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Experimental Models of Inherited PrP Prion Diseases

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The inherited prion protein (PrP) prion disorders, which include familial Creutzfeldt–Jakob disease, Gerstmann–Sträussler–Scheinker disease, and fatal familial insomnia, constitute ~10%–15% of all PrP prion disease cases in humans. Attempts to generate animal models of these disorders using transgenic mice expressing mutant PrP have produced variable results. Although many lines of mice develop spontaneous signs of neurological illness with accompanying prion disease–specific neuropathological changes, others do not. Furthermore, demonstrating the presence of protease-resistant PrP species and prion infectivity—two of the hallmarks of the PrP prion disorders—in the brains of spontaneously sick mice has proven particularly challenging. Here, we review the progress that has been made toward developing accurate mouse models of the inherited PrP prion disorders.

The prion protein (PrP) prion disorders are a group of invariably fatal neurodegenerative conditions that affect humans and animals. In these diseases, PrP undergoes a conformational rearrangement from a predominantly α-helical cellular isoform (PrP^C) into a misfolded, β-sheet-rich isoform (PrP^{Sc}) that aggregates and causes disease (Colby and Prusiner 2011). Like other prions, PrP^{Sc} is self-propagating and can catalyze its own formation by binding to PrP^C and templating its conversion to PrP^{Sc}. This process permits a cascade of PrP^{Sc} production and its subsequent spread throughout the brain, which ultimately results in the neuropathological changes associated with the PrP prion diseases—namely, spongiform (vacuolar) degeneration of the brain parenchyma, cerebral deposition of aggregated and misfolded PrP species, neuronal loss, and highly elevated levels of reactive astrocytic gliosis. The self-propagating nature of PrP^{Sc} underlies the infectious nature of the human PrP prion disorders, many of which have been successfully transmitted to primates and laboratory rodents.

PrP^C is a neuronal glycoprotein that is anchored to the outer leaflet of the plasma membrane by virtue of a glycophosphatidylinositol (GPI) anchor. Human (Hu) PrP is initially synthesized as a 253-residue precursor protein that contains an N-terminal signal peptide that directs the protein to the secretory pathway and a C-terminal signal sequence that is replaced by

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the GPI anchor. The mature, processed form of HuPrP consists of residues 23-231 and folds into a structure consisting of two domains: an α-helical C-terminal domain and a flexibly disordered N-terminal domain (Zahn et al. 2000), which contains a series of five octapeptide repeats. Whereas PrP^C is completely digested by proteases such as proteinase K (PK), PrP^{Sc} is partially resistant. PK-resistant PrP^{Sc} species of varying sizes were found in the vast majority of PrP prion disease cases, although PK-sensitive PrP^{Sc} isoforms have also been described (Safar et al. 2005).

MUTATIONS IN PrP CAUSE INHERITED PrP PRION DISORDERS

Approximately 10%–15% of PrP prion disease cases in humans are heritable and can be classified into three distinct disorders based on their clinical and pathological characteristics: familial Creutzfeldt–Jakob disease (fCJD), Gerstmann–Sträussler–Scheinker disease (GSS), and fatal familial insomnia (FFI). These diseases are caused by autosomal dominant mutations in the *PRNP* gene, which encodes PrP. In the inherited PrP prion disorders, mutations in PrP are thought to either directly promote the spontaneous misfolding of PrP^C into PrP^{Sc} or to stabilize PrP^{Sc} once it is formed. A large spectrum of PrP mutations has been identified in patients with genetic prion disease (Fig. 1). Disease-causing mutations occur throughout the mature, processed form of the protein and can be broken down into three categories: missense mutations; nonsense mutations that result in the production of truncated, GPI-anchorless PrP species; and mutations that increase or decrease the quantity of octapeptide repeats within the N-terminal domain. A common polymorphism also exists in HuPrP at codon 129, where either a methionine (M) or valine (V) residue can be present (Owen et al. 1990).

Like sporadic CJD, fCJD is a rapidly progressive dementia. Mutations that cause fCJD are preferentially located within the α-helical C-terminal domain of PrP (Fig. 1), suggesting that they may act by destabilizing the structure of PrP^C. Octapeptide repeat insertion (OPRI) and octapeptide repeat deletion (OPRD) mutations also cause fCJD. It should be noted that some of the putative fCJD-causing mutations depicted in Figure 1 have only been identified in a small number of patients, raising the possibility that they may constitute rare polymorphic variants identified by chance in patients with sporadic CJD. The neuropathological hallmarks of fCJD are cerebral spongiform degeneration and PrP^{Sc} deposits that do not typically contain PrP amyloid. "Stereotypical," variably glycosylated, and N-terminally truncated PK-resistant PrP species that are ~19–30 kDa in size, which are sometimes referred to as PrP27–30, are found in the brains of fCJD patients.

Compared to fCJD, GSS is a more slowly progressive disease, and patients tend to exhibit more cerebellar symptoms such as ataxia. GSS-causing mutations are located throughout the PrP sequence (Fig. 1). Although OPRI mutations cause both fCJD and GSS, longer insertions are typically associated with a GSS phenotype, whereas shorter insertions commonly result in a CJD phenotype. Truncation mutations that cause the production of GPI-anchorless PrP isoforms also cause GSS; interestingly, many of these cases have extensive deposition of PrP species surrounding blood vessels in the brain (cerebral amyloid angiopathy). The brains of GSS patients typically contain minimal spongiform degeneration but abundant PrP-containing amyloid plaques. The PK-resistant PrP species in GSS patients are usually both N- and C-terminally truncated and smaller in size, with molecular weights ranging from 7 to 11 kDa, than those observed in fCJD patients.

