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Efficient Transmission and Characterization of Creutzfeldt–Jakob Disease Strains in Bank Voles

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Transmission of prions between species is limited by the “species barrier,” which hampers a full characterization of human prion strains in the mouse model. We report that the efficiency of primary transmission of prions from Creutzfeldt–Jakob disease patients to a wild rodent species, the bank vole (Clethrionomys glareolus), is comparable to that reported in transgenic mice carrying human prion protein, in spite of a low prion protein–sequence homology between man and vole. Voles infected with sporadic and genetic Creutzfeldt–Jakob disease isolates show strain-specific patterns of spongiform degeneration and pathological prion protein–deposition, and accumulate protease-resistant prion protein with biochemical properties similar to the human counterpart. Adaptation of genetic Creutzfeldt–Jakob disease isolates to voles shows little or no evidence of a transmission barrier, in contrast to the striking barriers observed during transmission of mouse, hamster, and sheep prions to voles. Our results imply that in voles there is no clear relationship between the degree of homology of the prion protein of the donor and recipient species and susceptibility, consistent with the view that the prion strain gives a major contribution to the species barrier. The vole is therefore a valuable model to study human prion diversity and, being susceptible to a range of animal prions, represents a unique tool for comparing isolates from different species.

Introduction

Transmissible spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative diseases of humans and animals, caused by unconventional infectious agents known as prions. They are characterized by the accumulation of a disease-associated isoform (PrPSc) of the host-encoded cellular prion protein (PrPSc). According to the protein-only hypothesis, prions are composed mainly or exclusively of PrPSc. Although apparently devoid of any nucleic acid, prions exist as different infectious strains with characteristic pathogenetic properties [1], which can be characterized from their different disease phenotypes in an inbred animal host. The prion hypothesis equates strains to different self-propagating conformational variants of PrPSc [2], which parallel the diversity of physicochemical properties of PrPSc observed in human and animal prion diseases [3–6]. The electrophoretic mobility and the relative level of glycosylation of the protease-resistant fragment of PrPSc are the basis for the molecular classification of TSEs.

Sporadic Creutzfeldt–Jakob disease (sCJD) represents the most common human TSE [7], occurring worldwide with an incidence of about 1.7 cases per million people per year [8], and has an apparently spontaneous origin. Genetic Creutzfeldt–Jakob disease (gCJD) [9] is associated with mutations of the prion protein–gene and accounts for about 10% of Creutzfeldt–Jakob disease (CJD) cases [8]. Other genetic TSEs are Gerstmann–Straussler–Scheinker disease (GSS) and familial insomnia. The emergence of variant Creutzfeldt–Jakob disease (vCJD) [10], a new disease linked to bovine spongiform encephalopathy (BSE) [11,12], highlights the zoonotic potential of TSEs.

Prion diversity is revealed by transmission to laboratory animals, but this approach can be seriously limited by the “species barrier” effect, which hampers a full characterization of human prion strains in the mouse model [11,13]. Animal models that are suitable for studies with sCJD or gCJD therefore represent a major advance in understanding the extent to which various clinico-pathological forms represent different strains, and whether atypical forms are caused by novel prion strains.

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Prions are unconventional infectious agents that cause fatal neurodegenerative diseases. The transmission of prions between species is considered a rare event because it is limited by the “species barrier.” Nevertheless, in the past 10 y, more than 180 people worldwide died with variant Creutzfeldt-Jakob disease (vCJD) following consumption of bovine spongiform encephalopathy (BSE)–contaminated food. The vCJD crisis highlights the need for experimental approaches that are able to characterize human prions and to estimate the risk of animal prions for man. The authors used a new animal model, the bank vole, which appears to address these issues. They observed that these rodents are highly susceptible to sporadic Creutzfeldt-Jakob disease (sCJD) and genetic Creutzfeldt-Jakob disease (gCJD), as well as to several animal prions. Transmission to voles indicates that sCJD is caused by at least two distinct prion strains. Surprisingly, voles challenged with gCJD isolates do not show a species barrier, while prions from closely related rodent species encounter a clear barrier in transmitting to voles. Inoculation of voles with scrapie-related and BSE-related strains from several species suggests that the prion strain, and not the donor species, is the major determinant of prion transmissibility between different species. The authors conclude that the vole model is a valuable tool for comparing animal and human prions.

