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A naturally occurring variant of the human prion protein completely prevents prion disease

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Abstract

Mammalian prions, transmissible agents causing lethal neurodegenerative diseases, are composed of assemblies of misfolded cellular prion protein (PrP) [1]. A novel PrP variant, G127V, was under positive evolutionary selection during the epidemic of kuru, an acquired prion disease epidemic of the Fore population in Papua New Guinea, and appeared to provide strong protection against disease in the heterozygous state[2]. We have now investigated the protective role of this variant and its interaction with the common worldwide M129V PrP polymorphism; V^{127} was seen exclusively on a M^{129} *PRNP* allele. Here we demonstrate that transgenic mice expressing both variant and wild type human PrP are completely resistant to both kuru and classical CJD prions (which are closely similar) but can be infected with variant CJD prions, a human prion strain resulting from exposure to BSE prions to which the Fore were not exposed. Remarkably however, mice expressing only PrP V^{127} were completely resistant to all prion strains demonstrating a different molecular mechanism to M129V, which provides its relative protection against classical CJD and kuru in the heterozygous state. Indeed this single amino acid substitution (G→V) at a residue invariant in vertebrate evolution is as protective as deletion of the protein. Further study in transgenic mice expressing different ratios of variant and wild type PrP indicates that not only is PrP V^{127} completely refractory to prion conversion, but acts as a potent dose-dependent inhibitor of wild type prion propagation.

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Author contributions

EAA, JDFW and JC conceived and designed the study. MS and EAA cloned the transgene constructs. RH, AT and AJ generated and developed transgenic lines. MS, TJ, SH and EAA analysed and characterised the transgenic mice. AG analysed prion-infected mouse brains by immunoblotting. AR-L, JML and SB performed histological analyses and interpreted the pathology data. SM, JW, JDFW and MA characterised CJD cases and inocula. EAA supervised the study and collated data. EAA, JDFW and JC drafted the paper with contributions from all authors.

Competing financial interest

JC is a Director and JC and JDFW are shareholders of D-Gen Limited, an academic spin-out company in the field of prion diagnosis, decontamination and therapy. D-Gen supplied the ICSM35 antibody used in this study.

Prions cause fatal neurodegenerative conditions such as scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease (CJD) in humans[1]. The fundamental molecular process, seeded propagation of assemblies of misfolded host protein, is increasingly recognised as being of importance in all the major human neurodegenerative diseases[3]. There is a common polymorphism, present worldwide, in the substrate protein in human prion disease, human prion protein (PrP), where either methionine (M) or valine (V) is present at residue 129. MV heterozygosity provides relative protection against acquired, sporadic, and some inherited prion diseases[4-6] and may have been selected during the evolution of modern humans by ancestral prion disease epidemics[7]. This protective effect is thought to relate to inhibition of homotypic protein-protein interactions[5, 8] although residue 129 also influences the propagation of particular prion strains via conformational selection[9, 10]. Heterozygosity at another polymorphism, E219K, also provides resistance to CJD in Japan[11].

Kuru was a devastating epidemic prion disease transmitted by endocannibalism and restricted to a remote area in Papua New Guinea (PNG). We reported a novel PrP variant (G127V) amongst unaffected individuals which appeared be a resistance factor selected by the epidemic and unique to this region[2]. Given the proximity to residue 129, we considered that it may have a similar action to M129V, blocking homotypic interactions and exerting its protective effect only in the heterozygous state. No *PRNP* codon 127VV homozygotes were identified in the kuru-exposed population and V^{127} was always seen on an M^{129} allele[2]. We therefore generated multiple lines of transgenic mice, expressing only human PrP (HuPrP) on a congenic FVB/N $Prnp^{o/o}$ background, to investigate whether G127V was indeed protective and whether this protection was dependent on heterozygosity or was an intrinsic property of the variant protein. Additionally we investigated its interaction with the residue 129 polymorphism.

Two lines of transgenic mice homozygous for HuPrP V^{127} were studied: Tg(HuPrP $V^{127}M^{129}/V^{127}M^{129}$ $Prnp^{o/o}$)-183 ($V^{127}M^{129}$ Tg183) and Tg(HuPrP $V^{127}M^{129}/V^{127}M^{129}$ $Prnp^{o/o}$)-190 ($V^{127}M^{129}$ Tg190). PrP expression levels in homozygotes as compared to pooled normal human brain were 2-fold for $V^{127}M^{129}$ Tg183 and 1-fold for $V^{127}M^{129}$ Tg190. G127V heterozygous mice were derived by crossing these lines with FVB-congenic versions of Tg35 mice homozygous for HuPrP $G^{127}M^{129}$ [12-15] or Tg152 mice homozygous for HuPrP $G^{127}V^{129}$ [15-19] designated Tg(HuPrP $G^{127}M^{129}/G^{127}M^{129}$ $Prnp^{o/o}$)-35c ($G^{127}M^{129}$ Tg35c) and Tg(HuPrP $G^{127}V^{129}/G^{127}V^{129}$ $Prnp^{o/o}$)-152c ($G^{127}V^{129}$ Tg152c) respectively. $G^{127}M^{129}$ Tg35c and $G^{127}V^{129}$ Tg152c express wild type huPrP at 2- and 6-times respectively as compared to pooled normal human brain (table 1). Extended Data Fig. 1 shows relative PrP^{C} expression levels in all transgenic mice used in this study.

All transgenic lines were then challenged by intracerebral prion inoculation from well-characterised and previously transmitted human prion disease cases including all three *PRNP* codon 129 genotypes and comprising four cases of kuru, 12 cases of classical CJD and two cases of variant CJD (vCJD). Heterozygous HuPrP $G^{127}M^{129}/V^{127}M^{129}$ mice (Tg35c × Tg183 and Tg35c × Tg190) (table 1), having the genotype associated with disease resistance in the kuru-exposed human population, proved completely resistant to all four

kuru isolates (which included all three *PRNP* codon 129 genotypes and two molecular strain types) (Fig. 1a and table 2a) while mice expressing wild type HuPrP $G^{127}M^{129}/G^{127}M^{129}$ (Tg35c) (Fig. 1a, table 2a) or $G^{127}V^{129}/G^{127}V^{129}$ (Tg152c) (table 2b) were fully susceptible with 100% attack rates. This is consistent with the population genetic data suggesting kuru resistance of G127V individuals. We have previously reported that prion strains seen in kuru brain are indistinguishable from those seen in classical CJD patients[19]. Similarly, none of four classical CJD isolates from patients of *PRNP* genotype 129MM transmitted to either line of $G^{127}M^{129}/V^{127}M^{129}$ mice (table 2a and Fig. 1b), while all four transmitted uniformly to Tg35c mice expressing wild type huPrP (Fig. 1b and table 2a). Remarkably, however, occasional $G^{127}M^{129}/V^{127}M^{129}$ mice developed clinical disease, and a larger number showed evidence of subclinical infection (positive PrP immunohistochemistry and/or western blot for PrP^{Sc}), on challenge with vCJD prions (table 2d and Extended Data Fig. 2). vCJD is a novel BSE-derived prion strain[17, 20, 21] to which the population in the kuru-affected area of PNG were not exposed.

