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Towards authentic transgenic mouse models of heritable PrP prion diseases

Joel C. Watts^{1,2,4}, Kurt Giles^{1,2}, Matthew E.C. Bourkas⁴, Smita Patel¹, Abby Oehler¹, Marta Gavidia¹, Sumita Bhardwaj¹, Joanne Lee¹, and Stanley B. Prusiner^{1,2,3,*}

¹Institute for Neurodegenerative Diseases, University of California, San Francisco, San Francisco, CA 94143, USA

²Department of Neurology, University of California, San Francisco, San Francisco, CA 94143, USA

³Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94143, USA

⁴Tanz Centre for Research in Neurodegenerative Diseases and Department of Biochemistry, University of Toronto, Toronto, ON, Canada M5T 2S8

Abstract

Attempts to model inherited human prion disorders such as familial Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) disease, and fatal familial insomnia (FFI) using genetically modified mice have produced disappointing results. We recently demonstrated that transgenic (Tg) mice expressing wild-type bank vole prion protein (BVPrP) containing isoleucine at polymorphic codon 109 develop a spontaneous neurodegenerative disorder that exhibits many of the hallmarks of prion disease. To determine if mutations causing inherited human prion disease alter this phenotype, we generated Tg mice expressing BVPrP containing the D178N mutation, which causes FFI; the E200K mutation, which causes familial CJD; or an anchorless PrP mutation similar to mutations that cause GSS. Modest expression levels of mutant BVPrP resulted in highly penetrant spontaneous disease in Tg mice, with mean ages of disease onset ranging from ~120 to ~560 days. The brains of spontaneously ill mice exhibited prominent features of prion disease specific neuropathology that were unique to each mutation and distinct from Tg mice expressing wild-type BVPrP. An ~8 kDa proteinase K resistant PrP fragment was found in the brains of spontaneously ill Tg mice expressing either wild-type or mutant BVPrP. The spontaneously formed mutant BVPrP prions were transmissible to Tg mice expressing wild-type or mutant BVPrP as well as to Tg mice expressing mouse PrP. Thus, Tg mice expressing mutant BVPrP

*Corresponding author: Stanley B. Prusiner, Institute for Neurodegenerative Diseases, 675 Nelson Rising Lane, San Francisco, CA 94143-0518. Tel: (415) 476-4482; Fax: (415) 476-8386; stanley.prusiner@ucsf.edu.

Conflict of Interest

The authors declare that they have no conflict of interest.

COMPLIANCE WITH ETHICAL STANDARDS

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

exhibit many of the hallmarks of heritable prion disorders in humans including spontaneous disease, protease-resistant PrP, and prion infectivity.

Keywords

prion; transgenic mice; Creutzfeldt-Jakob; Gerstmann-Sträussler-Scheinker; fatal familial insomnia

INTRODUCTION

Prions are proteins with altered conformations that undergo self-propagation and produce neurodegeneration in both humans and animals [11, 43]. In humans, diseases such as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), and fatal familial insomnia (FFI) are caused by the accumulation of misfolded prion protein (PrP) in the brain. PrP is a glycosylphosphatidylinositol (GPI) anchored protein that is normally found on the surface of cells of the central nervous system (CNS). PrP can exist in distinct conformations: PrP^C is correctly folded with a predominantly alpha-helical structure [46], whereas PrP^{Sc} is misfolded, enriched in beta sheets, aggregation prone, and infectious. In prion disease, PrP^{Sc} propagates through a self-templating mechanism, in which additional copies of PrP^C are converted into PrP^{Sc}. This results in a cascade of PrP^{Sc} aggregates that form throughout the brain, which leads to vacuolation, reactive astrocytic gliosis, and neuronal death. The most commonly encountered forms of PrP^{Sc} are partially resistant to digestion with proteinase K (PK) [33]. Digestion of PrP^{Sc} with PK produces an N-terminally truncated PrP fragment referred to as PrP 27–30 [44].

The vast majority of human PrP prion diseases are caused by spontaneous formation of PrP^{Sc} in the brain. In sporadic prion diseases, PrP^{Sc} is thought to arise from spontaneous misfolding of wild-type (wt) PrP^C into PrP^{Sc}. In inherited prion diseases, a mutation in the gene encoding PrP (*PRNP*) is likely to enhance the spontaneous formation of PrP^{Sc}. Inherited prion diseases encompass ~10–15% of all cases and include familial CJD (fCJD), GSS, and FFI. These three diseases can be differentiated by (i) the clinical signs of disease observed in patients, (ii) the specific disease-causing mutation present in PrP, (iii) the location and type of neuropathological changes in the brain, and (iv) the biochemical characteristics of PrP^{Sc} [28]. The unique features of each disease are thought to arise from the generation of distinct strains of prions, which are encoded with conformational variation within PrP^{Sc} aggregates [5, 57]. Two of the most common mutations that cause inherited prion disease are D178N and E200K. The E200K mutation causes fCJD [18, 21], whereas D178N can cause either fCJD or FFI, depending on whether the mutation is found in *cis* to methionine (FFI) or valine (fCJD) at polymorphic codon 129 in human PrP [19]. Nonsense mutations such as Y226X and Q227X located near the GPI anchor attachment site (GPI) in PrP cause GSS and result in a profound cerebral PrP amyloidosis [25].

Attempts to model heritable PrP prion diseases using transgenic (Tg) mice expressing PrP containing a prion disease causing mutation have been met with limited success (reviewed in [65]). For example, certain lines of Tg or knock-in mice expressing mutant mouse (Mo) or human (Hu) PrP recapitulate some, but not all, of the hallmarks of the corresponding human

prion disease [6, 10, 14, 15, 22–24, 38, 67]. In contrast, other lines of mice expressing mutant PrP do not develop any clinical or biochemical signs of prion disease [2, 56, 63]. It has been particularly difficult to demonstrate that PK-resistant PrP is present in the brains of spontaneously ill animals.

We recently discovered that Tg mice expressing wt bank vole PrP (BVPrP) containing isoleucine at polymorphic codon 109 (I109) develop a spontaneous neurodegenerative disease that recapitulates many characteristics of authentic prion disease, which includes the aggregation of prions accompanied by infectivity [64]. PrP from bank voles can be readily converted to PrP^{Sc} by prions from many different species [1, 13, 39, 40, 62], suggesting that BVPrP may be a universal acceptor for prions. Taken together, these results imply that BVPrP is more prone to misfolding (both spontaneously and upon exposure to PrP^{Sc}) than PrP from other species. Thus, we speculated that BVPrP might be an improved starting point for the generation of Tg mouse models of fCJD, GSS, and FFI that yield better translational qualities than can be currently achieved.

Here, we describe the generation and characterization of Tg mice expressing BVPrP(I109) with mutations that cause fCJD, FFI, or GSS. All lines of mice expressing mutant BVPrP(I109) developed a completely penetrant spontaneous disease and exhibited mutation-specific prion neuropathology. Moreover, a highly PK-resistant PrP fragment was observed in the brains of all spontaneously ill animals expressing wt or mutant BVPrP(I109). We suggest that Tg mice expressing mutant BVPrP(I109) may represent better mouse models of inherited human prion disorders.

MATERIALS AND METHODS

Mice

Mouse husbandry and bioassays were carried out in accordance with the recommendations of the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Academies Press, Washington, DC). All animal experiments were performed under Biosafety Level 2 (BSL-2) conditions using protocols approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco: “Breeding colony and production of transgenic rats and mice (AN084871)” and “Incubation periods of prion and other neurodegenerative diseases (AN084950).” All mice were on an FVB/N strain background. Tg(BVPrP,I109)3581 and Tg(BVPrP,I109)3574 mice [64] were maintained with a PrP knockout (*Prnp*^{0/0}) genotype [7] unless otherwise stated, whereas Tg(MoPrP)4053 mice [8] were maintained with a wt (*Prnp*^{+/+}) genotype. Tg(BVPrP,I109)3581 mice with a *Prnp*^{0/+} or a *Prnp*^{+/+} genotype were generated by crossing with wt FVB/N mice.

Generation of transgenic mice

The D178N, E200K, and GPI (R232X) mutations were generated in the BVPrP(I109) open reading frame by site-directed mutagenesis and were then inserted into the cos.Tet cosmid vector [49], which drives the expression of transgenes in the CNS using the hamster PrP promoter, as previously described [64]. Purified transgene cassettes were microinjected into

the pronuclei of fertilized eggs from *Prnp*^{0/0} mice. Potential founders were identified and then backcrossed to *Prnp*^{0/0} mice to confirm germ-line transmission of the transgene. All Tg lines expressing mutant BVPrP(I109) were maintained in a hemizygous state.

The presence of spontaneous neurological illness in mice was assessed three times per week, and mice were euthanized once two or more neurological signs were present, as determined using the standard diagnostic criteria for prion disease in mice [9]. Survival curves were compared by the log-rank (Mantel-Cox) test using GraphPad Prism 5 software. Brains from spontaneously ill mice were removed and either snap frozen on dry ice and then stored at -80°C or immersion-fixed in 10% neutral buffered formalin.

Mouse inoculations

Brain homogenates (10% [wt/vol] in sterile calcium- and magnesium-free PBS) from spontaneously ill Tg mice were generated using a bead beater (PreCellys). Homogenates were diluted to 1% (wt/vol) using 5% (wt/vol) bovine serum album (BSA), and then 30 µL homogenate was injected into the right cerebral hemisphere of weanling mice at ~60 days of age using a 27-gauge syringe. For intraglossal injections into the tongue [3] and intraperitoneal inoculations, 5 and 100 µL of 1% (wt/vol) brain homogenate, respectively, were used. Inoculated animals were euthanized and their brains collected once two or more signs of neurological illness were apparent, as described above. For end-point titrations, the 1% (wt/vol) brain homogenate was subjected to serial tenfold dilutions in 5% BSA, and 30 µL of each dilution was intracerebrally inoculated into Tg(BVPrP,I109)3581 mice. The resultant incubation periods were compared to PBS-inoculated mice by one-way analysis of variance (ANOVA) with Tukey's post-test using GraphPad Prism 5 software.

