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Human Prion Protein (PrP) 219K Is Converted to PrP^{Sc} but Shows Heterozygous Inhibition in Variant Creutzfeldt-Jakob Disease Infection*[S]

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Prion protein gene (*PRNP*) E219K is a human polymorphism commonly occurring in Asian populations but is rarely found in patients with sporadic Creutzfeldt-Jakob disease (CJD). Thus the polymorphism E219K has been considered protective against sporadic CJD. The corresponding mouse prion protein (PrP) polymorphism variant (mouse PrP 218K) is not converted to the abnormal isoform (PrP^{Sc}) and shows a dominant negative effect on wild-type PrP conversion. To define the conversion activity of this human molecule, we herein established knock-in mice with human PrP 219K and performed a series of transmission experiments with human prions. Surprisingly, the human PrP 219K molecule was converted to PrP^{Sc} in variant CJD infection, and the conversion occurred more efficiently than PrP 219E molecule. Notably the knock-in mice with *PRNP* codon 219E/K showed the least efficient conversion compared with their hemizygotes with *PRNP* codon 219E/0 or codon 219K/0, or homozygotes with *PRNP* codon 219E/E or codon 219K/K. This phenomenon indicated heterozygous inhibition. This heterozygous inhibition was observed also in knock-in mice with *PRNP* codon 129M/V genotype. In addition to variant CJD infection, the human PrP 219K molecule is conversion-competent in transmission experiments with sporadic CJD prions. Therefore, the protective effect of *PRNP* E219K against sporadic CJD might be due to heterozygous inhibition.

Human prion diseases have been classified into infectious, inherited, and sporadic forms. Infectious human prion disease was demonstrated first in Kuru (1) and recently in Creutzfeldt-Jakob disease (CJD)[3] with dura mater-grafted CJD, pituitary hormone-associated CJD, and variant CJD (vCJD) (2, 3). Familial CJD, Gerstmann-Straeussler syndrome, and fatal familial insomnia are human inherited prion diseases (4). Sporadic CJD (sCJD) is of unknown etiology. These prion diseases are caused by the accumulation of an abnormal isoform (PrP^{Sc}) of prion protein (PrP), which is converted from the normal cellular isoform (PrP^{C}) (5). The human PrP contains 253 amino acids encoded by prion protein gene (*PRNP*), which is located on chromosome 20. Numerous point mutations or insertional mutations in the open reading frame of *PRNP* have been reported in inherited prion diseases. In addition, normal polymorphisms of *PRNP* appear to influence the susceptibility to sporadic or infectious prion diseases. Homozygosity at the polymorphic *PRNP* codon 129 (methionine or valine) may cause a predisposition to sporadic or iatrogenic CJD in Europeans (6, 7). All cases of vCJD are homozygous for methionine at *PRNP* codon 129 (129M/M) (8).

In 1994, we reported that glutamate to lysine substitution at codon 219 is a polymorphism occurring in the Japanese population (9). This is a common polymorphism (allele frequency; 6%), which was later found also in other populations in the East Asia, the South Asian subcontinent, and the Pacific region, but has not been reported in Europeans (10–12). It has been reported that the *PRNP* genotype at codon 219 influences the clinicopathological features of Gerstmann-Straeussler syndrome with a codon 102 mutation (13), and that the codon 219K genotype appears to have a protective effect for sCJD (14). In addition, the codon 218K variant (corresponding to the human 219K) in the murine prion protein gene (*prnp*) was not converted to PrP^{Sc} and also showed a dominant negative effect on wild-type PrP conversion both in scrapie-infected neuroblastoma cells (15) and in transgenic mice (16). This dominant negative effect of the mouse PrP 218K variant was proposed at first to be mediated by protein X (15) but was also observed in *in vitro* fibril formation without protein X (17). In contrast to the murine *prnp* 218K variant, the human PrP 219K molecule did

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S4.

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[3] The abbreviations used are: CJD, Creutzfeldt-Jakob disease; vCJD, variant CJD; sCJD, sporadic CJD; PrP, prion protein; PrP^{C}, normal cellular isoform of PrP; PrP^{Sc}, abnormal isoform of PrP; FDC, follicular dendritic cell; *PRNP*, human prion protein gene; *prnp*, murine prion protein gene.

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not prevent the onset of dura mater-grafted CJD (14) or familial CJD (18). Therefore, it remains unclear whether the human PrP 219K molecule is conversion-competent or not.

In the present study, we newly established knock-in mice expressing the human PrP 219K molecule (Ki-Hu219K/K) and compared the conversion activity with other human PrP polymorphic molecules (19). We found that human PrP 219K is readily converted to PrP^{Sc} in vCJD infection, and also report the inhibition of PrP conversion in the heterozygous knock-in models.