The key question with respect to whether the protective effect of V^{127} is via a similar mechanism to the M129V polymorphism was addressed by challenge of mice homozygous for HuPrP $V^{127}M^{129}$. While mice homozygous for wild type HuPrP $G^{127}M^{129}$ or $G^{127}V^{129}$ are both highly susceptible to CJD prions, remarkably mice homozygous for HuPrP $V^{127}M^{129}$ were completely resistant to all 18 human prion disease isolates, including vCJD prions, with no clinical transmissions or evidence of subclinical infection (Fig. 1a and b, table 3 and Extended Data Fig. 3).

We also challenged mice heterozygous at both residues 127 and 129 of HuPrP ($G^{127}V^{129}/V^{127}M^{129}$) produced by crossing Tg183 or Tg190 mice with Tg152c mice (tables 1 and 2B). The higher level of expression of HuPrP V^{129} in Tg152c mice as compared to the other lines meant that there was a marked difference in the ratio of expression of the variant ($V^{127}M^{129}$) and wild type ($G^{127}V^{129}$) HuPrP in these crosses (table 1). While Tg183 and Tg35c had closely similar levels of expression of $V^{127}M^{129}$ and $G^{127}M^{129}$ HuPrP respectively, such that the Tg35c × Tg183 cross closely modelled the human *PRNP* G127V genotype, the expression ratios of wild type $G^{127}V^{129}$ to variant $V^{127}M^{129}$ HuPrP were approximately 6:1 and 3:1 for the Tg152c × Tg190 and Tg152c × Tg183 crosses respectively (table 1, Extended Data Fig.1 and 4). Interestingly, in these crosses some transmissions of kuru and classical CJD prions were seen (table 2b, Extended Data Fig. 4 and 5). In the Tg152c × Tg183 crosses, with a 3-fold excess of wild type PrP, subclinical infections were seen with four out of the 12 isolates used, and one kuru isolate (I10336) was associated with clinical disease (table 2b). However with the Tg152c × Tg190 crosses, with a 6-fold excess of wild type PrP, transmissions were seen from all isolates, the majority resulting in clinical disease (table 2b).

To further investigate the effect of HuPrP V^{127} on prion propagation, we compared hemizygous Tg(HuPrP $G^{127}V^{129}$ $Prnp^{o/o}$)-152c mice with the Tg152c × Tg183 and Tg152c × Tg190 crosses (table 2c). The hemizygous line was challenged with two classical CJD and one kuru isolate that had resulted in a 100% attack rate in both crosses. Again, 100% attack rates were seen for all three isolates but the incubation periods were significantly shorter ($P < 0.0001$; table 2c) in the absence of HuPrP V^{127} than in either cross. These data, together

with comparison of the two crosses themselves (table 2b), are consistent with a dominant negative effect of HuPrP V^{127} expression. Although dominant negative inhibition has been reported for other natural polymorphic residues of mammalian PrP in transgenic mice[22-24] none have shown complete prevention of prion conversion when expressed at a 1:1 ratio with wild type PrP. Dominant negative effects on yeast prion propagation have also been reported[25].

Our transgenic modelling of the HuPrP G127V polymorphism demonstrates that it confers strong protection against prion disease in the heterozygous state. However, most importantly, the molecular basis of this effect is clearly distinct from that proposed for the well-established HuPrP M129V polymorphism which is protective against developing sporadic CJD only in the heterozygous state: inhibition of homotypic protein-protein interactions during the process of prion propagation. Here we demonstrate that HuPrP V^{127} is intrinsically resistant to prion conversion and indeed capable of inhibiting propagation of wild type prions in a dose-dependent manner. Mice expressing only HuPrP V^{127} appear as resistant to prion disease as PrP null mice[26] and understanding the structural basis of this effect may therefore provide critical insight into the molecular mechanism of mammalian prion propagation.

At its height when first recognised by Western medicine in 1957, kuru was a devastating epidemic largely affecting women and children and killing up to 2% of the population annually in some villages. Indeed some villages became largely devoid of young women of childbearing age. While collapse of the Fore population was prevented by cessation of endocannibalism in the late 1950s which interrupted the route of transmission and led to a gradual decline in incidence[27], our data suggest that if transmission had continued the epicentre of the affected region might have been repopulated with kuru-resistant individuals as a population genetic response to the epidemic. Understanding the structural basis of why HuPrP V^{127} is unable to propagate prions of multiple strain types may provide key insights into prion propagation and the development of rational therapeutics.

Methods

Ethics Statement

Storage and biochemical analyses of post-mortem human brain samples and transmission studies to mice were performed with written informed consent from patients with capacity to give consent. Where patients were unable to give informed consent, assent was obtained from their relatives in accordance with UK legislation and Codes of Practice. Samples were stored and used in accordance with the Human Tissue Authority Codes of Practice and in line with the requirements of the Human Tissue Authority licence held by UCL Institute of Neurology. This study was performed with approval from the Medical Research Advisory Committee of the Government of Papua New Guinea, the National Hospital for Neurology and Neurosurgery and the UCL Institute of Neurology Joint Research Ethics Committee (now National Research Ethics Service Committee, London – Queen Square) - REC references: 03/N036, 03/N038 and 03/N133. Work with mice was performed under approval and licence granted by the UK Home Office (Animals (Scientific Procedures) Act 1986);

Project Licence number 70/6454 which conformed to University College London institutional and ARRIVE guidelines (www.nc3rs.org.uk/ARRIVE/).