Proteinase K digestions

Frozen brains from asymptomatic or clinically ill mice were homogenized to 10% (wt/vol) in PBS as described above, and 9 vol of brain homogenate was added to 1 vol of 10x detergent buffer to give a final concentration of 0.5% (wt/vol) sodium deoxycholate and 0.5% (vol/vol) NP-40. Samples were incubated on ice with occasional vortexing for 20 min and then centrifuged at 1,000 × *g* for 5 min at 4°C. Protein concentration in the supernatant was determined using the bicinchoninic acid (BCA) assay, and then 1 mg of total protein was adjusted to a final volume of 400 µL of detergent buffer containing 50 µg/mL PK (Fermentas) for a final PK-to-protein ratio of 1:50. Samples were digested for 1 h at 37°C (with shaking), and the digestions were then terminated by the addition of PMSF to a concentration of 2 mM. Sarkosyl was added to a final concentration of 2% (vol/vol), and the samples were then subjected to ultracentrifugation at 100,000 × *g* for 1 h at 4°C. The supernatant was removed by gentle aspiration, and the pellet was resuspended in NuPAGE LDS loading buffer (containing β-mercaptoethanol), boiled, and then evaluated by immunoblotting as described below. Typically, 0.5 to 1 mg of PK-digested protein was loaded per lane.

Immunoblotting

Undigested brain extracts were prepared in NuPAGE LDS loading buffer containing β-mercaptoethanol and then boiled. Samples (digested or undigested) were electrophoresed on

10% NuPAGE or Bolt gels (Life Technologies) using the MES buffer system. Blots were then transferred to polyvinylidene fluoride (PVDF) using a wet blotting system and blocked for a minimum of 1 h with blocking buffer (5% [wt/vol] skim milk in Tris-buffered saline containing 0.05% [vol/vol] Tween-20 [TBST]). Blots were incubated overnight with primary antibody (anti-PrP humanized antibodies HuM-P [47] or HuM-D18 [66]) and then washed three times with TBST. Blots were exposed to horseradish peroxidase (HRP) conjugated secondary antibodies diluted in blocking buffer for 1–2 h and then washed 3 times with TBST. In some instances, HuM-P antibody that was conjugated directly to HRP was used. Blots were developed using the enhanced chemiluminescence system (GE Healthcare or Perkin-Elmer) and then exposed to X-ray film.

Cultured cells

Murine N2a neuroblastoma cells lacking PrP expression (N2a-PrP^{-/-}; clone 10–18) [34] were maintained in growth medium (DMEM containing 10% fetal bovine serum, 1x GlutaMax, and 0.2x penicillin-streptomycin [Life Technologies]) in a humidified 37°C/5% CO₂ environment. Prior to transfection, all wt and mutant BVPPr(I109) constructs were subcloned into the pcDNA3 mammalian expression vector and purified using endotoxin-free plasmid Maxiprep kits (Qiagen). For transient transfections, 5×10^5 cells were seeded in 6-well dishes, and ~24 h later cells were transfected in OptiMEM medium using Lipofectamine-2000 according to the manufacturer's instructions (Life Technologies). Each well received 2 µg of plasmid DNA and 4 µL of Lipofectamine-2000. Cells were exposed to the transfection mixture for 24 h, and the medium was subsequently replaced with fresh growth medium. Cells were washed twice with PBS and then lysed using lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5% [vol/vol] NP-40, 0.5% [wt/vol] sodium deoxycholate containing Halt protease inhibitor cocktail [ThermoFisher]) at 48 h post-transfection. Lysates were incubated on ice for 20 min and afterward centrifuged at $1,000 \times g$ for 5 min. The protein concentration in the supernatant fraction was determined using the BCA assay, and samples were then prepared in NuPAGE LDS loading buffer containing β-mercaptoethanol, boiled, and analyzed by immunoblotting as described above. For stable transfections, cells were trypsinized 24 h post-transfection and cultured in growth medium containing G418 at a concentration of 1 mg/mL. Cells were selected over a period of ~2 weeks, pooled, and then expanded. No clonal selection was performed. Stably transfected cells were maintained in medium containing 0.2 mg/mL G418.

Protein turnover assays

N2a-PrP^{-/-} cells stably transfected with either wt, D178N-mutant, or E200K-mutant BVPPr(I109) were plated at a density of 8×10^5 cells in 6 cm tissue culture dishes. Cells were cultured for ~48 h, and the medium was then replaced with growth medium containing cycloheximide (CHX) (30 µg/mL) and lysed as described above at various time points post-exposure. Immunoblotting was performed as described above, and films were subsequently scanned and analyzed by densitometry using ImageJ. The half-lives of wt and mutant BVPPr(I109) following CHX treatment were determined by fitting residual PrP levels to a one-phase decay model with a fixed plateau of 0 using GraphPad Prism 5 software.

ELISA

Relative BVPrP expression levels in Tg mice and transfected cells were determined by enzyme-linked immunosorbent assays (ELISA), which were performed as previously described [64] except that plates were read using a SpectraMax i3 plate reader (Molecular Devices). For brain samples, data represent the average expression level determined using two individual mice. For transfected N2a-PrP^{-/-} cells, data represent the mean BVPrP expression level observed within four biological replicates. Data were analyzed by one-way ANOVA with Tukey's post-test using GraphPad Prism 5 software.

Quantitative polymerase chain reaction (qPCR)

To measure relative *Prnp* mRNA levels, brains from young, asymptomatic Tg mice were collected in RNA^{later} solution (ThermoFisher). Brains were homogenized in PBS, and RNA was then extracted using the MasterPure RNA Purification Kit according to the manufacturer's instructions (Epicentre). cDNA was then generated using the Tetro cDNA Synthesis Kit (BioLine). For determining transgene copy numbers, genomic DNA was isolated from tail snips and further purified using the Genomic DNA Clean and Concentrator (Zymo Research). Quantitative PCR reactions were performed using the SensiFAST SYBR Lo-ROX Kit (BioLine), with final DNA and cDNA concentrations of 3 and 6 ng/μL, respectively, using a Stratagene Mx3005P instrument. The following primers were used, which are specific to the 3' untranslated region of hamster *Prnp*: 5'-ATACTGATGTGACCCTCTGACTTCC-3' (forward) and 5'-GCTGTAGAACCCTTCGCCTATTT-3' (reverse). For cDNA analysis, C_t values were generated by comparison to a cDNA sample prepared from the brain of a Syrian hamster. Relative mRNA levels in the brains of three distinct mice per line were calculated using the formula 2^{-C_t} , averaged, and then normalized to the mRNA levels found in Tg(BVPrP,I109)3574 mice. For copy number analysis, $7\Delta C_t$ values from three independent mice per line were generated by comparison to a Syrian hamster genomic DNA sample, converted to copy numbers using the formula 2^{-C_t} , and then averaged.

Neuropathology

Formalin-fixed brains were embedded in paraffin and then processed for immunohistochemistry as previously described [62]. The following primary antibodies were used: anti-GFAP rabbit polyclonal antibody Z0334 (Dako, 1:500 dilution) to detect astrocytic gliosis and HuM-P (1:500 dilution) to detect PrP^{Sc} deposition. Semiquantitative scoring of vacuolation and PrP^{Sc} deposition in the brains of spontaneously ill or inoculated Tg mice was performed by assigning the following scores to different brain regions: 0 (no vacuolation/PrP^{Sc} deposition observed), 1 (mild vacuolation/PrP^{Sc} deposition), 2 (moderate vacuolation/PrP^{Sc} deposition), or 3 (severe vacuolation/PrP^{Sc} deposition).

RESULTS

In our earlier studies, we were unable to find any highly PK-resistant PrP (i.e., PrP species resistant to digestion with 50 μg/mL PK) in the brains of spontaneously ill Tg mice expressing wt BVPrP(I109) [64]. However, by harvesting insoluble macromolecules using ultracentrifugation following limited PK digestion and increasing the sample size to 1 mg of

protein, we were able to detect an ~8 kDa PK-resistant PrP fragment in brain homogenates from spontaneously ill Tg(BVPrP,I109)3574 mice that was absent in homogenates from young, asymptomatic mice (Fig. 1a). This small PK-resistant PrP fragment was also observed in the brains of Tg(BVPrP,I109)3574 mice following inoculation with brain homogenate from a spontaneously ill Tg(BVPrP,I109)3581 mouse (Fig. 1b). The ~8 kDa PK-resistant PrP fragment was detectable using the antibody HuM-P, which recognizes residues 96–106 of BVPrP. The molecular weight and the apparent lack of N-linked glycosylation sites (as evidenced by the absence of multiple bands corresponding to different PrP glycoforms) likely indicate that the fragment is C-terminally truncated.