Although early studies with transgenic mice suggested that the degree of PrP sequence homology is responsible for the species-barrier effect [14], a number of studies since then, in addition to the results reported here, have challenged this view [11,15,16]. The main strategy for overcoming the species barrier from human to mice has been the generation of transgenic mouse lines over-expressing human PrP or chimeric mouse–human PrP [2,17–22]. Such mice usually propagate human prions more efficiently than wild-type mice, although with variable results depending on the prion strain. For example, vCJD transmits more readily to wild-type mice than to the humanized transgenic models. Recently, the term “transmission barrier” rather than “species barrier” has been proposed to account for the variable ability of prion strains to cross barriers between species [23]. To improve on the traditional mouse-based model and to deepen our understanding of the species barrier, we carried out transmission studies of human prions to the bank vole (Clethrionomys glareolus), a wild rodent species which has proved to be susceptible to prions from a range of sources [24] (U. Agrimi et al., unpublished data).

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the biochemical characteristics of human and vole PrPSc by comparing their electrophoretic mobility and glycoform pattern after protease digestion (Figure 1). Voles inoculated with type 1 human PrPSc (MM1 sCJD, MV1 sCJD, E200K gCJD, pattern after protease digestion (Figure 1). Voles inoculated comparing their electrophoretic mobility and glycoform PrPSc [2], these findings show that the subtype-specific pattern after digestion is believed to reflect the conformation of PrPSc fragment from voles inoculated with the mouse-adapted scrapie strain ME7 was intermediate between types 1 and 2 (Figure 1A, 1B, and 1D).

The relative amounts of the three major bands observed on immunoblot further characterizes subtype-specific PrPSc fragments [4,25,27]. The glycoform pattern of the human PrPSc was characterized by the expected dominance of the mono-glycosylated PrPSc fragment in sCJD and V210I gCJD, while E200K gCJD had similar levels of di-glycosylated and mono-glycosylated PrPSc fragments [27] (Figure 1A and 1C). Vole PrPSc was generally more glycosylated than human PrPSc. MM2 sCJD–affected voles showed similar levels of di-glycosylated PrPSc of the same size as the human counterpart (Figure 1A). In MM2 sCJD, the molecular weight of both human and vole unglycosylated PrPSc was of type 2, ~2 kDa lower than type 1 (Figure 1A). These PrPSc molecular patterns were unchanged after vole-to-vole transmission (Figure 1B). As the size of the protease-resistant unglycosylated fragment is believed to reflect the conformation of PrPSc [2], these findings show that the subtype-specific conformations of human PrPSc have been faithfully reproduced in voles. Interestingly, the molecular weight of this PrPSc fragment from voles inoculated with the mouse-adapted scrapie strain ME7 was intermediate between types 1 and 2 (Figure 1A, 1B, and 1D).

To characterize the disease phenotype of affected voles, we analyzed the patterns of vacuolar degeneration in selected areas of the brain, as represented by the “lesion profile” (Figure 2). The lesion profile is a well-established semiquantitative method for representing the targeting of vacuolation to different brain regions, and reliably discriminates between TSE strains in mice [11,28]. The MM1 sCJD, MV1 sCJD, V210I gCJD, and E200K gCJD cases produced identical patterns of vacuolar degeneration in voles (Figure 2A), characterized by grey-matter spongiform degeneration of the superior colliculi, thalamic nuclei, and hippocampus, and the retrosplenial and cingulate cortices. The medulla oblongata was less severely involved, while the cerebellum, hypothalamus, and septum showed only occasional vacuolation. Voles infected from the MM2 sCJD case (Figure 2B) displayed a pattern of vacuolation in the medulla, cerebellum, and thalamus similar to that observed with MM1/MV1 sCJD, but their lesion profile was characterized by a lower spongiform change in the hippocampus and cortices, while septal nuclei were more severely involved. Voles inoculated with mouse scrapie ME7 (Figure 2B) had a distinct lesion profile characterized by severe vacuolation of the hypothalamus, which was mainly spared by human prions.