EXPERIMENTAL PROCEDURES

Production of Humanized Knock-in Mouse with Homozygous, Heterozygous, or Hemizygous Genetic Background—Knock-in mice and transgenic mice were generated as reported previously (20). The open reading frame was replaced with human PrP gene with lysine at codon 219 (Fig. 1*A*). The 5′-primer was designed to incorporate a SmaI site. The PCR fragment was ligated to the mouse sequence using the SmaI site. Consequently, after processing of the N-terminal signal peptide during post-translational modification, the resulting molecule was identical with human PrP. The knock-in mice with human PrP 129M or 129V were already established (19, 21). We produced knock-in mouse crossed with the PrP knock-out mouse (20) to provide the hemizygous genetic background in the present study.

Sources of Prion Inocula and Transmission Experiment—Human brain tissues were obtained at autopsy from CJD patients after receiving informed consent for research use. Brain homogenate was prepared from four patients with vCJD (96/02, 96/07, 96/45, or 05/02), or two cases with sCJD (MM1 and MV1). The open reading frame of *PRNP* was analyzed by PCR direct sequencing (22). Human brain homogenates (10%) were prepared as described previously (23). Transmission studies were performed using 20 μl of the homogenates for intracerebral inoculation or 50 μl for intraperitoneal inoculation. Mice were sacrificed at 75 days post-inoculation for follicular dendritic cell (FDC) bioassay. Our previous study showed that the level of PrP^{Sc} accumulated in the spleen of knock-in mouse expressing chimeric human/mouse PrP with 129 M/M reached a plateau at 45 days post-inoculation (20). Thus, we decided to perform FDC assay at 75 days post-inoculation (19). Half of the spleen was immediately frozen for Western blotting, and the remaining half was fixed in 10% buffered formalin for the immunohistochemistry. Intracerebrally inoculated mice were sacrificed after the onset of the disease or examined when postmortem. One hemisphere of the brain was immediately frozen for Western blotting, and the other hemisphere was fixed in formalin for the immunohistochemistry.

Immunohistochemistry—Mouse tissues were fixed with 10% buffered formalin, and treated with 60% formic acid before embedding in paraffin. Tissue sections were processed for PrP immunohistochemistry using hydrolytic autoclaving pretreatment (24). The PrP-N antiserum (25) or ChW antiserum (19) were used as the primary antibody. A goat anti-rabbit immunoglobulin polyclonal antibody labeled with a peroxidase-conjugated dextran polymer, EnVision (DakoCytomation, Denmark), was used as the secondary antibody.

Western Blotting—PrP^{Sc} was extracted from either spleen or brain with collagenase treatment as previously described (26) with modifications. PrP^{C} was measured in the membrane fractions of the brain isolated from the knock-in mice. For the quantitative analysis, samples (corresponding to 7.5 mg wet weight of spleen tissue for PrP^{Sc}, or 500 μg wet weight of brain tissue for PrP^{C}) were subjected to 13.5% SDS-PAGE and transferred to polyvinylidene difluoride membrane. ChW antiserum or 3F4 antibody was used as the primary antibody. Anti-rabbit or anti-mouse EnVision was used as the secondary antibody. Enhanced chemiluminescence detection (GE Healthcare) was used to visualize Western blots. The signal intensities of the Western blots were quantified with Quantity One software using an imaging device, Vasa Doc 5000 (Bio-Rad). Western blot analysis was repeated at least three times, and signal intensities were expressed as mean ± S.E.

To check the protease resistance in each PrP polymorphism, we did the following experiment with the infected or uninfected knock-in mouse brains. Brain tissues of the Ki-Hu219K/K or Ki-Hu219E/E infected with MM1 prions or the uninfected control brain tissues were homogenized with 10 times the amount of the buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, and protease inhibitors (Complete, Roche Applied Science). The homogenates were centrifuged at 1000 × *g* for 10 min to discard the nuclear fraction. The supernatants were homogenized again with a glass homogenizer adding the Sarkosyl solution at a final concentration of 2%. The homogenate was centrifuged again at 2000 × *g* for 10 min. The final supernatant was digested at 37°C for 60 min with Proteinase K at concentrations of 0, 1, 3, 10, 30, 100, and 300 μg/ml. The digested samples were added with the equal volume of Laemmli sample buffer and boiled for 15 min for the Western blot analysis.

Statistical Analysis—Incubation times and the signal intensities of Western blots are expressed as mean ± S.E.