We next assessed the level of infectivity present in the brains of spontaneously ill Tg(BVPrP,I109) mice by performing an end-point titration in Tg(BVPrP,I109)3581 mice (hereafter referred to as Tg3581 mice). Inoculation of young Tg3581 mice with dilutions of 10% brain homogenate prepared from a spontaneously ill Tg3581 mouse ranging from 10^{-1} to 10^{-6} produced incubation periods that were significantly shorter than those observed in mice inoculated with phosphate-buffered saline (PBS) (Fig. 1c), revealing that high titres of prions are present in spontaneously ill Tg3581 mice. We also investigated different routes of disease transmission. Intracerebral, intraglossal, and intraperitoneal inoculation of brain homogenate from a spontaneously ill Tg3581 mouse into young Tg3581 mice accelerated the onset of neurological illness compared to uninoculated mice (Fig. 1d), demonstrating that prions that form spontaneously in the brains of Tg(BVPrP,I109) mice are neuroinvasive. Finally, since certain nontransmissible proteinopathies caused by the neuronal expression of truncated PrP molecules or the PrP^C paralog Doppel can be rescued by co-expression of wt PrP^C [37, 50], we tested whether the simultaneous presence of murine PrP had any effect on the manifestation of spontaneous disease in Tg3581 mice. The survival curves for Tg3581 mice lacking murine PrP expression or with one or two copies of the murine *Prnp* gene were indistinguishable (Fig. 1e), suggesting that wt murine PrP^C does not protect against the neurodegenerative phenotype displayed by expression of BVPrP(I109). Cumulatively, these results argue that the brains of spontaneously ill Tg(BVPrP,I109) mice contain prions that exhibit many of the properties of authentic human prions.

Since several point mutations in human PrP cause prion disease with complete or near-complete penetrance [36], we and others attempted to create mouse models of heritable PrP prion diseases using Tg mice. Disappointingly, only modest success was achieved [65]. The promiscuity of bank vole PrP offered a new approach that might mimic the heritable human prion diseases. To test this hypothesis, we produced Tg mice expressing BVPrP harboring pathogenic human point mutations on a *Prnp*^{0/0} background. Three different HuPrP mutations were engineered into BVPrP(I109): (i) D178N, which is predicted to model FFI since the sequence of BVPrP contains methionine at codon 129; (ii) E200K, which is predicted to model fCJD; and (iii) an R232X mutation (“ GPI”) that is similar to the C-terminal nonsense PrP mutations that have been observed in some GSS patients. We obtained four lines of Tg(BVPrP,I109,D178N) mice, five lines of Tg(BVPrP,I109,E200K) mice, and one line of Tg(BVPrP,I109, GPI) mice with germ-line transmission of the transgene. Mutant BVPrP(I109) expression was detected in the brains of all the Tg lines (Fig. 2a). PrP was underglycosylated in the brains of Tg(BVPrP,I109, GPI) mice, as was observed in Tg mice expressing GPI-anchorless MoPrP [52]. Surprisingly, based on

transgene copy numbers, the levels of mutant BVPrP expression in the brain, as determined by ELISA, were lower than expected compared with Tg mice expressing wt BVPrP(I109) (Table 1). For example, a transgene copy number (determined by qPCR) of ~20 generated a wt BVPrP(I109) expression level of ~4x the levels present in non-Tg mice, whereas similar copy numbers resulted in expression levels that were ~10-, 2-, and 8-fold lower in D178N-, E200K-, and GPI-mutant mice, respectively (Table 1, Fig. 2b). Similarly, comparable amounts of brain *Prnp* mRNA levels, as determined by qPCR, resulted in much lower PrP protein levels in mice expressing mutant BVPrP(I109) than in mice expressing wt BVPrP(I109) (Fig. 2c), indicating that the decreased protein levels are not due to reduced *Prnp* transcription.

Lower levels of D178N- and E200K-mutant BVPrP(I109) protein compared to wt BVPrP(I109) were also observed following transient or stable transfection of N2a neuroblastoma cells in which the endogenous murine *Prnp* gene was ablated using CRISPR/Cas9 technology (N2a-PrP^{-/-} cells) (Fig. 2d) [34] with equal amounts of plasmid DNA. In transiently transfected N2a-PrP^{-/-} cells, levels of D178N- and E200K-mutant BVPrP(I109) were ~9- and 2-fold lower than wt BVPrP(I109) (Fig. 2e), which is similar to that observed in the Tg mice. Treatment of stably transfected N2a-PrP^{-/-} cells with CHX to shut off protein synthesis revealed that turnover of D178N- and E200K-mutant BVPrP(I109) occurs more rapidly than wt BVPrP(I109) (Fig. 2f, g). The half-lives of wt, D178N-mutant, and E200K-mutant BVPrP(I109) were estimated to be ~17, 2, and 6 h, respectively, suggesting that the lower steady-state levels of mutant BVPrP(I109) observed in the brains of the Tg mice are due to increased protein turnover rates.

Despite the physiological or subphysiological expression levels of mutant BVPrP(I109), all the lines of Tg mice developed age-dependent signs of spontaneous neurological illness (Table 1, Fig. 3a–c). All the examined mice expressing mutant BVPrP(I109) (198 out of 198) developed spontaneous disease. For the Tg mice expressing E200K-mutant BVPrP(I109), there was a clear inverse relationship between BVPrP expression level and mean age of disease onset. Compared to Tg mice expressing wt BVPrP(I109), much lower levels of D178N- or E200K-mutant BVPrP(I109) were required to produce spontaneous disease with a given mean age of onset (Fig. 3d). A highly PK-resistant PrP fragment of ~8 kDa was found in brain homogenates from spontaneously ill Tg mice expressing mutant BVPrP(I109) but not in homogenates from young, asymptomatic mice (Fig. 3e). Levels of this PK-resistant PrP fragment varied greatly with the three mutations; the highest amounts were observed in GPI-mutant mice, whereas lower levels were found in D178N-mutant mice, and much lower amounts were found in the E200K-mutant animals (Fig. S1).

The D178N, E200K, and GPI mutations generated distinct neuropathological profiles in spontaneously ill mice. Clustered, coarse PrP^{Sc} deposition was observed in the brains of spontaneously ill Tg(BVPrP,I109,D178N) mice (Fig. 4a, b), whereas small, rounded PrP^{Sc} aggregates were observed in Tg(BVPrP,I109,E200K) mice (Fig. 4c, d). The brains of spontaneously ill mice expressing GPI-mutant BVPrP(I109) contained large, dense-core PrP^{Sc} plaques as well as smaller or more diffuse aggregates (Fig. 4e–g). The dense core plaques stained positive with Thioflavin S (Fig. 4h), indicating the presence of PrP amyloids, whereas the smaller deposits were negative. No cerebral amyloid angiopathy

(CAA) was observed in the GPI-mutant BVPrP(I109) mice. Large numbers of PrP^{Sc} deposits were present throughout the brain in GPI-mutant mice, whereas PrP^{Sc} deposits were found in varying numbers among different brain regions of D178N- and E200K-mutant mice (Fig. 4s). Tg3581 mice expressing wt BVPrP(I109) exhibited two distinct types of PrP^{Sc} deposition [64]. Clusters of punctate PrP^{Sc} deposits (Fig. 4i) similar to those observed in Tg(BVPrP,I109,D178N) mice were found in all animals analyzed, whereas large, plaque-like PrP^{Sc} deposits (Fig. 4j) were found in ~40% of spontaneously ill mice. No plaque-like PrP^{Sc} deposits were observed in any of the Tg mice expressing D178N- or E200K-mutant BVPrP(I109). The distribution of PrP^{Sc} deposition in Tg3581 mice was most similar to that in Tg(BVPrP,I109,D178N) mice (Fig. 4s).

All the spontaneously ill Tg mice expressing mutant BVPrP(I109) exhibited spongiform degeneration (vacuolation) within their brains (Fig. 4k–n). However, each mutation produced a distinct vacuolation profile (Fig. 3t). For instance, vacuolation levels were generally low in the brains of GPI-mutant mice (except in the hippocampus) despite the high level of PrP^{Sc} deposition throughout the brain. In the D178N-mutant mice, the strongest vacuolation was observed in the frontal cortex, caudate nucleus (striatum), and the hypothalamus, whereas prominent midbrain vacuolation was unique to E200K-mutant mice (Fig. 3t). The cerebral vacuolation pattern in Tg3581 mice expressing wt BVPrP(I109) was similar to that in Tg(BVPrP,I109,E200K) mice (Fig. 3t). Reactive astrocytic gliosis was also observed in the brains of all spontaneously ill mice (Fig. 3o–r). These results demonstrate that spontaneously ill mice expressing mutant BVPrP(I109) exhibit all the neuropathological hallmarks of prion disease and that each mutation produces distinct neuropathological changes that can be distinguished from those present in mice expressing wt BVPrP(I109).

Passage of brain homogenate from spontaneously sick Tg mice expressing wt BVPrP(I109) into young mice expressing BVPrP(I109) resulted in remarkably rapid disease transmission with incubation periods of ~40 days [64]. To determine if a similar phenomenon occurs with Tg mice expressing mutant BVPrP(I109), we inoculated ~2-month-old Tg mice expressing D178N-, E200K-, or GPI-mutant BVPrP(I109) with brain homogenates from spontaneously ill mice expressing the same mutant PrP. For D178N and E200K mutations, inoculation experiments were performed using lines that developed spontaneous disease with longer mean ages of onset to permit a bigger transmission window. For D178N, three distinct samples from spontaneously ill mice transmitted with mean incubation times between ~40 and ~60 days (Table 2), which represents an ~120-day acceleration of disease compared to uninoculated mice (Fig. 5a). For E200K, brain homogenates from four different spontaneously ill mice transmitted with mean incubations between ~40 and ~70 days (Table 2), which represents an ~350-day acceleration of disease compared to uninjected mice (Fig. 5f). Two distinct samples from GPI-mutant mice transmitted disease to young mice expressing GPI-mutant BVPrP(I109) with mean incubation periods of ~100 days (Table 2), which represents an ~250-day acceleration of disease (Fig. 5k).

