Title: A domain responsible for spontaneous conversion of bank vole prion protein

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ABSTRACT

Bank vole is a small rodent that shows high susceptibility to infection with diverse prion strains. To determine whether the increased susceptibility of bank voles to prion diseases can be attributed to the intrinsic nature of bank vole prion protein (PrP) or to host factors other than PrP, we produced transgenic mice overexpressing bank vole PrP. These transgenic mice spontaneously developed neurological illness with spongiform changes and the accumulation of abnormal PrP in the brain. Then, we produced transgenic mice overexpressing chimeric mouse/bank vole PrP, which differs from mouse PrP only at two residues located at the C-terminus, to determine the minimum essential domain for the induction of spontaneous generation of abnormal PrP. These transgenic mice also developed spontaneous neurological illness with spongiform changes and the accumulation of abnormal PrP in the brain. In addition, knock-in mice expressing bank vole PrP at the same level as that of wild-type mice did not develop spontaneous disease but showed high susceptibility to infection with diverse prion strains, similarly to bank voles. Taken together, these findings show that bank vole PrP has a high propensity for the conformational conversion both in spontaneous disease and in prion infection, probably due to the characteristic structural properties of the C-terminal domain.

KEYWORDS: prion, prion disease, bank vole, misfolding

INTRODUCTION

Prion diseases are fatal transmissible neurodegenerative diseases including Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia in humans, or bovine spongiform encephalopathy, chronic wasting disease, and scrapie in animals. Accumulation of an abnormal protease-resistant isoform of prion protein (PrP^{res}), which is generated by conformational conversion of the normal cellular isoform (PrP^{C}), is one of the pathognomonic features of prion diseases (25). In most human cases of CJD, the initial formation of abnormal PrP isoform occurs spontaneously. However, the molecular mechanisms of the spontaneous generation of abnormal PrP isoform remain unknown.

Bank vole (*Myodes glareolus*) is a small rodent that has been used in various research areas such as virus infection, diabetes, seizures, etc. (26). Pioneering studies have revealed that bank voles are highly susceptible to the transmission of a broad range of human and animal prion strains (5,6,21,24). Furthermore, transgenic mice overexpressing bank vole PrP (Tg-Bv) spontaneously developed neurological illness with the accumulation of partially protease-resistant PrP^{res} (30). Of note, Tg-Bv mice with isoleucine at polymorphic residue 109 of the Bv PrP (Figure 1; residue numbering according to Bv PrP is used throughout the text), denoted as Tg-Bv(109I), developed spontaneous disease, whereas Tg-Bv mice with methionine at residue 109, denoted as Tg-Bv(109M), remained healthy for longer than 500 days. In addition, Tg-Bv(109I) harboring a pathogenic human point mutations also developed spontaneous diseases with mutation-specific neuropathological features as mouse models of inherited human prion diseases (32).

Here we newly established transgenic mouse lines overexpressing Bv PrP with M109 and found that these Tg-Bv(109M) mice spontaneously developed neurological disease with the accumulation of partially protease-resistant PrP^{res}. Moreover, we also established transgenic

mouse lines overexpressing chimeric mouse/bank vole PrP, denoted as Tg-Mo/Bv, to determine the minimum essential domain for inducing the spontaneous generation of PrP^{res}. Although the chimeric PrP was almost the same as mouse PrP except for two residues located near the C-terminus, these Tg-Mo/Bv mice also spontaneously developed neurological illness with the accumulation of partially protease-resistant PrP^{res}. These data suggest that the Bv PrP-specific C-terminal structure may induce the spontaneous formation of PrP^{res}.

MATERIALS AND METHODS

Ethics statement

Brain tissues were obtained at autopsy after receiving written informed consent for research use from all participants. Animal experiments were performed in strict accordance with the Regulations for Animal Experiments and Related Activities at Tohoku University, the regulations outlined in the Guide for the Care and Use of Laboratory Animals of the National Institute of Animal Health, the Regulations for Animal Experimentation at Nagoya City University, and Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions by Ministry of Education, Culture, Sports, Science and Technology in Japan, Notice No. 71. The protocol was approved by the Institutional Animal Care and Use Committees of Tohoku University (2013IDOU-434), the National Institute of Animal Health (07-113,08-43), and Nagoya City University (09117).

Production of transgenic mice and knock-in mice

Tg-Bv(109M) mice, Tg-Mo/Bv mice, and knock-in mice expressing bank vole PrP with M109 (Ki-Bv(109M) mice) were produced as described previously (13,14). Tg-Mo/Bv mice expressed chimeric Mo/Bv PrP which had the Bv PrP sequence downstream of the *Bst*EII site at codon 189.

Tg-Bv(109M) mice lacking endogenous mouse PrP were generated by repeated backcrosses with PrP knock-out mice.

Sources of prion inocula

Human brain tissues were collected from patients with sporadic CJD (MM1, MM2C, and VV2 subtypes), acquired CJD, or variant CJD. The diagnosis of CJD, the *PRNP* genotype, and PrP^{res} type were confirmed by immunohistochemistry, western blot analysis, and *PRNP* sequence analysis as described (11,12). The subtyping of CJD was carried out according to the classification by Parchi *et al.* (23). Details of the acquired CJD patient have been described elsewhere (9,18). A mouse-adapted Gerstmann-Sträussler-Scheinker syndrome strain, Fukuoka-1 (29), was maintained in C57BL/6 mice, and the inoculum was prepared from the terminally ill mice.

