon R, Torchia M, Cohen FE, DeArmond SJ, Prusiner SB. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell*. 1995; 83(1):79–90. [PubMed: 7553876]
38. Perrier V, Kaneko K, Safar J, Vergara J, Tremblay P, DeArmond SJ, Cohen FE, Prusiner SB, Wallace AC. Dominant-negative inhibition of prion replication in transgenic mice. *Proc Natl Acad Sci U S A*. 2002; 99(20):13079–13084. [PubMed: 12271119]
39. Geoghegan JC, Miller MB, Kwak AH, Harris BT, Supattapone S. Trans-dominant inhibition of prion propagation in vitro is not mediated by an accessory cofactor. *PLoS Pathog*. 2009; 5(7):e1000535. [PubMed: 19649330]
40. Christen B, Hornemann S, Damberger FF, Wuthrich K. Prion protein NMR structure from tammar wallaby (*Macropus eugenii*) shows that the beta2-alpha2 loop is modulated by long-range sequence effects. *J Mol Biol*. 2009; 389(5):833–845. [PubMed: 19393664]
41. Perez DR, Damberger FF, Wuthrich K. Horse prion protein NMR structure and comparisons with related variants of the mouse prion protein. *Journal of molecular biology*. 2010; 400(2):121–128. [PubMed: 20460128]
42. Telling GC, Haga T, Torchia M, Tremblay P, DeArmond SJ, Prusiner SB. Interactions between wild-type and mutant prion proteins modulate neurodegeneration in transgenic mice. *Genes & Dev*. 1996; 10:1736–1750. [PubMed: 8698234]
43. Chiesa R, Drisaldi B, Quaglio E, Migheli A, Piccardo P, Ghetti B, Harris DA. Accumulation of protease-resistant prion protein (PrP) and apoptosis of cerebellar granule cells in transgenic mice expressing a PrP insertional mutation. *Proc Natl Acad Sci U S A*. 2000; 97(10):5574–5579. [PubMed: 10805813]
44. Bian J, Nazor KE, Angers R, Jernigan M, Seward T, Centers A, Green M, Telling GC. GFP-tagged PrP supports compromised prion replication in transgenic mice. *Biochem Biophys Res Commun*. 2006; 340(3):894–900. [PubMed: 16386707]
45. Barmada SJ, Harris DA. Visualization of prion infection in transgenic mice expressing green fluorescent protein-tagged prion protein. *J Neurosci*. 2005; 25(24):5824–5832. [PubMed: 15958749]
46. Shmerling D, Hegyi I, Fischer M, Blattler T, Brandner S, Gotz J, Rulicke T, Flechsig E, Cozzio A, von Mering C, et al. Expression of amino-terminally truncated PrP in the mouse leading to ataxia and specific cerebellar lesions. *Cell*. 1998; 93(2):203–214. [PubMed: 9568713]
47. Baumann F, Tolnay M, Brabeck C, Pahnke J, Klotz U, Niemann HH, Heikenwalder M, Rulicke T, Burkle A, Aguzzi A. Lethal recessive myelin toxicity of prion protein lacking its central domain. *Embo J*. 2007; 26(2):538–547. [PubMed: 17245436]
48. Li A, Christensen HM, Stewart LR, Roth KA, Chiesa R, Harris DA. Neonatal lethality in transgenic mice expressing prion protein with a deletion of residues 105–125. *Embo J*. 2007; 26(2):548–558. [PubMed: 17245437]
49. Scott M, Foster D, Mirenda C, Serban D, Coufal F, Wälchli M, Torchia M, Groth D, Carlson G, DeArmond SJ, et al. Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell*. 1989; 59:847–857. [PubMed: 2574076]
50. Angers R, Christiansen J, Nalls AV, Kang HE, Hunter N, Hoover E, Mathiason CK, Sheetz M, Telling GC. Structural effects of PrP polymorphisms on intra- and interspecies prion transmission. *Proc Natl Acad Sci U S A*. 2014; 111(30):11169–11174. [PubMed: 25034251]
51. Telling GC. Nucleic acid-free mutation of prion strains. *Prion*. 2010; 4(4):252–255. [PubMed: 20948302]

52. Büeler H, Fischer M, Lang Y, Bluethmann H, Lipp H-P, DeArmond SJ, Prusiner SB, Aguet M, Weissmann C. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature*. 1992; 356:577–582. [PubMed: 1373228]
53. Gombojav A, Shimauchi I, Horiuchi M, Ishiguro N, Shinagawa M, Kitamoto T, Miyoshi I, Mohri S, Takata M. Susceptibility of transgenic mice expressing chimeric sheep, bovine and human PrP genes to sheep scrapie. *J Vet Med Sci*. 2003; 65(3):341–347. [PubMed: 12679564]
54. Kupfer L, Eiden M, Buschmann A, Groschup MH. Amino acid sequence and prion strain specific effects on the in vitro and in vivo convertibility of ovine/murine and bovine/murine prion protein chimeras. *Biochimica et biophysica acta*. 2007; 1772(6):704–713. [PubMed: 17145171]
55. Scott MR, Safar J, Telling G, Nguyen O, Groth D, Torchia M, Koehler R, Tremblay P, Walthers D, Cohen FE, et al. Identification of a prion protein epitope modulating transmission of bovine spongiform encephalopathy prions to transgenic mice. *Proc Natl Acad Sci U S A*. 1997; 94(26): 14279–14284. [PubMed: 9405603]
56. Bishop MT, Hart P, Aitchison L, Baybutt HN, Plinston C, Thomson V, Tuzi NL, Head MW, Ironside JW, Will RG, et al. Predicting susceptibility and incubation time of human-to-human transmission of vCJD. *Lancet neurology*. 2006; 5(5):393–398. [PubMed: 16632309]