The brains of inoculated mutant Tg(BVPrP,I109) mice contained a similar ~8 kDa highly PK-resistant PrP fragment to those fragments observed in spontaneously ill animals (Fig. 5b, g, l). In general, the neuropathological features of the spontaneous illness were maintained upon second passage in mutant BVPrP(I109) Tg mice. For example, clusters of coarse PrP^{Sc}

deposits were observed in the inoculated D178N mice (Fig. 5c), whereas large numbers of plaque-like PrP^{Sc} deposits were found in the brains of inoculated GPI mice (Fig. 5m). Inoculated E200K mice exhibited synaptic-like PrP^{Sc} deposit that were much smaller than the rounded deposits observed in spontaneously ill animals (Fig. 5h). Abundant vacuolation was found in inoculated E200K mice (Fig. 5i) and was also present in the inoculated D178N mice (Fig. 5d), whereas little to no vacuolation was observed in the brains of inoculated GPI mice, even in the hippocampus (Fig. 5n) where robust vacuolation was observed in spontaneously ill GPI mice. Astrocytic gliosis was most prominent in the inoculated E200K (Fig. 5j) and GPI mice (Fig. 5o), whereas only mild gliosis was present in the inoculated D178N mice (Fig. 5e).

The unique, mutation-specific neuropathological features observed both in spontaneously ill Tg mice and following disease transmission led us to suggest that each mutation determines the formation of a distinct strain of prions. To test this hypothesis, we inoculated Tg3581 mice expressing wt BVPrP(I109) with brain homogenates from spontaneously ill mice expressing each of the three mutant BVPrP(I109) proteins. Mean incubation periods between ~34 and ~76 days were obtained (Table 2), which represented a substantial acceleration of disease compared to PBS-inoculated and uninoculated mice (Fig. 6a). The brains of all the inoculated Tg3581 mice exhibited the ~8 kDa highly PK-resistant PrP fragment, which was absent in brains isolated from age-matched uninoculated control animals (Fig. 6b). Inoculation with brain homogenate from a spontaneously ill D178N mouse produced PrP^{Sc} deposits similar to those observed in spontaneously ill and inoculated Tg(BVPrP,I109,D178N) mice (Fig. 6c). In contrast, small synaptic-like PrP^{Sc} deposits that resembled those present in inoculated Tg(BVPrP,I109,E200K) mice were found in the brains of Tg3581 mice inoculated with homogenate from a spontaneously ill E200K mouse (Fig. 6d). These synaptic-like deposits were also similar to those found in Tg3581 mice inoculated with homogenate from spontaneously ill Tg mice expressing wt BVPrP(I109) [64]. Plaque-like PrP^{Sc} deposits with dense cores were not observed in the brains of GPI-inoculated Tg3581 mice. Instead, small plaque-like clusters of PrP^{Sc} aggregates were observed in the hippocampus (Fig. 6e), which could be clearly distinguished from those present in Tg3581 mice inoculated with D178N or E200K samples. Despite the distinct morphologies of PrP^{Sc} aggregates, the distribution of cerebral PrP^{Sc} deposition was not substantially different between mice inoculated with D178N, E200K, or GPI samples (Fig. S2a). Vacuolation and astrocytic gliosis were most intense in D178N- and E200K-inoculated Tg3581 mice (Fig. 6f, g, i, j) and were also present to a lesser degree in GPI-inoculated animals (Fig. 6h, k) (Fig. S2b). The distribution of vacuolation in the brains of Tg3581 mice inoculated with D178N or E200K samples was essentially the same (Fig. S2b).

To further address the question of unique strains, we inoculated Tg(MoPrP)4053 mice that overexpress wt MoPrP [8] but do not develop a late-onset neurodegenerative disorder [12] with brain homogenates from spontaneously ill mice expressing D178N- or E200K-mutant BVPrP(I109). Near-complete disease transmission was observed in the inoculated Tg(MoPrP)4053 mice, with mean incubation periods ranging from 304 to 421 days (Table 2). The brains of mice inoculated with brain homogenate from spontaneously ill mutant Tg(BVPrP,I109) mice exhibited variable levels of an ~8 kDa highly PK-resistant PrP fragment (Fig. 7a). This fragment was absent in asymptomatic Tg(MoPrP)4053 mice

euthanized at ~660 days post-inoculation (dpi) with PBS. While D178N- and E200K-inoculated Tg(MoPrP)4053 mice both exhibited vacuolation in the hippocampus (Fig. 7d, g), the morphologies of PrP^{Sc} deposition were unique for each mutation. PrP^{Sc} deposits were much larger in the D178N-inoculated Tg(MoPrP)4053 mice (Fig. 7b, c) compared with the E200K-inoculated animals, which exhibited smaller, clustered deposits (Fig. 7e, f) that were similar to those present in Tg(MoPrP)4053 mice that developed disease ~300 days post-inoculation with homogenate from spontaneously ill Tg mice expressing wt BVPPrP(I109) [64]. The distribution of PrP^{Sc} aggregates in the brain and the pattern of cerebral vacuolation were essentially identical for D178N- and E200K-inoculated Tg(MoPrP)4053 mice (Fig. S3).

DISCUSSION

One of the most remarkable features of the prion disorders is that these illnesses are both infectious and genetic. In some instances, mutant prion proteins causing inherited prion diseases produce clinical illness when studied in Tg mouse models, whereas the corresponding wt proteins do not [10, 14, 22, 38]. That said, developing authentic mouse models of inherited PrP prion disorders has been disappointing: low levels of PrP^{Sc} and prion infectivity as well as prolonged incubation times and minimal neuropathological changes have typically been observed. The remarkable promiscuity of BVPrP(I109) prompted us to examine several aspects of inherited human PrP prion disease that were previously resistant to investigation in Tg mice. We constructed unique transgenes encoding mutant BVPrP(I109) containing the D178N, E200K, or GPI human PrP point mutations. Tg mice expressing the mutant BVPrPs recapitulated many of the hallmarks of inherited human prion disorders, including spontaneous neurodegeneration and prion infectivity.

A comparison of other Tg or knock-in mouse models expressing D178N-, E200K-, or GPI-mutant BVPrP(I109) reveals that utilizing the BVPrP backbone enhanced the development of spontaneous disease (Table S1). For example, Tg(FFI-26) mice, which express D178N-mutant MoPrP at 2x [6], require ~200 days to develop spontaneous disease, whereas a similar incubation period was achieved with 0.4–0.5x expression of D178N-mutant BVPrP(I109). Although the D178N-mutant BVPrP(I109) mice developed a transmissible illness characterized by the presence of a PK-resistant PrP fragment, no disease transmissibility or highly PK-resistant PrP was observed in the brains of Tg(FFI-26) mice. While Tg(MHu2M,E199K) mice, which express a chimeric mouse/human PrP containing the E200K mutation at 2x [15], develop spontaneous disease with incubation periods comparable to the E200K-mutant BVPrP(I109) mice, the presence of disease transmissibility and highly PK-resistant PrP is less clear in this line. Perhaps the strongest evidence for the BVPrP backbone facilitating the generation of spontaneous disease occurs with the GPI mutation. Approximately 50% of Tg8015 mice, which express GPI-anchorless MoPrP at 1.7x, develop spontaneous signs of neurological illness with a mean incubation period of ~600 days [52], whereas 100% of Tg24600 mice, which express GPI-mutant BVPrP(I109) at three times lower levels, develop spontaneous disease ~200 days more rapidly. Thus, we infer that BVPrP(I109) is a superior substrate for studying mutation-induced effects on PrP misfolding and prion formation in Tg mice.

Compared with Tg mice expressing wt BVPrP(I109) [64], mice expressing mutant BVPrP required much lower PrP levels to elicit similar incubation periods, arguing strongly that these mutations promote the formation of prions. A “two-hit” model may explain these observations. We hypothesize that the first “hit” is the presence of isoleucine at polymorphic codon 109 of BVPrP. Tg mice expressing BVPrP(I109) developed spontaneous disease, whereas mice expressing BVPrP(M109) remained healthy for more than 500 days [64], suggesting that I109 promotes the spontaneous misfolding of BVPrP. The expression of a disease-causing mutation may constitute a second “hit” that further accelerates the misfolding of the protein and modifies the phenotypic expression of disease. The turnover rates for D178N- and E200K-mutant BVPrP(I109) were accelerated compared to wt BVPrP(I109), suggesting that the mutations destabilize the structure of PrP. This destabilization may augment the likelihood of PrP misfolding and spontaneous prion formation.

All the spontaneously ill wt and mutant BVPrP(I109) Tg mice exhibited an ~8 kDa highly PK-resistant PrP fragment in their brains. This is a major discrepancy from the brains of FFI and CJD(E200K) patients, which exhibit the higher molecular weight, PK-resistant PrP^{Sc} species PrP 27–30 [57]. Whether this ~8 kDa PrP fragment is the pathogenic and/or transmissible PrP species in the Tg mice remains to be determined. Similarly sized PK-resistant PrP fragments have been observed in the brains of some GSS patients [41, 54, 55], bank voles inoculated with GSS prions [42], patients with variably protease-sensitive prionopathy (VPSPr) [68], or in sheep with Nor98 atypical scrapie [27], providing evidence that this fragment is relevant to disease. Spontaneously generated PrP 27–30 has not been observed in the brains of any Tg mice expressing wt or mutant PrP [10, 15, 22, 38, 51, 58, 64], the reason for which is unclear.

Transmission of spontaneously generated mutant BVPrP(I109) prions to Tg mice expressing wt BVPrP(I109) or MoPrP provided some evidence that each sequence specifies the formation of a distinct strain of prions. The morphologies of cerebral PrP^{Sc} deposition were unique to each mutation, arguing for conformationally distinct PrP prions. However, the overall distribution of PrP^{Sc} deposition and the pattern of cerebral vacuolation were highly similar between mice inoculated with the various mutant BVPrP(I109) samples. Moreover, the PK-resistant PrP fragments observed in Tg mice expressing wt or mutant BVPrP(I109) or upon disease transmission were similar, if not indistinguishable, suggesting that the PrP^{Sc} aggregates are structurally analogous and exhibit similar neurotoxic properties. It may be possible to biochemically differentiate the various ~8 kDa PK-resistant PrP species using other prion-strain-specific assays, such as the conformational stability assay [29], or by mass spectrometry. In humans, fCJD(E200K) and FFI prions exhibit distinct sizes of protease-resistant cores in immunoblots upon digestion with PK. Following removal of N-linked sugars, PK-resistant PrP species from fCJD(E200K) patients migrate to ~21 kDa, whereas FFI prions migrate to ~19 kDa [57]. Our inability to decipher a difference in the molecular weight of the PK-resistant PrP fragments between Tg mice expressing D178N- or E200K-mutant BVPrP(I109) implies that the structural consequences of these mutations in our Tg mice are distinct from those that occur in fCJD(E200K) and FFI patients.