Transmission experiments

Intracerebral inoculation was performed as described (15). The inoculated mice were sacrificed at a predefined clinical endpoint, or at the time point showing intercurrent illness. One hemisphere of the brain was fixed in 10% buffered formalin for immunohistochemistry, and the other hemisphere was immediately frozen for western blotting. Incubation times are expressed as mean ± SEM.

Immunohistochemistry

Formalin-fixed mouse brain tissues were treated with 60% formic acid for 1 hour to inactivate the infectivity and embedded in paraffin. Tissue sections were pretreated by hydrolytic autoclaving before PrP immunohistochemistry (11). The anti-PrP antiserum PrP-N (10) and

rabbit anti-glial fibrillary acidic protein (GFAP) polyclonal antibody (Dako) were used as the primary antibodies. Goat-anti-rabbit immunoglobulin polyclonal antibody labelled with the peroxidase-conjugated dextran polymer, EnVision^{+} (Dako) was used as the secondary antibody.

Western blotting

Brain tissues were homogenized in homogenize buffer (100 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, and 50 units/ml DNase I) and incubated for 1 h at 4°C. These 10% brain homogenates were centrifuged at 900*g* for 10 min at 4°C, and the supernatants were subjected to PK-digestion. The PK-digestion was carried out by adding Sarkosyl and PK (final concentrations: 2% Sarkosyl, and 0.5 or 20 μg/ml PK) and incubation for 30 min at 37°C. The PK-digestion was stopped by adding Pefabloc SC (Roche Diagnostics) (final concentration: 2 mM). The PK-digested proteins were precipitated by adding a butanol/methanol mixture (1:5) and centrifuged at 19,000*g* for 10 min at 4°C. The pellets were resuspended in Laemmli's sample buffer (60 mM Tris-HCl pH 6.8, 5% glycerol, 2% SDS, 5% 2-mercaptoethanol, and 0.01% bromophenol blue) and boiled for 10 min. Protein samples were subjected to SDS-PAGE and western blotting as reported (1). Anti-PrP monoclonal antibodies SAF32 and SAF83 (Bertin Pharma) were used as the primary antibodies. Anti-mouse EnVision^{+} was used as the secondary antibody.

RESULTS

Spontaneous illness of Tg-Bv(109M) mice

To determine whether the increased susceptibility of bank voles to prion diseases can be attributed to the intrinsic nature of Bv PrP or to host factors other than PrP, we produced two lines (B495$^{+/+}$ and D920$^{+/+}$) of transgenic mice expressing Bv PrP with M109, denoted as Tg-Bv(109M). These Tg-Bv(109M) B495$^{+/+}$ mice and D920$^{+/+}$ mice showed high levels of PrP

expression (4-6 times and 8 times the level of wild-type mice, respectively) (Figure 2a). Noteworthy, these Tg-Bv(109M) mice spontaneously developed neurological dysfunctions including ataxia, lethargy, kyphosis, and weight loss. The mean age at onset was 450 ± 26 days in Tg-Bv(109M) B495$^{+/+}$ mice or 238 ± 4 days in Tg-Bv(109M) D920$^{+/+}$ mice (Table 1). Neuropathological examination of the brains from clinically ill mice revealed spongiform changes, the accumulation of PrP, and gliosis (Figure 2b). Spongiform changes and PrP deposition were most evident in the cerebral cortex and thalamus, and were sometimes observed also in the basal ganglia. Western blot analysis of proteinase K (PK)-resistant PrP^{res} in the brain revealed that the affected mice harbored partially PK-resistant PrP^{res} (Figure 2c). These PrP^{res} bands were visible after digestion with 0.5 μg/ml of PK but were eliminated after digestion with 20 μg/ml of PK, similarly to those reported in spontaneously diseased Tg-Bv(109I) mice (30). Tg-Bv(109M) D920$^{+/+}$ mice harbored more PrP^{res} compared with Tg-Bv(109M) B495$^{+/+}$ mice. The migration pattern of the partially PK-resistant PrP^{res} was distinct from that of typical PrP^{res} and was represented as a single band located around 23-24 kDa. The partially PK-resistant PrP^{res} lacked the epitope for the SAF32 antibody that recognizes the N-terminal octapeptide repeat region of PrP. The ~8 kDa PrP^{res} fragments, which have been reported in Tg-Bv(109I) mice (32), were not observed (data not shown). Meanwhile, Tg-Bv(109M) B495$^{+/+}$ mice inoculated with a mouse-adapted human prion strain Fukuoka-1 (29) produced typical PrP^{res}, which was resistant to 20 μg/ml of PK. Thus, Tg-Bv(109M) mice spontaneously developed neurological illness associated with the accumulation of partially protease-resistant PrP^{res}.