57. Hadlow WJ. Scrapie and kuru. *Lancet*. 1959; 2:289–290.
58. Gajdusek DC, Gibbs CJ Jr, Alpers M. Experimental transmission of a kuru-like syndrome to chimpanzees. *Nature*. 1966; 209:794–796. [PubMed: 5922150]
59. Gibbs CJ Jr, Gajdusek DC, Asher DM, Alpers MP, Beck E, Daniel PM, Matthews WB. Creutzfeldt-Jakob disease (spongiform encephalopathy): transmission to the chimpanzee. *Science*. 1968; 161:388–389. [PubMed: 5661299]
60. Brown P, Gibbs CJ Jr, Rodgers-Johnson P, Asher DM, Sulima MP, Bacote A, Goldfarb LG, Gajdusek DC. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. *Ann Neurol*. 1994; 35:513–529. [PubMed: 8179297]
61. Gibbs CJ, Jr, Gajdusek DC, Amyx H. Strain variation in the viruses of Creutzfeldt-Jakob disease and kuru. In: Prusiner SB, Hadlow WJ, editors. *Slow Transmissible Diseases of the Nervous System*. Vol. 2. New York: Academic Press; 1979. p. 87-110.
62. Tateishi J, Sato Y, Ohta M. Creutzfeldt-Jakob disease in humans and laboratory animals. In: Zimmerman HM, editor. *Progress in Neuropathology*. Vol. 5. New York: Raven Press; 1983. p. 195-221.
63. Tateishi J, Kitamoto T. Inherited prion diseases and transmission to rodents. *Brain Pathol*. 1995; 5:53–59. [PubMed: 7767491]
64. Manuelidis E, Kim J, Angelo J, Manuelidis L. Serial propagation of Creutzfeldt-Jakob disease in guinea pigs. *Proc Natl Acad Sci USA*. 1976; 73:223–227. [PubMed: 1108016]
65. Manuelidis E, Gorgacz EJ, Manuelidis L. Interspecies transmission of Creutzfeldt-Jakob disease to Syrian hamsters with reference to clinical syndromes and strains of agent. *Proc Natl Acad Sci USA*. 1978; 75:3422–3436. [PubMed: 277944]
66. Scott MR, Köhler R, Foster D, Prusiner SB. Chimeric prion protein expression in cultured cells and transgenic mice. *Protein Sci*. 1992; 1:986–997. [PubMed: 1338978]
67. Kong Q, Zheng M, Casalone C, Qing L, Huang S, Chakraborty B, Wang P, Chen F, Cali I, Corona C, et al. Evaluation of the human transmission risk of an atypical bovine spongiform encephalopathy prion strain. *Journal of virology*. 2008; 82(7):3697–3701. [PubMed: 18234793]
68. Beringue V, Herzog L, Reine F, Le Dur A, Casalone C, Vilotte JL, Laude H. Transmission of atypical bovine prions to mice transgenic for human prion protein. *Emerg Infect Dis*. 2008; 14(12): 1898–1901. [PubMed: 19046515]
69. Bishop MT, Will RG, Manson JC. Defining sporadic Creutzfeldt-Jakob disease strains and their transmission properties. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(26):12005–12010. [PubMed: 20547859]
70. Hill AF, Joiner S, Wadsworth JD, Sidle KC, Bell JE, Budka H, Ironside JW, Collinge J. Molecular classification of sporadic Creutzfeldt-Jakob disease. *Brain*. 2003; 126(Pt 6):1333–1346. [PubMed: 12764055]

71. Parchi P, Castellani R, Capellari S, Ghetti B, Young K, Chen SG, Farlow M, Dickson DW, Sima AAF, Trojanowski JQ, et al. Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. *Ann Neurol*. 1996; 39:767–778. [PubMed: 8651649]
72. Polymenidou M, Stoeck K, Glatzel M, Vey M, Bellon A, Aguzzi A. Coexistence of multiple PrP^{Sc} types in individuals with Creutzfeldt-Jakob disease. *Lancet Neurol*. 2005; 4(12):805–814. [PubMed: 16297838]
73. Mastrianni JA, Capellari S, Telling GC, Han D, Bosque P, Prusiner SB, DeArmond SJ. Inherited prion disease caused by the V210I mutation: transmission to transgenic mice. *Neurology*. 2001; 57(12):2198–2205. [PubMed: 11756597]
74. Mastrianni J, Nixon F, Layzer R, DeArmond SJ, Prusiner SB. Fatal sporadic insomnia: fatal familial insomnia phenotype without a mutation of the prion protein gene. *Neurology*. 1997; 48(Suppl):A296.