Because the ~8 kDa PK-resistant PrP fragment is predicted to be C-terminally truncated, it is likely to exclude the mutant D178N or E200K residues. Thus, the ~8 kDa fragment may represent a core prion structure that is prone to form spontaneously when residue I109 of BVPPrP is present, potentially in conjunction with other BVPPrP-specific residues. It should be noted that BVPPrP(I109) is also capable of adopting other PK-resistant conformations, such as PrP 27–30 [13, 62]. In this scenario, the D178N, E200K, and GPI mutations are likely to slightly modify the conformation of BVPPrP(I109), resulting in distinct morphologies of PrP^{Sc} deposits that are self-propagating. Alternatively, each mutation may selectively stabilize a specific PrP^{Sc} aggregate conformation that arises stochastically due to expression of BVPPrP(I109). Indeed, both FFI and fCJD(E200K) have sporadic equivalents that exhibit nearly identical clinical, pathological, and biochemical features but without the presence of a mutation in PrP [16, 32]. In support of this notion, the PrP^{Sc} deposits in Tg mice expressing wt BVPPrP(I109) were more heterogeneous than those present in the Tg mice expressing mutant BVPPrP(I109), where each mutation defined a specific pathology. Moreover, PrP^{Sc} deposits similar to those found in the D178N- and GPI-mutant mice have been observed in Tg(BVPPrP,I109) mice [64]. However, the small, rounded PrP^{Sc} deposits found in the brains of E200K-mutant mice were unique to these animals, suggesting that this mutation specifies a distinct aggregate conformation that does not readily form with wt BVPPrP(I109).

Further studies are required to determine whether Tg mice expressing mutant BVPPrP(I109) recapitulate any of the clinicopathological characteristics of the corresponding human prion diseases. For example, it will be interesting to investigate whether the D178N-mutant BVPPrP(I109) Tg mice exhibit alterations to sleep–wake cycles, a predominant clinical feature of FFI. Such disturbances have been found in two mouse models expressing FFI-mutant MoPrP despite the absence of FFI-like PK-resistant PrP species in their brains [6, 23]. Of the three mutations investigated, the pathological phenotype elicited by the GPI mutation most closely resembled that of the associated human disease. Like GSS patients with mutations that create GPI-anchorless PrP [25], the brains of Tg mice expressing GPI-mutant BVPPrP(I109) exhibited abundant PrP-containing amyloid plaques and an ~8 kDa PK-resistant PrP fragment. Tg mice expressing GPI-anchorless MoPrP also developed a similar phenotype [52]. Interestingly, CAA was observed in a GSS patient with the Y226X mutation but not in a patient with the Q227X mutation, suggesting that the retention of tyrosine at residue 226 may restrict the formation of PrP deposits within blood vessels [25]. This finding is in accordance with our results: we observed no PrP-CAA in the brains of ill Tg24600 mice, likely because the GPI mutation we employed (R232X) retains the Y226 residue.

The abbreviated incubation periods and the relatively uniform ages of disease onset observed in some of the D178N- and E200K-mutant BVPPrP(I109) lines suggest that they may be useful for testing the efficacy of candidate prion disease therapeutics. Small molecules such as cpd-B, 2-aminothiazoles, anle138b, and polythiophenes prolong the life span of mice inoculated with mouse-passaged prion strains [4, 17, 20, 26, 30, 59]. However, ~99% of human prion disease cases result from the spontaneous formation of prions in the brain and not the introduction of preformed PrP^{Sc} prions. Whether the same compounds are effective at blocking the initial steps of prion generation is unknown. Finally, it has become

increasingly clear that the principles of PrP biology are also applicable to many other proteins including A β , tau, and α -synuclein, all three of which can acquire many of the features of PrP prions, including transmissibility and the existence of strains [31, 35, 45, 48, 53, 60, 61]. Thus, Tg mice expressing mutant B^VPrP(I109) may be suitable for deciphering the molecular mechanisms governing prion formation, which may have widespread implications across multiple human neurodegenerative disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Agrimi U, Nonno R, Dell'Omo G, Di Bari MA, Conte M, Chiappini B, Esposito E, Di Guardo G, Windl O, Vaccari G, et al. Prion protein amino acid determinants of differential susceptibility and molecular feature of prion strains in mice and voles. *PLoS Pathog.* 2008; 4:e1000113. [PubMed: 18654630]
2. Asante EA, Gowland I, Grimshaw A, Linehan JM, Smidak M, Houghton R, Osiguwa O, Tomlinson A, Joiner S, Brandner S, et al. Absence of spontaneous disease and comparative prion susceptibility of transgenic mice expressing mutant human prion proteins. *J Gen Virol.* 2009; 90:546–558. [PubMed: 19218199]
3. Bartz JC, Kincaid AE, Bessen RA. Rapid prion neuroinvasion following tongue infection. *J Virol.* 2003; 77:583–591. [PubMed: 12477862]
4. Berry DB, Lu D, Geva M, Watts JC, Bhardwaj S, Oehler A, Renslo AR, DeArmond SJ, Prusiner SB, Giles K. Drug resistance confounding prion therapeutics. *Proc Natl Acad Sci USA.* 2013; 110:E4160–E4169. [PubMed: 24128760]
5. 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]
6. Bouybayoune I, Mantovani S, Del Gallo F, Bertani I, Restelli E, Comerio L, Tapella L, Baracchi F, Fernández-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:e1004796. [PubMed: 25880443]
7. Büeler H, Fisher 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]
8. Carlson GA, Ebeling C, Yang S-L, Telling G, Torchia M, Groth D, Westaway D, DeArmond SJ, Prusiner SB. Prion isolate specified allotypic interactions between the cellular and scrapie prion proteins in congenic and transgenic mice. *Proc Natl Acad Sci USA.* 1994; 91:5690–5694. [PubMed: 7911243]

9. Carlson GA, Kingsbury DT, Goodman PA, Coleman S, Marshall ST, DeArmond S, Westaway D, Prusiner SB. Linkage of prion protein and scrapie incubation time genes. *Cell*. 1986; 46:503–511. [PubMed: 3015416]
10. Chiesa R, Piccardo P, Ghetti B, Harris DA. Neurological illness in transgenic mice expressing a prion protein with an insertional mutation. *Neuron*. 1998; 21:1339–1351. [PubMed: 9883727]
11. Colby DW, Prusiner SB. Prions. *Cold Spring Harb Perspect Biol*. 2011; 3:a006833. [PubMed: 21421910]
12. Colby DW, Wain R, Baskakov IV, Legname G, Palmer CG, Nguyen H-OB, Lemus A, Cohen FE, DeArmond SJ, Prusiner SB. Protease-sensitive synthetic prions. *PLoS Pathog*. 2010; 6:e1000736. [PubMed: 20107515]
13. Di Bari MA, Nonno R, Castilla J, D'Agostino C, Pirisinu L, Riccardi G, Conte M, Richt J, Kunkle R, Langeveld J, et al. Chronic wasting disease in bank voles: characterisation of the shortest incubation time model for prion diseases. *PLoS Pathog*. 2013; 9:e1003219. [PubMed: 23505374]
14. 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; 60:598–609. [PubMed: 19038218]
15. 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:e1002350. [PubMed: 22072968]
16. Gambetti P, Kong Q, Zou W, Parchi P, Chen SG. Sporadic and familial CJD: classification and characterisation. *Br Med Bull*. 2003; 66:213–239. [PubMed: 14522861]
17. Giles K, Berry DB, Condello C, Hawley RC, Gallardo-Godoy A, Bryant C, Oehler A, Elepano M, Bhardwaj S, Patel S, et al. Different 2-aminothiazole therapeutics produce distinct patterns of scrapie prion neuropathology in mouse brains. *J Pharmacol Exp Ther*. 2015; 355:2–12. [PubMed: 26224882]
18. Goldfarb LG, Mitrova E, Brown P, Toh BH, Gajdusek DC. Mutation in codon 200 of scrapie amyloid protein gene in two clusters of Creutzfeldt-Jakob disease in Slovakia. *Lancet*. 1990; 336:514–515.
19. Goldfarb LG, Petersen RB, Tabaton M, Brown P, LeBlanc AC, Montagna P, Cortelli P, Julien J, Vital C, Pendelbury WW. Fatal familial insomnia and familial Creutzfeldt-Jakob disease: disease phenotype determined by a DNA polymorphism. *Science*. 1992; 258:806–808. [PubMed: 1439789]
20. Herrmann US, Schutz AK, Shirani H, Huang D, Saban D, Nuvolone M, Li B, Ballmer B, Åslund AKO, Mason JJ, et al. Structure-based drug design identifies polythiophenes as antiprion compounds. *Sci Transl Med*. 2015; 7:299ra123.
21. Hsiao K, Meiner Z, Kahana E, Cass C, Kahana I, Avrahami D, Scarlato G, Abramsky O, Prusiner SB, Gabizon R. Mutation of the prion protein in Libyan Jews with Creutzfeldt-Jakob disease. *N Engl J Med*. 1991; 324:1091–1097. [PubMed: 2008182]
22. Hsiao KK, Scott M, Foster D, Groth DF, DeArmond SJ, Prusiner SB. Spontaneous neurodegeneration in transgenic mice with mutant prion protein. *Science*. 1990; 250:1587–1590. [PubMed: 1980379]
23. 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; 63:438–450. [PubMed: 19709627]
24. 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 USA*. 2013; 110:14759–14764. [PubMed: 23959875]
25. Jansen C, Parchi P, Capellari S, Vermeij AJ, Corrado P, Baas F, Strammiello R, van Gool WA, van Swieten JC, Rozemuller AJM. Prion protein amyloidosis with divergent phenotype associated with two novel nonsense mutations in *PRNP*. *Acta Neuropathol*. 2010; 119:189–197. [PubMed: 19911184]
26. Kawasaki Y, Kawagoe K, Chen CJ, Teruya K, Sakasegawa Y, Doh-ura K. Orally administered amyloidophilic compound is effective in prolonging the incubation periods of animals cerebrally

