Cross-sequence Transmission of Sporadic Creutzfeldt-Jakob Disease Creates a New Prion Strain*

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Atsushi Kobayashi‡, Masahiro Asano§, Shirou Mohri¶, and Tetsuyuki Kitamoto∥

From the ‡Division of CJD Science and Technology, Department of Prion Research, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan and §Prion Disease Research Center, National Institute of Animal Health, Tsukuba, Ibaraki 305-0856, Japan

The genotype (methionine or valine) at polymorphic codon 129 of the human prion protein (PrP) gene and the type (type 1 or type 2) of abnormal isoform of PrP (PrPSc) are major determinants of the clinicopathological phenotypes of sporadic Creutzfeldt-Jakob disease (sCJD). Here we found that the transmission of sCJD prions from a patient with valine homozygosity (129V/V) and type 2 PrPSc (sCJD-VV2 prions) to mice expressing human PrP with methionine homozygosity (129M/M) generated unusual PrPSc intermediate in size between type 1 and type 2. The intermediate type PrPSc was seen in all examined dura mater graft-associated CJD cases with 129M/M and plaque-type PrP deposits (p-dCJD). p-dCJD prions and sCJD-VV2 prions exhibited similar transmissibility and neuropathology, and the identical type of PrPSc when inoculated into PrP-humanized mice with 129M/M or 129V/V. These findings suggest that p-dCJD could be caused by cross-sequence transmission of sCJD-VV2 prions.

Creutzfeldt-Jakob disease (CJD), kuru, scrapie, and bovine spongiform encephalopathy are lethal transmissible neurodegenerative diseases caused by an abnormal isoform of prion protein (PrPSc), which is converted from the normal cellular isoform (PrPC) (1). The genotype (methionine or valine) at polymorphic codon 129 of the human prion protein (PrP) gene and the type (type 1 or type 2) of PrPSc in the brain are major determinants of the clinicopathological phenotypes of sporadic CJD (sCJD) (2–5). Type 1 and type 2 PrPSc are distinguishable according to the size of the proteinase K-resistant core of PrPSc (PrPScK) (21 and 19 kDa, respectively), reflecting differences in the proteinase K cleavage site (at residues 82 and 97, respectively) (2, 3). The genotype at codon 129 also influences the susceptibility to variant CJD (vCJD), iatrogenic CJD, and kuru (6–11). A transmission study using transgenic mice expressing human PrP revealed that the congruency of the genotype at codon 129 between the inoculum and the inoculated transgenic mice determines the susceptibility to sCJD prions (12). Transmission of sCJD prions to mice with an incongruent genotype (referred to as cross-sequence transmission) results in a relatively long incubation period.

The potential for cross-sequence transmission should be considered in the iatrogenic transmission of CJD via cadaveric pituitary hormones, dura mater and corneal grafts, or contaminated neurosurgical instruments. More than half of the reported cases of dura mater graft-associated CJD (dCJD) occurred in Japan, where 123 cases have been recognized as of February 2006 (13–16). The dural grafts used in Japan were manufactured by German companies (13–15). In Europe, 28.4% of sCJD patients are valine homozygotes (129V/V) or methionine/valine heterozygotes at codon 129 (129M/V) (5). Meanwhile, the population data show a high prevalence (91.6%) of methionine homozygosity (129M/M) in Japanese people (17). These data raise the possibility that part of the Japanese dCJD cases might have been caused by cross-sequence transmission of sCJD prions. In fact, there are two distinct phenotypes in dCJD, with the majority represented by a non-plaque type of dCJD (np-dCJD) and the minority by a plaque-type dCJD (p-dCJD) (18–21). The clinicopathological features of np-dCJD are similar to those of sCJD with 129M/M and type 1 PrPSc (sCJD-MM1) (14). In contrast, p-dCJD cases show unique features characterized by (i) the absence or late occurrence of myoclonus and periodic synchronous discharges on electroencephalogram; (ii) a long incubation period after grafting and a clinical course of long duration, and (iii) plaque-type PrP deposits in the brain (18–25). Although we have classified p-dCJD cases into MM1, the clinicopathological features of p-dCJD are quite different from those of sCJD-MM1 or np-dCJD (18–21). The reason for the existence of two distinct phenotypes in dCJD has remained elusive.