RESULTS

Knock-in Mouse as a Model to Provide the Physiological Expression Level of Recombinant PrP—In comparison with transgenic technology, knock-in mice produced by the homologous recombination technique have the advantage of providing a constant expression level (20, 27). Therefore, as reported previously (19), the expression level of PrP^{C} in the spleens of the heterozygous knock-in mice (Ki-Hu129M/V) was the same as that of the homozygous knock-in mice (Ki-Hu129M/M or Ki-Hu129V/V). It is impossible to establish a heterozygous animal model without the homologous recombination technique. However, a transgenic model with the *PRNP* 129M/V genotype was reported previously, but this model (Tg45/152) showed uneven expression (M:V = 1:1.5) and overexpression of the gene (4–6 fold) (28). In addition to the advantage described above, here we present that the expression levels of PrP^{C} in the brains of the hemizygous knock-in mice (Ki-Hu129M/0 or Ki-Hu129V/0) showed almost half the intensities of those seen in the homozygous mice (Ki-Hu129M/M or Ki-Hu129V/V) (Fig. 1, *B* and *C*). Thus, in this study, we can analyze two different expression levels of recombinant PrP: 1 copy of gene expression in the hemizygous knock-in mice and 2 copies of

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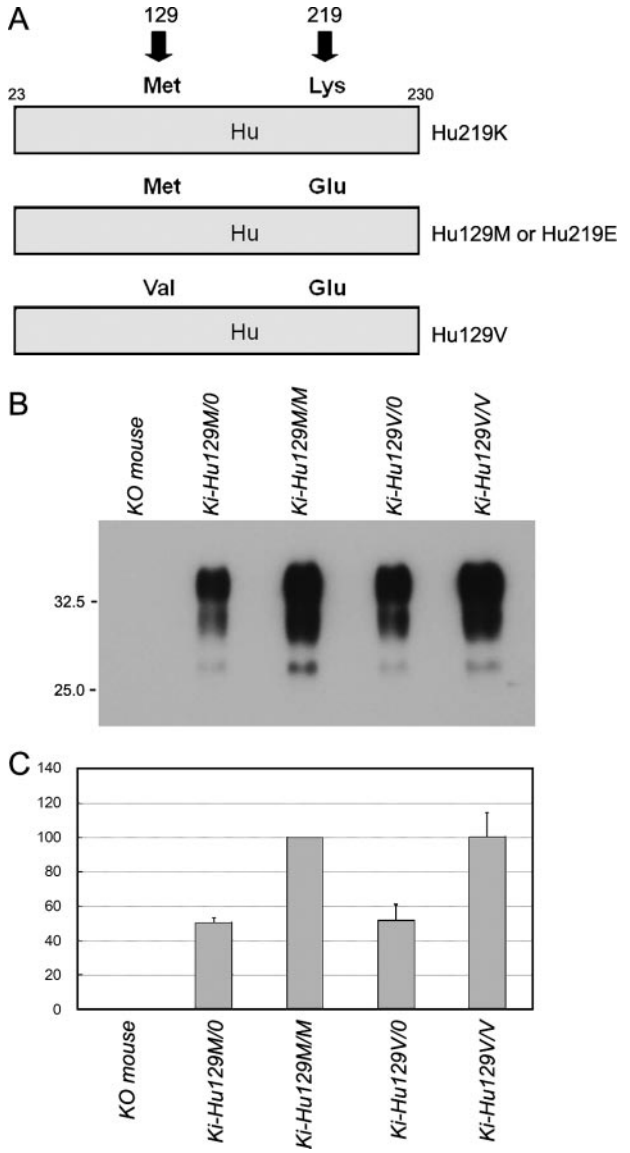

FIGURE 1. **Characterization of the knock-in mice.** *A*, the open reading frames of the knock-in vectors. The Hu219K vector encodes methionine at codon 129 and lysine at codon 219. The Hu129M (a synonym for Hu219E) and the Hu129V vectors were reported previously (19). *B*, Western blot analysis of the membrane fraction of the brains from the knock-out mouse, the hemizygous knock-in mice, and the homozygous knock-in mice. The position of molecular size standards is shown on the *left* (kilodaltons). *C*, the comparatively corrected signal intensities (numbers/mm^2) are presented. We assigned a signal intensity of 100/mm^2 for Ki-Hu129M/M. The corrected signal intensities are as follows. Ki-Hu129M/0, 50.7 ± 2.7; Ki-Hu129V/0, 51.8 ± 9.3; and Ki-Hu129V/V, 100.5 ± 13.9/mm^2 (mean ± S.E.).

gene expression in the homozygous or heterozygous knock-in mice.

Human PrP 219K Molecule Is Conversion-competent in vCJD Infection—At first, we examined the transmission experiment with vCJD prions, since a case of vCJD patient was reported in Japan (29). When considering the possibility of secondary infection from human to human transmission in Japan, it is important to know whether the Japanese populations with the codon 219E/E, E/K or K/K genotype are susceptible to vCJD prions. In secondary vCJD infection, a direct intracerebral route of exposure is only likely to occur during neurosurgical procedures, whereas a peripheral route of infection via blood transfusion, tissue transplantation or general surgery is far more likely. Thus, we analyzed the susceptibility to vCJD prions using the FDC assay after the peripheral route of infection in a murine model.