- infected with prion diseases in a prion strain-dependent manner. *J Virol.* 2007; 81:12889–12898. [PubMed: 17881452]
27. Klingeborn M, Wik L, Simonsson M, Renstrom LH, Ottinger T, Linne T. Characterization of proteinase K-resistant N- and C-terminally truncated PrP in Nor98 atypical scrapie. *J Gen Virol.* 2006; 87:1751–1760. [PubMed: 16690942]
 28. Kong, Q.; Surewicz, WK.; Petersen, RB.; Zou, W.; Chen, SG.; Gambetti, P.; Parchi, P.; Capellari, S.; Goldfarb, L.; Montagna, P., et al. Inherited prion diseases. In: Prusiner, SB., editor. *Prion Biology and Diseases. 2.* Cold Spring Harbor Laboratory Press; City: 2004. p. 673-775.
 29. Legname G, Nguyen H-OB, Peretz D, Cohen FE, DeArmond SJ, Prusiner SB. Continuum of prion protein structures enciphers a multitude of prion isolate-specified phenotypes. *Proc Natl Acad Sci USA.* 2006; 103:19105–19110. [PubMed: 17142317]
 30. Lu D, Giles K, Li Z, Rao S, Dolgih E, Gever JR, Geva M, Elepano ML, Oehler A, Bryant C, et al. Biaryl amides and hydrazones as therapeutics for prion disease in transgenic mice. *J Pharmacol Exp Ther.* 2013; 347:325–338. [PubMed: 23965382]
 31. Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, Lee VM. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science.* 2012; 338:949–953. [PubMed: 23161999]
 32. Mastrianni JA, Nixon R, Layzer R, Telling GC, Han D, DeArmond SJ, Prusiner SB. Prion protein conformation in a patient with sporadic fatal insomnia. *N Engl J Med.* 1999; 340:1630–1638. [PubMed: 10341275]
 33. McKinley MP, Bolton DC, Prusiner SB. A protease-resistant protein is a structural component of the scrapie prion. *Cell.* 1983; 35:57–62. [PubMed: 6414721]
 34. Mehrabian M, Brethour D, MacIsaac S, Kim JK, Gunawardana CG, Wang H, Schmitt-Ulms G. CRISPR-Cas9-based knockout of the prion protein and its effect on the proteome. *PLoS ONE.* 2014; 9:e114594. [PubMed: 25490046]
 35. Meyer-Luehmann M, Coomaraswamy J, Bolmont T, Kaeser S, Schaefer C, Kilger E, Neuenschwander A, Abramowski D, Frey P, Jaton AL, et al. Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. *Science.* 2006; 313:1781–1784. [PubMed: 16990547]
 36. Minikel EV, Vallabh SM, Lek M, Estrada K, Samocha KE, Sathirapongsasuti JF, McLean CY, Tung JY, Yu LPC, Gambetti P, et al. Quantifying prion disease penetrance using large population control cohorts. *Sci Transl Med.* 2016; 8:322ra329.
 37. Moore RC, Mastrangelo P, Bouzamondo E, Heinrich C, Legname G, Prusiner SB, Hood L, Westaway D, DeArmond SJ, Tremblay P. Doppel-induced cerebellar degeneration in transgenic mice. *Proc Natl Acad Sci USA.* 2001; 98:15288–15293. [PubMed: 11734625]
 38. 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; 24:2472–2480. [PubMed: 15962001]
 39. 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:e12. [PubMed: 16518470]
 40. Orrú CD, Groveman BR, Raymond LD, Hughson AG, Nonno R, Zou W, Ghetti B, Gambetti P, Caughey B. Bank vole prion protein as an apparently universal substrate for RT-QuIC-based detection and discrimination of prion strains. *PLoS Pathog.* 2015; 11:e1004983. [PubMed: 26086786]
 41. Parchi P, Chen SG, Brown P, Zou W, Capellari S, Budka H, Hainfellner J, Reyes PF, Golden GT, Hauw JJ, et al. Different patterns of truncated prion protein fragments correlate with distinct phenotypes in P102L Gerstmann-Straussler-Scheinker disease. *Proc Natl Acad Sci USA.* 1998; 95:8322–8327. [PubMed: 9653185]
 42. Pirisinu L, Di Bari MA, D'Agostino C, Marcon S, Riccardi G, Pileggi A, Cohen ML, Appleby BS, Gambetti P, Ghetti B, et al. Gerstmann-Sträussler-Scheinker disease subtypes efficiently transmit in bank voles as genuine prion diseases. *Sci Rep.* 2016; 6:20443. [PubMed: 26841849]
 43. Prusiner SB. A unifying role for prions in neurodegenerative diseases. *Science.* 2012; 336:1511–1513. [PubMed: 22723400]

44. Prusiner SB, Groth DF, Bolton DC, Kent SB, Hood LE. Purification and structural studies of a major scrapie prion protein. *Cell*. 1984; 38:127–134. [PubMed: 6432339]
45. Prusiner SB, Woerman AL, Rampersaud R, Watts JC, Berry DB, Patel S, Oehler A, Lowe JK, Kravitz SN, Geschwind DH, et al. Evidence for α -synuclein prions causing multiple system atrophy in humans with signs of Parkinson's disease. *Proc Natl Acad Sci USA*. 2015; 112:E5308–E5317. [PubMed: 26324905]
46. Riek R, Hornemann S, Wider G, Billeter M, Glockshuber R, Wüthrich K. NMR structure of the mouse prion protein domain PrP(121–231). *Nature*. 1996; 382:180–182. [PubMed: 8700211]
47. Safar JG, Scott M, Monaghan J, Deering C, Didorenko S, Vergara J, Ball H, Legname G, Leclerc E, Solforsoli L, et al. Measuring prions causing bovine spongiform encephalopathy or chronic wasting disease by immunoassays and transgenic mice. *Nat Biotechnol*. 2002; 20:1147–1150. [PubMed: 12389035]
48. Sanders DW, Kaufman SK, DeVos SL, Sharma AM, Mirbaha H, Li A, Barker SJ, Foley AC, Thorpe JR, Serpell LC, et al. Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron*. 2014; 82:1271–1288. [PubMed: 24857020]
49. 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]
50. 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; 93:203–214. [PubMed: 9568713]
51. 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 USA*. 2009; 106:304–309. [PubMed: 19073920]
52. Stöhr J, Watts JC, Legname G, Oehler A, Lemus A, Nguyen H-OB, Sussman J, Wille H, DeArmond SJ, Prusiner SB, et al. Spontaneous generation of anchorless prions in transgenic mice. *Proc Natl Acad Sci USA*. 2011; 108:21223–21228. [PubMed: 22160704]
53. Stöhr J, Watts JC, Mensinger ZL, Oehler A, Grillo SK, DeArmond SJ, Prusiner SB, Giles K. Purified and synthetic Alzheimer's amyloid beta (A β) prions. *Proc Natl Acad Sci USA*. 2012; 109:11025–11030. [PubMed: 22711819]
54. Tagliavini F, Prelli F, Ghiso J, Bugiani O, Serban D, Prusiner SB, Farlow MR, Ghetti B, Frangione B. Amyloid protein of Gerstmann-Sträussler-Scheinker disease (Indiana kindred) is an 11 kd fragment of prion protein with an N-terminal glycine at codon 58. *EMBO J*. 1991; 10:513–519. [PubMed: 1672107]
55. Tagliavini F, Prelli F, Porro M, Rossi G, Giaccone G, Farlow MR, Dlouhy SR, Ghetti B, Bugiani O, Frangione B. Amyloid fibrils in Gerstmann-Sträussler-Scheinker disease (Indiana and Swedish kindreds) express only PrP peptides encoded by the mutant allele. *Cell*. 1994; 79:695–703. [PubMed: 7954833]
56. 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]
57. 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]
58. Torres JM, Castilla J, Pintado B, Gutiérrez-Adan A, Andréoletti O, Aguilar-Calvo P, Arroba AI, Parra-Arrondo B, Ferrer I, Manzanares J, et al. Spontaneous generation of infectious prion disease in transgenic mice. *Emerg Infect Dis*. 2013; 19:1938–1947. [PubMed: 24274622]
59. Wagner J, Ryazanov S, Leonov A, Levin J, Shi S, Schmidt F, Prix C, Pan-Montojo F, Bertsch U, Mitteregger-Kretschmar G, et al. Anle138b: a novel oligomer modulator for disease-modifying therapy of neurodegenerative diseases such as prion and Parkinson's disease. *Acta Neuropathol*. 2013; 125:795–813. [PubMed: 23604588]
60. Watts JC, Condello C, Stöhr J, Oehler A, Lee J, DeArmond SJ, Lannfelt L, Ingelsson M, Giles K, Prusiner SB. Serial propagation of distinct strains of A β prions from Alzheimer's disease patients. *Proc Natl Acad Sci USA*. 2014; 111:10323–10328. [PubMed: 24982139]