To identify the origin of p-dCJD, we inoculated sCJD prions into mice expressing human PrP with either 129M/M or 129V/V. Here we report that cross-sequence transmission of sCJD prions from a patient with 129V/V and type 2 PrPSc manufactured by German companies (13–15) occurred in Japan, where 123 cases have been recognized as of February 2006 (13–16). The dural grafts used in Japan were manufactured by German companies (13–15). In Europe, 28.4% of sCJD patients are valine homozygotes (129V/V) or methionine/valine heterozygotes at codon 129 (129M/V) (5). Meanwhile, the population data show a high prevalence (91.6%) of methionine homozygosity (129M/M) in Japanese people (17). These data raise the possibility that part of the Japanese dCJD cases might have been caused by cross-sequence transmission of sCJD prions. In fact, there are two distinct phenotypes in dCJD, with the majority represented by a non-plaque type of dCJD (np-dCJD) and the minority by a plaque-type dCJD (p-dCJD) (18–21). The clinicopathological features of np-dCJD are similar to those of sCJD with 129M/M and type 1 PrPSc (sCJD-MM1) (14). In contrast, p-dCJD cases show unique features characterized by (i) the absence or late occurrence of myoclonus and periodic synchronous discharges on electroencephalogram; (ii) a long incubation period after grafting and a clinical course of long duration, and (iii) plaque-type PrP deposits in the brain (18–25). Although we have classified p-dCJD cases into MM1, the clinicopathological features of p-dCJD are quite different from those of sCJD-MM1 or np-dCJD (18–21). The reason for the existence of two distinct phenotypes in dCJD has remained elusive.

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Cross-sequence Transmission of sCJD Prions

### Table 1

| Inoculum      | Incubation period |
|---------------|-------------------|
|               | Ki-Hu129M/M (1x)  | Tg+Ki-Hu129M/M (1.2x) | Tg+Ki-Hu129M4R/M4R (9.8x) | Ki-Hu129V/V (1x) | Tg+Ki-Hu129V/V (2.1x) |
| sCJD-MM1 H3   | 467 ± 24 (8/8)    | 429 ± 6 (6/6)         | 175 ± 4 (9/9)              | 542, 648 (2/5)   | 288 ± 9 (4/4)         |
| sCJD-VV2 AK   | ND                 | 723 ± 79 (4/4)        | 505 ± 14 (5/5)             | 312 ± 7 (4/4)    | 183 ± 5 (11/11)       |

*The expression levels of human PrP in the brains.

| n | number of diseased animals; n0, number of inoculated animals.

a Three animals died of causes other than prion disease.

b ND, not done.

### EXPERIMENTAL PROCEDURES

**Production of Knock-in Mice and Transgenic Mice**—The production of knock-in mice expressing human PrP with 129M/M (Ki-Hu129M/M) and Ki-Hu129V/V mice has been reported previously (8). Knock-in mice expressing human PrP with 129M/M and four octapeptide repeats (Ki-Hu129M4R/M4R, transgenic mice expressing human PrP with 129M and four octapeptide repeats (Tg-Hu129M4R), and Tg-Hu129M mice were generated as described (26, 27). To evaluate the effect of overexpression of the PrP gene, Ki-Hu129M4R/M4R, Ki-Hu129M/M, and Ki-Hu129V/V mice were crossed with Tg-Hu129M4R, Tg-Hu129M, or Tg-Hu129V mice (8). The expression levels of human PrP in the brains from Tg+Ki-Hu129M4R/M4R, Tg+Ki-Hu129M/M, and Tg+Ki-Hu129V/V mice were 9.8x, 1.2x, and 2.1x, respectively, the levels observed in Ki-Hu129M/M or Ki-Hu129V/V mice.