75. Notari S, Xiao X, Espinosa JC, Cohen Y, Qing L, Aguilar-Calvo P, Kofskey D, Cali I, Cracco L, Kong Q, et al. Transmission characteristics of variably protease-sensitive prionopathy. *Emerg Infect Dis*. 2014; 20(12):2006–2014. [PubMed: 25418590]
76. Diack AB, Ritchie DL, Peden AH, Brown D, Boyle A, Morabito L, MacLennan D, Burgoyne P, Jansen C, Knight RS, et al. Variably protease-sensitive prionopathy, a unique prion variant with inefficient transmission properties. *Emerg Infect Dis*. 2014; 20(12):1969–1979. [PubMed: 25418327]
77. Giles K, Glidden DV, Patel S, Korth C, Groth D, Lemus A, DeArmond SJ, Prusiner SB. Human prion strain selection in transgenic mice. *Annals of neurology*. 2010; 68(2):151–161. [PubMed: 20695008]
78. Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet*. 1996; 347:921–925. [PubMed: 8598754]
79. Collinge J, Palmer MS, Sidle KC, Hill AF, Gowland I, Meads J, Asante E, Bradley R, Doey LJ, Lantos PL. Unaltered susceptibility to BSE in transgenic mice expressing human prion protein. *Nature*. 1995; 378(6559):779–783. [PubMed: 8524411]
80. Collinge J, Sidle KC, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of ‘new variant’ CJD. *Nature*. 1996; 383(6602):685–690. [PubMed: 8878476]
81. Scott MR, Will R, Ironside J, Nguyen HO, Tremblay P, DeArmond SJ, Prusiner SB. Compelling transgenic evidence for transmission of bovine spongiform encephalopathy prions to humans. *Proc Natl Acad Sci U S A*. 1999; 96(26):15137–15142. [PubMed: 10611351]
82. Mead S, Poulter M, Uphill J, Beck J, Whitfield J, Webb TE, Campbell T, Adamson G, Deriziotis P, Tabrizi SJ, et al. Genetic risk factors for variant Creutzfeldt-Jakob disease: a genome-wide association study. *Lancet neurology*. 2009; 8(1):57–66. [PubMed: 19081515]
83. Plinston C, Hart P, Chong A, Hunter N, Foster J, Piccardo P, Manson JC, Barron RM. Increased susceptibility of human-PrP transgenic mice to bovine spongiform encephalopathy infection following passage in sheep. *Journal of virology*. 2011; 85(3):1174–1181. [PubMed: 21084466]
84. Plinston C, Hart P, Hunter N, Manson JC, Barron RM. Increased susceptibility of transgenic mice expressing human PrP to experimental sheep bovine spongiform encephalopathy is not due to increased agent titre in sheep brain tissue. *The Journal of general virology*. 2014; 95(Pt 8):1855–1859. [PubMed: 24828334]
85. Asante EA, Linehan JM, Gowland I, Joiner S, Fox K, Cooper S, Osiyawa O, Gorry M, Welch J, Houghton R, et al. Dissociation of pathological and molecular phenotype of variant Creutzfeldt-Jakob disease in transgenic human prion protein 129 heterozygous mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103(28):10759–10764. [PubMed: 16809423]
86. Mead S, Whitfield J, Poulter M, Shah P, Uphill J, Campbell T, Al-Dujaily H, Hummerich H, Beck J, Mein CA, et al. A novel protective prion protein variant that colocalizes with kuru exposure. *The New England journal of medicine*. 2009; 361(21):2056–2065. [PubMed: 19923577]
87. Asante EA, Smidak M, Grimshaw A, Houghton R, Tomlinson A, Jeelani A, Jakubcova T, Hamdan S, Richard-Londt A, Linehan JM, et al. A naturally occurring variant of the human prion protein completely prevents prion disease. *Nature*. 2015; 522(7557):478–481. [PubMed: 26061765]

88. Hsiao K, Baker HF, Crow TJ, Poulter M, Owen F, Terwilliger JD, Westaway D, Ott J, Prusiner SB. Linkage of a prion protein missense variant to Gerstmann-Sträussler syndrome. *Nature*. 1989; 338:342–345. [PubMed: 2564168]
89. Manson JC, Jamieson E, Baybutt H, Tuzi NL, Barron R, McConnell I, Somerville R, Ironside J, Will R, Sy MS, et al. A single amino acid alteration (101L) introduced into murine PrP dramatically alters incubation time of transmissible spongiform encephalopathy. *EMBO*. 1999; 18(23):6855–6864.
90. Hsiao KK, Groth D, Scott M, Yang S-L, Serban H, Rapp D, Foster D, Torchia M, DeArmond SJ, Prusiner SB. Serial transmission in rodents of neurodegeneration from transgenic mice expressing mutant prion protein. *Proc Natl Acad Sci USA*. 1994; 91:9126–9130. [PubMed: 7916462]
91. Kaneko KBH, Wille H, Zhang H, Groth D, Torchia M, Tremblay P, Safar J, Prusiner SB, DeArmond SJ, Baldwin MA, Cohen FE. A Synthetic Peptide Initiates Gerstmann-Straussler-Scheinker (GSS) Disease in Transgenic Mice. *J Mol Biol*. 2000; 295(4):997–1007. [PubMed: 10656806]
92. Tremblay P, Ball HL, Kaneko K, Groth D, Hegde RS, Cohen FE, DeArmond SJ, Prusiner SB, Safar JG. Mutant PrP^{Sc} conformers induced by a synthetic peptide and several prion strains. *Journal of virology*. 2004; 78(4):2088–2099. [PubMed: 14747574]
93. Cohen FE, Pan K-M, Huang Z, Baldwin M, Fletterick RJ, Prusiner SB. Structural clues to prion replication. *Science*. 1994; 264:530–531. [PubMed: 7909169]
94. Tzaban S, Friedlander G, Schonberger O, Horonchik L, Yedidia Y, Shaked G, Gabizon R, Taraboulos A. Protease-sensitive scrapie prion protein in aggregates of heterogeneous sizes. *Biochemistry*. 2002; 41(42):12868–12875. [PubMed: 12379130]
95. Korth C, Stierli B, Streit P, Moser M, Schaller O, Fischer R, Schulz-Schaeffer W, Kretzschmar H, Raeber A, Braun U, et al. Prion (PrP^{Sc})-specific epitope defined by a monoclonal antibody. *Nature*. 1997; 389:74–77.