It was surprising that positive PrP immunolabeling was observed in the FDC of the spleens from Ki-Hu219K/K intraperitoneally inoculated with vCJD prions (Fig. 2*A*). To compare the conversion efficiency between PrP 219E and PrP 219K, we prepared knock-in mice with the following genotypes: Ki-Hu219E/K, Ki-Hu219E/0, Ki-Hu219K/0, Ki-Hu219E/E, and Ki-Hu219K/K. These knock-in mice were inoculated with vCJD prions from 2 different patients and were sacrificed at 75 days post-inoculation to analyze PrP^{Sc} in the FDC by immunohistochemistry or Western blotting. The immunohistochemical analysis showed positive FDC staining in almost all knock-in mice inoculated with the vCJD prions (supplemental Fig. S1*A*). Although the vCJD infection was established in almost all mice irrespective of the codon 219 genotype, the numbers of positively stained FDCs differed in each knock-in mouse (Fig. 2*A*). Therefore, we counted the total number of lymphoid follicles and the number of positively stained FDCs in all mice. The positive rate in the FDC assay was lowest in Ki-Hu219E/K, followed by Ki-Hu219E/0, Ki-Hu219E/E and Ki-Hu219K/0, and was highest in Ki-Hu219K/K (Fig. 2*B*). Western blot analysis showed that the quantity of PrP^{Sc} was highest in the spleens of Ki-Hu219K/K, followed by Ki-Hu219K/0, Ki-Hu219E/E and Ki-Hu219E/0, and was lowest in Ki-Hu219E/K (Fig. 2, C and D). Despite the different genotype in the knock-in mice (219K/K) and vCJD prions (219E/E), the most effective conversion was observed in Ki-Hu219K/K mice. Even the hemizygous Ki-Hu219K/0 showed more PrP^{Sc} accumulation than did Ki-Hu219E/E. This observation in Western blot was reproducible in a transmission experiment using another vCJD inoculum (vCJD96/07) (supplemental Fig. S1*B*). In both transmission experiments, the heterozygous model showed the lowest efficiency of conversion.

Heterozygous Inhibition Is Also Observed in the PRNP Codon 129M/V Genotype—As reported previously (19), Ki-Hu129M/M and Ki-Hu129M/V mice were susceptible to vCJD prions as revealed by FDC assay, but Ki-Hu129V/V mice were not. Because the human PrP 129V molecule is conversion-incompetent in vCJD infection, it was the best model to compare the amount of PrP^{Sc} between Ki-Hu129M/V and Ki-Hu129M/0 to examine the influence of heterozygosity. We prepared knock-in mice with the following genotypes: Ki-Hu129M/V, Ki-Hu129M/0, Ki-Hu129M/M (a synonym for Ki-Hu219E/E), and Ki-Hu129V/V. All of these knock-in mice had Glu at codon 219 of *PRNP* (Fig. 1*A*). These mice were inoculated intraperitoneally with vCJD prions (vCJD05/02). In Western blot analysis, Ki-Hu129M/M and Ki-Hu129M/V had PrP^{Sc} in the spleen, but Ki-Hu129V/V did not (Fig. 3, *A* and *B*). However, the amount of PrP^{Sc} in the spleens of Ki-Hu129M/V was consistently less than that in Ki-Hu129M/0. This observation in Western blot was reproducible in a transmission experiment using another vCJD inoculum (vCJD96/07) (supplemental Fig. S2). Therefore, the conversion-incompetent human PrP 129V molecule also appears to show an inhibitory effect on the accumulation of human PrP 129M PrP^{Sc}.