Next, we examined the influence of coexisting endogenous mouse PrP on the spontaneous generation of Bv PrP^{res}. Heterologous PrP molecules can interfere with each other during PrP^{res} formation (8,16). Therefore, Tg-Bv(109M) B495$^{+/+}$ mice were crossed with PrP knock-out mice to produce Tg-Bv mice lacking endogenous mouse PrP, denoted as Tg-Bv(109M) B495$^{-/-}$. As

shown in Table 1, Tg-Bv(109M) B495$^{-/-}$ mice also developed neurological illness spontaneously, with a mean incubation period of 488 ± 19 days. Histopathological examination of the brain revealed that the neuropathological changes in the Tg-Bv(109M) B495$^{-/-}$ mice were more severe than those of Tg-Bv(109M) B495$^{+/+}$ mice (Figure 2b). In the affected Tg-Bv(109M) B495$^{-/-}$ mice, spongiform changes and PrP deposition were observed not only in the cerebral cortex, thalamus and the basal ganglia but also in the cerebellar cortex and hippocampus. In addition, the PrP deposition in each brain region was more extensive compared with that of Tg-Bv(109M) B495$^{+/+}$ mice. Western blot analysis of PrP^{res} also revealed that Tg-Bv(109M) B495$^{-/-}$ mice harbored larger amounts of PrP^{res} compared with Tg-Bv(109M) B495$^{+/+}$ mice, despite the fact that Tg-Bv(109M) B495$^{-/-}$ mice expressed smaller amounts of PrP than Tg-Bv(109M) B495$^{+/+}$ mice (Figures 2a, c). Thus, the elimination of coexisting endogenous mouse PrP enhanced the accumulation of Bv PrP^{res} and exacerbated the neuropathological changes, while the mean age at onset was not affected.

We then examined whether the spontaneously generated PrP^{res} in Tg-Bv(109M) mice was transmissible to wild-type mice (NZW or C57BL/6). After intracerebral challenge with brain homogenates from the clinically ill Tg-Bv(109M) B495$^{+/+}$ mice, none of the inoculated mice developed neurological symptoms or PrP^{res} accumulation (Table 2). In addition, disease transmission from Tg-Bv(109M) B495$^{-/-}$ mice to wild-type mice was also not observed, though Tg-Bv(109M) B495$^{-/-}$ mice harbored larger amounts of PrP^{res} compared with Tg-Bv(109M) B495$^{+/+}$ mice. Thus, the spontaneously generated PrP^{res} in Tg-Bv(109M) mice was not transmissible to wild-type mice.

Spontaneous illness of Tg-Mo/Bv mice

To determine the domain responsible for the high propensity for spontaneous conversion of Bv

PrP, we produced two lines ($J642^{+/+}$ and $J643^{+/+}$) of transgenic mice expressing chimeric mouse/bank vole PrP, denoted as Tg-Mo/Bv. These mice carried chimeric PrP that differed from Mo PrP only at two residues (aspartic acid to glutamic acid at residue 227, D227E; and arginine to serine at residue 230, R230S). The expression levels of PrP in Tg-Mo/Bv $J642^{+/+}$ mice and Tg-Mo/Bv $J643^{+/+}$ mice were 2-4 times and 4 times the level of wild-type mice, respectively (Figure 3a). Of note, one out of 14 (7%) Tg-Mo/Bv $J642^{+/+}$ mice and 7 out of 17 (41%) Tg-Mo/Bv $J643^{+/+}$ mice spontaneously developed neurological dysfunctions similar to those observed in Tg-Bv(109M) mice (Table 3). The affected mice exhibited spongiform changes, PrP accumulation, and gliosis in the brain (Figure 3b). These neuropathological features in the diseased Tg-Mo/Bv mice were the same as those of the spontaneously ill Tg-Bv(109M) mice. Western blot analysis of PK-resistant PrP^{res} in the brain revealed that the affected Tg-Mo/Bv mice harbored partially protease-resistant PrP^{res} similar to those of Tg-Bv(109M) mice (Figure 3c). The unusual PrP^{res} band was visible after weak PK digestion and located around 23-24 kDa. In addition, the partially protease-resistant PrP^{res} in Tg-Mo/Bv mice also lacked the epitope for the SAF32 antibody. Thus, only the two amino acid substitutions at the C-terminus of Mo PrP to Bv PrP-specific residues (D227E and R230S) contributed to the spontaneous generation of PrP^{res}.

Absence of spontaneous illness in Ki-Bv(109M) mice

The findings described above, together with data from a previous study (30), suggest that Bv PrP itself has a propensity for spontaneous PrP^{res} generation regardless of the host factors in bank voles. On the other hand, spontaneous prion disease has not been reported in bank voles. Although the reason for this discrepancy remains elusive, a possible relationship to the lower expression level of Bv PrP in bank voles compared with Tg-Bv mice (30) is worth considering.

To test this possibility, we established knock-in mice expressing Bv PrP with M109, denoted as Ki-Bv(109M). Ki-Bv(109M) mice expressed Bv PrP at exactly the same level as that of wild-type mice (Figure 4a). In these Ki-Bv(109M) mice, neither the development of clinical symptoms nor the accumulation of PrP^{res} was observed even at very advanced age over 900 days. Thus, the expression of Bv PrP at a physiological level did not induce spontaneous disease.

General susceptibility of Ki-Bv(109M) to a broad range of prion strains

To confirm that the general susceptibility of bank voles to the transmission of diverse prion strains (5,6,21,24) can also be attributed to the intrinsic nature of Bv PrP regardless of the host factors in bank voles, we performed a transmission study using Ki-Bv(109M) mice. Although the increased susceptibility of Tg-Bv(109M) mice to the transmission of diverse prion strains has already been reported (7,31), the influence of the expression level or the expression site of PrP on the increased susceptibility could not be excluded. Therefore, we examined whether Ki-Bv(109M) mice can also show general susceptibility to diverse prion strains as well as bank voles. As shown in Table 4, almost all prion strains examined could be transmitted to Ki-Bv(109M) mice. Notably, despite the fact that the MM2C subtype of sporadic CJD is not transmissible even to PrP-humanized knock-in mice (3,17,19), Ki-Bv(109M) mice intracerebrally inoculated with sporadic CJD MM2C prions developed disease with a 100% attack rate. The neuropathological properties of the affected Ki-Bv(109M) mice differed from those of spontaneously ill Tg-Bv(109M) mice (data not shown). Moreover, the PrP^{res} band patterns in the affected Ki-Bv(109M) mice were quite different from the partially protease-resistant PrP^{res} in the spontaneously ill Tg-Bv(109M) mice and basically reflected those of the inoculated PrP^{res} (Figure 4b). Thus, Bv PrP had a high propensity for the conformational conversion also in prion infection. Nevertheless, the spontaneously generated