The symptoms of FFI include a progressive insomnia with hallucinations and, ultimately, dementia. A single mutation in PrP (D178N) is known to cause FFI. However, FFI only manifests when the mutation is in *cis* to methionine at polymorphic codon 129; when the D178N mutation occurs in conjunction with valine at codon 129, an fCJD phenotype is present. Pathology in FFI patients is normally restricted to the thalamus, where spongiosis and extensive neuronal loss are apparent. In cases with longer disease duration, cortical pathology is also observed. The brains of FFI patients exhibit low,

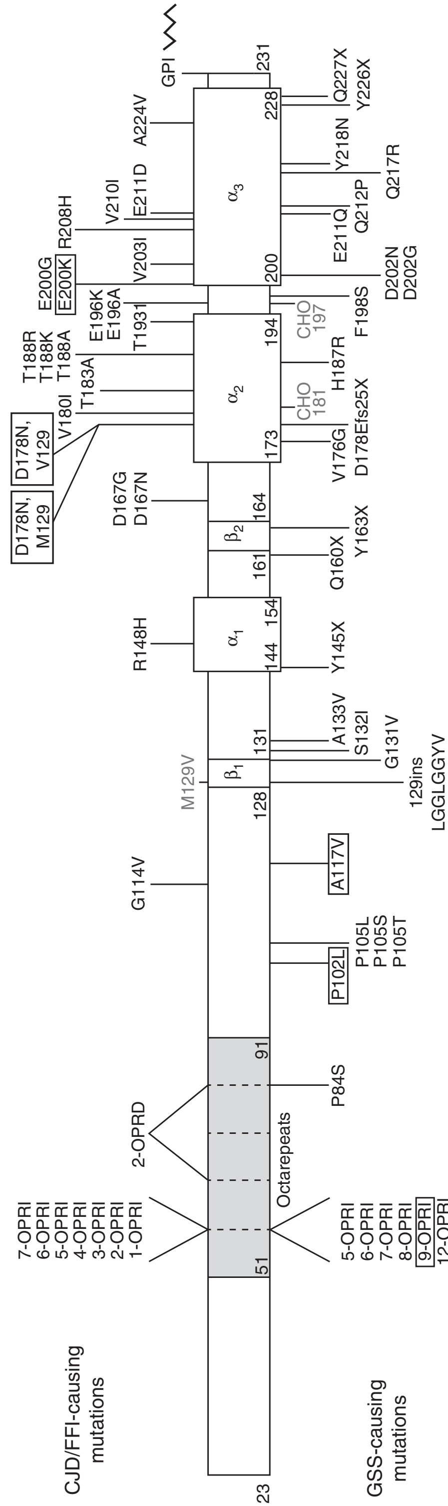

Figure 1. Mutations in the prion protein (PrP) causing inherited human prion disease. Schematic representation of the domain structure of human PrP^C lacking the N- and C-terminal signal sequences (residues 23-231). The amino acid boundaries of the octapeptide repeat domain, the three α-helices, and the two short β-strands are indicated. The location of N-glycosylation sites (CHO) at residues 181 and 197 are also shown. A common polymorphism (M129V) is depicted *above* the domain structure. GSS-causing mutations are listed *below* the domain structure, whereas CJD- and FFI-causing mutations are denoted *above* the domain structure. Mutations that were successfully modeled using Tg mice are shown in boxes.

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but detectable, levels of "stereotypical" PK-resistant PrP^{Sc} (PrP27–30).

DESIGNING MOUSE MODELS OF INHERITED PrP PRION DISORDERS

The discovery that mutations in HuPrP cause inherited PrP prion disorders prompted the investigation of whether animal models of these diseases could be generated by the targeted expression of mutant PrP in the brain. Two general strategies were used when trying to generate mouse models of the inherited PrP prion diseases: transgenic (Tg) mice and knock-in mice. In knock-in mouse models, the endogenous wild-type (WT) mouse PrP locus is replaced with a mutant version via gene targeting (homologous recombination) in embryonic stem cells. Knock-in mice express mutant PrP at physiological levels under the control of their endogenous regulatory elements, which should ensure the correct spatiotemporal expression pattern. In Tg models, fertilized mouse embryos are microinjected with DNA encoding a transgene cassette that drives expression of mutant PrP in the brain. In all Tg mouse models generated to date, Syrian hamster or mouse PrP promoter elements were used to specify the neuronal expression of mutant PrP. In Tg models, multiple copies of the transgene cassette are typically inserted into the mouse genome, resulting in overexpression of mutant PrP. This has the advantage that disease phenotypes can be obtained more rapidly than in knock-in models. However, integration artifacts can occur following the random insertion of transgenes into the genome, and high levels of even WT PrP overexpression can elicit non-prion-disease-specific pathology (Westaway et al. 1994).