61. Watts JC, Giles K, Oehler A, Middleton L, Dexter DT, Gentleman SM, DeArmond SJ, Prusiner SB. Transmission of multiple system atrophy prions to transgenic mice. *Proc Natl Acad Sci USA*. 2013; 110:19555–19560. [PubMed: 24218576]
62. Watts JC, Giles K, Patel S, Oehler A, DeArmond SJ, Prusiner SB. Evidence that bank vole PrP is a universal acceptor for prions. *PLoS Pathog*. 2014; 10:e1003990. [PubMed: 24699458]
63. Watts JC, Giles K, Serban A, Patel S, Oehler A, Bhardwaj S, Guan Y, Greicius M, Miller BL, DeArmond SJ, et al. Modulation of Creutzfeldt-Jakob disease prion propagation by the A224V mutation. *Ann Neurol*. 2015; 78:540–553. [PubMed: 26094969]
64. Watts JC, Giles K, Stöhr J, Oehler A, Bhardwaj S, Grillo SK, Patel S, DeArmond SJ, Prusiner SB. Spontaneous generation of rapidly transmissible prions in transgenic mice expressing wild-type bank vole prion protein. *Proc Natl Acad Sci USA*. 2012; 109:3498–3503. [PubMed: 22331873]
65. Watts JC, Prusiner SB. Mouse models for studying the formation and propagation of prions. *J Biol Chem*. 2014; 289:19841–19849. [PubMed: 24860095]
66. Williamson RA, Peretz D, Pinilla C, Ball H, Bastidas RB, Rozenshteyn R, Houghten RA, Prusiner SB, Burton DR. Mapping the prion protein using recombinant antibodies. *J Virol*. 1998; 72:9413–9418. [PubMed: 9765500]
67. 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; 29:10072–10080. [PubMed: 19675240]
68. Zou WQ, Puoti G, Xiao X, Yuan J, Qing L, Cali I, Shimoji M, Langeveld JP, Castellani R, Notari S, et al. Variably protease-sensitive prionopathy: a new sporadic disease of the prion protein. *Ann Neurol*. 2010; 68:162–172. [PubMed: 20695009]

Fig. 1.

Further characterization of spontaneously ill Tg(BVPrP,I109) mice. Immunoblots of detergent-insoluble, PK-resistant PrP in the brains of asymptomatic or clinically ill Tg(BVPrP,I109)3574 mice at the indicated ages (**a**), or clinically ill Tg(BVPrP,I109)3574 mice at the indicated days post-inoculation (dpi) with brain homogenate from a spontaneously ill Tg3581 mouse (**b**). PrP was detected with the antibody HuM-P. Molecular weight markers are indicated in kDa. (**c**) Resultant incubation periods following inoculation of Tg3581 mice with PBS or with the indicated dilutions of 10% brain homogenate from a spontaneously ill Tg3581 mouse. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, compared to PBS-inoculated mice, as determined by one-way ANOVA. ns, not significant. (**d**) Kaplan-Meier survival curves for uninoculated Tg3581 mice (black line, $n = 9$) or Tg3581 mice inoculated intracerebrally (solid gray line, $n = 8$), intraglossally (dashed gray line, $n = 8$), or intraperitoneally (dotted gray line, $n = 8$) with brain homogenate from a spontaneously ill Tg3581 mouse. (**e**) Kaplan-Meier survival curves for uninoculated Tg3581 mice with a *Prnp*^{0/0} (solid line, $n = 9$), *Prnp*^{0/+} (dashed line, $n = 12$), or *Prnp*^{+/+} (dotted line, $n = 12$) genotype. There was no significant difference in the survival curves ($P = 0.76$) as determined by the log-rank test.

Fig. 2.

PrP protein levels in Tg mice and cultured cells expressing mutant BVPrP molecules. **(a)** Immunoblots of brain homogenates from young, asymptomatic wt FVB mice or various lines of Tg mice expressing D178N-, E200K-, or GPI-mutant BVPrP. PrP was detected using the antibodies HuM-D18 (left blot) or HuM-P (right blot). Similar transgene DNA copy numbers **(b)** or *Pmp* mRNA levels **(c)** (as determined by qPCR) resulted in lower levels of BVPrP protein in Tg mice expressing D178N- (blue line) or E200K- (red line) mutant BVPrP(I109) compared with Tg mice expressing wt BVPrP(I109) (black line). In panels b and c, each data point represents a distinct line of Tg mice. **(d)** Immunoblots of cell lysates from N2a-PrP^{-/-} cells transiently (left blot) or stably (right blot) transfected with equal amounts of empty vector (e.v.) or plasmids encoding the indicated proteins. PrP was detected using the antibody HuM-D18, and membranes were reprobed with an anti-actin

antibody. (e) Quantification of relative PrP levels in N2a-PrP^{-/-} cells transiently transfected with vectors encoding the indicated proteins, as determined by ELISA (n = 4 each). ***P < 0.001 compared to cells transfected with wt BVPrP(I109). (f) Immunoblots of residual PrP levels in cell lysates from stably transfected N2a-PrP^{-/-} cells expressing either wt (top blot), D178N-mutant (middle blot), or E200K-mutant (bottom blot) BVPrP(I109) following treatment with cycloheximide (CHX) for the indicated amounts of time. PrP was detected using the antibody HuM-P. (g) Quantification of residual PrP levels in cell lysates from stably transfected N2a-PrP^{-/-} cells following CHX treatment (n = 3 for each time point). The half-lives for wt (black), D178N-mutant (blue), or E200K-mutant (red) BVPrP(I109) were calculated to be 17.6, 2.2, or 5.9 h, respectively. In the immunoblots (a, d, f), molecular weight markers are indicated in kDa.

Fig. 3.

Spontaneous neurological illness and protease-resistant PrP in Tg mice expressing mutant BVPPr(I109). (**a–c**) Kaplan-Meier survival curves for various lines of Tg mice expressing either D178N- (**a**), E200K- (**b**), or GPI-mutant (**c**) BVPPr(I109). (**d**) To achieve a similar disease incubation period, lower levels of PrP expression are required in mice expressing D178-mutant (blue) or E200K-mutant (red) BVPPr(I109) compared with mice expressing wt BVPPr(I109) (black). (**e**) Immunoblot of detergent-insoluble, PK-resistant PrP in the brains of Tg mice expressing BVPPr containing D178N (left blot), E200K (middle blot), or GPI (right blot) mutations. No PK-resistant PrP was observed in young asymptomatic Tg mice, whereas an ~8 kDa PK-resistant PrP species was present in spontaneously ill Tg mice at the specified ages. PrP was detected with the antibody HuM-P, and molecular weight markers are indicated in kDa.

Fig. 4.

Neuropathological characterization of spontaneously ill Tg mice expressing wild-type or mutant BVPrP(I109). Brain sections from spontaneously ill mice were stained immunohistochemically for PrP^{Sc} deposition using the HuM-P antibody (a–g, i, j), Thioflavin S (h), hematoxylin and eosin (H&E) staining to assess vacuolation (k–n), or with an anti-GFAP antibody to visualize astrocytic gliosis (o–r). PrP^{Sc} deposition was observed in the stratum oriens layer of the hippocampus (a) and the frontal cortex (b) of Tg(BVPrP,I109,D178N) mice; the brainstem (c) and nucleus accumbens (d) of Tg(BVPrP,I109,E200K) mice; the frontal cortex (e), hippocampus (f), and cerebellum (g) of a Tg(BVPrP,I109, GPI) mouse; and the thalamus of a Tg(BVPrP,I109) mouse (i, j). g, granule cell layer of the cerebellum; m, molecular layer of the cerebellum. (h) A Thioflavin S positive dense-core plaque in the frontal cortex of a spontaneously ill

Tg(BVPrP,I109, GPI) mouse is shown. Vacuolation and astrocytic gliosis in the caudate nucleus (striatum) (**k, o**) and thalamus (**l, p**) of spontaneously ill Tg(BVPrP,I109,D178N) mice; the frontal cortex (**m, q**) of a spontaneously ill Tg(BVPrP,I109,E200K) mouse; and the hippocampus (**n, r**) of a spontaneously ill Tg(BVPrP,I109, GPI) mouse. Sections are from a 187-day-old Tg15972 (D178N) mouse (a, k, o); a 183-day-old Tg15965 (D178N) mouse (b); a 420-day-old Tg14210 (E200K) mouse (c); a 194-day-old Tg4253 (E200K) mouse (d); 408-day-old Tg24600 (GPI) mice (e–h, n, r); a 250-day-old Tg3581 mouse (i, j); a 177-day-old Tg15464 (D178N) mouse (l, p); or a 402-day-old Tg14210 (E200K) mouse (m, q). Scale bars represent 20 μm (a–j) or 40 μm (k–r). Semiquantitative PrP^{Sc} deposition (**s**) and vacuolation (**t**) scoring (data are represented as mean $\pm$ SEM) within the indicated brain regions from spontaneously ill Tg15965 (D178N) (blue, n = 5), Tg4253 (E200K) (red, n = 3), Tg24600 (GPI) (green, n = 4), or Tg3581 (black, n = 5) mice. Fc, frontal cortex; Cd, caudate nucleus; Na, nucleus accumbens; Pc, parietal cortex; Hp, hippocampus; Th, thalamus; Hy, hypothalamus; Mb, midbrain; Cb, cerebellum; Bs, brainstem

Fig. 5.