**Human Brain Inocula**—Brain tissues were obtained at autopsy from CJD patients after receiving informed consent for research use. The diagnosis of CJD and the type of PrPSc were confirmed by neuropathological examination, PrPSc immunohistochemistry, and Western blotting as described (28, 29). The genotype and the absence of mutations in the coding region of the PrP gene were determined by sequence analysis (30).

**Transmission Experiments**—Human brain homogenates (10%) and mouse brain homogenates (10%) were prepared as described (27). Transmission studies were performed using 20 μl of the homogenates for intracerebral inoculation or 50 μl for intraperitoneal inoculation (8, 29). Intracerebrally inoculated mice were sacrificed after the onset of disease, and their brains were immediately frozen or fixed in 10% buffered formalin. Intraperitoneally inoculated mice were sacrificed at 75 days post-inoculation, and their spleens were immediately frozen or formalin-fixed.

**Immunohistochemistry**—Formalin-fixed mouse brains and spleens were treated with 60% formic acid for 1 h to inactivate the infectivity and embedded in paraffin. Tissue sections were pretreated by hydrolytic autoclaving before PrP immunohistochemistry (28). The PrP-N antiserum was used as the primary antibody (31). Goat anti-rabbit immunoglobulins polyclonal antibody labeled with the peroxidase-conjugated dextran polymer, EnVision+ (DakoCytomation) were used as the secondary antibody.

**Western Blotting**—PrPSc was extracted from human brains or mouse brains and spleens with collagenase treatment as described (32) with some modifications. For deglycosylation of PrP, samples were treated with PNGase F (New England Biolabs). Samples were subjected to 13.5% SDS-PAGE and Western blotting as described (8). The 3F4 monoclonal antibody (Signet Laboratories) and the ChW antiserum (8) were used as the primary antibodies. Anti-mouse EnVision+ and anti-rabbit EnVision+ were used as the secondary antibodies.

**Statistical Analysis**—Incubation times are expressed as mean ± S.E.

### RESULTS

**Transmission of sCJD Prions to PrP-humanized Mice with 129M/M or 129V/V**—To investigate whether strain-dependent traits of sCJD prions can be inherited through cross-sequence transmission, we performed intracerebral inoculation of a brain homogenate from a sCJD-MM1 patient (H3) or that from a sCJD-VV2 patient (AK) into PrP humanized mice with the 129M/M or 129V/V genotype. For sCJD-MM1 prions, the incubation times of Ki-Hu129V/V mice were 542 and 648 days (number of diseased animals/number of inoculated animals = 2/5), which were longer than those of Ki-Hu129M/M mice (467 ± 24 days, 8/8) (Table 1). For sCJD-VV2 prions, the mean incubation time of Tg+Ki-Hu129M/M mice was 723 ± 79 days (4/4), which was longer than that of Ki-Hu129V/V mice (312 ± 7 days, 4/4). Immunohistochemical analysis showed diffuse synaptic-type PrP deposits in the brains of both Tg+Ki-Hu129M/M and Tg+Ki-Hu129V/V mice inoculated with sCJD-MM1 prions (Fig. 1A). In contrast, Tg+Ki-Hu129V/V mice inoculated with sCJD-VV2 prions showed small plaque-type PrP deposits in the cerebral white matter and granular to diffuse synaptic-type deposits in the gray matter. The plaque-type PrP deposits were more prominent in the brains from Tg+Ki-Hu129M/M mice inoculated with sCJD-VV2 prions, in which larger plaque-type PrP deposits spread throughout the cerebral gray matter and thalamus rather than the white matter. Western blot analysis revealed that the size of PrPSc in the brains was maintained after cross-sequence transmission of sCJD-MM1 prions but not sCJD-VV2 prions (Fig. 1, B and C). Tg+Ki-Hu129V/V mice inoculated with sCJD-MM1 prions produced type 1 PrPSc (hereafter denoted as VV[MM1]1 PrPSc:host genotype[inoculated prions]type of generated PrPSc), which were identical in size to MM[MM1]1 PrPSc from Tg+Ki-Hu129M/M mice.
However, Tg+Ki-Hu129M/M mice inoculated with sCJD-VV2 prions produced unusual PrP\textsuperscript{res} that were larger than VV[\textsuperscript{VV2}]\textsuperscript{2} PrP\textsuperscript{res} from Tg+Ki-Hu129V/V mice but smaller than MM[\textsuperscript{MM1}]\textsuperscript{1} or VV[\textsuperscript{MM1}]\textsuperscript{1} PrP\textsuperscript{res}. We designated this intermediate-sized PrP\textsuperscript{res} as MM[\textsuperscript{VV2}]\textsuperscript{2Sh}, PrP\textsuperscript{res} with an upward size shift (Sh+) from the inoculated type 2 template. The shift in the size of PrP\textsuperscript{res} and the prominent plaque formation in Tg+Ki-Hu129M/M mice indicated that the strain-dependent traits of sCJD-VV2 prions were modified through cross-sequence transmission.