Another consideration is the PrP sequence used as the backbone for the disease-causing mutation. Successful Tg models of the inherited PrP prion disorders were generated using mouse (Mo) PrP or chimeric Mo/Hu PrP as the starting point. Interestingly, attempts to generate Tg models using mutant HuPrP have failed, suggesting that HuPrP is less prone to misfolding than MoPrP or that interactions between MoPrP and other mouse-specific factors are important for the generation of prions. Recently, Tg models were generated using bank vole (BV) PrP. Bank voles (*Myodes glareolus*) are highly susceptible to human prions, and BVPrP is prone to misfolding spontaneously or upon exposure to prions from many different species (Nonno et al. 2006; Watts et al. 2012, 2014; Orrú et al. 2015).

MOUSE MODELS OF GSS

The greatest success in modeling inherited PrP prion disorders in mice has been achieved with GSS-causing mutations (Table 1). Expression of PrP containing P102L, A117V, 9-OPRI, or GPI-anchorless mutations in the brains of Tg mice has resulted in a spontaneous neurodegenerative disease phenotype with accompanying GSS-specific neuropathological changes, and in some instances, the generation of small GSS-like PK-resistant PrP fragments.

P102L

The first inherited prion disease mutation to be successfully modeled using Tg mice was P102L, which is the most common cause of GSS (Hsiao et al. 1989). Tg174 mice overexpressing MoPrP with the mouse equivalent of the mutation (P101L) developed signs of spontaneous neurological illness, including ataxia and rigidity, with a mean age of onset of ∼200 d (Hsiao et al. 1990). The brains of spontaneously ill mice exhibited the hallmark neuropathological changes observed in GSS patients, including spongiform degeneration, PrP-containing amyloid plaques, and reactive astrocytic gliosis (Hsiao et al. 1989, 1994), but did not contain any highly PK-resistant PrP (i.e., resistant to degradation by a PK concentration of 20 µg/mL or higher). However, it was subsequently determined that disease-specific PrP conformers could be detected in the brains of spontaneously ill mice by digestion with PK at 4°C ("cold PK") followed by precipitation with phosphotungstic acid (PTA) (Tremblay et al. 2004; Nazor et al. 2005). Spontaneous disease has been observed in eight independent lines of Tg mice overexpressing MoPrP(P101L) at levels at least three times higher than those

Table 1. Mouse models of GSS

Mutation	Line	Type of mouse	PrP sequence	PrP expression level	Spontaneous signs of neurologic illness?	Incubation period to onset of clinical disease (d)	Prion disease–specific neuropathological changes?	Highly PK-resistant PrP?[a]	Disease is transmissible?	Reference
P102L	101LL	Knock-in	Mouse	1×	No	N/A	No	No	N/A	Manson et al. 1999
	Tg174[b]	Transgenic	Mouse	8×	Yes	∼200	Yes	No	Yes	Hsiao et al. 1990
	Tg87[b]	Transgenic	Mouse	8×	Yes	∼150	Yes	No	Yes	Hsiao et al. 1994
	Tg196[b]	Transgenic	Mouse	2×	No	N/A	No	No	N/A	Hsiao et al. 1994
	Tg2866	Transgenic	Mouse	8×	Yes	∼150	Yes	No	Yes	Telling et al. 1996b
	Tg2247[b]	Transgenic	Mouse	8×	Yes	∼230	Yes	No	Yes	Telling et al. 1996b
	Tg2862[b]	Transgenic	Mouse	32×	Yes	∼320	Yes	No	Not reported	Telling et al. 1996b
	Tg69	Transgenic	Chimeric mouse/human	2×	Yes	∼360	Yes	No	Not reported	Telling et al. 1995
	Tg(GSS)2	Transgenic	Mouse	0.5×–1×	No	N/A	No	No	N/A	Nazor et al. 2005
	Tg(GSS)6	Transgenic	Mouse	3×	Yes	∼600	Yes	No	ND	Nazor et al. 2005
	Tg(GSS)12	Transgenic	Mouse	6×	Yes	∼430	Yes	No	ND	Nazor et al. 2005
	Tg(GSS)22	Transgenic	Mouse	12×	Yes	∼160	Yes	No	Yes	Nazor et al. 2005
	113LBoPrP-Tg009	Transgenic	Cow	1×	No	N/A	No	No	N/A	Torres et al. 2013
	113LBoPrP-Tg037	Transgenic	Cow	6×	Yes	∼190	Yes	No	Yes	Torres et al. 2013
	Tg27	Transgenic	Human (M129)	3×	No	N/A	No	No	N/A	Asante et al. 2009
A117V	E15727	Transgenic	Hamster	4×	Yes	∼570	Not reported	No	No	Hegde et al. 1999
	Tg(A116V)	Transgenic	Mouse (M128V)	4×–6×	Yes	∼150	Yes	No	Not reported	Yang et al. 2009
	Tg31	Transgenic	Human (V129)	3×	No	N/A	No	No	N/A	Asante et al. 2013
9-OPRI	PG14	Transgenic	Mouse (3F4 epitope tag)	1×	Yes	∼240	No	No	No	Chiesa et al. 1998
ΔGPI	tg44+/+	Transgenic	Mouse	0.13×	No	N/A	No	No	N/A	Chesebro et al. 2010
	Tg8423	Transgenic	Mouse (C-terminal myc tag)	0.3×	No	N/A	Mild	No	ND	Stöhr et al. 2011
	Tg8015	Transgenic	Mouse (C-terminal myc tag)	1.7×	Yes	∼600	Yes	Yes	Yes	Stöhr et al. 2011
	Tg24600	Transgenic	Bank vole (I109)	0.5×	Yes	∼420	Yes	Yes	Yes	Watts et al. 2016

GSS, Gerstmann–Sträussler–Scheinker disease; PK, proteinase K; N/A, not applicable; ND, not determined.
[a]Highly PK-resistant PrP is defined as PrP that is resistant to digestion with PK at a concentration of 20 μg/mL or higher.
[b]These lines also express endogenous WT MoPrP; all other lines express only mutant PrP.