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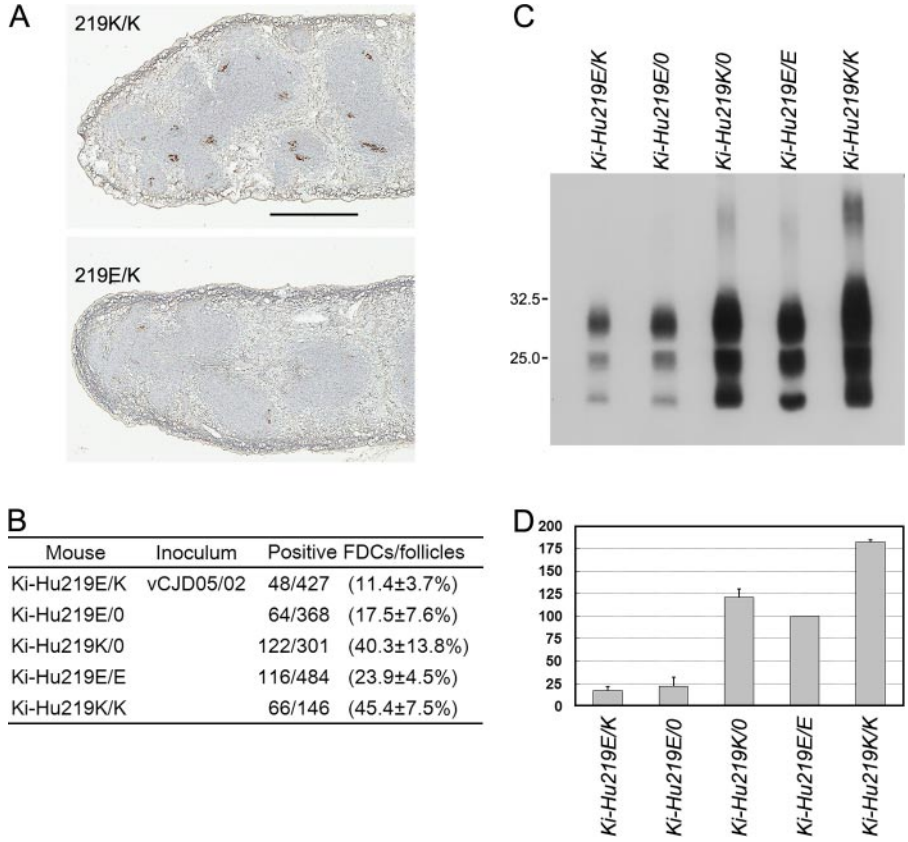

B

Mouse	Inoculum	Positive FDCs/follicles	
Ki-Hu219E/K	vCJD05/02	48/427	(11.4±3.7%)
Ki-Hu219E/0		64/368	(17.5±7.6%)
Ki-Hu219K/0		122/301	(40.3±13.8%)
Ki-Hu219E/E		116/484	(23.9±4.5%)
Ki-Hu219K/K		66/146	(45.4±7.5%)

FIGURE 2. **Transmission experiment using the knock-in mice with codon 219 polymorphism.** *A*, immunohistochemistry analysis of the spleen from Ki-Hu219K/K or from Ki-Hu219E/K. The follicular dendritic cells were positively immunolabeled with PrP antibody. *Scale bar*, 500 μm. *B*, summary of the immunohistochemical analysis. We used the hemizygous models (Ki-Hu219E/0 and Ki-Hu219K/0), the homozygous models (Ki-Hu219E/E and Ki-Hu219K/K), and the heterozygous model (Ki-Hu219E/K) intraperitoneally inoculated with vCJD05/02. We counted the total number of positive FDCs and the total number of lymphoid follicles in the spleens from all mice. The positive rate of FDCs for each animal is expressed as mean % value ± S.D. *C*, Western blot analysis of PrP^{Sc} from the knock-in mice inoculated with vCJD05/02. The position of molecular size standards is shown on the *left* (kilodaltons). *D*, the comparatively corrected signal intensities (numbers/mm^2) are presented. We assigned a signal intensity of 100/mm^2 for Ki-Hu219E/E. The corrected signal intensities are as follows. Ki-Hu219E/K, 17.6 ± 3.9; Ki-Hu219E/0, 21.9 ± 10.1; Ki-Hu219K/0, 121.0 ± 8.4; and Ki-Hu219K/K, 182.1 ± 2.1/mm^2 (mean ± S.E.).

Transmission Studies via the Intracerebral Administration—It was established that the human PrP 219K molecule is converted in vCJD infection, but it remains uncertain as to whether this molecule could be converted by infection with other prions. To examine the transmissibility of other prions, we performed intracerebral inoculation of 10% brain homogenates from a patient with sCJD. In the transmission experiment using sCJD prions, all Ki-Hu219K/K showed PrP^{Sc} accumulation in the brain. Compared with Ki-Hu219E/E (467 ± 24 days), Ki-Hu219K/K showed a longer incubation period (573 ± 103 days) after inoculation with sCJD-MM1 prions (129M/M, 219E/E, and type 1 PrP^{Sc}). It is significant that human PrP 219K could be converted by sCJD-MM1 or MV1 prions (129M/V, 219E/E, and type 1 PrP^{Sc}) (Fig. 4*A*). Western blot analysis of the brains from Ki-Hu219K/K showed a similar PrP^{Sc} isoform on blot to that seen in Ki-Hu219E/E (Fig. 4*B*). Thus the human PrP 219K is conversion-competent also in sCJD prion infection.