Intermediate Type PrP\textsuperscript{res} in Human p-dCJD Cases—Tg+Ki-Hu129M/M mice inoculated with sCJD-VV2 prions showed plaque-type Prp deposits in the brains despite their 129M/M genotype. CJD patients with the 129M/M genotype and plaque-type PrP deposits have been mainly recognized in vCJD and p-dCJD. To date, we have classified vCJD cases into MM2B and p-dCJD cases into MM1. However, there has been an exceptional case of p-dCJD that showed the accumulation of PrP\textsuperscript{res} intermediate in size between type 1 and type 2 (20). The occurrence of plaque-type PrP deposits and MM[\textsuperscript{VV2}]\textsuperscript{2Sh} PrPres in Tg+Ki-Hu129M/M mice inoculated with sCJD-VV2 prions raised the possibility that p-dCJD could be caused by cross-sequence transmission of sCJD-VV2 prions. To address this possibility, we re-examined carefully the size of PrP\textsuperscript{res} in the brains from three p-dCJD patients. Western blot analysis showed that the size of PrP\textsuperscript{res} from p-dCJD cases was smaller than that of MM1 PrP\textsuperscript{res} from sCJD-MM1 or np-dCJD cases (Fig. 2, A and B). Therefore, it appeared that the intermediate type PrP\textsuperscript{res} reported by Kretzschmar and colleagues (20) is not rare but rather a common form in human p-dCJD cases. We designated this intermediate type PrP\textsuperscript{res} observed in p-dCJD cases as MM\textsubscript{i} PrP\textsuperscript{res}.