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found in WT mice, with mean incubation periods ranging from 150 to 600 d (Table 1) (Hsiao et al. 1994; Telling et al. 1996b; Nazor et al. 2005). Removing the expression of endogenous WT MoPrP both accelerated and unified the disease incubation period in Tg mice expressing MoPrP(P101L) (Telling et al. 1996b), suggesting that the presence of WT PrP can hinder the misfolding of mutant PrP or delay disease progression.

Tg mice expressing MoPrP(P101L) at low levels (i.e., 0.5×–2×) and knock-in mice expressing physiological levels of MoPrP(P101L) did not develop spontaneous disease (Hsiao et al. 1994; Manson et al. 1999; Nazor et al. 2005), likely because the disease incubation period exceeds the normal life span of a mouse. Similarly, Tg mice expressing the bovine PrP equivalent of the P102L mutation (P113L) at 1× levels did not develop a spontaneous illness, whereas mice expressing the mutant protein at 8× levels developed spontaneous disease in <200 d (Torres et al. 2013). Interestingly, Tg mice expressing human PrP (HuPrP) containing the P102L mutation at 3× levels failed to develop a spontaneous neurodegenerative illness (Asante et al. 2009), potentially suggesting that elements within the sequence of HuPrP may restrict the spontaneous formation of prions.

Brain homogenates from spontaneously ill Tg mice expressing high levels of MoPrP (P101L) are capable of transmitting disease to Tg196 mice, which express low levels of MoPrP(P101L), indicating that the misfolded PrP conformers in the brains of spontaneously ill mice are infectious (Hsiao et al. 1994; Telling et al. 1996b; Tremblay et al. 2004). In contrast, no disease transmission was observed when samples from spontaneously sick mice were inoculated into non-Tg mice or Tg mice overexpressing WT MoPrP (Hsiao et al. 1994; Telling et al. 1996b; Tremblay et al. 2004), whereas only nine of 348 inoculated hamsters developed disease (Hsiao et al. 1994). One interpretation is that the P101L mutation creates a barrier that hinders the transmission of the spontaneously formed prions to animals expressing WT MoPrP. Another possible interpretation is that the misfolded MoPrP(P101L) conformers are only capable of accelerating disease kinetics in mice that are inherently prone to developing spontaneous disease, as opposed to the true generation of prion infectivity. This idea is supported by two observations: (1) A small proportion of Tg196 mice develop a late-onset spontaneous disease (Kaneko et al. 2000); and (2) no disease transmission was observed following inoculation of Tg mice expressing MoPrP(P101L) at 0.5×–1× levels (which do not develop late-onset spontaneous disease) with brain homogenate from spontaneously ill Tg mice expressing higher levels of the protein (Nazor et al. 2005). However, it should be noted that not all cases of P102L GSS are transmissible (Tateishi 1996).

A117V

The A117V mutation, which occurs within the hydrophobic tract region of PrP, is another common cause of GSS (Tateishi et al. 1990; Hsiao et al. 1991). This mutation has been shown to increase the levels of transmembrane topological variants of PrP (Hegde et al. 1998). Tg mice overexpressing hamster PrP containing the A117V mutation at 4× levels developed a late-onset spontaneous neurodegenerative illness with an incubation period of ~570 d (Table 1) (Hegde et al. 1999). Similarly, Tg mice expressing MoPrP with the mouse equivalent of the mutation (A116V) with fourfold to sixfold overexpression developed spontaneous disease in only ~150 d (Yang et al. 2009). However, no spontaneous disease was observed in Tg mice expressing A117V-mutant HuPrP with threefold PrP overexpression (Asante et al. 2013). The brains of spontaneously ill A116V-mutant MoPrP mice exhibited prion disease–specific neuropathology, including mild vacuolation and PrP plaques that were most prominent in the cerebellar cortex (Yang et al. 2009). Although levels of detergent-insoluble PrP were higher in mice expressing MoPrP(A116V) (Yang et al. 2009), no highly PK-resistant PrP species were observed in any of the lines. Transmissibility of the spontaneous disease has not yet been demonstrated, although it should be noted that the transmissibility of GSS cases with the A117V mutation has only recently

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been demonstrated using Tg mice expressing HuPrP(A117V) and only after long incubation periods (Asante et al. 2013).