We also summarize the transmission data of vCJD prions with the intracerebral inoculation (Fig. 4*A*). In vCJD (vCJD05/02) infection, Ki-Hu219K/K mice showed PrP^{Sc} accumulation in the brain after the incubation period of 412 ± 6 days. Ki-Hu129M/M (the same mouse as Ki-Hu219E/E) showed a longer incubation period compared with Ki-Hu219K/K. In addition to these homozygous models, the Ki-Hu129M/V heterozygous mice show no clinical signs and still are alive after >740 days of incubation (Fig. 4*A*). Therefore, heterozygous inhibition was similarly observed as in intracerebral infections.

DISCUSSION

It was not expected that the Hu219K PrP molecule was converted to the abnormal isoform. Therefore, it should be important to check the protease sensitivity in the normal isoforms and abnormal isoforms of Hu219K and Hu219E. At first, we checked the protease sensitivity of the uninfected or infected brain samples with MM1 prion (supplemental Fig. S3). The pattern in the protease sensitivity of PrP^{C} and the protease resistance of PrP^{Sc} was almost the same in Ki-Hu219E/E and Ki-Hu219K/K. In addition to this result, the positive rate in the immunohistochemical analysis of the FDC assay infected with vCJD prions correlated to the quantitative data in the Western blot (Fig. 2 and supplemental Fig. S4). In addition, Ki-Hu219K/K mice showed a shorter incubation period compared with Ki-Hu129M/M (Ki-Hu219E/E) in the central nervous system infection. Therefore, it was concluded that the Hu219K molecule is readily converted in vCJD prions compared with Hu219E. From our data, the Hu219K molecule might be more suitable as a substrate to amplify vCJD prions by the PMCA technique (30) compared with Hu219E (31). Although many transmission studies have shown the importance of homology at the polymorphic codons between PrP^{C} and PrP^{Sc} for efficient conversion (32), vCJD prions represent an exception as far as *PRNP* codon 219 is concerned. This exception can be explained by the fact that bovine PrP has a amino acid substitution of glutamine corresponding to codon 219. The structure of PrP^{Sc} with Hu219K might have a similar structure of BSE PrP^{Sc} with 219Q compared with that of Hu219E. Based on the codon 219 substitution, we can design a better PrP^{C} substrate to amplify vCJD prions.

Despite the conversion competence of the Hu219K and Hu219E PrP molecules, heterozygous inhibition was observed in Ki-Hu219E/K as well as Ki-Hu129M/V mice with conversion-competent 129M and conversion-incompetent 129V molecules. In addition to the peripheral route infection in the FDC assay, Ki-Hu129M/V mice showed heterozygous inhibition of

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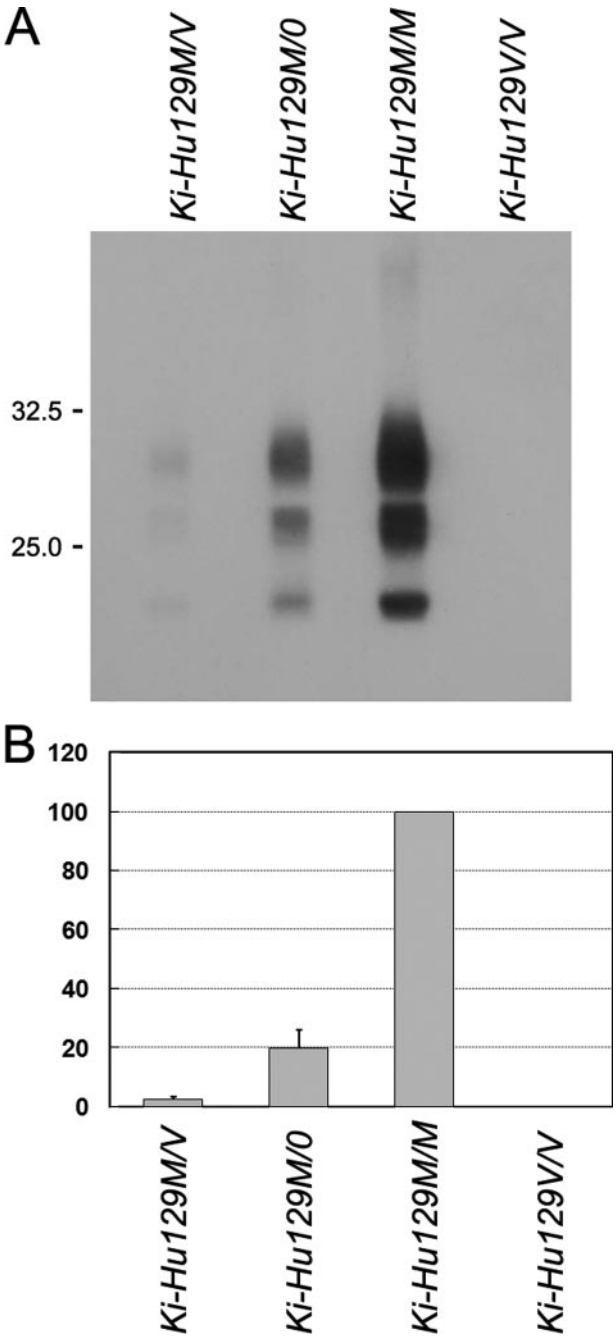