PrP^{res} from Tg-Bv(109M) $B495^{+/+}$ mice or Tg-Bv(109M) $B495^{-/-}$ mice was not transmissible even to Ki-Bv(109M) mice (Table 4).

DISCUSSION

The present study consists of three major findings. First, Tg-Bv(109M) mice model, overexpressing the Bv PrP, showed spontaneous generation of PrP^{res}. Second, Tg-Mo/Bv mice overexpressing chimeric Mo/Bv PrP with two C-terminal Bv PrP-specific residues also showed spontaneous generation of PrP^{res}. Finally, Ki-Bv(109M) model, which expresses the Bv PrP in equivalent levels to the wild-type mice, lacked spontaneous generation of PrP^{res} but showed general susceptibility to infection with diverse prion strains. These findings suggest that Bv PrP has a high propensity for the conformational conversion both in spontaneous disease and in prion infection, and that the C-terminal domain of Bv PrP may play an important role in this property.

Since spontaneous generation of PrP^{res} cannot be observed in bank voles or Ki-Bv(109M) mice, overexpression of Bv PrP is necessary for the spontaneous generation of PrP^{res}. Meanwhile, it is unlikely that the spontaneous generation of PrP^{res} in Tg-Bv(109M) mice or Tg-Mo/Bv mice is solely due to the overexpression of PrP. Spontaneous generation of subclinical levels of prion infectivity has also been reported in aged *tga20* mice that express wild-type mouse PrP at very high levels (28). In *tga20*, however, the spontaneous generation of prion infectivity occurred in much older mice with lower attack rates in spite of the higher expression level of PrP. Therefore, the present data, together with data previously reported (30), suggest that the spontaneous generation of PrP^{res} in Tg-Bv(109M) mice or Tg-Mo/Bv mice can be attributed to the intrinsic structural properties of Bv PrP or chimeric Mo/Bv PrP.

The present study reveals a contribution of two Bv PrP-specific residues (E227 and S230) to

the spontaneous generation of PrP^{res}. Among eight residues that differ between Bv PrP and mouse PrP, Bv PrP shares six residues with hamster PrP except for E227 and S230 (Figure 1). Therefore, the potential importance of the two C-terminal residues in the characteristic properties of Bv PrP has been suggested (31). Indeed, in the present study, Tg-Mo/Bv mice carrying chimeric PrP with the two C-terminal Bv PrP-specific residues showed spontaneous generation of PrP^{res}. Interestingly, two asparagine (N) residues at 155 and 170 of Bv PrP have been implicated in the high susceptibility to prion infection (2). Furthermore, transgenic mice expressing mouse PrP with two amino acid substitutions at residues 170 and 174 (serine to asparagine at residue 170, S170N; and asparagine to threonine at residue 174, N174T) showed spontaneous generation of PrP^{res} (28). Therefore, the N170 and the two C-terminal residues (E227 and S230) of Bv PrP may exert synergetic effects on the induction of the spontaneous generation of PrP^{res}. Indeed, residues 169-174 and residues 227-231 have been proposed to compose an interface that provides lateral interactions between neighboring PrP molecules (27). These findings raise the possibility that the Bv PrP-specific C-terminal residues may affect the structural properties of the interface (4) and cause an increase of intermolecular PrP interactions. These changes may accelerate the formation of PrP^{res}. Since the increase of intermolecular PrP interactions can also accelerate the propagation of exogenously inoculated PrP^{res}, the general susceptibility of bank voles to infection with diverse prion strains may also be explained by the peculiar C-terminal structure of Bv PrP. Watts *et al*. reported that Tg-Bv(109I) harboring pathogenic human point mutations developed spontaneous diseases with mutation-specific neuropathological features (32). In this regard, Tg mice expressing human PrP harboring pathogenic point mutations combined with the C-terminal Bv PrP-specific residues might become optimal mouse models of inherited human prion diseases because the PrP amino acid sequences of these mice would be almost identical to those of human patients except for the two

C-terminal residues.