9-OPRI

Two families presenting with GSS and a nine-octapeptide repeat insertion (9-OPRI) within the N-terminal domain of PrP have been described (Owen et al. 1992; Krasemann et al. 1995). Tg mice expressing physiological levels of MoPrP with the 9-OPRI mutation have been generated (Chiesa et al. 1998). These mice, termed PG14, develop spontaneous signs of neurological illness (such as ataxia) with a mean onset of ∼240 d, and the incubation period is not strongly modulated by the presence or absence of endogenous WT MoPrP (Chiesa et al. 2000). The principal neuropathological finding in spontaneously ill PG14 mice is loss of granule cells within the cerebellum, as well as some accompanying "synaptic-like" PrP deposition in the molecular layer and reactive astrocytic gliosis (Chiesa et al. 1998). No obvious spongiosis is present in the brains of PG14 mice. Although the mutant PrP in PG14 mice is detergent-insoluble and mildly PK-resistant (Chiesa et al. 1998, 2000), no disease transmission was observed when brain extracts from spontaneously ill mice were injected into non-Tg mice, Tg mice expressing WT MoPrP, or Tg mice expressing 9-OPRI-mutant MoPrP at lower levels that do not develop spontaneous disease (Chiesa et al. 2003). These results imply that prions are not formed in the brains of PG14 mice, and the spontaneous disease may be better characterized as a "PrP proteinopathy."

GPI-Anchorless PrP

Two GSS cases were identified with either Y226X or Q227X mutations in *PRNP* (Jansen et al. 2010). These mutations result in the production of nearly full-length GPI-anchorless PrP ("ΔGPI") species and cause a profound PrP amyloidosis in the brain. In earlier studies, Tg mice expressing GPI-anchorless MoPrP were generated to examine the necessity of the PrP GPI anchor for the propagation of prions (Chesebro et al. 2005). These mice express very low levels of PrP(ΔGPI) and did not exhibit any spontaneous signs of neurological illness (Chesebro et al. 2010). Later, Tg mice expressing GPI-anchorless MoPrP at higher levels (∼1.7-fold higher than PrP levels in non-Tg mice) were generated (Stöhr et al. 2011). Approximately 50% of these mice, termed Tg8015, developed a late-onset spontaneous neurological disorder (Table 1). The brains of spontaneously ill Tg8015 mice exhibited a large number of PrP-amyloid deposits, and a highly PK-resistant PrP fragment of ∼10 kDa, similar to fragments found in GSS cases, was observed in brain extracts from sick mice. Moreover, brain homogenates from spontaneously ill Tg8015 mice accelerated disease when inoculated into young Tg8015 mice and transmitted disease to Tg mice overexpressing WT MoPrP, confirming the spontaneous generation of GPI-anchorless prions (Stöhr et al. 2011).

Because Tg mice expressing membrane-anchored, WT bank vole PrP (BVPrP) containing isoleucine at polymorphic codon 109 (I109) developed a spontaneous prion disease (Watts et al. 2012), Tg mice expressing GPI-anchorless BVPrP(I109) were also generated. All of these mice, termed Tg24600, developed spontaneous disease, with a mean age of onset of ∼420 d (Table 1), despite the fact that PrP levels were about three times lower than in the brains of Tg8015 mice (Watts et al. 2016). The brains of spontaneously ill Tg24600 mice contained abundant PrP deposits (including PrP-containing amyloid plaques) (Fig. 2) and an ∼ 8-kDa highly PK-resistant PrP fragment. Moreover, brain extracts from sick mice accelerated disease when inoculated into young Tg24600 mice or Tg mice expressing WT BVPrP(I109). Thus, Tg mice expressing GPI-anchorless MoPrP or BVPrP recapitulate the pathological and biochemical hallmarks of the associated GSS cases, suggesting that they may be excellent models for testing candidate GSS therapeutics.

Y145X

A nonsense mutation at codon 145 of PrP (Y145X) causes vascular and parenchymal dep-

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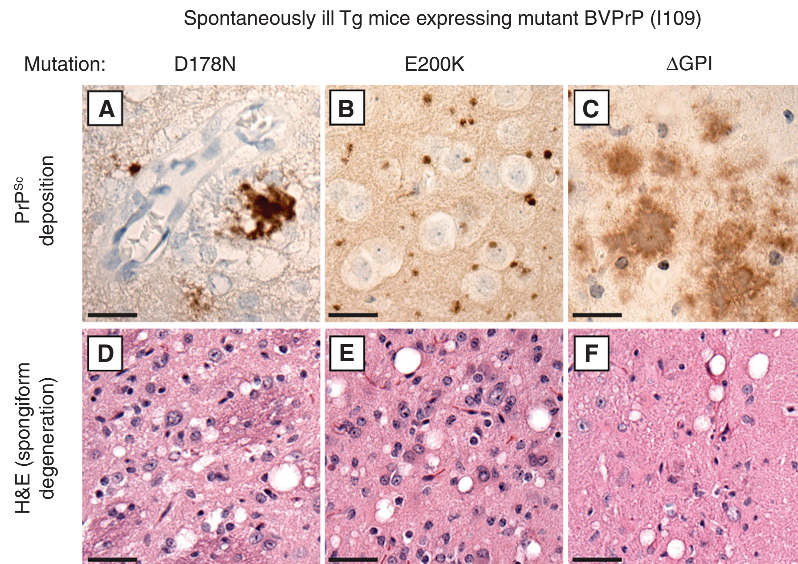

Figure 2. Prion disease–specific neuropathology in Tg mice expressing mutant bank vole PrP. (*A–C*) PrP^{Sc} deposition, as determined by immunohistochemistry with the antibody HuM-P, and (*D–F*) spongiform degeneration, as revealed by hematoxylin and eosin (H&E) staining, are apparent in brain sections prepared from spontaneously ill Tg mice expressing D178N-mutant (*A*,*D*), E200K-mutant (*B*,*E*), or ΔGPI-mutant (*C*,*F*) BVPrP(I109). Unique patterns of PrP^{Sc} deposition were observed with each mutation: clustered coarse deposits with D178N, small round deposits with E200K, and "plaque-like" deposits with ΔGPI. Scale bars, 20 μm (*A–C*); 40 μm (*D–F*).

osition of PrP amyloid in GSS patients (Kitamoto et al. 1993; Ghetti et al. 1996). Attempts to model this disease using Tg mice have been unsuccessful: Two independent lines of Tg mice expressing MoPrP with the equivalent mutation (Y144X) did not exhibit any mutant protein expression and did not develop spontaneous disease (Fischer et al. 1996; Muramoto et al. 1997).