Generation of transgenic mice

The 759bp human PrP ORF was amplified by PCR with pfu polymerase from genomic DNA prepared from the brain of a patient with the 127V polymorphism on the 129M allele, using forward primer 5′-GTCGACCAGTCATTATGGCGAACCTT-3′ and reverse primer 5′-CTCGAGAAGACCTTCCTCATCCCACT-3′. Restriction sites Sal I and Xho I (underlined) were introduced in the forward and reverse primers respectively for cloning. The blunt ended PCR fragment generated by pfu polymerase was subcloned into Sma I digested pSP72 vector and sequenced to ensure that no spurious alterations had been introduced by the PCR and to confirm the presence of valine-127 and methionine-129 polymorphisms matching the patient DNA template. The amplified human PrP ORF with the confirmed polymorphisms was then isolated by Sal I and Xho I digestion. Subsequent subcloning into the Sal I site of the cosmid vector SHaCosTt, packaging and preparation of high quality DNA of the Not I transgene insert was as previously reported[12]. Microinjection of the purified Not I DNA fragment was carried out according to standard protocol into single cell eggs of *Prnp* null mice which had been backcrossed onto an FVB/N genetic background. Genotyping was performed by PCR and PrP expression levels estimated by western blot analysis as previously reported [12, 29]. Two homozygous lines were established for variant HuPrP $V^{127}M^{129}$ described as Tg(HuPrP $V^{127}M^{129}/V^{127}M^{129}$ $Prnp^{o/o}$)-183 ($V^{127}M^{129}$ Tg183) and Tg(HuPrP $V^{127}M^{129}/V^{127}M^{129}$ $Prnp^{o/o}$)-190 ($V^{127}M^{129}$ Tg190), with transgene expression levels of 2 and 1 × respectively, that of pooled 10% (w/v) normal human brain homogenate (table 1). FVB-congenic versions of Tg35 mice homozygous for HuPrP $G^{127}M^{129}$ [12-15] and Tg152 mice homozygous for HuPrP $G^{127}V^{129}$ [15-19] were used as wild type human PrP-expressing controls, designated Tg(HuPrP $G^{127}M^{129}/G^{127}M^{129}$ $Prnp^{o/o}$)-35c ($G^{127}M^{129}$ Tg35c) and Tg(HuPrP $G^{127}V^{129}/G^{127}V^{129}$ $Prnp^{o/o}$)-152c ($G^{127}V^{129}$ Tg152c) respectively. The *PRNP* codon 127-129 genotypes and relative expression levels of wild type and variant PrP in the parental transgenic lines and in the F1 crosses are shown in table 1.

Transmission Studies

Strict bio-safety protocols were followed. Inocula were prepared, using disposable equipment for each inoculum, in a microbiological containment level 3 laboratory and inoculations performed within a class 1 microbiological safety cabinet as described previously[12, 19]. Ten mice per group of control $G^{127}M^{129}$ Tg35c and $G^{127}V^{129}$ Tg152c lines and 15 per group of newly generated $V^{127}M^{129}$ Tg183, $V^{127}M^{129}$ Tg190 lines and their respective crosses with Tg35c and Tg152c, were inoculated (see below) with human brain homogenates from neuropathologically confirmed patients comprising, four kuru cases, eight sporadic CJD cases, four iatrogenic CJD cases and two cases of vCJD. The genotype of each mouse was confirmed by PCR of ear punch DNA prior to inclusion and all mice were uniquely identified by sub-cutaneous transponders. Disposable cages were used and all cage lids and water bottles were also uniquely identified by transponder and remained with each cage of mice throughout the incubation period. Care of the mice was according to institutional guidelines. Mice (female, aged 6-8 weeks) were randomly assigned to

experimental groups and anaesthetised with a mixture of halothane and O_2, and intracerebrally inoculated into the right parietal lobe with 30 μl of a 1% (w/v) brain homogenate prepared in Dulbecco's phosphate buffered saline lacking Ca^{2+} or Mg^{2+} ions (D-PBS). All mice were thereafter examined daily for early indicators of clinical prion disease including piloerection, sustained erect ears, intermittent generalised tremor, unsustained hunched posture, rigid tail, mild loss of coordination,and clasping hind legs when lifted by the tail. Definite diagnosis of clinical prion disease (triggering experimental end point) was reached if mice exhibited any two early indicator signs in addition to one confirmatory sign, or any two confirmatory signs. The confirmatory signs included ataxia,impairment of righting reflex, dragging of hind limbs, sustained hunched posture, or significant abnormal breathing. Mice were killed (by CO_2 asphyxiation) if they exhibited any signs of distress or once a diagnosis of prion disease was established. At post-mortem brains from inoculated mice were removed, divided sagittally with half frozen and half fixed in 10% buffered formol saline. Subsequent immunohistochemical or biochemical investigations were performed blind to sample provenance.

Neuropathology and Immunohistochemistry

Fixed brain was immersed in 98% formic acid for 1 hour and paraffin wax embedded. Serial sections of 4 μm thickness were pre-treated by boiling for 10 min in a low ionic strength buffer (2.1 mM Tris, 1.3 mM EDTA, 1.1 mM sodium citrate, pH 7.8) before exposure to 98% formic acid for 5 min. Abnormal PrP accumulation was examined using anti-PrP monoclonal antibody ICSM 35 (D-Gen Ltd, London) on a Ventana automated immunohistochemical staining machine (Ventana Medical Systems Inc., Tucson, Arizona) using proprietary secondary detection reagents (Ventana Medical Systems Inc) before development with 3′3 diaminobenzedine tetrachloride as the chromogen[30]. Harris haematoxylin and eosin staining was done by conventional methods. Appropriate controls were used throughout.

Immunoblotting

Preparation of brain homogenates (10% (w/v) in D-PBS), proteinase K digestion (75 μg/ml for 1 h at 37°C) and subsequent immunoblotting was performed as described previously[28, 30]. Blots were probed with anti-PrP monoclonal antibody ICSM 35 (D-Gen Ltd, London) in conjunction with an anti-mouse IgG-alkaline phosphatase conjugate and development in chemiluminescent substrate (CDP-Star; Tropix Inc). Primary screening of brain homogenates was performed blind to sample identity.

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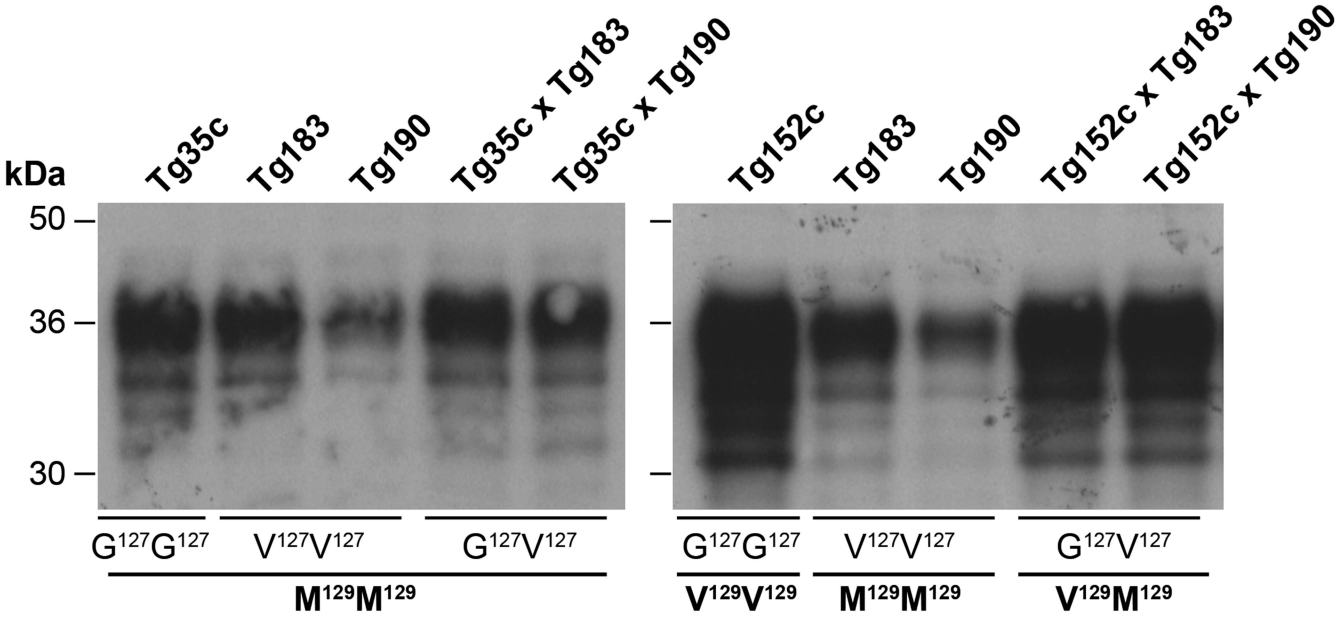
Extended Data