The present study shows that Bv PrP with M109 is also conversion-prone, as with Bv PrP with I109 (30). However, spontaneously generated PrP^{res} in the clinically ill Tg-Bv(109M) mice was not transmissible to wild-type mice nor Ki-Bv(109M) mice in the present study. Notably, Watts *et al*. reported that spontaneous generation of infectious PrP^{res} could be observed in Tg-Bv(109I) mice but not in Tg-Bv(109M) mice (30). Therefore, polymorphism at residue 109 may affect the efficiency of PrP^{res} formation as well as infectivity properties. The residue 109 in Bv PrP corresponds to the residue 108 in mouse PrP, which is a well-known polymorphic residue (leucine or phenylalanine, L or F) combined with another polymorphic residue 189 (threonine or valine, T or V) (33). These polymorphic residues determine the incubation time of scrapie infection in mice (20). In the structural model of PrP^{res}, residue 109 is located at the interface that provides longitudinal interactions between N-terminal β helices of neighboring PrP molecules stacked into fibrils (27). These findings raise the possibility that the polymorphism at residue 109 may affect the structural properties of the N-terminal interface and alter the longitudinal intermolecular PrP interactions. Thus, I109 in Bv PrP might accelerate PrP^{res} formation by increasing the intermolecular PrP interactions. The difference in the propensity for spontaneous PrP^{res} formation between the previously established Tg-Bv(109M) mouse line (30) and our Tg mouse line may relate to the transgenic vector used. Watts *et al.* (30) used a transgenic vector with Syrian hamster PrP gene backbone containing a long 5' promoter region, whereas our transgenic vector had mouse PrP gene backbone with a relatively short 5' promoter region (14). Therefore, the different transgenic vectors, especially the difference in the length of 5' promoter region, might affect the cell population expressing PrP or the expression level of PrP in each cell. These differences in PrP expression patterns might account for the different propensity for spontaneous generation of PrP^{res}.

Ki-Bv(109M) mice can be a useful animal model for the transmission of diverse prion strains including the MM2C subtype of sporadic CJD. Patients with sporadic CJD are classified by the polymorphism at residue 129 of PrP (methionine or valine, M or V) and the type of PrP^{res} in the brain (types 1 or 2) (23). Among the six sporadic CJD subtypes, only MM2C lacked transmissibility to PrP-humanized knock-in mice (3,17,19). By contrast, in the present study, MM2C could be transmitted to Ki-Bv(109M) mice with a 100% attack rate. The neuropathological and biochemical properties of the affected Ki-Bv(109M) mice were different from those of the spontaneously ill Tg-Bv(109M) mice, indicating that the disease observed in Ki-Bv(109M) mice was not due to the spontaneous generation of Bv PrP^{res}. Transmission of the sporadic CJD MM2 subtype has also been reported in bank voles and Tg-Bv(109M) mice, though it was not specified whether the inoculum was MM2C or MM2T (21,31). Taken together, these findings support the notion that Bv PrP is a universal acceptor for a wide range of prion strains (22,31) irrespective of the host factors or expression levels. On the other hand, the VV2 subtype of sporadic CJD was not transmissible to Ki-Bv(109M) mice in the present study. The lack of susceptibility to the VV2 subtype or MV2 subtype, collectively designated as the V2 CJD strain (3), has also been reported in bank voles (21). Although the reason for the relative resistance of Bv PrP against infection with the V2 CJD strain needs to be addressed in the future, it is noteworthy that Ki-Bv(109M) mice were susceptible to infection with a CJD isolate from a patient with an acquired form of CJD caused by transmission of the V2 CJD strain to a 129M/M individual (acquired CJD MMiK (9,18)). Adaptation of the V2 CJD strain to human PrP with M129 might facilitate the PrP^{res} propagation using Bv PrP, which also has M129.

In conclusion, Bv PrP has a high propensity for conformational conversion both in spontaneous disease and in prion infection, probably due to the characteristic C-terminal structure. The detailed mechanisms of the induction of the spontaneous conversion by the

C-terminal structure, particularly by the interactions between neighboring PrP molecules using the C-terminal interface, need to be clarified in the future.

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DECLARATIONS OF INTEREST

There is no conflict of interest.

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FIGURE LEGENDS

Figure 1. Comparison of PrP amino acid sequences. Bv PrP contains three α-helices spanning residues 144–156, 172–194, and 200–225, and a two-stranded antiparallel β-sheet of residues 128–131 and 161–164 (30). Eight amino acids differ between mature Bv PrP and mouse PrP, but six (blue) out of eight are common between Bv PrP and hamster PrP. Only two C-terminal residues (E227 and S230; red) are specific to Bv PrP. Bv PrP shows M109I polymorphism at residue 109.

Figure 2. Spontaneous illness of Tg-Bv(109M) mice. (**a**) Expression levels of PrP in clinically healthy Tg-Bv(109M) mice. Brain sample, equivalent to 50 μg in wet weight, was loaded in each lane and immunoblotted with the SAF32 antibody. (**b**) Neuropathology of the spontaneously diseased Tg-Bv(109M) mice. The affected mice showed spongiform changes, granular PrP deposition, and GFAP-positive astrogliosis in the cerebral cortex (Cx) and thalamus (Th). Age-matched unaffected Tg-Bv(109M) $D920^{+/+}$ mice did not show these neuropathological changes. Scale bars: 50 μm in HE-stained section and immunohistochemistry for PrP, or 100 μm in immunohistochemistry for GFAP. (**c**) Western blot analysis of proteinase K (PK)-resistant PrP^{res} in the brain of Tg-Bv(109M) mice using the SAF83 or SAF32 antibody. Brain homogenates were digested with 20 μg/ml or 0.5 μg/ml of PK.

Figure 3. Spontaneous illness of Tg-Mo/Bv mice. (**a**) Expression levels of PrP in clinically healthy Tg-Mo/Bv mice. A brain sample, equivalent to 50 μg in wet weight, was loaded in each lane and immunoblotted with the SAF32 antibody. (**b**) Neuropathology of the spontaneously diseased Tg-Mo/Bv mice. The affected mice showed spongiform changes, granular PrP deposition, and GFAP-positive astrogliosis in the cerebral cortex (Cx) and thalamus (Th).