Passage of spontaneously formed prions in Tg mice expressing mutant BVPPr(I109). (**a, f, k**) Kaplan-Meier survival curves for the passage of spontaneously formed D178N- (**a**), E200K- (**f**), or GPI-mutant (**k**) BVPPr(I109) prions in Tg mice expressing the same mutant BVPPr(I109) allele. Solid lines represent uninoculated mice, whereas dashed and dotted lines indicate mice inoculated with brain homogenates from two distinct spontaneously ill mice. (**b, g, i**) Immunoblots of detergent-insoluble, PK-resistant PrP in the brains of clinically ill Tg15972 (D178N) mice (**b**), Tg14210 (E200K) mice (**g**), or Tg24600 (GPI) mice (**i**) at the indicated dpi with brain homogenate from a spontaneously ill Tg mouse expressing the same mutant BVPPr(I109) allele. PrP was detected with the antibody HuM-P, and molecular weight markers are indicated in kDa. (**c-e, h-j, m-o**) Neuropathological characterization of inoculated mice. Coarse PrP^{Sc} deposition (**c**), spongiform degeneration (**d**), and mild astrocytic gliosis (**e**) in the parietal cortex of a clinically ill Tg15972 (D178N) mouse at 57 dpi with brain extract from a spontaneously ill Tg15972 (D178N) mouse. Synaptic-like PrP^{Sc} deposition (**h**), spongiform degeneration (**i**), and intense astrocytic gliosis (**j**) in the hippocampus of a clinically ill Tg14210 (E200K) mouse at 41 dpi with brain extract from a spontaneously ill Tg7253 (E200K) mouse. Large PrP^{Sc} deposits (**m**), absence of spongiform degeneration (**n**), and moderate astrocytic gliosis (**o**) in the hippocampus of a clinically ill Tg24600 (GPI) mouse at 74 dpi with brain extract from a spontaneously ill Tg24600 (GPI) mouse. Sections were stained with H&E (**d, i, n**) or with antibodies to PrP (HuM-P; **c, h, m**) or GFAP (**e, j, o**). Scale bars represent 20 μm (**c, h, m**) or 40 μm (**d, e, i, j, n, o**).

Fig. 6.

Spontaneously formed mutant BVPPr(I109) prions transmit disease to Tg mice expressing wt BVPPr(I109). **(a)** Kaplan-Meier survival curves for uninoculated Tg3581 mice (solid black line, $n = 9$), Tg3581 mice inoculated with PBS (dashed black line, $n = 12$), and Tg3581 mice inoculated with brain homogenate from a spontaneously ill Tg15972 (D178N) mouse (blue line, $n = 8$), a Tg7253 (E200K) mouse (red line, $n = 8$), or a Tg24600 (ΔGPI) mouse (green line, $n = 8$). **(b)** Immunoblot of detergent-insoluble, PK-resistant PrP in the brains of asymptomatic uninoculated Tg3581 mice at 100 days of age ("None") or clinically ill Tg3581 mice at the indicated dpi with brain homogenate from a spontaneously ill Tg15972 (D178N), Tg7253 (E200K), or Tg24600 (ΔGPI) mouse. PrP was detected with the antibody HuM-P. Molecular weight markers are indicated in kDa. **(c–k)** Neuropathological characterization of inoculated Tg3581 mice. Coarse PrP^{Sc} deposition in the frontal cortex **(c)** as well as spongiform degeneration **(f)** and astrocytic gliosis **(i)** in the hippocampus of a clinically ill Tg3581 mouse at 47 dpi with brain extract from a spontaneously ill Tg15972 (D178N) mouse. Synaptic-like PrP^{Sc} deposition **(d)**, spongiform degeneration **(g)**, and astrocytic gliosis **(j)** in the hippocampus of a clinically ill Tg3581 mouse at 33 dpi with brain extract from a spontaneously ill Tg7253 (E200K) mouse. Compact granular PrP^{Sc} deposition **(e)**, mild spongiform degeneration **(h)**, and mild astrocytic gliosis **(k)** in the hippocampus of a clinically ill Tg3581 mouse at 41 dpi with brain extract from a spontaneously ill Tg24600 (ΔGPI) mouse. Sections were stained with H&E (f–h) or with antibodies to PrP (HuM-P; c–e) or GFAP (i–k). Scale bars represent 20 μm (c–e) or 40 μm (f–k).

Fig. 7. Spontaneously formed mutant BVP^{PrP}(I109) prions transmit disease to Tg(MoPrP) mice. **(a)** Immunoblot of detergent-insoluble, PK-resistant PrP in the brains of asymptomatic Tg(MoPrP)4053 mice at the indicated dpi with PBS, or clinically ill Tg(MoPrP)4053 mice at the indicated dpi with brain homogenate from a spontaneously ill Tg15972 (D178N) or Tg7271 (E200K) mouse. All samples were run on the same blot; the vertical black line indicates two unrelated samples that were removed. PrP was detected with the antibody HuM-P. Molecular weight markers are indicated in kDa. **(b–g)** Neuropathological characterization of inoculated Tg(MoPrP)4053 mice. Clusters of large PrP^{Sc} deposits **(b, c)** and spongiform degeneration **(d)** in the hippocampus of clinically ill Tg(MoPrP)4053 mice at 327 **(b, d)** or 370 **(c)** dpi with brain extract from a spontaneously ill Tg15972 (D178N) mouse. Clusters of smaller PrP^{Sc} deposits **(e, f)** and spongiform degeneration **(g)** in the hippocampus of clinically ill Tg(MoPrP)4053 mice at 320 **(e, g)** or 292 **(f)** dpi with brain

extract from a spontaneously ill Tg7271 (E200K) mouse. Sections were stained with H&E (d, g) or with the antibody HuM-P to detect PrP^{Sc} deposition (b, c, e, f). Scale bars represent 40 μ m (d, g) or 20 μ m (b, c, e, f).

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Table 1

Spontaneous neurologic illness in Tg mice expressing mutant BVPrP(I109)

Mutation	Line	Transgene copy number ^a	Relative <i>Prnp</i> mRNA level (- fold) ± SEM ^b	Relative PrP expression level (- fold) ^c	Mean age of disease onset ± SEM (d)	Signs of neurologic dysfunction (n/n ₀) ^d
wt	Tg3574	17	1.0 ± 0.3	3.6 ^e	340 ± 26 ^e	14/14 ^e
	Tg3581	25	1.5 ± 0.7	5.3 ^e	218 ± 9 ^e	19/19 ^e
D178N	Tg15972	20	1.6 ± 0.4	0.4	240 ± 11	24/24
	Tg15464	ND ^f	ND ^f	0.4	221 ± 9	23/23
	Tg15465	ND ^f	ND ^f	0.4	197 ± 8	22/22
E200K	Tg15965	33	1.6 ± 0.2	0.5	179 ± 8	22/22
	Tg14205	ND ^f	ND ^f	0.6	555 ± 29	12/12
	Tg14210	8	0.9 ± 0.1	1.2	460 ± 31	12/12
	Tg7253	17	1.8 ± 0.3	1.7	268 ± 16	24/24
	Tg4253	31	2.8 ± 0.4	2.4	162 ± 7	24/24
GPI	Tg7271	50	2.1 ± 0.4	2.7	119 ± 2	24/24
	Tg24600	22	1.6 ± 0.2	0.5	415 ± 15	11/11

^aTransgene copy numbers were determined by qPCR on genomic DNA samples using Syrian hamster DNA as a control.^b*Prnp* mRNA levels were determined by qPCR on brain cDNA samples and then normalized to *Prnp* mRNA levels in Tg3574 mice.^cRelative PrP expression levels were determined by ELISA relative to expression of wt MoPrP in FVB mouse brain.^dn, number of positive mice; n₀, number of examined mice.^eData previously reported in Ref. [64].^fND, not determined.

Table 2

Passage of brain homogenates from spontaneously ill Tg mice expressing mutant BVPPrP(D1109)

Tg line (mutation)	Inoculum	Serial transmission to mice expressing homotypic PrP				Transmission to Tg(BVPPrP ₁₁₀₉)3581 mice		Transmission to Tg(MoPrP ₄₀₅₃) mice	
		Age at onset of spontaneous illness (d)	Recipient line (mutation)	Mean incubation period $\pm$ SEM (d)	Signs of neurologic dysfunction (n/n ₀) ^a	Mean incubation period $\pm$ SEM (d)	Signs of neurologic dysfunction (n/n ₀) ^a	Mean incubation period $\pm$ SEM (d)	Signs of neurologic dysfunction (n/n ₀) ^a
Tg15972 (D178N)		187	Tg15972 (D178N)	43 $\pm$ 6	8/8	46 $\pm$ 3	8/8	332 $\pm$ 20	7/7
Tg15965 (D178N)		183	Tg15972 (D178N)	62 $\pm$ 1	8/8	76 $\pm$ 4	8/8	ND ^b	ND ^b
Tg15965 (D178N)		203	Tg15972 (D178N)	60 $\pm$ 2	8/8	67 $\pm$ 4	7/7	421 $\pm$ 54 ^c	5/7
Tg14210 (E200K)		464	Tg14210 (E200K)	68 $\pm$ 1	8/8	50 $\pm$ 2	7/7	ND ^b	ND ^b
Tg7253 (E200K)		282	Tg14210 (E200K)	41 $\pm$ 1	8/8	36 $\pm$ 1	8/8	356 $\pm$ 10	5/5
Tg4253 (E200K)		194	Tg14210 (E200K)	51 $\pm$ 1	5/5	40 $\pm$ 0	7/7	ND ^b	ND ^b
Tg7271 (E200K)		131	Tg14210 (E200K)	46 $\pm$ 2	7/7	34 $\pm$ 1	8/8	304 $\pm$ 8	7/7
Tg24600 (GPI)		349	Tg24600 (GPI)	100 $\pm$ 5	6/6	47 $\pm$ 5	5/5	ND ^b	ND ^b
Tg24600 (GPI)		408	Tg24600 (GPI)	110 $\pm$ 9	8/8	42 $\pm$ 1	8/8	ND ^b	ND ^b

^a n, number of positive mice; n₀, number of examined mice.

^b ND, not determined.

^c Incubation period calculated from five ill mice. Two mice remained healthy at 601 dpi.