96. Nazor KE, Kuhn F, Seward T, Green M, Zwald D, Purro M, Schmid J, Biffiger K, Power AM, Oesch B, et al. Immunodetection of disease-associated mutant PrP, which accelerates disease in GSS transgenic mice. *EMBO J*. 2005; 24(13):2472–2480. [PubMed: 15962001]
97. Uptain SM, Lindquist S. Prions as protein-based genetic elements. *Annu Rev Microbiol*. 2002; 56:703–741. [PubMed: 12142498]
98. Piccardo P, King D, Telling G, Manson JC, Barron RM. Dissociation of prion protein amyloid seeding from transmission of a spongiform encephalopathy. *J Virol*. 2013; 87(22):12349–12356. [PubMed: 24027305]
99. Legname G, Baskakov IV, Nguyen HO, Riesner D, Cohen FE, DeArmond SJ, Prusiner SB. Synthetic mammalian prions. *Science*. 2004; 305(5684):673–676. [PubMed: 15286374]
100. Asante EA, Grimshaw A, Smidak M, Jakubcova T, Tomlinson A, Jeelani A, Hamdan S, Powell C, Joiner S, Linehan JM, et al. Transmission Properties of Human PrP 102L Prions Challenge the Relevance of Mouse Models of GSS. *PLoS Pathog*. 2015; 11(7):e1004953. [PubMed: 26135918]
101. Friedman-Levi Y, Meiner Z, Canello T, Frid K, Kovacs GG, Budka H, Avrahami D, Gabizon R. Fatal prion disease in a mouse model of genetic E200K Creutzfeldt-Jakob disease. *PLoS Pathog*. 2011; 7(11):e1002350. [PubMed: 22072968]
102. Chiesa R, Piccardo P, Ghetti B, Harris DA. Neurological illness in transgenic mice expressing a prion protein with an insertional mutation. *Neuron*. 1998; 21(6):1339–1351. [PubMed: 9883727]
103. Chiesa R, Piccardo P, Quaglio E, Drisaldi B, Si-Hoe SL, Takao M, Ghetti B, Harris DA. Molecular distinction between pathogenic and infectious properties of the prion protein. *Journal of virology*. 2003; 77(13):7611–7622. [PubMed: 12805461]
104. Dossena S, Imeri L, Mangieri M, Garofoli A, Ferrari L, Senatore A, Restelli E, Balducci C, Fiordaliso F, Salio M, et al. Mutant prion protein expression causes motor and memory deficits and abnormal sleep patterns in a transgenic mouse model. *Neuron*. 2008; 60(4):598–609. [PubMed: 19038218]
105. Bouybayoune I, Mantovani S, Del Gallo F, Bertani I, Restelli E, Comerio L, Tapella L, Baracchi F, Fernandez-Borges N, Mangieri M, et al. Transgenic fatal familial insomnia mice indicate prion infectivity-independent mechanisms of pathogenesis and phenotypic expression of disease. *PLoS Pathog*. 2015; 11(4):e1004796. [PubMed: 25880443]

106. Jackson WS, Borkowski AW, Faas H, Steele AD, King OD, Watson N, Jasanoff A, Lindquist S. Spontaneous generation of prion infectivity in fatal familial insomnia knockin mice. *Neuron*. 2009; 63(4):438–450. [PubMed: 19709627]
107. Jackson WS, Borkowski AW, Watson NE, King OD, Faas H, Jasanoff A, Lindquist S. Profoundly different prion diseases in knock-in mice carrying single PrP codon substitutions associated with human diseases. *Proc Natl Acad Sci U S A*. 2013; 110(36):14759–14764. [PubMed: 23959875]
108. Hsiao KK, Cass C, Schellenberg GD, Bird T, Devine-Gage E, Wisniewski H, Prusiner SB. A prion protein variant in a family with the telencephalic form of Gerstmann-Sträussler-Scheinker syndrome. *Neurology*. 1991; 41:681–684. [PubMed: 1674116]
109. Hegde RS, Mastrianni JA, Scott MR, DeFea KA, Tremblay P, Torchia M, DeArmond SJ, Prusiner SB, Lingappa VR. A transmembrane form of the prion protein in neurodegenerative disease. *Science*. 1998; 279:827–834. [PubMed: 9452375]
110. Yang W, Cook J, Rassbach B, Lemus A, DeArmond SJ, Mastrianni JA. A New Transgenic Mouse Model of Gerstmann-Straussler-Scheinker Syndrome Caused by the A117V Mutation of PRNP. *J Neurosci*. 2009; 29(32):10072–10080. [PubMed: 19675240]
111. Asante EA, Linehan JM, Smidak M, Tomlinson A, Grimshaw A, Jeelani A, Jakubcova T, Hamdan S, Powell C, Brandner S, et al. Inherited prion disease A117V is not simply a proteinopathy but produces prions transmissible to transgenic mice expressing homologous prion protein. *PLoS Pathog*. 2013; 9(9):e1003643. [PubMed: 24086135]
112. Buschmann A, Pfaff E, Reifenberg K, Muller HM, Groschup MH. Detection of cattle-derived BSE prions using transgenic mice overexpressing bovine PrP(C). *Arch Virol Suppl*. 2000; (16): 75–86. [PubMed: 11214936]
113. Castilla J, Gutierrez Adan A, Brun A, Pintado B, Ramirez MA, Parra B, Doyle D, Rogers M, Salguero FJ, Sanchez C, et al. Early detection of PrPres in BSE-infected bovine PrP transgenic mice. *Arch Virol*. 2003; 148(4):677–691. [PubMed: 12664293]
114. Jacobs JG, Langeveld JP, Biacabe AG, Acutis PL, Polak MP, Gavier-Widen D, Buschmann A, Caramelli M, Casalone C, Mazza M, et al. Molecular discrimination of atypical bovine spongiform encephalopathy strains from a geographical region spanning a wide area in Europe. *J Clin Microbiol*. 2007; 45(6):1821–1829. [PubMed: 17442800]
115. Casalone C, Zanusso G, Acutis P, Ferrari S, Capucci L, Tagliavini F, Monaco S, Caramelli M. Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. *Proc Natl Acad Sci U S A*. 2004; 101(9):3065–3070. [PubMed: 14970340]