Spongiform changes in positive mice with MM1 sCJD, MV1 sCJD, and V210I gCJD were generally mild, and this was consistent with the absence of clinical disease. The lesion profiles paralleled those observed in voles (Figure 2D) and are

| Table 1. Survival Times of Voles and C3H Mice Infected with Human TSEs |
|------------------|------------------|------------------|------------------|
| Inoculum Voles C3H Mice |
| Inoculum | Voles | Survival Time (Days ± SD) | Clinical Disease | Infection |
| MM1 sCJD | 188 ± 22 | 16/16 | 258–774 | 0/7 | 3/7 |
| Vole-passaged MM1 sCJD | 129 ± 8 | 14/14 | |
| Mouse-passaged MM1 sCJD | 188 ± 17 | 9/9 | 450–815 | 0/9 | 3/9 |
| MV1 sCJD | 179 ± 10 | 20/20 | 290–695 | 0/7 | 3/7 |
| Vole-passaged MV1 sCJD | 128 ± 15 | 15/15 | |
| Mouse-passaged MV1 sCJD | 187 ± 33 | 8/8 | 351–872 | 0/13 | 0/13 |
| MM2 sCJD | 408 ± 80 | 9/9 | 555–796 | 0/8 | 4/8 |
| Vole-passaged MM2 sCJD | 339 ± 27 | 20/20 | |
| MV2 sCJD | >500 | 0/20 | 367–871 | 0/7 | 0/7 |
| V2 sCJD | >500 | 0/18 | 562–862 | 0/8 | 0/8 |
| E200K/MM1 gCJD | 158 ± 13 | 19/19 | 533–686 | 0/4 | 0/4 |
| Vole-passaged E200k gCJD | 143 ± 12 | 13/13 | |
| V210/MM1 gCJD | 157 ± 8 | 20/20 | 258–774 | 0/6 | 3/6 |
| Vole-passaged V210l gCJD | 151 ± 24 | 12/12 | |
| P102L/MM1 gSs | >500 | 0/20 | 546–774 | 0/4 | 0/4 |
| ME7 | 230 ± 46 | 7/7 | 169 ± 6 | 22/22 | |
| Vole-passaged ME7 | 83 ± 8 | 13/13 | |

*When mice did not show clinical signs, the range of survival time is reported.

Vole infection in clinically healthy mice that died or were sacrificed at more than 250-d post-inoculation was assessed by Western blotting or histopathology.

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in agreement with previous reports of sCJD-infected mice [11,29].

Analysis of the distribution of PrPSc in the brain, assessed by paraffin-embedded tissue (PET) blot (Figure 3), further strengthened the similarity of type 1 sCJD and gCJD, which showed indistinguishable patterns of PrPSc accumulation, primarily in the cerebral cortex, striatum, thalamus, substantia nigra, hippocampus, and in the nuclei of the visual pathways. On the other hand, MM2 sCJD was characterized by a stronger PrPSc accumulation in the septum, lateral habenular nuclei, and hypothalamus, with only sparse labeling in the cerebral cortex and striatum. Immunohistochemistry for PrPSc showed mainly a punctate or synaptic staining in type 1 sCJD and in gCJD, while MM2 sCJD was characterized by a coarse, mostly intracellular, pattern and by frequent staining of the rim of vacuoles (Figure S1).

C3H Mice Do Not Show Disease upon Serial Passage of sCJD, but Propagate Infectivity with Strain Properties Identical to the Original Human Inocula

During adaptation to a new host, prions can either maintain their original strain properties, as assessed by passage back into the original species, or can change in a manner consistent with the isolation of variant strains with shorter incubation periods in the new host [30]. In an attempt to adapt sCJD to C3H mice, we set up secondary transmission experiments. C3H mice infected with mouse-passaged MM1 sCJD and MV1 sCJD did not show clinical signs of prion disease and died or were sacrificed after long survival times (Table 1). Only three out of nine mice inoculated with mouse-passaged MV1 sCJD had low levels of PrPSc (unpublished data), while none of the mice inoculated with MM1 sCJD showed evidence of prion infection. However, in another study of MM1 sCJD in different mouse strains, it was possible to transmit infection from mouse to mouse, but the incubation periods remained extremely long (M. Bruce, unpublished data). Lack of adaptation after successful primary transmission was unexpected, although a similar phenomenon was recently reported [22] in transgenic mice expressing human PrP with Val at codon 129 infected with BSE. Our findings suggest that C3H mice propagate sCJD prions at a level insufficient for sustaining the disease upon serial passage.