Transmission of p-dCJD Prions to PrP-humanized Mice with 129M/M or 129V/V—To investigate the transmissibility of p-dCJD prions, we performed intracerebral inoculation of brain homogenates from p-dCJD patients (KR, KD, TV) into PrP-humanized mice with the 129M/M or 129V/V genotype. We had already established Ki-Hu129M\textsubscript{4R}/M\textsubscript{4R} mice expressing human PrP with 129M/M and four octapeptide repeats before we produced Ki-Hu129M/M mice expressing human PrP with five octapeptide repeats. The four or five octapeptide repeats of human PrP are polymorphisms unassociated with CJD (33). For sCJD-MM1 prions, these Ki-Hu129M\textsubscript{4R}/M\textsubscript{4R} mice showed long incubation times of \(600\) days in our preliminary experiment (data not shown). Therefore, they were crossed with Tg-Hu129M\textsubscript{4R} mice to overexpress the human PrP gene. Because these Tg+Ki-Hu129M\textsubscript{4R}/M\textsubscript{4R} mice were highly susceptible to...
sCJD-MM1 prions (175 ± 4 days, 9/9) (Table 1), we used them as PrP-humanized mice with the 129M/M genotype in this experiment at first. All of Tg+Ki-Hu129M4R/M4R and Ki-Hu129V/V mice inoculated with p-dCJD prions developed disease (Table 2). The mean incubation times of Tg+Ki-Hu129M4R/M4R mice were 398 ± 6 days, 9/9 (Table 2). The mean incubation times of Tg+Ki-Hu129M/M mice was 9.8-fold higher than that of Ki-Hu129V/V mice, the mean incubation times of Tg+Ki-Hu129M4R/M4R mice were longer than those of Ki-Hu129V/V mice (259 ± 6 and 304 ± 13 days). For np-dCJD, the mean incubation times of Tg+Ki-Hu129M4R/M4R mice were 161 ± 5 (5/5) and 208 ± 2 (5/5) days. Immunohistochemical analysis of the brains from Tg+Ki-Hu129M4R/M4R mice inoculated with p-dCJD prions showed a few plaque-type PrP deposits similar to those in Tg+Ki-Hu129M4R/M4R mice inoculated with sCJD-VV2 prions (Fig. 3A). Ki-Hu129V/V mice inoculated with p-dCJD prions showed small plaque-type PrP deposits in the cerebral white matter and granular to diffuse synaptic-type deposits in the gray matter similar to those in Ki-Hu129V/V mice inoculated with sCJD-VV2 prions. There was no plaque-type PrP deposits in the brains from Tg+Ki-Hu129M4R/M4R mice inoculated with np-dCJD prions (Fig. 3B). Western blot analysis revealed that Tg+Ki-Hu129M4R/M4R mice inoculated with p-dCJD prions produced the intermediate type PrPres (MM1[M4R][M4R][M4R][M4R]) [PrPres], which were identical in size to MM1[M4R][M4R][V2][2Sh+][PrPres] (Fig. 3, C and D). Furthermore, Ki-Hu129V/V mice inoculated with p-dCJD prions produced type 2 PrPres (VV[MM1][MM1][2PrPres]), which were identical in size to VV[V2][2PrPres]. Thus, p-dCJD prions and sCJD-VV2 prions were similar in the transmissibility, the patterns of PrP deposition, and the size of PrPres (the intermediate type in Tg+Ki-Hu129M4R/M4R or type 2 in Ki-Hu129V/V) in PrP-humanized mice.

On the basis of the following facts, we considered that the number of octapeptide repeats of PrP did not affect the size of PrPres, Tg+Ki-Hu129M4R/M4R mice inoculated with sCJD-MM1 prions produced M4R[M4R][M4R][M4R] [PrPres] identical in size to MM1[M4R][1PrPres] from Ki-Hu129M/M mice (Fig. 3E). Furthermore, Tg+Ki-Hu129M4R/M4R mice inoculated with sCJD-VV2 prions produced M4R[M4R][V2][2Sh+][PrPres] identical in size to MM1[V2][2Sh+][PrPres] from Tg+Ki-Hu129M/M mice (Fig. 3E).

We inoculated intracerebrally a brain homogenate from a p-dCJD patient (KD) into Ki-Hu129M/M mice besides Tg+Ki-

### Table 2

| Inoculum Genotype | Mean Incubation Period (days ± S.E.) | Ki-Hu129V/V | Tg+Ki-Hu129M4R/M4R |
|-------------------|-------------------------------------|-------------|---------------------|
| p-dCJD            |                                     |             |                     |
| KR 129M/M         | 420 ± 10 (5/5)                      | 259 ± 6 (6/6) |
| KD 129M/M         | 398 ± 10 (5/5)                      | 304 ± 13 (6/6) |
| TV 129M/M         | 584 ± 65 (5/5)                      | ND          |

| sCJD-VV2 prions   |                                      |             |                     |
|-------------------|-------------------------------------|-------------|---------------------|
| AK 129V/V         | 505 ± 14 (5/5)                      | 312 ± 7 (4/4) |

*a* n, number of diseased animals; *n*°, number of inoculated animals.  
b ND, not done.