FIGURE 3. **Transmission experiment using the knock-in mice with the codon 129 polymorphism.** *A*, Western blot analysis of PrP^{Sc} from the knock-in mice with the 129 polymorphism. The hemizygous model (Ki-Hu129M/0), the homozygous models (Ki-Hu129M/M or Ki-Hu129V/V), or the heterozygous model (Ki-Hu129M/V) were intraperitoneally inoculated with vCJD05/02. The position of molecular size standards is shown on the *left* (kilodaltons). *B*, the comparatively corrected signal intensities (numbers/mm^2) are presented. We assigned a signal intensity of 100/mm^2 for Ki-Hu129M/M. The corrected signal intensities are as follows. Ki-Hu129M/V, 2.6 ± 0.7; and Ki-Hu129M/0, 19.7 ± 6.3/mm^2 (mean ± S.E.).

A

Mouse	Inoculum	Incubation period
Ki-Hu129M/M	sCJD MM1(H3)	467±24 (8/8)
Ki-Hu219K/K	sCJD MM1(H3)	573±103 (5/5)
Ki-Hu219K/K	sCJD MV1(Su)	643±146 (5/5)
Ki-Hu219K/K	vCJD05/02	412± 6 (3/4)
Ki-Hu129M/M	vCJD05/02	607, 622, >700 (2/5)
Ki-Hu129M/V	vCJD05/02	>742 (0/5)

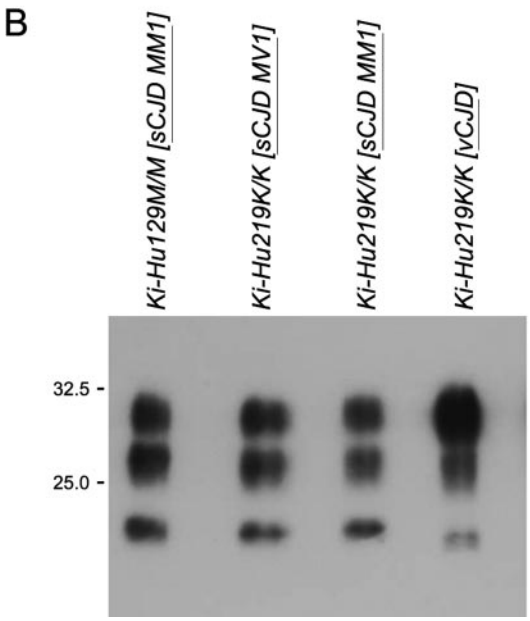

FIGURE 4. **Transmission experiment in Ki-Hu219K/K inoculated intracerebrally with sCJD prions.** *A*, summary of the transmission experiments. The MM1 inoculum is the same in both Ki-Hu219K/K and Ki-Hu129M/M. The vCJD inoculum is also the same in Ki-Hu219K/K, Ki-Hu129M/M, and Ki-Hu129M/V. The incubation period was expressed by days (number of positive transmissions/number of total animals). *B*, Western blot analysis of PrP^{Sc} in the brains. The knock-in mouse [*the inoculated sample*] is designated *above each lane*. The position of molecular size standards is shown on the *left* (kilodaltons).

vCJD prion infection in the central nervous system. Therefore, the heterozygous inhibition is a universal feature of prion infections both in peripheral infection and central nervous system infection.

The effect of *PRNP* polymorphisms have been studied recently in an *in vitro* model (17, 33), in which fibril formation revealed the β-oligomer state (34). It was suggested that the β-oligomer was not on the pathway to amyloid formation and that the refolding and dissociation of the β-oligomer into the α-monomer most likely preceded the fibril formation. The kinetics of dissociation of the β-oligomer was 100-fold slower in the 129M/V heterogenous β-oligomer than those in either the 129M or 129V homogenous β-oligomer (33). The inhibition of amyloid formation was also reported in a fibrillization model mixed with murine wild-type PrP with 218Q and murine PrP 218K molecule (17). In this system, the murine PrP 218K molecule was converted into a fibril, but the conversion efficiency was lowered. It was interesting that the murine PrP 218K molecules were incorporated into fibrils as often as the wild-type molecules. In another model (35), transgenic mice expressing conversion-incompetent PrP-Fc_2 showed a reduced conversion of wild-type PrP by dimeric PrP-Fc_2. Interestingly, the dimeric PrP-Fc_2 was also incorporated in protease-resistant fibrils.