Extended Data Figure 1. Immunoblots showing relative PrP^C expression levels in transgenic mice

The provenance of each brain sample is designated above each lane and molecular markers are indicated on the left. The *PRNP* codon 127 (G, glycine, V, valine) or codon 129 (M, methionine, V, valine) genotypes of the transgenic mice are designated below. Transgenic mouse brains were analysed by enhanced chemiluminescence without proteinase-K digestion and equal amounts of total protein loaded in each well and probed with anti-PrP monoclonal antibody ICSM 35. Wild type human PrP expression levels of $G^{127}M^{129}$/$G^{127}M^{129}$ Tg35c and $G^{127}V^{129}$/$G^{127}V^{129}$ Tg152c mice are 2- and 6-fold higher respectively, than seen in 10 % (w/v) pooled human brain homogenate. Homozygous $V^{127}M^{129}$ Tg183 and Tg190 mice have 2-fold higher or equivalent PrP expression levels respectively compared to 10 % (w/v) pooled human brain homogenate.

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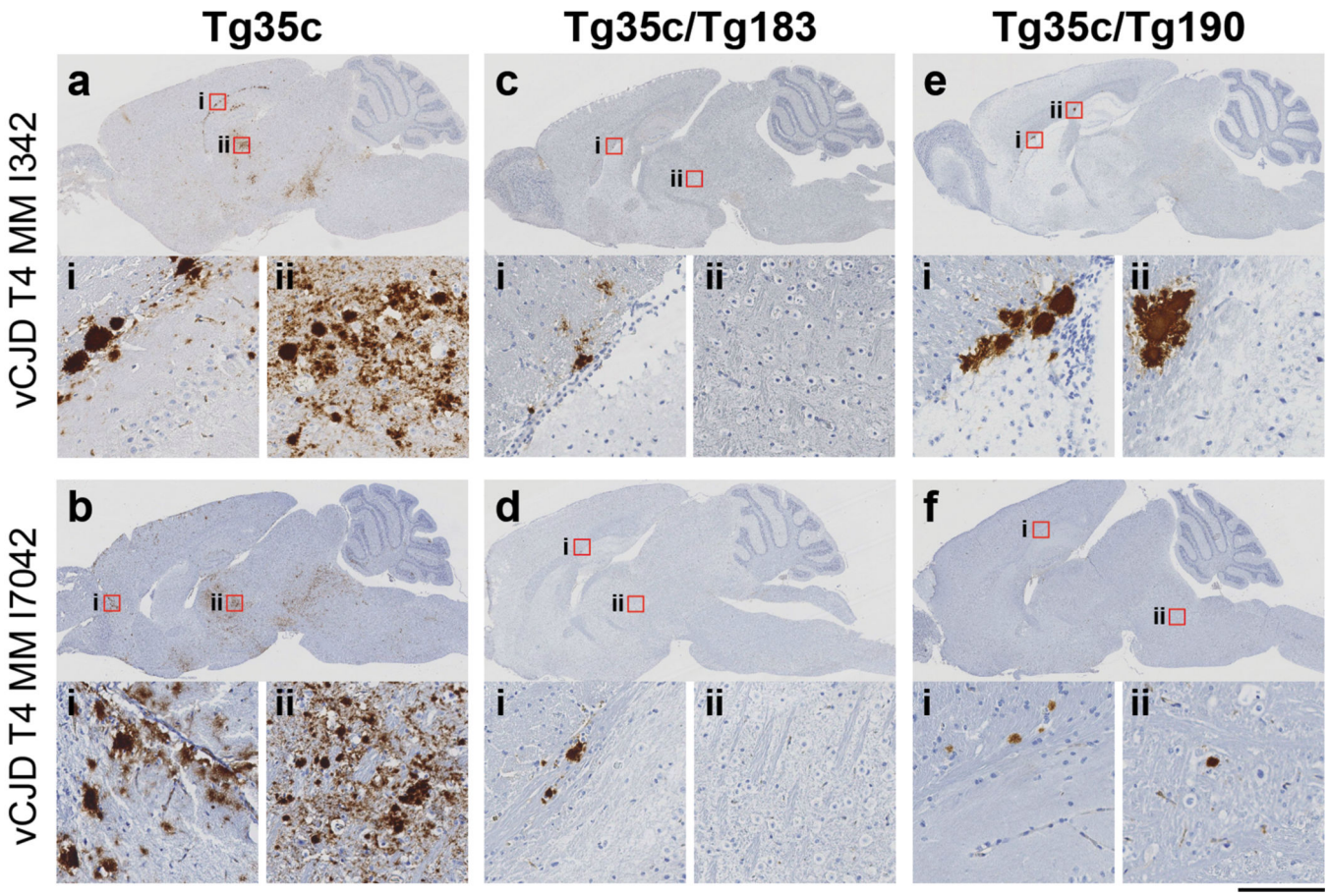
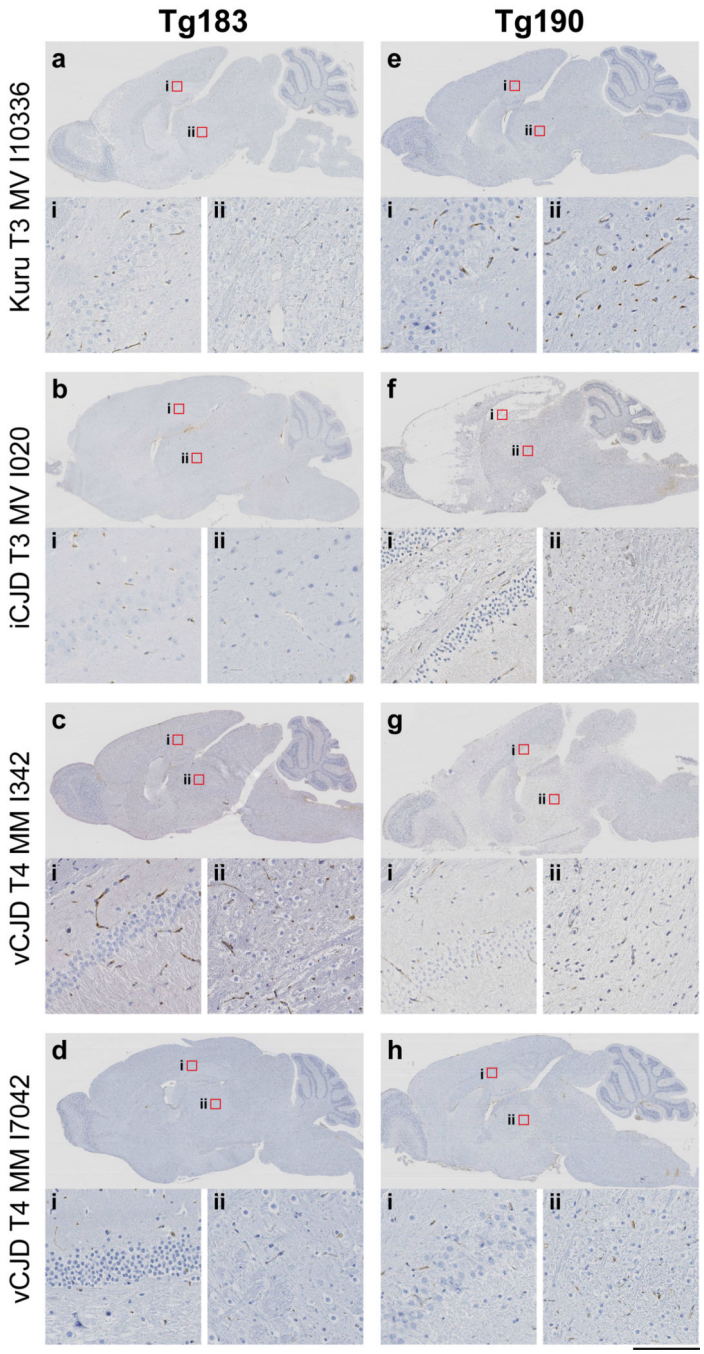