To address this hypothesis, we inoculated bank voles with the same mouse-passaged sCJD used for secondary transmissions in mice. Voles had very short survival times (188 and 187 d post-infection [dpi], respectively; see Table 1) and a 100% attack rate, showing that the brains of mice inoculated with sCJD contained high levels of infectivity, incompatible with persistence of the original human inoculum. Strikingly,
the survival times, the biochemical characteristics of PrPSc, and the brain-lesion profiles of voles inoculated with mouse-passaged sCJD were identical to those observed after direct transmission of the original sCJD isolates (Table 1; Figures 1A and 2C). From these experiments, we conclude that a subset of mice infected with type 1 sCJD propagated high levels of infectious prions which carried strain-specific information similar to that of the original human prions. Recently, several experimental approaches have emphasized the possible occurrence of a subclinical carrier-state in recipient animals, after either prion interspecies [31,32] or intraspecies [33,34] transmissions, and implications for public health have been emphasized [23,35]. Our data add further concern because they indicate that animals remaining clinically healthy after interspecies transmission may replicate prions that transmit inefficiently to individuals of the same species but are highly infectious for a third species.

Transmission Barrier Depends on Prion Strain, rather than PrP Homology

Differences in amino acid sequence between donor PrPSc and recipient PrPC have been proposed to be one of the main factors contributing to prion species barriers [14]. However, the reasons for the extraordinary sensitivity of voles to sCJD and gCJD, compared to the low susceptibility of mice, are not easily deduced from the comparison of PrP sequences (Figure 4). Indeed, the prion proteins of voles and mice display high homology and have several amino acid substitutions compared to human PrP. Taking into consideration the central portion of the PrP sequence, which has been proposed to play a major role in determining the species barrier [17], the vole and the mouse prion proteins differ at three positions, which are Met109Asn154Asn169 in the vole and Leu108Tyr153Ser168 in the mouse. The corresponding human positions are Met109His154Ser169, suggesting that homology at position 109 between the vole and the human PrP sequences might play a role in the ease of transmission observed with some human TSEs.

It is worth noting that all animal models suitable for the study of sCJD and gCJD prions are Met at the corresponding human residue 109. Even single amino acid substitutions at this position of the prion protein can influence the efficiency of transmission to mice [36,37] and voles [24]. To further evaluate the relative contributions of TSE agent strain and PrP sequence homology in determining the transmission barrier, we inoculated bank voles with scrapie-related and BSE-related strains from species with different PrP sequences, such as C57Bl mice, VM mice, hamsters, and sheep (Figure 5). While the short survival times observed overall in these transmission studies suggest that the high susceptibility of voles to TSEs is not restricted to human prions (Figure 5A), the decrease in survival time observed in secondary transmissions implies that scrapie-related and BSE-related strains, unlike gCJD, encounter a clear transmission barrier in voles (Figure 5B).
The extent of each transmission barrier was inferred by the reduction in survival times between primary and secondary transmissions (Figure 5C and 5D). The variable transmission barriers observed with different prion strains from the same donor species (Figure 5C) clearly point to the prion strain as a determinant of the outcome of interspecies transmission in bank voles, as opposed to PrP sequence homology with the donor species. Conversely, the magnitude of the transmission barrier varied according to the original source of the prion isolate, irrespective of the donor species (Figure 5D).

Discussion

We studied the transmission characteristics of human prions in a new animal model, the bank vole, which showed an unforeseen susceptibility to sCJD and gCJD. Some subtypes of sCJD and gCJD gave very short survival times and faithful propagation of their molecular phenotype in voles. These findings are unprecedented for wild-type animal models and comparable to what has been reported in humanized transgenic mouse lines. These features make the vole model a valuable and inexpensive tool for in vivo bioassays of the most common forms of sCJD and gCJD and for the characterization of human prion strains in relation to the clinico-pathological phenotype of patients. On this point, we infer the existence of at least two different prion strains in MM1/MV1 and MM2 sCJD, based on the survival times and the disease phenotype observed after primary and secondary transmission. The lack of transmission up to 500 dpi for MV2 and VV2 sCJD sources suggests that they could represent further sCJD strains. Genetic CJD with V210I and E200K mutations produced disease phenotypes similar to each other and indistinguishable from that induced by type 1 sCJD, and thus may be caused by a strain similar to the MM1/MV1 subtype. Overall, biological strain typing in voles concurs with the clinico-pathological classification of sCJD and gCJD [25,38].