116. Richt JA, Hall SM. BSE case associated with prion protein gene mutation. *PLoS Pathog*. 2008; 4(9):e1000156. [PubMed: 18787697]
117. Buschmann A, Groschup MH. Highly bovine spongiform encephalopathy-sensitive transgenic mice confirm the essential restriction of infectivity to the nervous system in clinically diseased cattle. *J Infect Dis*. 2005; 192(5):934–942. [PubMed: 16088845]
118. Beringue V, Bencsik A, Le Dur A, Reine F, Lai TL, Chenais N, Tilly G, Biacabe AG, Baron T, Vilotte JL, et al. Isolation from cattle of a prion strain distinct from that causing bovine spongiform encephalopathy. *PLoS Pathogens*. 2006; 2(10):e112. [PubMed: 17054396]
119. Beringue V, Andreoletti O, Le Dur A, Essalmani R, Vilotte JL, Lacroix C, Reine F, Herzog L, Biacabe AG, Baron T, et al. A bovine prion acquires an epidemic bovine spongiform encephalopathy strain-like phenotype on interspecies transmission. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2007; 27(26):6965–6971. [PubMed: 17596445]
120. Buschmann A, Gretzschel A, Biacabe AG, Schiebel K, Corona C, Hoffmann C, Eiden M, Baron T, Casalone C, Groschup MH. Atypical BSE in Germany--proof of transmissibility and biochemical characterization. *Veterinary microbiology*. 2006; 117(2–4):103–116. [PubMed: 16916588]
121. Capobianco R, Casalone C, Suardi S, Mangieri M, Miccolo C, Limido L, Catania M, Rossi G, Di Fede G, Giaccone G, et al. Conversion of the BASE prion strain into the BSE strain: the origin of BSE? *PLoS Pathogens*. 2007; 3(3):e31. [PubMed: 17352534]

122. Wadsworth JD, Asante EA, Desbruslais M, Linehan JM, Joiner S, Gowland I, Welch J, Stone L, Lloyd SE, Hill AF, et al. Human prion protein with valine 129 prevents expression of variant CJD phenotype. *Science*. 2004; 306(5702):1793–1796. [PubMed: 15539564]
123. Beringue V, Le Dur A, Tixador P, Reine F, Lepourry L, Perret-Liaudet A, Haik S, Vilotte JL, Fontes M, Laude H. Prominent and persistent extraneural infection in human PrP transgenic mice infected with variant CJD. *PLoS ONE*. 2008; 3(1):e1419. [PubMed: 18183299]
124. Espinosa JC, Herva ME, Andreoletti O, Padilla D, Lacroux C, Cassard H, Lantier I, Castilla J, Torres JM. Transgenic mice expressing porcine prion protein resistant to classical scrapie but susceptible to sheep bovine spongiform encephalopathy and atypical scrapie. *Emerg Infect Dis*. 2009; 15(8):1214–1221. [PubMed: 19751582]
125. Westaway D, DeArmond SJ, Cayetano-Canlas J, Groth D, Foster D, Yang S-L, Torchia M, Carlson GA, Prusiner SB. Degeneration of skeletal muscle, peripheral nerves, and the central nervous system in transgenic mice overexpressing wild-type prion proteins. *Cell*. 1994; 76:117–129. [PubMed: 8287472]
126. Rubenstein R, Bulgin MS, Chang B, Sorensen-Melson S, Petersen RB, LaFauci G. PrP(Sc) detection and infectivity in semen from scrapie-infected sheep. *The Journal of general virology*. 2012; 93(Pt 6):1375–1383. [PubMed: 22323531]
127. Crozet C, Flamant F, Bencsik A, Aubert D, Samarut J, Baron T. Efficient transmission of two different sheep scrapie isolates in transgenic mice expressing the ovine PrP gene. *Journal of virology*. 2001; 75(11):5328–5334. [PubMed: 11333913]
128. Crozet C, Bencsik A, Flamant F, Lezmi S, Samarut J, Baron T. Florid plaques in ovine PrP transgenic mice infected with an experimental ovine BSE. *EMBO Rep*. 2001; 2(10):952–956. [PubMed: 11571272]
129. Vilotte JL, Soulier S, Essalmani R, Stinnakre MG, Vaiman D, Lepourry L, Da Silva JC, Besnard N, Dawson M, Buschmann A, et al. Markedly increased susceptibility to natural sheep scrapie of transgenic mice expressing ovine prp. *Journal of virology*. 2001; 75(13):5977–5984. [PubMed: 11390599]
130. Tamguney G, Miller MW, Giles K, Lemus A, Glidden DV, DeArmond SJ, Prusiner SB. Transmission of scrapie and sheep-passaged bovine spongiform encephalopathy prions to transgenic mice expressing elk prion protein. *The Journal of general virology*. 2009; 90(Pt 4):1035–1047. [PubMed: 19264659]
131. Goldmann W, Hunter N, Smith G, Foster J, Hope J. PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. *J Gen Virol*. 1994; 75:989–995. [PubMed: 7909834]
132. Cordier C, Bencsik A, Philippe S, Betemps D, Ronzon F, Calavas D, Crozet C, Baron T. Transmission and characterization of bovine spongiform encephalopathy sources in two ovine transgenic mouse lines (TgOvPrP4 and TgOvPrP59). *The Journal of general virology*. 2006; 87(Pt 12):3763–3771. [PubMed: 17098996]
133. Saijo E, Kang HE, Bian J, Bowling KG, Browning S, Kim S, Hunter N, Telling GC. Epigenetic dominance of prion conformers. *PLoS Pathog*. 2013; 9(10):e1003692. [PubMed: 24204258]
134. Foster JD, Dickinson AG. The unusual properties of CH 1641, a sheep-passaged isolate of scrapie. *Vet Rec*. 1988; 123:5–8. [PubMed: 3140468]