Extended Data Figure 2. Immunohistochemical analysis of transgenic mouse brain following challenge with vCJD prions

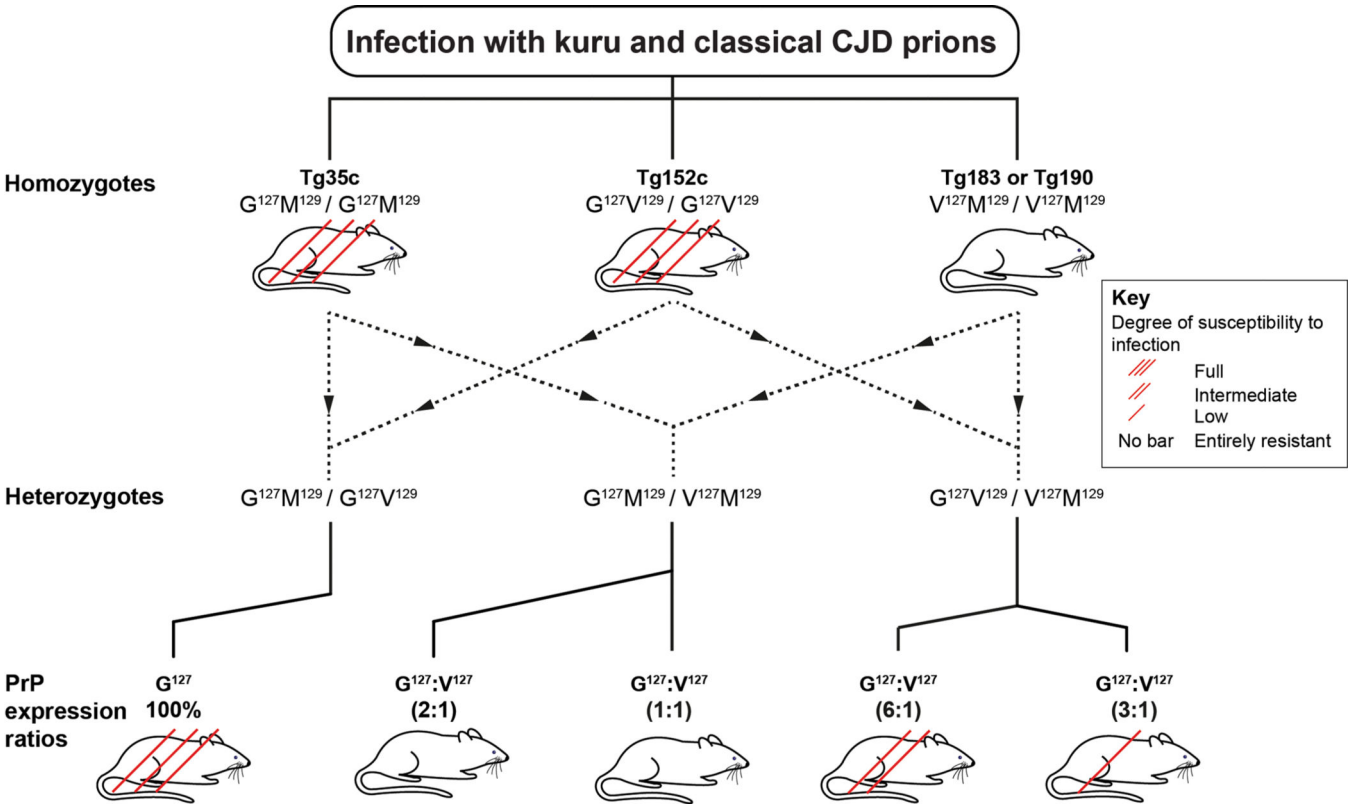
All mice were intracerebrally challenged with the same vCJD prion isolates (I342 and I7042). Abnormal PrP deposition in fixed post-mortem brain from affected mice was detected using anti-PrP monoclonal antibody ICSM 35. (a-b) Homozygous Tg35c mice (expressing $G^{127}M^{129}/G^{127}M^{129}$ wild type PrP only) show intense and widespread PrP plaque deposits. Magnified areas show (ai) hippocampus, (bi) frontal cortex and (aii and bii) thalamus. In contrast, heterozygous $G^{127}M^{129}/V^{127}M^{129}$ Tg35c/Tg183 mice expressing equivalent levels of $G^{127}M^{129}$ and $V^{127}M^{129}$ PrP (c-*d*) show only weak PrP deposition in the corpus callosum (ci and di) with no abnormal PrP deposition detected in other brain areas, for example, in the thalamus (cii and dii). Heterozygous $G^{127}M^{129}/V^{127}M^{129}$ Tg35c/Tg190 mice which express a lower level of $V^{127}M^{129}$ PrP relative to wild type $G^{127}M^{129}$ PrP (e-f) show greater levels of PrP deposition than seen in Tg35c/Tg183 mice following challenge with the same vCJD prion isolates, (ei, eii and fi) corpus callosum; (fii) pons. Scale bar, upper panels (a-f) 2 mm; magnified panels, 100 μm.