MOUSE MODELS OF FFI

Several attempts were made to model FFI using genetically modified mice (Table 2). The first attempt used knock-in mice (ki-3F4-FFI) in which the WT MoPrP open reading frame was replaced with a mutant allele carrying the D177N mutation, which is the mouse PrP equivalent of the D178N mutation in FFI patients (Jackson et al. 2009), as well as a two-residue substitution to confer immunoreactivity to the 3F4 antibody. Some of the ki-3F4-FFI mice developed late-onset neurological illness with accompanying neuronal loss and gliosis in the thalamus, which is the principal target area in FFI patients, although no highly PK-resistant PrP species and no PrP deposits were observed in the brain (Jackson et al. 2009). Inoculation of Tg*a*20 mice overexpressing WT MoPrP or knock-in mice expressing 3F4-tagged MoPrP with brain extracts from spontaneously sick ki-3F4-FFI mice resulted in disease transmission, confirming the generation of prion infectivity.

Tg mice expressing D177N-mutant MoPrP have also been created. FFI-26 mice, which express mutant PrP at 2× levels, developed a progressive neurological disease at ~200 d of age that was characterized by ataxia and kyphosis (Bouybayoune et al. 2015). Tg mice expressing D177N-mutant PrP at 1× also developed spontaneous disease, whereas mice with 0.5× expression did not. In FFI-26 mice, co-expression of WT endogenous MoPrP had no effect on disease onset. Mild thalamic and cerebellar atrophy was observed in the brains of aged FFI-

Table 2. Mouse models of FFI

Mutation	Line	Type of mouse	PrP sequence	PrP expression level	Spontaneous signs of neurologic illness?	Incubation period to onset of clinical disease (d)	Prion disease–specific neuropathological changes?	Highly PK-resistant PrP?[a]	Disease is transmissible?	Reference
D178N,M129	ki-3F4-FFI	Knock-in	Mouse (3F4 epitope tag)	$< 1\times$	Yes	Not reported	Mild	No	Yes	Jackson et al. 2009
	FFI-K5	Transgenic	Mouse (3F4 epitope tag)	$0.7\times$	No	N/A	Mild	Not reported	Not reported	Bouybayoune et al. 2015
	FFI-10	Transgenic	Mouse	$1\times$	Yes	$\sim$540	Not reported	Not reported	Not reported	Bouybayoune et al. 2015
	FFI-15	Transgenic	Mouse	$0.5\times$	No	N/A	No	Not reported	Not reported	Bouybayoune et al. 2015
	FFI-26	Transgenic	Mouse	$2\times$	Yes	$\sim$200	Mild	No	No	Bouybayoune et al. 2015
	Tg15972	Transgenic	Bank vole (I109)	$0.4\times$	Yes	$\sim$240	Yes	Yes	Yes	Watts et al. 2016
	Tg15464	Transgenic	Bank vole (I109)	$0.4\times$	Yes	$\sim$220	Yes	Yes	ND	Watts et al. 2016
	Tg15465	Transgenic	Bank vole (I109)	$0.4\times$	Yes	$\sim$200	Yes	Yes	ND	Watts et al. 2016
	Tg15965	Transgenic	Bank vole (I109)	$0.5\times$	Yes	$\sim$180	Yes	Yes	Yes	Watts et al. 2016

FFT, Fatal familial insomnia; PK, proteinase K; N/A, not applicable; ND, not determined.
[a]Highly PK-resistant PrP is defined as PrP that is resistant to digestion with PK at a concentration of 20 μg/mL or higher.

26 mice, as was some "synaptic-like" PrP deposition, but no spongiform degeneration was detected. The D177N-mutant PrP in FFI-26 mice exhibited increased detergent insolubility and PK resistance compared to WT PrP, but no highly PK-resistant PrP species were present. No transmission was observed following inoculation of non-Tg or Tg*a*20 mice with brain homogenates from diseased FFI-26 mice, arguing that the pathogenic changes observed in FFI-26 mice are not related to the generation of prion infectivity (Bouybayoune et al. 2015).

Four independent lines of Tg mice expressing BVPrP(I109) containing the D178N mutation at low levels (0.4×–0.5×) developed a highly penetrant spontaneous neurological illness with incubation periods ranging from ∼180 to ∼240 d (Table 2) (Watts et al. 2016). The brains of spontaneously ill mice exhibited spongiform degeneration, reactive astrocytic gliosis, and clustered coarse PrP deposits similar to those that have been observed in some FFI patients (Almer et al. 1999). Moreover, a highly PK-resistant PrP species with a molecular weight of ∼8 kDa was found in symptomatic mice but not in young, asymptomatic animals. The disease could be transmitted to Tg mice expressing WT BVPrP(I109) and to Tg4053 mice overexpressing WT MoPrP.