Based on these previous findings, we propose a possible mechanism to explain the heterozygous inhibition. This mechanism is entirely based on the distinct structure of each PrP^{Sc} molecule (36, 37) (Fig. 5). At first, PrP^{C} is converted to PrP^{Sc},

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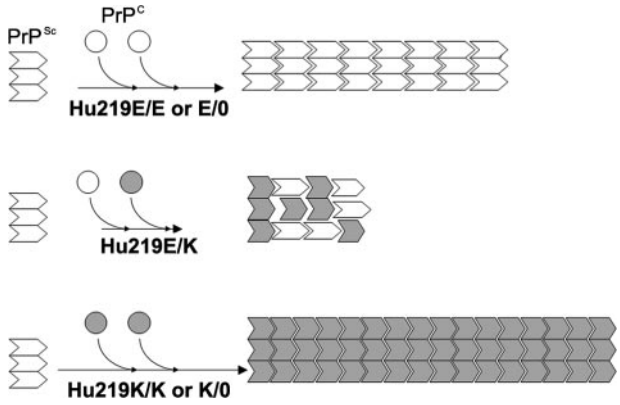

FIGURE 5. **A possible mechanism underlying the heterozygous inhibition in vCJD infection.** *Upper*, PrP^{Sc} proliferation model in Ki-Hu219E/E or Ki-Hu219E/0. There is only one PrP^{Sc}. The Hu219E PrP^{Sc} is piled up into the protease-resistant amyloid fibril. *Middle*, the heterozygous inhibition model. There are two distinct structural PrP^{Sc} in the same mouse. It takes time to pile up amyloid fibrils like a stone fence, because there are two distinct types of PrP^{Sc} blocks. Thus, PrP^{Sc} formation can be inhibited in the heterozygous animals. *Lower*, PrP^{Sc} proliferation model in Ki-Hu219K/K or Ki-Hu219K/0. The initial seed is Hu219E PrP^{Sc}. However, the resulting Hu219K PrP^{Sc} acts as a new seed, and the increasing Hu219K PrP^{Sc} is piled up rapidly into the protease-resistant amyloid fibrils. There was no or negligible Hu219E PrP^{Sc} as a decelerator. In this figure, each *circle* or *block* designates the following: *open circles*, Hu219E PrP^{C}; *open hexagonal blocks*, Hu219E PrP^{Sc}; *filled circles*, Hu219K PrP^{C}; and *filled hexagonal blocks*, Hu219K PrP^{Sc}.

then the converted PrP^{Sc} is piled up into amyloid fibrils according to the nucleated polymerization hypothesis (38). In the homozygous and hemizygous animals, there is only one structural PrP^{Sc}. This means that the same blocks (the same structural PrP^{Sc}) are piled up into amyloid fibrils with no other influence on fibril formation and elongation. However, in the heterozygous animals, there are at least two distinct structural PrP^{Sc} composed of the Hu219E or Hu219K molecule. To form and elongate amyloid fibrils in the heterozygous animal, it takes time to pile up amyloid fibrils, because there are two types of blocks (PrP^{Sc}) with a distinct structure (Fig. 5). The two different-shaped PrP^{Sc} may act as decelerators of each other. In the FDC assay of Ki-Hu129M/V, the amyloid fibril formation of Hu129M was inhibited by the Hu129V molecule, which was conversion-incompetent. This phenomenon corresponds to the dominant negative effect as reported previously. We can explain the dominant negative phenomenon by this decelerator hypothesis if the conversion-incompetent Hu129V molecules significantly reduce the rate of Hu129M amyloid formation and elongation.

The decelerator hypothesis may explain some unusual phenomena in prion infections. We propose that PrP^{Sc} molecules with different amino acid sequences act as decelerators in the process of amyloid formation. Because PrP molecules with different amino acid sequences are likely to have different conformations, we can infer that PrP^{Sc} molecules with different conformations act as decelerators in the process of amyloid formation. This decelerator hypothesis can explain the phenomenon described as interference (39), which is observed in an animal inoculated with two different prion strains. A distinct strain should have a distinct conformation of PrP^{Sc}. This PrP^{Sc} with a distinct conformation may inhibit another type of PrP^{Sc} amyloid formation. Recently, it has been reported that the prion interference is due to a reduction of the strain-specific PrP^{Sc} level (40). Our decelerator hypothesis can account for such prion interference.

In this report, we clearly show heterozygous inhibition of PrP^{Sc} formation using a knock-in mouse model of prion infection. *PRNP* heterozygosity may be important in determining resistance to human prion diseases (12). Although the incubation period after the intracerebral transmission of sCJD prions in Ki-Hu219E/K remains to be determined, the present study suggests that the absence of patients with the 219E/K *PRNP* genotype in sCJD might be due to heterozygous inhibition, because Hu219K is conversion-competent also in sCJD prions infection.

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