Age-matched unaffected Tg-Mo/Bv J643$^{+/+}$ mice did not show these neuropathological changes. Scale bars: 50 μm in HE-stained section and immunohistochemistry for PrP, or 100 μm in immunohistochemistry for GFAP. (**c**) Western blot analysis of proteinase K (PK)-resistant PrP^{res} in the brain of Tg-Mo/Bv mice using the SAF83 or SAF32 antibody. Brain homogenates were digested with 20 μg/ml or 0.5 μg/ml of PK.

Figure 4. General susceptibility of Ki-Bv(109M) to a broad range of prion strains. (**a**) Expression levels of PrP in Ki-Bv(109M) mice. A brain sample, equivalent to 50 μg in wet weight, was loaded in each lane and immunoblotted with the SAF32 antibody. (**b**) Western blot analysis of proteinase K (PK)-resistant PrP^{res} in the brain of Ki-Bv(109M) mice inoculated with diverse prion strains using the SAF83 antibody. Brain homogenates were digested with 20 μg/ml of PK.

Table 1. Spontaneous illness of Tg-Bv(109M) mice.

Mouse line	PrP expression level (times [a])	Mean age at onset (days ± SEM)	Attack rate (n/n^0 [b])
Tg-Bv(109M) B495+/+	4-6	450 ± 26	4/4
Tg-Bv(109M) D920+/+	8	238 ± 4	12/15
Tg-Bv(109M) B495-/-	4	488 ± 19	19/20

[a] Comparison with the level of wild-type mice.

[b] n, number of mice positive for PrP accumulation in the brain in the immunohistochemical analysis; n^0, number of examined mice.

Table 2. Transmission of spontaneously generated PrP^{res} from Tg-Bv(109M) mice to wild-type mice.

Inoculum	Mouse	Incubation period (days)	Attack rate (n/n^{0} [a])
Tg-Bv(109M) B495+/+	NZW	> 831	0/5
	C57BL/6	> 855	0/5
Tg-Bv(109M) B495-/-	NZW	> 855	0/5
	C57BL/6	> 831	0/2

[a] n, number of mice positive for PrP accumulation in the brain in the immunohistochemical analysis; n^{0}, number of inoculated mice.

Table 3. Spontaneous illness of Tg-Mo/Bv mice.

Mouse line	PrP expression level (times [a])	Mean age at onset (days ± SEM)	Attack rate (n/n^{0} [b])
Tg-Mo/Bv J642+/+	2-4	960	1/14
Tg-Mo/Bv J643+/+	4	689 ± 47	7/17

[a] Comparison with the level of wild-type mice.

[b] n, number of mice positive for PrP accumulation in the brain in the immunohistochemical analysis; n^{0}, number of examined mice.

Table 4. Transmission of diverse prion strains to Ki-Bv(109M) mice.

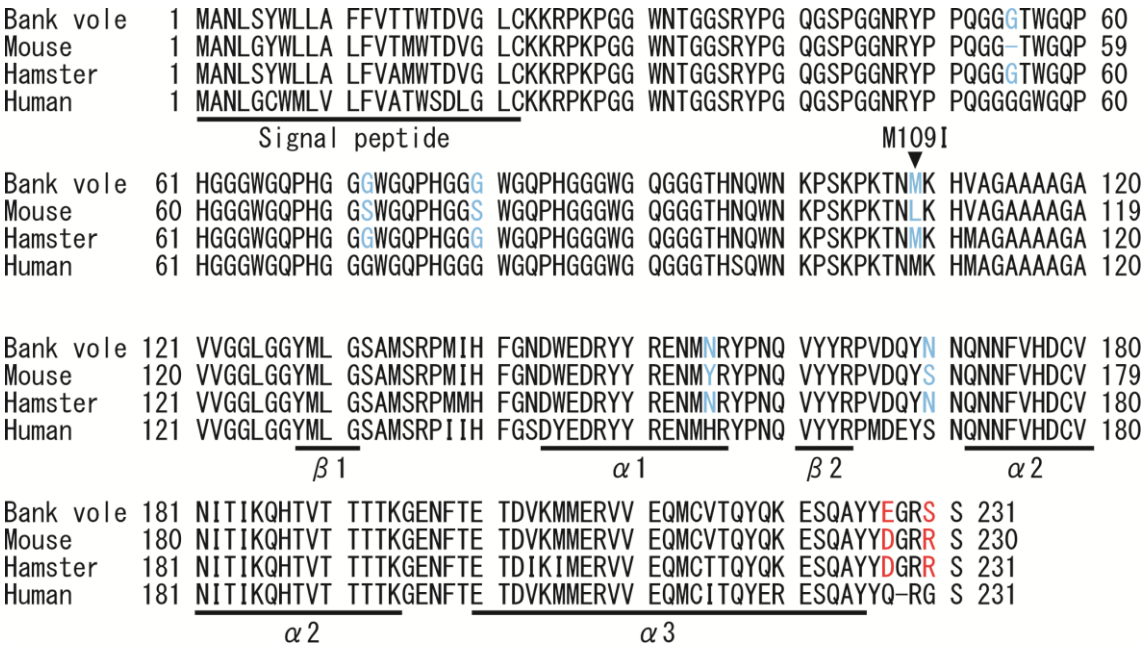
Inoculum		Mean incubation period (days ± SEM)	Attack rate (n/n^0 [a])
Sporadic CJD	MM1 [b]	296 ± 11	3/3
	MM2C	508 ± 58	5/5
	VV2	> 657	0/4
Acquired CJD	MMiK [c]	416 ± 20	5/6
Variant CJD		489 ± 27	4/4
Mouse-adapted Gerstmann-Sträussler-Scheinker syndrome	Fukuoka-1 [d]	334 ± 17	6/6
Spontaneously ill Tg-Bv(109M) B495+/+		> 832	0/10
Spontaneously ill Tg-Bv(109M) B495-/-	#1	> 791	0/4
	#2	> 791	0/6

[a] n, number of mice positive for PrP accumulation in the brain in the immunohistochemical analysis; n^0, number of inoculated mice.