Both the ki-3F4-FFI and FFI-26 models also display behavioral abnormalities. Using automated mouse behavioral analysis, it was determined that ki-3F4-FFI mice exhibit increased sleep interruption as measured by twitching during rest and extended periods of inactivity, possibly from a lack of uninterrupted sleep (Jackson et al. 2009). Moreover, compared to controls, FFI-26 mice exhibited numerous sleep abnormalities such as defective circadian organization, increased disruptions to sleep continuity, and abnormal entry into REM sleep (Bouybayoune et al. 2015). Collectively, these studies indicate that mouse models of FFI may recapitulate some of the key clinical hallmarks of the disease in addition to pathological markers. However, none of the FFI models developed to date produce PrP27–30, which is found in all patients with FFI.

MOUSE MODELS OF fCJD

Of the large number of purported fCJD-causing mutations in *PRNP* (Fig. 1), only two (E200K and D178N) have resulted in clear spontaneous disease with prion disease–specific neuropathological changes when inserted into PrP and expressed in the brains of Tg mice. Although highly PK-resistant PrP species were found in the brains of some of these lines, they do not resemble those present in corresponding fCJD patients.

E200K

Early attempts to model fCJD caused by the E200K mutation were unsuccessful. Despite overexpressing the MoPrP equivalent of the mutation (E199K) at 8× levels, no spontaneous disease was observed in Tg5182 mice, although it should be noted that endogenous WT MoPrP was also present in these mice, which may have restricted the formation of prions (Telling et al. 1996b). Furthermore, Tg mice overexpressing HuPrP containing the E200K mutation at 3× levels showed no signs of spontaneous neurological illness or prion disease–specific neuropathological changes (Asante et al. 2009).

Although Tg mice expressing MoPrP (E199K) did not develop spontaneous disease, Tg mice expressing a chimeric Mo/Hu PrP containing the E199K mutation at 2× levels exhibited hind-limb paralysis and kyphosis beginning at ∼200 d of age and eventually died around ∼365 d (Friedman-Levi et al. 2011). Co-expression of endogenous WT MoPrP did not have a major effect on disease manifestation (Friedman-Levi et al. 2013). Some PrP deposition was apparent throughout the brain, but only minimal spongiform degeneration was observed (Friedman-Levi et al. 2011). Some atypical PK-resistant PrP species were found in the brains of sick mice (Friedman-Levi et al. 2011, 2013). Inoculation of non-Tg mice with brain homogenates from spontaneously ill Tg mice resulted in some instances of disease transmission, but the transmission efficiency was highly variable (Friedman-Levi et al. 2011).

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Knock-in mice expressing E199K-mutant MoPrP (also containing the 3F4 epitope tag) at physiological levels exhibited some clinical abnormalities, such as reduced performance on the rotarod test and decreased burrowing behavior, but were not reported to develop profound clinical signs of a neurodegenerative disease (Jackson et al. 2013). In contrast to the ki-3F4-FFI mice, the ki-3F4-CJD mice exhibited clear spongiosis and punctate PrP deposits in the brain with aging. Some mildly PK-resistant PrP was present in ki-3F4-CJD mice, but the highly PK-resistant PrP species observed in CJD(E200K) patients was not observed. Passage of brain extracts from ki-3F4-CJD mice into Tg*a*20 mice or non-Tg mice resulted in efficient disease transmission, confirming that prions were formed spontaneously in the brains of these knock-in mice.

Like with the D178N mutation, addition of the E200K mutation to BVPrP(I109) reduced the amount of protein overexpression required to produce spontaneous disease. In fact, spontaneous disease was observed in all of the mice generated, including mice that expressed physiological levels of mutant PrP (Table 3) (Watts et al. 2016). Like the ki-3F4-CJD mice, Tg mice expressing E200K-mutant BVPrP(I109) exhibited small, rounded PrP aggregates in the brain as well as spongiform degeneration. A highly PK-resistant PrP fragment of ∼8 kDa was observed in the brains of spontaneously ill animals and also upon transmission of the disease to Tg mice expressing WT BVPrP(I109) or MoPrP. In both the knock-in and BVPrP(I109) Tg models, FFI- and CJD-causing mutations caused distinct spontaneous pathologies, as well as unique pathologies, following transmission, providing evidence that the D178N and E200K mutations cause the formation of different prion strains.

D178N

Tg mice expressing MoPrP containing the mouse equivalent of the D178N mutation (D177N) along with an M128V substitution were generated to model inherited CJD cases caused by the *PRNP* D178N,V129 haplotype. In homozygous "Tg(CJD)" mice with approximately twofold overexpression of D177N-mutant PrP, signs of spontaneous neurological illness such as ataxia, kyphosis, and foot clasping began to appear at ∼150 d of age and ultimately resulted in death of the mice by ∼300 d of age (Dossena et al. 2008). Detergent-insoluble and mildly PK-resistant PrP species were observed in Tg(CJD) mice and were accompanied by some PrP deposition and reactive astrocytic gliosis in the brain. However, no spongiform degeneration was observed. Brain extracts from spontaneously ill Tg(CJD) mice did not transmit disease to non-Tg mice, Tg mice overexpressing WT MoPrP, or to Tg mice expressing lower levels of D177N-mutant MoPrP(V128) (Bouybayoune et al. 2015).