Extended Data Figure 3. Immunohistochemical analysis of homozygous *PRNP* $V^{127}M^{129}$/$V^{127}M^{129}$ transgenic mouse brain following challenge with human prions

Mice were intracerebrally challenged with kuru, classical and variant CJD prions. Following prolonged (>600 days) post inoculation periods, abnormal PrP deposition in fixed post-mortem brain was examined using anti-PrP monoclonal antibody ICSM 35. (a-d) $V^{127}M^{129}/V^{127}M^{129}$ Tg183 mice with 2-fold overexpression of $V^{127}M^{129}$ PrP. (*E-H*) $V^{127}M^{129}/V^{127}M^{129}$ Tg190 mice expressing endogenous levels of $V^{127}M^{129}$ PrP. Red square boxes in the main panels (a-h) define the area of magnified images of hippocampus

(i) and thalamus (ii) shown in the lower panels. Scale bar, a-h, 2 mm; magnified panels i and ii, 100 μm. The lack of detection of abnormal PrP deposition in brain indicates that the mice are not subclinically infected with prions.



Extended Data Figure 4. Schematic diagram summarising transmissions of kuru and classical CJD prions to transgenic mice expressing human PrP

$G^{127}M^{129}/G^{127}M^{129}$ Tg35c mice or $G^{127}V^{129}/G^{127}V^{129}$ Tg152c mice are homozygous for wild type human PrP alleles and are fully susceptible to kuru and classical CJD prions. $V^{127}M^{129}/V^{127}M^{129}$ Tg183 or $V^{127}M^{129}/V^{127}M^{129}$ Tg190 transgenic mice are homozygous for the variant $V^{127}M^{129}$ allele found only in humans from the kuru-exposed population of Papua New Guinea and are entirely resistant to infection with kuru and classical CJD prions. The levels of PrP expression in the brain of these homozygous transgenic mice relative to a pooled human brain homogenate are 1× (Tg190 mice) 2× (Tg35c and Tg183 mice) and 6× (Tg152c mice). Generation of F1 mice through inter-breeding the various homozygous lines produces different combinations of the various human PrP alleles leading to differences in the relative expression levels of the various prion proteins in brain. The PrP expression ratios from the two PrP alleles in the crosses are shown in parentheses above the cartoon mice. Full, intermediate or low susceptibility of the mice to infection with kuru and classical CJD prions is indicated by three, two or one diagonal red bar, respectively, drawn across the mice. Mice with no red bar are entirely resistant to infection with kuru and classical CJD prions.

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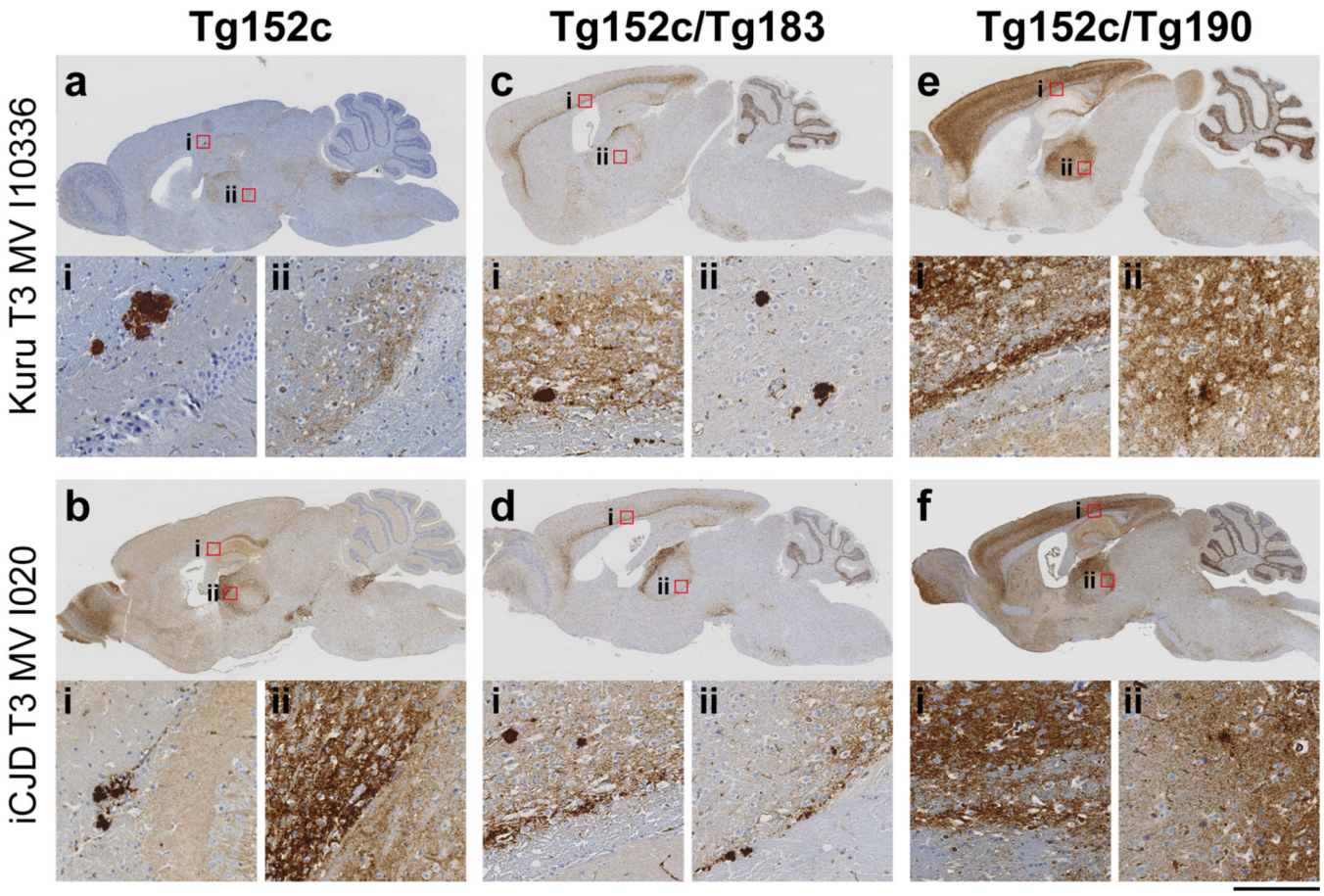

Extended Data Figure 5. Immunohistochemical analysis of homozygous *PRNP* $G^{127}V^{129}$/$G^{127}V^{129}$ and heterozygous $G^{127}V^{129}/V^{127}M^{129}$ transgenic mouse brain following challenge with human prions

Mice were intracerebrally challenged with kuru and classical CJD prions and abnormal PrP deposition in fixed post-mortem brain from affected mice was examined using anti-PrP monoclonal antibody ICSM 35. Red square boxes labelled i and ii in panels a-f mark brain areas that are magnified and displayed below; (i) cortex and (ii) thalamus. (a-b) Wild type $G^{127}V^{129}/G^{127}V^{129}$ Tg152c mice. (c-d) Heterozygous $G^{127}V^{129}/V^{127}M^{129}$ Tg152c/Tg183 mice. (e-f) Heterozygous $G^{127}V^{129}/V^{127}M^{129}$ Tg152c/190 mice Scale bar, a-f, 2 mm; magnified panels i and ii, 100 μm. The detection of abnormal PrP deposition in brain indicates that the mice are infected with prions.