The transmission studies presented here recapitulate several features of prion interspecies transmission, such as variable transmission barriers with different strains from the same donor species [11,15] and the occurrence of prion infections in clinically healthy animals [31,32]. The susceptibility of voles to different prion sources allowed us to study several transmission barriers in a single animal model. Surprisingly, V210I gCJD and E200K gCJD transmit in secondary transmission with mean survival times similar to the first passage. These findings show that some prion strains, but not all, could transmit to a different species (i.e., with a different PrP sequence) in the absence of a species barrier.

Figure 3. Regional Distribution of Protease-Resistant PrPSc in Voles following Transmission of sCJD and gCJD
PET blots of coronal sections of the forebrain (telencephalon in [A] and diencephalon in [B]), midbrain (C), and hindbrain (D) in voles infected with MM2 sCJD, MM1 sCJD, MV1 sCJD, V210I gCJD, and E200K gCJD. At the lower part of the figure, the labeled coronal sections of a negative control brain are shown. NC, neocortex; Sp, septum; St, striatum; Hp, hippocampus; Th, thalamus; Hy, hypothalamus; SC, superior colliculus; GN, geniculate nuclei; SN, substantia nigra; Cb, cerebellum; MO, medulla oblongata.
DOI: 10.1371/journal.ppat.0020012.g003

Figure 4. Alignment of Human, Vole, and Mouse Prion Protein–Amino Acid Sequences
The sequence numbers of the human (Homo sapiens) amino acids are indicated and refer to the residue under the final digit. In the vole (C. glareolus) and the mouse (Mus musculus) sequences, identical residues to the human are indicated as dots.
DOI: 10.1371/journal.ppat.0020012.g004
Scott and colleagues recently proposed that the majority of the so-called species barriers are actually strain barriers [39]. Our results provide further evidence that the outcome of interspecies transmission of prions cannot be predicted by the degree of PrP sequence homology between two species, but depends mainly on the prion strain. In fact, prion strains coming from the same species display variable transmission barriers in voles (Figure 5C) as reported in mice [15] and transgenic models [16,20], consistent with the view that sequence homology alone is not sufficient to explain the species-barrier effect. Furthermore, transmission barriers in voles seem to vary according to the prion strain, and also when a given strain is propagated in different species (Figure 5D). Several studies with mammalian and yeast prions point to an intimate relationship between prion strains and transmission barriers [16,40–42]. This interpretation is consistent with recent studies with mammalian prion amyloids [43] and yeast prions [44], which suggest that the PrP sequence of any individual species dictates the range of possible PrP conformations and hence the susceptibility to different prion strains. In this context, it is possible to speculate that the vole PrP sequence is particularly prone to faithfully reproduce the conformation that characterizes PrPSc of some human CJD strains, as supported by the fact that gCJD cases do not encounter apparent transmission barriers. It is, however, possible that mechanisms other than the PrP primary sequence contribute to the species barrier, for instance binding of PrPc [45] or PrPSc [46,47] to other proteins or to cellular factors. Transgenic mice expressing vole PrP are being generated and will be challenged with human prions in order to investigate whether the transmission barrier encountered in voles depends on vole PrP sequence or on other host factors.

Our findings also have important implications for public-health issues related to the zoonotic potential of prion diseases. The observation that prion infections in clinically healthy individuals of one species may be highly infectious for another species shows that a possible threat to human health can derive from animals in which a prion infection could not be easily detected by current diagnostic methods. Furthermore, the finding that prions can be transmitted between species with different PrP sequences, in the absence of a transmission barrier, underlines the difficulty of predicting the impact on public health of ruminant TSEs, such as BSE, BASE (bovine amyloidotic spongiform encephalopathy) [48], scrapie, and chronic wasting disease. The vole model, being susceptible to a range of prion strains, represents a major advance for the characterization of TSE isolates from different species and may aid in the development of control strategies for TSEs.
Materials and Methods

Animals. The research protocol has been approved by the Service for Biotechnology and Animal Welfare of the Istituto Superiore di Sanità and authorized by the Italian Ministry of Health, according to Legislative Decree 116/92, which has implemented in Italy the European Directive 86/609/EEC on laboratory animal protection. Bank voles (Istituto Superiore di Sanità breeding colony) and C3H mice (Charles River, Como, Italy) were housed in standard cages and treated according to Legislative Decree 116/92 guidelines, and animal welfare was routinely checked by veterinarians from the Service for Biotechnology and Animal Welfare. All animals were individually identified by a passive integrated transponder.