135. Baron T, Biacabe AG. Molecular behaviors of “CH1641-like” sheep scrapie isolates in ovine transgenic mice (TgOvPrP4). *Journal of virology*. 2007; 81(13):7230–7237. [PubMed: 17442721]
136. Kang HE, Weng CC, Saijo E, Saylor V, Bian J, Kim S, Ramos L, Angers R, Langenfeld K, Khaychuk V, et al. Characterization of Conformation-Dependent Prion Protein Epitopes. *The Journal of biological chemistry*. 2012
137. Bencsik A, Philippe S, Debeer S, Crozet C, Calavas D, Baron T. Scrapie strain transmission studies in ovine PrP transgenic mice reveal dissimilar susceptibility. *Histochem Cell Biol*. 2007; 127(5):531–539. [PubMed: 17361441]
138. Baron T, Crozet C, Biacabe AG, Philippe S, Verchere J, Bencsik A, Madec JY, Calavas D, Samarut J. Molecular analysis of the protease-resistant prion protein in scrapie and bovine

- spongiform encephalopathy transmitted to ovine transgenic and wild-type mice. *Journal of virology*. 2004; 78(12):6243–6251. [PubMed: 15163717]
139. Le Dur A, Beringue V, Andreoletti O, Reine F, Lai TL, Baron T, Bratberg B, Vilotte JL, Sarradin P, Benestad SL, et al. A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes. *Proc Natl Acad Sci U S A*. 2005; 102(44):16031–16036. [PubMed: 16239348]
  140. Simmons MM, Konold T, Simmons HA, Spencer YI, Lockey R, Spiropoulos J, Everitt S, Clifford D. Experimental transmission of atypical scrapie to sheep. *BMC Vet Res*. 2007; 3:20. [PubMed: 17725818]
  141. Wadsworth JD, Joiner S, Linehan JM, Balkema-Buschmann A, Spiropoulos J, Simmons MM, Griffiths PC, Groschup MH, Hope J, Brandner S, et al. Atypical scrapie prions from sheep and lack of disease in transgenic mice overexpressing human prion protein. *Emerg Infect Dis*. 2013; 19(11):1731–1739. [PubMed: 24188521]
  142. Cassard H, Torres JM, Lacroux C, Douet JY, Benestad SL, Lantier F, Lugan S, Lantier I, Costes P, Aron N, et al. Evidence for zoonotic potential of ovine scrapie prions. *Nature communications*. 2014; 5:5821.
  143. Mathiason CK, Powers JG, Dahmes SJ, Osborn DA, Miller KV, Warren RJ, Mason GL, Hays SA, Hayes-Klug J, Seelig DM, et al. Infectious prions in the saliva and blood of deer with chronic wasting disease. *Science*. 2006; 314(5796):133–136. [PubMed: 17023660]
  144. Browning SR, Mason GL, Seward T, Green M, Eliason GA, Mathiason C, Miller MW, Williams ES, Hoover E, Telling GC. Transmission of prions from mule deer and elk with chronic wasting disease to transgenic mice expressing cervid PrP. *J Virol*. 2004; 78(23):13345–13350. [PubMed: 15542685]
  145. Bartz JC, Marsh RF, McKenzie DI, Aiken JM. The host range of chronic wasting disease is altered on passage in ferrets. *Virology*. 1998; 251(2):297–301. [PubMed: 9837794]
  146. Angers RC, Browning SR, Seward TS, Sigurdson CJ, Miller MW, Hoover EA, Telling GC. Prions in skeletal muscles of deer with chronic wasting disease. *Science*. 2006; 311(5764):1117. [PubMed: 16439622]
  147. Meade-White K, Race B, Trifilo M, Bossers A, Favara C, Lacasse R, Miller M, Williams E, Oldstone M, Race R, et al. Resistance to chronic wasting disease in transgenic mice expressing a naturally occurring allelic variant of deer prion protein. *J Virol*. 2007; 81(9):4533–4539. [PubMed: 17314157]
  148. LaFauci G, Carp RI, Meeker HC, Ye X, Kim JI, Natelli M, Cedeno M, Petersen RB, Kascak R, Rubenstein R. Passage of chronic wasting disease prion into transgenic mice expressing Rocky Mountain elk (*Cervus elaphus nelsoni*) PrPC. *The Journal of general virology*. 2006; 87(Pt 12): 3773–3780. [PubMed: 17098997]
  149. Tamguney G, Giles K, Bouzamondo-Bernstein E, Bosque PJ, Miller MW, Safar J, DeArmond SJ, Prusiner SB. Transmission of elk and deer prions to transgenic mice. *Journal of virology*. 2006; 80(18):9104–9114. [PubMed: 16940522]
  150. Kong Q, Huang S, Zou W, Vanegas D, Wang M, Wu D, Yuan J, Zheng M, Bai H, Deng H, et al. Chronic wasting disease of elk: transmissibility to humans examined by transgenic mouse models. *J Neurosci*. 2005; 25(35):7944–7949. [PubMed: 16135751]
  151. Sigurdson CJ, Manco G, Schwarz P, Liberski P, Hoover EA, Hornemann S, Polymenidou M, Miller MW, Glatzel M, Aguzzi A. Strain fidelity of chronic wasting disease upon murine adaptation. *Journal of virology*. 2006; 80(24):12303–12311. [PubMed: 17020952]
  152. Raymond GJ, Raymond LD, Meade-White KD, Hughson AG, Favara C, Gardner D, Williams ES, Miller MW, Race RE, Caughey B. Transmission and adaptation of chronic wasting disease to hamsters and transgenic mice: evidence for strains. *J Virol*. 2007; 81(8):4305–4314. [PubMed: 17287284]
  153. Meyerett C, Michel B, Pulford B, Spraker TR, Nichols TA, Johnson T, Kurt T, Hoover EA, Telling GC, Zabel MD. In vitro strain adaptation of CWD prions by serial protein misfolding cyclic amplification. *Virology*. 2008

154. Green KM, Castilla J, Seward TS, Napier DL, Jewell JE, Soto C, Telling GC. Accelerated high fidelity prion amplification within and across prion species barriers. *PLoS Pathog.* 2008; 4(8):e1000139. [PubMed: 18769716]