### DISCUSSION

Our data comprised of four major findings. First, transmission of sCJD-VV2 prions to Tg+Ki-Hu129M/M mice generated unusual PrPres (MM1[V2]2Sh+ [PrPres]) intermediate in size between type 1 and type 2. Second, the intermediate type MM1 PrPres was seen in all examined p-dCJD cases. Third, Ki-Hu129V/V mice inoculated with p-dCJD prions showed short incubation times and the accumulation of type 2 PrPres with a downward size shift from the intermediate type template. Finally, Ki-Hu129V/V mice intraperitoneally inoculated with MM1[V2]2Sh+ prions showed high susceptibility and the accumulation of type 2 PrPres with a downward size shift from type 2Sh+ template. These findings suggest that p-dCJD could be caused by transmission of sCJD-VV2 prions to individuals with the 129M/M genotype (cross-sequence transmission).
Our results clearly demonstrate that p-dCJD prions and np-dCJD prions are distinct strains. On careful examination, the intermediate type PrPres was found to be a common form in p-dCJD cases. Moreover, the characteristic features of p-dCJD including the intermediate type PrPres, the long incubation period, and plaque-type PrP deposits in the brain were maintained after transmission to PrP-humanized mice with 129M/M. Meanwhile, the humanized mice with 129M/M inoculated with np-dCJD prions showed type 1 PrPres, a short incubation period, and synaptic-type PrP deposits in the brain. These results demonstrate that p-dCJD and np-dCJD are distinct subtypes of d-CJD caused by distinct prion strains. The long incubation times of the mice inoculated with p-dCJD prions suggest the frightening possibility that the numbers of p-dCJD patients may increase in the future.

sCJD cases with the 129M/V genotype and type 2 PrPSc (sCJD-MV2) and sCJD-VV2 cases account for 25% of the total sCJD cases and show the characteristic neuropathological changes highlighted by plaque-type PrP deposits (5). To date, some similarities among sCJD-MV2, sCJD-VV2, and p-dCJD have been described in clinicopathological, biochemical, and transmission studies (18–21, 29). Although the results of the present study show that cross-sequence transmission of sCJD-VV2 prions can cause phenotypes similar to those of p-dCJD, further studies are needed to clarify whether sCJD-MV2 prions can cause a p-dCJD like phenotype in humanized mice with 129M/M.

Besides p-dCJD, a few iatrogenic CJD cases with the 129M/M genotype and plaque-type PrP deposits in the brain have been reported in human growth hormone-related CJD (34, 35). The present results lead us to surmise that the human growth hormone-related CJD cases with 129M/M and plaque-type PrP deposits might be caused by the cross-sequence transmission of sCJD-VV2 prions.

**FIGURE 3.** p-dCJD prions and sCJD-VV2 prions exhibited similar transmissibility and neuropathology and identically sized PrPres in PrP-humanized mice. A, immunohistochemical analysis of the brains from Tg + Ki-Hu129M/+/M mice inoculated with p-dCJD or sCJD-VV2 prions showed a few plaque-type PrP deposits (arrowheads). B, diffuse synaptic-type PrP deposits in the brains from Tg + Ki-Hu129M/+/M mice inoculated with p-dCJD prions or sCJD-VV2 prions showed small plaque-type PrP deposits in the cerebral white matter and synaptic-type deposits in the gray matter. C, Western blot analysis of the brains from PrP-humanized mice after proteinase K digestion. D, for deglycosylation of PrPres, proteinase K-digested samples were treated with PNGase F. E, for deglycosylation of PrPres, proteinase K-digested samples were treated with PNGase F. F, Tg + Ki-Hu129M/+/M mice inoculated with sCJD-MM2 prions produced PrPres identical in size to M4R/MMM1 PrPres from Tg + Ki-Hu129M/+/M mice. Tg + Ki-Hu129M/+/M mice inoculated with sCJD-VV2 prions produced PrPres identical in size to M4R/MMM1 PrPres from Tg + Ki-Hu129M/+/M mice.
Cross-sequence Transmission of sCJD Prions