[b] According to the classification of sporadic CJD by Parchi *et al.* (23).

[c] Acquired CJD caused by transmission of the V2 CJD strain (VV2 or MV2) to a 129M/M individual. Details of the clinicopathological and biochemical features of the patient have been reported elsewhere (9,18).

[d] Tateishi *et al.* (29).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Mouse 1 MANLGYWLLA LFVTMWTDVG LCKKRPKPGG WNTGGSRYPG QGSPGGNRYP PQGG-TWGQP 59
Hamster 1 MANLSYWLLA LFVAMWTDVG LCKKRPKPGG WNTGGSRYPG QGSPGGNRYP PQGGGTWGQP 60
Human 1 MANLGCWMLV LFVATWSDLG LCKKRPKPGG WNTGGSRYPG QGSPGGNRYP PQGGGGWGQP 60
Signal peptide
M109I
Bank vole 61 HGGGWGQPHG GGWGQPHGGG WGQPHGGGWG QGGGTHNQWN KPSKPKTNMK HVAGAAAAGA 120
Mouse 60 HGGGWGQPHG GSWGQPHGGS WGQPHGGGWG QGGGTHNQWN KPSKPKTNLK HVAGAAAAGA 119
Hamster 61 HGGGWGQPHG GGWGQPHGGG WGQPHGGGWG QGGGTHNQWN KPSKPKTNMK HMAGAAAAGA 120
Human 61 HGGGWGQPHG GGWGQPHGGG WGQPHGGGWG QGGGTHSQWN KPSKPKTNMK HMAGAAAAGA 120
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Hamster 121 VVGGLGGYML GSAMSRPMMH FGNDWEDRYY RENMNRYPNQ VYYRPVDQYN NQNNFVHDCV 180
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β1
α1
β2
α2
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Mouse 180 NITIKQHTVT TTTKGENFTE TDVKMMERVV EQMCVTQYQK ESQAYYDGRR S 230
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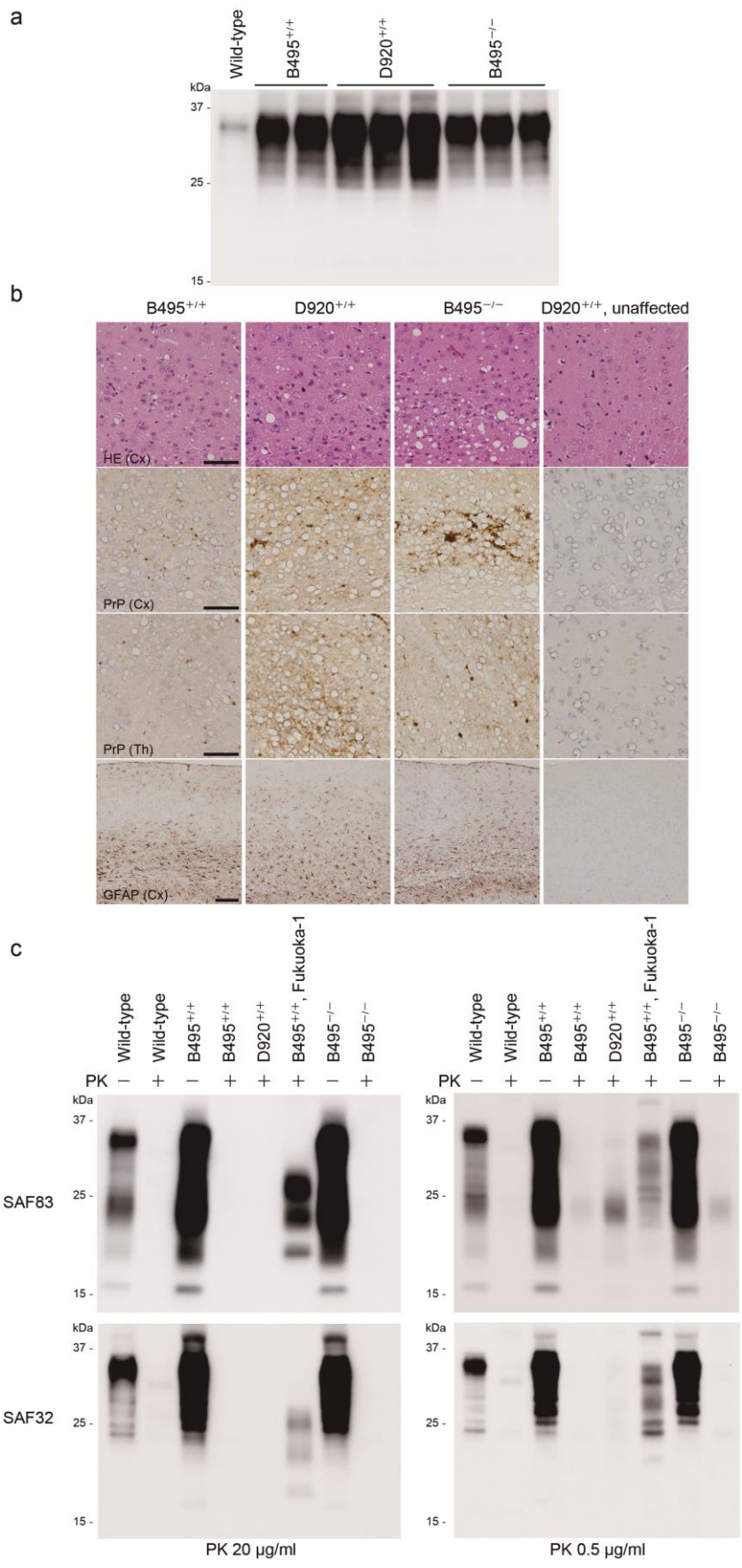
α2
α3