Brain inocula. Patient CJD diagnoses were confirmed by histopathology, immunohistochemistry, and Western blotting [27]. Human brain samples for transmission were collected from areas showing pathology for the genes and gene products discussed in this paper. Accession Numbers Found at DOI: 10.1371/journal.ppat.0020012.sg001 (1.7 MB PDF).

Transmission of Human Prions to Voles

Neurodegeneration and PrP Sc deposition in voles. Voles and mice were infected with the prion agent, and brain samples for transmission were collected from areas showing pathology, immunohistochemistry, and Western blotting [27]. Human PrP Sc was analyzed together with vole PrPSc in Western blotting as above.

Histopathology, immunohistochemistry, and PET blot. At post-mortem, each brain was divided into two parts by a sagittal paramedian cut. The smaller portion was immediately frozen and stored at −20°C for Western blotting. The remaining part was immersed and fixed in 10% formal saline for 4 d. The brains were trimmed at standard coronal levels, decontaminated with formic acid for 1 h, and embedded in paraffin. Sections (6 µm thick) were cut for haematoxylin and eosin staining, immunohistochemistry, and PET blot, randomly mixed and coded for pathological assessment.

For the construction of lesion profiles, vacuolar changes were scored in nine grey-matter areas of the brain on haematoxylin and eosin-stained sections, as described by H. Fraser and A. G. Dickinson [49]. Vacularization scores are derived from at least six individual voles per group and from three individual mice per group, and are reported as mean ± standard error of the mean.

For PrP immunohistochemistry, sections were collected on silanized slides (Dako-Cytomation, Glostrup, Denmark). After treatment at 60°C for 24 h, sections were hydrated, pretreated with 98% formic acid for 1 min, followed by hydrating autolocating for 30 min at 12°C and finally incubating overnight. Incubation with antibodies, plus avidin–biotin complex treatment and revelation, were carried out with Dako-AutoStainer (Dako-Cytomation). Sections were treated with 6% normal goat serum (Vector, Burlingame, California, United States) in PBS for 30 min. Immunohistochemical detection of PrP was performed with mAb SAF84 (Sp-Bio, Montigny-Le-Bretoumeaux, France) at 2 µg/ml in PBS with 3% of normal goat serum (Vector) for 45 min. After washing with PBS, sections were treated with ABC Complex (Vector) for 45 min and with diaminobenzidine (Dako-Cytomation) for 7 min. Sections were counterstained with Mayer's haematoxylin. In each run, positive- and negative-control sections were included.

Sections for PET blot were collected on prewetted 0.45-µm-pore nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Membranes were dried for 24 h at 55°C. Membrane treatments, proteinase K digestion (50 µg/ml), and immunodetection were performed as described [50]. Monoclonal Ab SAF84 (1 µg/ml) was used as primary antibody.

Western blot. Brain tissues were homogenized (10% v/v) in 100 mM Tris-HCl (pH 7.4) containing 2% sarcosyl (Sigma, St. Louis, Missouri, United States). The homogenates were incubated for 30 min at 37°C with gentle shaking, proteinase K (50 µg/ml; Sigma) was added, and then the homogenates were further incubated for 1 h at 37°C with gentle shaking. Proteinase treatment was stopped with 3 mM PMSF (Sigma). Treated homogenates were denatured by adding an equal volume of 2% NuPage sample buffer (Invitrogen, Carlsbad, California, United States) and heating for 10 min at 90°C. After centrifugation at 12,000 rpm for 5 min in a microfuge, 10 µl of each sample was loaded onto 12% bis-Tris polyacrylamide gels (Invitrogen). Precision Plus Strep-tagged molecular markers (Bio-Rad, Hercules, California, United States) were loaded in each gel. After electrophoresis and Western blotting on polyvinylidene difluoride membranes (Millipore, Bedford, Massachusetts, United States), the blots were blocked in PBS containing 0.05% Tween 20 and 0.5% non-fat milk powder for 1 h. PrPSc from voles and mice was detected with mAb SAF84 (0.4 µg/ml) for 1 h at room temperature. When human PrPSc was analyzed together with PrPSc isolated in combination with mAb 3F4 (1:5,000). Horseradish peroxidase-conjugated anti-mouse immunoglobulin (Pierce Biotechnology, Rockford, Illinois, United States) was used as secondary antibody (1:80,000 for 1 h). The membranes were developed with an enhanced chemiluminescence method (SuperSignal Femto, Pierce Biotechnology) and detected with the VersaDoc imaging system (Bio-Rad). Apparent molecular weights and glycoform patterns were determined with QuantityOne software (Bio-Rad). For glycoform analysis, values are derived from PrPSc of human inocula and from five individual voles per group (means ± standard error of the mean).