155. Kurt TD, Perrott MR, Wilusz CJ, Wilusz J, Supattapone S, Telling GC, Zabel MD, Hoover EA. Efficient in vitro amplification of chronic wasting disease PrPRES. *J Virol.* 2007; 81(17):9605–9608. [PubMed: 17553879]
156. Kurt TD, Telling GC, Zabel MD, Hoover EA. Trans-species amplification of PrP(CWD) and correlation with rigid loop 170N. *Virology.* 2009; 387(1):235–243. [PubMed: 19269662]
157. Haley NJ, Seelig DM, Zabel MD, Telling GC, Hoover EA. Detection of CWD prions in urine and saliva of deer by transgenic mouse bioassay. *PLoS One.* 2009; 4(3):e4848. [PubMed: 19293928]
158. Tamguney G, Miller MW, Wolfe LL, Sirochman TM, Glidden DV, Palmer C, Lemus A, DeArmond SJ, Prusiner SB. Asymptomatic deer excrete infectious prions in faeces. *Nature.* 2009; 461(7263):529–532. [PubMed: 19741608]
159. Haley NJ, Mathiason CK, Zabel MD, Telling GC, Hoover EA. Detection of sub-clinical CWD infection in conventional test-negative deer long after oral exposure to urine and feces from CWD + deer. *PLoS One.* 2009; 4(11):e7990. [PubMed: 19956732]
160. Pulford B, Spraker TR, Wyckoff AC, Meyerett C, Bender H, Ferguson A, Wyatt B, Lockwood K, Powers J, Telling GC, et al. Detection of PrPCWD in feces from naturally exposed Rocky Mountain elk (*Cervus elaphus nelsoni*) using protein misfolding cyclic amplification. *J Wildl Dis.* 2012; 48(2):425–434. [PubMed: 22493117]
161. Henderson DM, Manca M, Haley NJ, Denkers ND, Nalls AV, Mathiason CK, Caughey B, Hoover EA. Rapid antemortem detection of CWD prions in deer saliva. *PLoS One.* 2013; 8(9):e74377. [PubMed: 24040235]
162. Angers RC, Seward TS, Napier D, Green M, Hoover E, Spraker T, O'Rourke K, Balachandran A, Telling GC. Chronic wasting disease prions in elk antler velvet. *Emerging Infectious Diseases.* 2009; 15(5):696–703. [PubMed: 19402954]
163. Race B, Meade-White K, Race R, Chesebro B. Prion infectivity in fat of deer with chronic wasting disease. *J Virol.* 2009; 83(18):9608–9610. [PubMed: 19570855]
164. Johnson C, Johnson J, Clayton M, McKenzie D, Aiken J. Prion protein gene heterogeneity in free-ranging white-tailed deer within the chronic wasting disease affected region of Wisconsin. *J Wildl Dis.* 2003; 39(3):576–581. [PubMed: 14567218]
165. Raymond GJ, Bossers A, Raymond LD, O'Rourke KI, McHolland LE, Bryant PK 3rd, Miller MW, Williams ES, Smits M, Caughey B. Evidence of a molecular barrier limiting susceptibility of humans, cattle and sheep to chronic wasting disease. *Embo J.* 2000; 19(17):4425–4430. [PubMed: 10970836]
166. Heaton MP, Leymaster KA, Freking BA, Hawk DA, Smith TP, Keele JW, Snelling WM, Fox JM, Chitko-McKown CG, Laegreid WW. Prion gene sequence variation within diverse groups of U.S. sheep, beef cattle, and deer. *Mamm Genome.* 2003; 14(11):765–777. [PubMed: 14722726]
167. O'Rourke KI, Spraker TR, Hamburg LK, Besser TE, Brayton KA, Knowles DP. Polymorphisms in the prion precursor functional gene but not the pseudogene are associated with susceptibility to chronic wasting disease in white-tailed deer. *The Journal of general virology.* 2004; 85(Pt 5): 1339–1346. [PubMed: 15105552]
168. Schatzl HM, Wopfner F, Gilch S, von Brunn A, Jager G. Is codon 129 of prion protein polymorphic in human beings but not in animals? *Lancet.* 1997; 349(9065):1603–1604. [PubMed: 9174569]
169. O'Rourke KI, Baszler TV, Miller JM, Spraker TR, Sadler-Riggelman I, Knowles DP. Monoclonal antibody F89/160.1. 5 defines a conserved epitope on the ruminant prion protein. *J Clin Microbiol.* 1998; 36(6):1750–1755. [PubMed: 9620413]
170. O'Rourke KI, Besser TE, Miller MW, Cline TF, Spraker TR, Jenny AL, Wild MA, Zebarth GL, Williams ES. PrP genotypes of captive and free-ranging Rocky Mountain elk (*Cervus elaphus nelsoni*) with chronic wasting disease. *J Gen Virol.* 1999; 80(Pt 10):2765–2769. [PubMed: 10573173]
171. Hamir AN, Gidlewski T, Spraker TR, Miller JM, Creekmore L, Crocheck M, Cline T, O'Rourke KI. Preliminary observations of genetic susceptibility of elk (*Cervus elaphus nelsoni*) to chronic

- wasting disease by experimental oral inoculation. *J Vet Diagn Invest.* 2006; 18(1):110–114. [PubMed: 16566268]
172. O'Rourke KI, Spraker TR, Zhuang D, Greenlee JJ, Gidlewski TE, Hamir AN. Elk with a long incubation prion disease phenotype have a unique PrP^d profile. *Neuroreport.* 2007; 18(18):1935–1938. [PubMed: 18007190]
  173. Green KM, Browning SR, Seward TS, Jewell JE, Ross DL, Green MA, Williams ES, Hoover EA, Telling GC. The elk PRNP codon 132 polymorphism controls cervid and scrapie prion propagation. *J Gen Virol.* 2008; 89(Pt 2):598–608. [PubMed: 18198392]
  174. Race B, Meade-White K, Miller MW, Fox KA, Chesebro B. In vivo comparison of chronic wasting disease infectivity from deer with variation at prion protein residue 96. *J Virol.* 2011; 85(17):9235–9238. [PubMed: 21697479]