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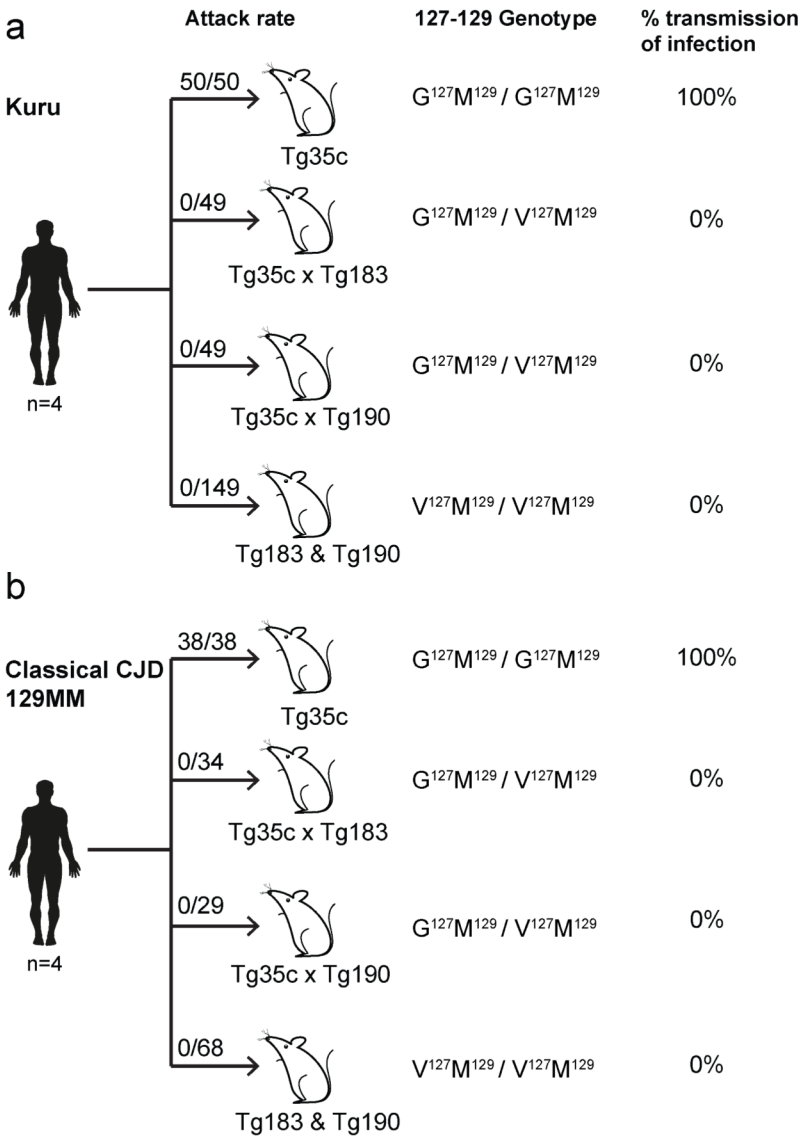

Figure 1. Transmission rates of human prions to transgenic mice homo- or heterozygous for human PrP V^{127}

Transgenic mice were intracerebrally inoculated with brain homogenate from patients with kuru (a) or classical CJD (b). Codon 127 and 129 *PRNP* genotypes of the recipient mice are shown (G, glycine, M, methionine, V, valine). $G^{127}M^{129}$ is a wild type human allele, and the $V^{127}M^{129}$ allele is seen only in humans from the kuru-exposed population of Papua New Guinea. Attack rate reports the total of clinically affected and sub-clinically infected mice as a proportion of the number of inoculated mice after prolonged (>600 days) post-inoculation periods. Primary prion transmission data are reported in tables 2 and 3.

Table 1

Transgenic mice expressing G^{127} and V^{127} human PrP

Transgenic mice	*PRNP* Codon 127-129 genotype	Total HuPrP* expression level (x)	WT† G^{127} allele expression level (x)	Variant‡ V^{127} allele expression level (x)	PrP Ratio G^{127} : V^{127}
Parental lines:					
Tg35c	$G^{127}M^{129}/G^{127}M^{129}$	2	2	-	-
Tg152c	$G^{127}V^{129}/G^{127}V^{129}$	6	6	-	-
Tg183	$V^{127}M^{129}/V^{127}M^{129}$	2	-	2	-
Tg190	$V^{127}M^{129}/V^{127}M^{129}$	1	-	1	-
F1 crosses:					
Tg35c × Tg183	$G^{127}M^{129}/V^{127}M^{129}$	2	1	1	1 : 1
Tg35c × Tg190	$G^{127}M^{129}/V^{127}M^{129}$	1.5	1	0.5	2 : 1
Tg152c × Tg183	$G^{127}V^{129}/V^{127}M^{129}$	4	3	1	3 : 1
Tg152c × Tg190	$G^{127}V^{129}/V^{127}M^{129}$	3.5	3	0.5	6 : 1
Tg152c $^{+/o}$ (Hemi)§	$G^{127}V^{129}$	3	3	-	-

WT = wild type

*PrP expression level is relative to pooled 10% (w/v) normal human brain homogenate

†Wild type alleles are either $G^{127}M^{129}$ or $G^{127}V^{129}$

‡Variant allele is $V^{127}M^{129}$

§Generated by crossing Tg152c with FVB/PrP-null mice

Table 2

Transmission of human prions to transgenic mice heterozygous for human PrP V^{127}

Inoculum			Transmission data					
Aetiology	Source Code	Human PrP^{Sc} type*	Attack rate†	Incubation period, (days ± SEM) or (days p.i)	Attack rate†	Incubation period, (days ± SEM) or (days p.i)	Attack rate†	Incubation period, (days ± SEM) or (days p.i)
A			Tg35c $G^{127}M^{129}/G^{127}M^{129}$		Tg35c × Tg183 $G^{127}M^{129}/V^{127}M^{129}$		Tg35c × Tg190 $G^{127}M^{129}/V^{127}M^{129}$	
kuru	I516	T3 VV	14/14	509 ± 56 (3)	0/11	>518-605	0/13	>517-605
kuru	I520	T3 VV	12/12	558 ± 4 (4)	0/14	>525-622	0/14	>460-622
kuru	I10336	T3 MV	13/13	454 ± 14 (10)	0/14	>522-606	0/11	>504-607
kuru	I518	T2 MM	11/11	493 ± 32 (7)	0/12	>545-620	0/13	>544-603
iCJD (GH)	I035	T1 MM	10/10	221 ± 3 (10)	0/10	>462-603	0/8	>506-602
sCJD	I11058	T1 MM	8/8	231 ± 3 (8)	0/8	>467-605	0/7	>487-605
iCJD (DM)	I026	T2 MM	10/10	256 ± 9 (8)	0/8	>507-601	0/7	>520-602
sCJD	I7040	T2 MM	10/10	233 ± 3 (10)	0/8	>582-604	0/7	>600-604