Through cross-sequence transmission, sCJD-VV2 prions acquired new conformational properties as reflected by the upward shift of the size of PrP\textsuperscript{res}. A similar shift of the PrP\textsuperscript{res} size through cross-sequence transmission has been reported in mice inoculated with vCJD prions (29, 36) or hamster scrapie strain Sc237 (37, 38). Moreover, the altered size of mouse-passaged Sc237 PrP\textsuperscript{res} reverts to those of hamster-passaged Sc237 PrP\textsuperscript{res} through transmission to hamsters (37). In accordance with these findings, the intermediate type PrP\textsuperscript{res} reverted to type 2 when MM[VV]\textsuperscript{2sh+} prions or p-dCJD prions were transmitted to the humanized mice with 129/V/V in this study. The most plausible explanation for these findings is that adaptation to the new host PrPC and/or selection of a PrP\textsuperscript{Sc} subpopulation from the whole heterogeneous population result in the emergence of a new prion strain with altered conformational properties, and that the emerging prion strain retains the memory of the parental prions within its conformational properties and/or its PrP\textsuperscript{Sc} subpopulation. Therefore, if the emerging prion strain is transmitted to the original host, the parental prions may re-emerge and become dominant.

The above concept is supported by the “traceback” phenomenon (8), e.g. knock-in mice and transgenic mice expressing bovine PrP are highly susceptible to vCJD prions as well as bovine spongiform encephalopathy prions (8, 39). Consistent with a report using transgenic mice expressing human PrP with 129M or 129V (12), the humanized mice with 129/V/V in our study were more susceptible to sCJD-VV2 prions than the humanized mice with 129M/M. Furthermore, the humanized mice with 129/V/V showed high susceptibility to MM[VV]\textsuperscript{2sh+} prions and p-dCJD prions despite cross-sequence transmission. These phenomena can be explained as follows. Because MM[VV]\textsuperscript{2sh+} prions and p-dCJD prions retained the memory of the parental sCJD-VV2 prions, the humanized mice with 129/V/V were highly susceptible to these prions as well as sCJD-VV2 prions. Our results demonstrate that traceback studies can be a powerful tool to identify the origin of prions.

In this study, the strain-dependent traits of sCJD-MM1 prions were inherited through cross-sequence transmission without any modification. The humanized mice with 129/V/V produced type 1 PrP\textsuperscript{res} after inoculation with sCJD-MM1 prions. Because sCJD-VV1 cases are extremely rare (at most 1–2% of the total number of sCJD cases) and characterized by early onset (mean age at onset, 39.3 years) (5), our results raise the possibility that CJD cases classified as VV1 may include cases caused by iatrogenic transmission of sCJD-MM1 prions or food-borne infection by type 1 prions from animals, e.g. chronic wasting disease prions in cervid. In fact, two CJD-VV1 patients who hunted deer or consumed venison have been reported (40, 41). The results of the present study emphasize the need for traceback studies and careful re-examination of the biochemical properties of sCJD-VV1 prions.

In conclusion, cross-sequence transmission of sCJD-VV2 prions generates a new prion strain with altered conformational properties and disease phenotypes as p-dCJD prions. Furthermore, the newly generated prions have unique transmissibility including the traceback phenomenon. In the future, if atypical prion strains emerge through cross-sequence transmission, especially from animals, traceback studies will enable us to identify the origin of the prions.

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