  175. Keane DP, Barr DJ, Bochsler PN, Hall SM, Gidlewski T, O'Rourke KI, Spraker TR, Samuel MD. Chronic wasting disease in a Wisconsin white-tailed deer farm. *J Vet Diagn Invest.* 2008; 20(5): 698–703. [PubMed: 18776116]
  176. Duque Velasquez C, Kim C, Herbst A, Daude N, Garza MC, Wille H, Aiken J, McKenzie D. Deer Prion Proteins Modulate the Emergence and Adaptation of Chronic Wasting Disease Strains. *Journal of virology.* 2015; 89(24):12362–12373. [PubMed: 26423950]
  177. Gossert AD, Bonjour S, Lysek DA, Fiorito F, Wuthrich K. Prion protein NMR structures of elk and of mouse/elk hybrids. *Proc Natl Acad Sci U S A.* 2005; 102(3):646–650. [PubMed: 15647363]
  178. Sigurdson CJ, Nilsson KP, Hornemann S, Heikenwalder M, Manco G, Schwarz P, Ott D, Rulicke T, Liberski PP, Julius C, et al. De novo generation of a transmissible spongiform encephalopathy by mouse transgenesis. *Proc Natl Acad Sci U S A.* 2009; 106(1):304–309. [PubMed: 19073920]
  179. Kurt TD, Bett C, Fernandez-Borges N, Joshi-Barr S, Hornemann S, Rulicke T, Castilla J, Wuthrich K, Aguzzi A, Sigurdson CJ. Prion Transmission Prevented by Modifying the beta2-alpha2 Loop Structure of Host PrP^C. *J Neurosci.* 2014; 34(3):1022–1027. [PubMed: 24431459]
  180. Sigurdson CJ, Nilsson KP, Hornemann S, Manco G, Fernandez-Borges N, Schwarz P, Castilla J, Wuthrich K, Aguzzi A. A molecular switch controls interspecies prion disease transmission in mice. *J Clin Invest.* 2010; 120(7):2590–2599. [PubMed: 20551516]
  181. Kurt TD, Jiang L, Fernandez-Borges N, Bett C, Liu J, Yang T, Spraker TR, Castilla J, Eisenberg D, Kong Q, et al. Human prion protein sequence elements impede cross-species chronic wasting disease transmission. *J Clin Invest.* 2015; 125(4):1485–1496. [PubMed: 25705888]
  182. Khan MQ, Sweeting B, Mulligan VK, Arslan PE, Cashman NR, Pai EF, Chakrabarty A. Prion disease susceptibility is affected by beta-structure folding propensity and local side-chain interactions in PrP. *Proceedings of the National Academy of Sciences of the United States of America.* 2010; 107(46):19808–19813. [PubMed: 21041683]
  183. Jewell JE, Conner MM, Wolfe LL, Miller MW, Williams ES. Low frequency of PrP genotype 225SF among free-ranging mule deer (*Odocoileus hemionus*) with chronic wasting disease. *The Journal of general virology.* 2005; 86(Pt 8):2127–2134. [PubMed: 16033959]
  184. Wolfe LL, Fox KA, Miller MW. "Atypical" chronic wasting disease in PRNP genotype 225FF mule deer. *J Wildl Dis.* 2014; 50(3):660–665. [PubMed: 24807352]
  185. Johnson C, Johnson J, Vanderloo JP, Keane D, Aiken JM, McKenzie D. Prion protein polymorphisms in white-tailed deer influence susceptibility to chronic wasting disease. *The Journal of general virology.* 2006; 87(Pt 7):2109–2114. [PubMed: 16760415]
  186. Bessen RA, Robinson CJ, Seelig DM, Watschke CP, Lowe D, Shearin H, Martinka S, Babcock AM. Transmission of chronic wasting disease identifies a prion strain causing cachexia and heart infection in hamsters. *PLoS ONE.* 2011; 6(12):e28026. [PubMed: 22174765]
  187. Crowell J, Hughson A, Caughey B, Bessen RA. Host Determinants of Prion Strain Diversity Independent of Prion Protein Genotype. *Journal of virology.* 2015; 89(20):10427–10441. [PubMed: 26246570]
  188. Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCardle L, Chree A, Hope J, Birkett C, et al. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature.* 1997; 389:498–501. [PubMed: 9333239]

189. 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(6):977–984. [PubMed: 15207045]
190. 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(10):1527–1535. [PubMed: 17176567]
191. Barria MA, Telling GC, Gambetti P, Mastrianni JA, Soto C. Generation of a new form of human PrP(Sc) in vitro by interspecies transmission from cervid prions. *J Biol Chem.* 2011; 286(9): 7490–7495. [PubMed: 21209079]
192. Sandberg M, Al-Doujaily H, Sigurdson C, Glatzel M, O'Malley C, Powell C, Asante EA, Linehan JM, Brandner S, Wadsworth JD, et al. Chronic wasting disease prions are not transmissible to transgenic mice over-expressing human prion protein. *The Journal of general virology.* 2010
193. Marsh RF, Kincaid AE, Bessen RA, Bartz JC. Interspecies transmission of chronic wasting disease prions to squirrel monkeys (*Saimiri sciureus*). *Journal of virology.* 2005; 79(21):13794–13796. [PubMed: 16227298]
194. Race B, Meade-White KD, Miller MW, Barbican KD, Rubenstein R, LaFauci G, Cervenakova L, Favara C, Gardner D, Long D, et al. Susceptibilities of nonhuman primates to chronic wasting disease. *Emerg Infect Dis.* 2009; 15(9):1366–1376. [PubMed: 19788803]
195. Davenport KA, Henderson DM, Bian J, Telling GC, Mathiason CK, Hoover EA. Insights into Chronic Wasting Disease and Bovine Spongiform Encephalopathy Species Barriers by Use of Real-Time Conversion. *J Virol.* 2015; 89(18):9524–9531. [PubMed: 26157118]