B			Tg152c $G^{127}V^{129}/G^{127}V^{129}$		Tg152c × Tg183 $G^{127}V^{129}/V^{127}M^{129}$		Tg152c × Tg190 $G^{127}V^{129}/V^{127}M^{129}$	
kuru	I516	T3 VV	9/9	218 ± 1 (6)	0/13	>447-608	9/15	543, 592
kuru	I520	T3 VV	9/9	196 ± 7 (7)	0/18	>491-616	13/13	498 ± 17 (5)
kuru	I10336	T3 MV	15/15	212 ± 3 (11)	15/15	456 ± 3 (15)	15/15	316 ± 4 (14)
kuru	I518	T2 MM	11/11	211 ± 4 (8)	3/11	>494-620	13/14	468 ± 13 (9)
sCJD	I280	T2 VV	8/8	203 ± 5 (4)	0/4	>600-602	10/10	559 ± 14 (8)
sCJD	I278	T2 VV	6/6	236 ± 8 (6)	0/8	>424-602	8/9	582
sCJD	I284	T2 MV	9/9	338 ± 4 (8)	0/9	>410-602	5/7	>545-608
sCJD	I1478	T2 MV	10/10	248 ± 8 (8)	0/6	>451-608	2/7	>482-603
sCJD	I7394	T3 VV	9/9	213 ± 1 (8)	7/7	>537-677	10/10	427 ± 8 (10)
sCJD	I764	T3 MV	10/10	219 ± 5 (7)	9/10	>368-588	10/10	461 ± 7 (9)
iCJD (GH)	I2651	T3 VV	8/8	200 ± 3 (6)	0/6	>567-607	7/7	538 ± 2 (5)
iCJD (GH)	I020	T3 MV	9/9	211 ± 2 (6)	7/7	>489-602	10/10	399 ± 8 (9)

C			Tg152c × Tg183 $G^{127}V^{129}/V^{127}M^{129}$		Tg152c × Tg190 $G^{127}V^{129}/V^{127}M^{129}$		Tg152c+/o (Hemi) $G^{127}V^{129}$	
iCJD (GH)	I020	T3 MV	7/7	>489-602	10/10	399 ± 8‡ (9)	14/14	252 ± 3‡ (14)
sCJD	I7394	T3 VV	7/7	>537-677	10/10	427 ± 8‡ (10)	12/12	245 ± 5‡ (12)
kuru	I10336	T3 MV	15/15	456 ± 3 (15)	15/15	316 ± 4‡ (14)	15/15	222 ± 2‡ (15)

D			Tg35c $G^{127}M^{129}/G^{127}M^{129}$		Tg35c × Tg183 $G^{127}M^{129}/V^{127}M^{129}$		Tg35c × Tg190 $G^{127}M^{129}/V^{127}M^{129}$	
vCJD	I342	T4 MM	15/15	>426-603	9/15	467, 556	17/18	419±17 (3)
vCJD	I7042	T4 MM	14/14	559, 561	4/13	>496-607	10/12	596

iCJD, iatrogenic CJD; sCJD, sporadic CJD; GH, growth hormone; DM, dura mater

*According to classification of Hill et al.[28]

†Attack rate is defined as the total number of both clinically *affected* and sub-clinically *infected* mice as a proportion of the total number of *inoculated* mice. Sub-clinical prion infection was assessed by immunoblotting and/or immunohistochemical examination of brain. Incubation periods are reported for clinically affected mice in days; where n ≥ 3 the mean ± SEM is reported with the number of mice contributing to the mean shown in parentheses, otherwise individual incubation times are given. In groups where no clinical transmission of prion disease was observed, the attack rate represents subclinical infection only and the interval between inoculation and death (from either senescence, culling due to inter-current illness or termination of the experiment) is reported as >x-y days.

‡Mean incubation periods for all 3 isolates are significantly lower in Tg152c hemizygotes than in Tg190 × Tg152c heterozygotes (P<0.0001; two-tailed unpaired t-test).

Table 3

Transmission of human prions to transgenic mice homozygous for human PrP V^{127}

Inoculum			Transmission data			
Aetiology	Source Code	Human PrP^{Sc} type*	Attack rate†	Incubation period, (days p.i)	Attack rate†	Incubation period, (days p.i)
			Tg183 $V^{127}M^{129}/V^{127}M^{129}$		Tg190 $V^{127}M^{129}/V^{127}M^{129}$	
kuru	I516	T3 VV	0/19	>488-604	0/22	>391-609
kuru	I520	T3 VV	0/19	>391-609	0/19	>370-609
kuru	I10336	T3 MV	0/21	>405-607	0/24	>463-617
kuru	I518	T2 MM	0/12	>439-600	0/13	>432-603
vCJD	I342	T4 MM	0/8	>553-605	0/10	>506-604
vCJD	I7042	T4 MM	0/11	>522-609	0/14	>446-607
iCJD (GH)	I035	T1 MM	0/10	>434-622	0/10	>450-602
sCJD	I11058	T1 MM	0/8	>411-609	0/9	>454-612
iCJD (DM)	I026	T2 MM	0/9	>381-602	0/8	>516-602
sCJD	I7040	T2 MM	0/8	>524-601	0/6	>564-600
sCJD	I280	T2 VV	0/9	>532-602	0/9	>425-601
sCJD	I278	T2 VV	0/7	>530-602	0/7	>417-601
sCJD	I284	T2 MV	0/6	>549-603	0/6	>466-609
sCJD	I1478	T2 MV	0/7	>517-602	0/7	>418-617
sCJD	I7394	T3 VV	0/8	>500-600	0/8	>600-603
sCJD	I764	T3 MV	0/10	>447-602	0/8	>484-607
iCJD (GH)	I2651	T3 VV	0/7	>563-606	0/7	>487-599
iCJD (GH)	I020	T3 MV	0/6	>546-603	0/6	>572-600

(days p.i), days post inoculation; vCJD, variant CJD; sCJD, sporadic CJD; iCJD, iatrogenic CJD; GH, growth hormone; DM, dura mater

*According to classification of Hill et al.[28]

†Attack rate is defined as the total of clinically *affected* and sub-clinically *infected* mice as a proportion of the number of *inoculated* mice. Sub-clinical prion infection was assessed by immunoblotting and/or immunohistochemical examination of brain. As no clinical transmission of prion disease was observed the interval between inoculation and death (from either senescence, culling due to inter-current illness or termination of the experiment) is reported as >x-y days.