Figure 1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D920+/+
B495−/−
kDa
37 -
25 -
15 -
b
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D920+/+, unaffected
HE (Cx)
PrP (Cx)
PrP (Th)
GFAP (Cx)
c
Wild-type
Wild-type
B495+/+
B495+/+
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B495−/−
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SAF32
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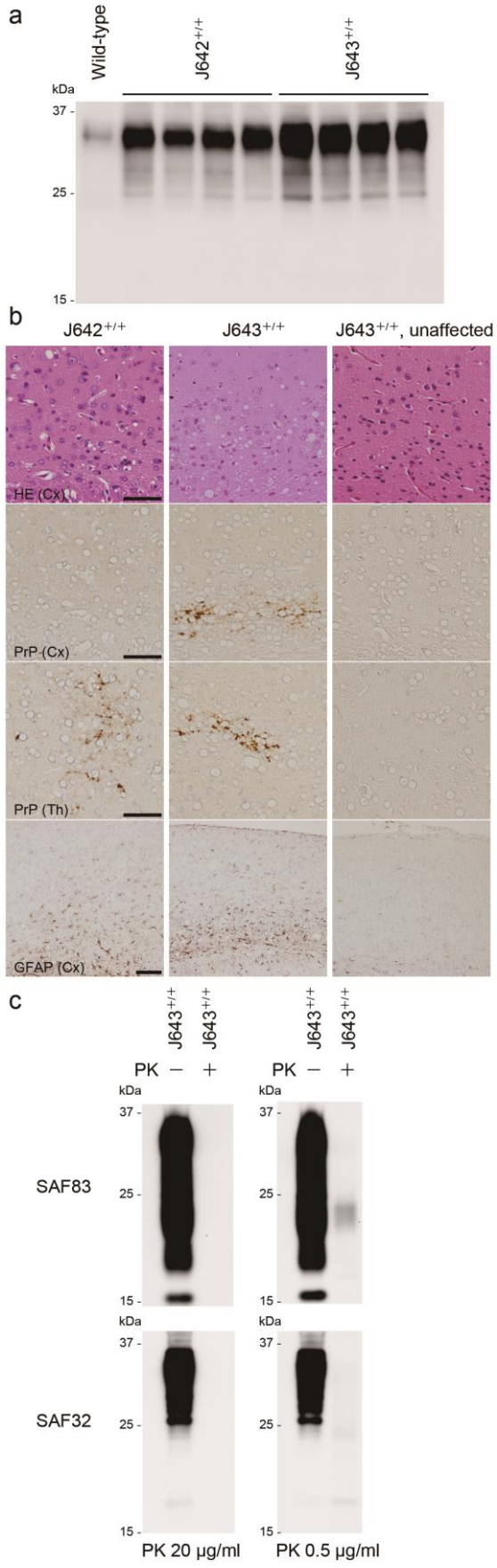
PK 0.5 µg/ml

Figure 2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kDa
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25 -
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b
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PrP (Cx)
PrP (Th)
GFAP (Cx)
c
J643+/+
J643+/+
J643+/+
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kDa
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25 -
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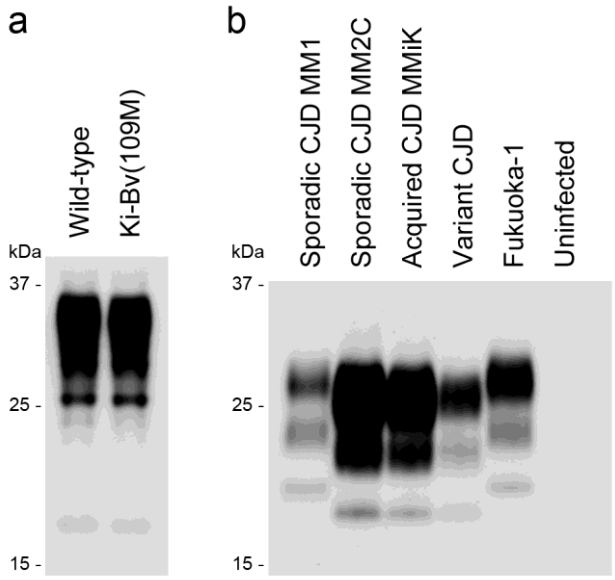
SAF83
SAF32
PK 20 µg/ml
PK 0.5 µg/ml

Figure 3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Wild-type
Ki-Bv(109M)
kDa
37 -
25 -
15 -
b
Sporadic CJD MM1
Sporadic CJD MM2C
Acquired CJD MMiK
Variant CJD
Fukuoka-1
Uninfected
kDa
37 -
25 -
15 -

Figure 4