Samples that were negative by this standard protocol were further analyzed after PrPSc concentration. In this case, 10% (w/v) homogenates were added with 100 mM Tris-HCl (pH 7.4) containing Sarcosyl (Sigma) to obtain a final 5% (w/v) homogenate with 10% Sarcosyl. The homogenates were incubated for 20 min at room temperature and then centrifuged at 22,000 g for 20 min (TLA 100.3 rotor, Beckman Instruments, Fullerton, California, United States). Supernatants were added with proteinase K (50 µg/ml; Sigma) and incubated for 1 h at 37°C with gentle shaking. Proteinase treatment was stopped with 3 mM PMSF (Sigma). The treated homogenates were ultracentrifuged at 210,000 g for 40 min (TLA 100.3 rotor, Beckman Instruments), and the pellets were resuspended in 100 µl of distilled water and desiccated in speed-vacuum (Speed Vac Sc 110, Savant, Holbrook, New York, United States) overnight. The final pellets were resuspended in NuPage sample buffer and were analyzed by Western blotting as above.

Supporting Information

Figure S1. Neurodegeneration and PrPSc Deposition in Voles Infected with MM2 sCJD and MM1 sCJD

Accession Numbers

The GenBank (http://www.ncbi.nlm.nih.gov/Genbank) accession numbers for the genes and gene products discussed in this paper are C57Bl mice PrP (M18070), hamsters PrP (M14054), human PrP (M18099), VM mice PrP (M18071), vole PrP (AF367624), and sheep PrP (M31313).
Transmission of Human Prions to Voles