T183A

A T183A mutation in *PRNP* has been found in multiple individuals with CJD (Nitrini et al. 1997; Grasbon-Frodl et al. 2004). This mutation abolishes the first glycosylation site in PrP by altering the consensus sequence for N-glycan attachment. Tg mice expressing Syrian hamster PrP containing the T183A mutation were generated, but these mice did not develop spontaneous disease (DeArmond et al. 1997).

A224V

A previously unreported A224V mutation was found *in cis* to valine at codon 129 in a patient with CJD (Watts et al. 2015). Tg mice expressing A224V-mutant HuPrP(V129) at levels up to ∼3× did not develop any spontaneous signs of neurological illness with aging and did not exhibit any detergent-insoluble or PK-resistant PrP species in their brains (Watts et al. 2015).

REMAINING CHALLENGES

A critical unresolved issue is determining why the brains of spontaneously ill Tg mice expressing PrP with a fCJD- or FFI-causing mutation do not exhibit the principal biochemical hallmark of these diseases. None of the mouse models of inherited PrP prion disorders created to date develop "stereotypical," highly PK-resis-

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Table 3. Mouse models of familial CJD

Mutation	Line	Type of mouse	PrP sequence	PrP expression level	Spontaneous signs of neurologic illness?	Incubation period to onset of clinical disease (d)	Prion disease–specific neuropathological changes?	Highly PK-resistant PrP?[a]	Disease is transmissible?	Reference
D178N,V129	CJD-A21	Transgenic	Mouse	2×	Yes	~150	Mild	No	No	Dossena et al. 2008
E200K	ki-3F4-CJD	Knock-in	Mouse (3F4 epitope tag)	1×	No	Not reported	Yes	No	Yes	Jackson et al. 2013
	Tg23	Transgenic	Human	3×	No	N/A	No	No	N/A	Asante et al. 2009
	Tg5182	Transgenic	Mouse	8×	No	N/A	No	No	N/A	Telling et al. 1996b
	TgMHu2ME199K	Transgenic	Chimeric mouse/human	2×	Yes	~200	Yes	Yes	Yes	Friedman-Levi et al. 2011
	Tg14210	Transgenic	Bank vole (I109)	1.2×	Yes	~460	Yes	Yes	Yes	Watts et al. 2016
	Tg7253	Transgenic	Bank vole (I109)	1.7×	Yes	~270	Yes	Yes	Yes	Watts et al. 2016
	Tg4253	Transgenic	Bank vole (I109)	2.4×	Yes	~160	Yes	Yes	Yes	Watts et al. 2016
	Tg7271	Transgenic	Bank vole (I109)	2.7×	Yes	~120	Yes	Yes	Yes	Watts et al. 2016

CJD = Creutzfeldt–Jakob disease; PK, proteinase K; N/A, not applicable.
[a]Highly PK-resistant PrP is defined as PrP that is resistant to digestion with PK at a concentration of 20 μg/mL or higher.

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tant PrP species (i.e., PrP27–30) in their brains, despite the presence of obvious prion disease–specific neuropathological changes. The reason behind this conundrum is unclear, but possibilities include inherent differences between the brains of mice and humans or the necessity for extended incubation periods to generate PrP27–30 spontaneously, which cannot be achieved within the normal life span of a mouse. Familial CJD and FFI prions have been successfully transmitted to Tg mice expressing chimeric Mo/HuPrP, resulting in the presence of fCJD- or FFI-specific PrP27–30 species in the brain (Telling et al. 1995, 1996a). This finding indicates that mice are capable of propagating such PK-resistant PrP species, and their nonexistence in Tg mouse models may indicate an inability for them to be formed in the absence of a template. For fCJD, only a small subset of mutations were investigated in Tg mice, and it remains possible that investigating additional, perhaps rarer mutations may be necessary for generating a completely faithful model.

Other challenges include successively modeling the selective targeting of certain brain regions and neuronal populations specified by distinct mutations in *PRNP* and fully recapitulating some of the critical clinical aspects of these disorders. Although some progress has certainly been made in these areas (Jackson et al. 2009; Bouybayoune et al. 2015), it is clear that there is room for the creation of superior models. "Next generation" animal models of the inherited PrP prion disorders may need to take advantage of PrP sequences that are more prone to misfolding (such as BVPrP) or exploit the advantages offered by Tg rats, which were used to generate a potentially more authentic model of AD (Cohen et al. 2013).

CONCLUDING REMARKS

Although much progress has been made toward generating accurate mouse models of the inherited PrP prion disorders, most of the currently available models do not fully recapitulate the entire spectrum of clinical, biochemical, and pathological hallmarks of the corresponding human disease. Development of more translational animal models not only will provide a system for studying the earliest events in prion generation and spread in vivo, but also will create a paradigm for assessing the efficacy of candidate therapeutics designed to interfere with prion formation. Individuals predisposed to developing an inherited PrP prion disorder are likely to benefit the most from the creation of drugs that halt prion formation and/or replication because they can be identified early in life and treatment can commence long before any irreversible neuropathological changes have occurred in the brain. Thus, mouse models of the inherited PrP prion disorders will likely play a critical role in current and future prion disease drug discovery efforts.

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