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References

1. Bruce M (1993) Scrapie strain variation and mutation. Br Med Bull 49: 822–832. 
2. Telling GC, Parchi P, DeArmond SJ, Cortelli P, Montagna P, et al. (1996) Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. Science 274: 2079–2083.
3. Parchi P, Castellani R, Capellari S, Ghetti B, Young K, et al. (1996) Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. Ann Neurol 39: 669–680.
4. Collinge J, Siddique K, Ironside J, Hill AF (1996) Molecular analysis of a new variant strain and the etiology of “new variant” CJD. Nature 383: 685–690.
5. Hill AF, Siddique K, Joiner S, Keyes P, Martin TC, et al. (1998) Molecular screening of sheep for bovine spongiform encephalopathy. Neurosci Lett 255: 159–162.
6. Safar J, Wille H, Itti V, Groth D, Serban H, et al. (1998) Eight prion strains have PrPSc with different conformations. Nat Med 4: 1157–1165.
7. Pocchiari M (1994) Prions and related neurological diseases. Mol Aspects Med 15: 195–291.
8. Ladogana A, Puopolo M, Croes EA, Budka H, Jarius C, et al. (2005) Genetic prion disease: The EUROCJD experience. Hum Genet 118: 166–174.
9. Will RG, Ironside JW, Zeidler M, Couzens SN, Estebereiko K, et al. (1998) A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 347: 921–925.
10. Bruce M, Will RG, Ironside JW, McConnell I, Drummond D, et al. (1997) Transmissions to mice indicate that “new variant” CJD is caused by the BSE agent. Nature 398: 498–501.
11. Hill AF, Desbruslais M, Joiner S, Siddique KCL, Gowland I, et al. (1997) The same prion strain causes CJD and BSE. Nature 389: 448–450.
12. Brown P, Gibbs CJ, Rodgers-Johnson P, Asher DM, Sulima MP, et al. (1994) Human spongiform encephalopathy: The National Institutes of Health series of 300 cases of experimentally transmitted disease. Ann Neurol 35: 513–529.
13. Prusiner SB, Scott M, Foster D, Pan KM, Groth D, et al. (1996) Transgenic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. Cell 65: 673–686.
14. Bruce M, Chree A, McConnell I, Foster J, Pearson G, et al. (1994) Transmission of bovine spongiform encephalopathy and scrapie to mice: Strain variation and the species barrier. Philos Trans R Soc Lond B Biol Sci 343: 513–529.
15. Pocchiari M, Weissmann C (1998) The prion’s perplexing persistence. Nature 392: 763–764.
16. Moore RG, Hope J, McBride PA, McConnell I, Selfridge J, et al. (1998) Mice with gene targeted prion protein alterations show that Prnp, Sinc and Prni are congruent. Nat Genet 18: 118–125.
17. Barron RM, Baybutt H, Tuzzi NL, McCormack J, King D, et al. (2005) Polyomorphisms at codons 108 and 198 in murine PrP play distinct roles in the control of scrapie incubation time. J Gen Virol 86: 852–868.
18. Gambetti P, Kong K, Zou W, Parchi P, Chen SG (2005) Sporadic and familial CJD: Classification and characterisation. Br Med Bull 66: 213–239.
19. Scott MR, Peretz D, Nguyen HO, Dearmond SJ, Prusiner SB (2005) Transmission barriers for bovine, ovine, and human prions in transgenic mice. J Virol 79: 5259–5271.
20. Barron RM, Thomson V, Jamieson E, Melton DW, Ironside J, et al. (2001) Changing a single amino acid in the N-terminus of murine PrP alters TSE incubation time across three species barriers. EMBO J 20: 5070–5078.
21. Chien P, Weissman JS (2001) Conformational diversity in a yeast prion dictates its seeding specificity. Nature 410: 223–227.
22. Chien P, DePace AH, Collins SR, Weissman JS (2003) Generation of prion transmission barriers by mutational control of amyloid conformations. Nature 424: 948–951.
23. Jones EM, Sureswicz WK (2005) Fibril conformation as the basis of species and strain-dependent seeding specificity of mammalian prion amyloids. Cell 121: 65–72.
24. Tanaka M, Chien P, Yonekura K, Weissman JS (2005) Mechanism of cross-species prion transmission: An infectious conformation compatible with two highly divergent yeast prion proteins. Cell 121: 49–62.
25. Collinge J, Siddique K, Ironside J, Hill AF (1995) Unaltered susceptibility to BSE in transgenic mice expressing human prion protein. Nature 378: 779–783.
26. Asante EA, Linehan JM, Desbruslais M, Joiner S, Gowland I, et al. (2002) BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. EMBO J 21: 6358–6366.
27. Korth C, Kaneko K, Groth D, Heye N, Telling G, et al. (2003) Abbreviated incubation times for human prions in mice expressing a chimeric mouse-human prion protein transgene. Proc Natl Acad Sci U S A 91: 9936–9940.
28. Collinge J, Siddique K, Ironside J, Hill AF (1995) Unaltered susceptibility to BSE in transgenic mice expressing human prion protein. Nature 378: 779–783.
29. Asante EA, Linehan JM, Desbruslais M, Joiner S, Gowland I, et al. (2002) BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. EMBO J 21: 6358–6366.
30. Cartoni C, Schininà ME, Maras B, Nonno R, Vaccari G, et al. (2005) Identification of the pathological prion protein allootypes in scrapie-infected heterozygous bank voles (Clethrionomys glareolus) by high-performance liquid chromatography-mass spectrometry. J Chromatogr A 1081: 122–126.
31. Parchi P, Giese A, Capellari S, Brown P, Schulte-Schaeffer W, et al. (1999) Identification of scrapie-induced conformational changes in prion proteins. J Biol Chem 274: 7195–7196.
32. Casalone C, Zanusso G, Saccomani M, Taviani S, et al. (2004) Identification of a second familial bovine amyloidotic spongiform encephalopathy: Molecular similarities with sporadic Creutzfeldt-Jakob disease. Proc Natl Acad Sci U S A 101: 3065–3070.
33. Fraser H, Dickinson AG (1968) The sequential development of the brain lesions of scrapie in three strains of mice. J Comp Pathol 78: 301–311.
34. Schulz-Schaeffer WJ, Tse MG, Nourse J, Drose W, Hause-Reitner D, et al. (2000) The paraffin-embedded tissue blot detects PrPSc early in the incubation time in prion diseases. Am J Pathol 156: 51–56.