RESEARCH ARTICLE

Sporadic Creutzfeldt–Jakob Disease MM1+2C and MM1 are Identical in Transmission Properties

Atsushi Kobayashi[1]*; Yuichi Matsuura[2]; Toru Iwaki[3]; Yasushi Iwasaki[4]; Mari Yoshida[4]; Hitoshi Takahashi[5]; Shigeo Murayama[6]; Masaki Takao[6]; Shinsuke Kato[7]; Masahito Yamada[8]; Shirou Mohri[1]; Tetsuyuki Kitamoto[1]

[1] Department of Neurological Science, Tohoku University Graduate School of Medicine, Sendai, Japan.

[2] Influenza and Prion Disease Research Center, National Institute of Animal Health, Tsukuba, Ibaraki, Japan.

[3] Department of Neuropathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

[4] Department of Neuropathology, Institute for Medical Science of Ageing, Aichi Medical University, Nagakute, Aichi, Japan.

[5] Department of Pathology, Brain Research Institute, University of Niigata, Niigata, Japan.

[6] Department of Neuropathology, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Itabashi-ku, Tokyo, Japan.

[7] Division of Neuropathology, Department of Pathology, School of Medicine, Faculty of Medicine, Tottori University, Yonago, Japan.

[8] Department of Neurology and Neurobiology of Ageing, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan.

Abstract

The genotype (methionine, M or valine, V) at polymorphic codon 129 of the *PRNP* gene and the type (1 or 2) of abnormal prion protein in the brain are the major determinants of the clinicopathological features of sporadic Creutzfeldt–Jakob disease (CJD), thus providing molecular basis for classification of sporadic CJD, that is, MM1, MM2, MV1, MV2, VV1 or VV2. In addition to these "pure" cases, "mixed" cases presenting mixed neuropathological and biochemical features have also been recognized. The most frequently observed mixed form is the co-occurrence of MM1 and MM2, namely MM1+2. However, it has remained elusive whether MM1+2 could be a causative origin of dura mater graft-associated CJD (dCJD), one of the largest subgroups of iatrogenic CJD. To test this possibility, we performed transmission experiments of MM1+2 prions and a systematic neuropathological examination of dCJD patients in the present study. The transmission properties of the MM1+2 prions were identical to those of MM1 prions because MM2 prions lacked transmissibility. In addition, the neuropathological characteristics of MM2 were totally absent in dCJD patients examined. These results suggest that MM1+2 can be a causative origin of dCJD and causes neuropathological phenotype similar to that of MM1.

INTRODUCTION

Creutzfeldt–Jakob disease (CJD), a fatal transmissible neurodegenerative disease, is caused by an abnormal infectious isoform of prion protein (PrP^{Sc}), which is generated by a conformational change of the normal cellular isoform (PrP^{C}) (31). The conformational conversion of PrP^{C} occurs due to either one of three causes: spontaneous conversion in sporadic CJD (sCJD), pathogenic mutations of the *PRNP* gene in genetic CJD, or PrP^{Sc} infection in iatrogenic CJD and variant CJD.

The genotype (methionine, M or valine, V) at polymorphic codon 129 of the *PRNP* gene and the type (1 or 2) of PrP^{Sc} in the brain are the major determinants of the clinicopathological fea-

tures of sCJD and permit molecular classification of sCJD, that is, MM1, MM2, MV1, MV2, VV1 or VV2 (26). The PrP^{Sc} types 1 and 2 are distinguishable according to the size of the proteinase K-resistant core of PrP^{Sc} (21 and 19 kDa, respectively), reflecting differences in the proteinase K-cleavage site (at residues 82 and 97, respectively) (27). In addition, MM2 cases can be further divided into two subgroups, cortical form (MM2C) or thalamic form (MM2T), based on the neuropathological characteristics (26). Similarly, MV2 cases can also be divided into two neuropathologically distinct subgroups, cortical form (MV2C) or kuru plaque form (MV2K) (28). On the other hand, the MM1 and MV1 subgroups are indistinguishable in clinicopathological, biochemical and transmission properties, and are merged into one subgroup

as MM/MV1 (4, 26). Similarly, MM2C and MV2C are also indistinguishable and are merged into one subgroup as MM/MV2C. As a consequence, six subgroups are recognized in the current classification of sCJD, that is, MM/MV1, MM/MV2C, MM2T, MV2K, VV1 and VV2 (30). In addition to these "pure" subgroups, "mixed" cases presenting mixed neuropathological features and more than one PrP^{Sc} type in the same brain have also been reported (5, 26, 28, 32, 33, 35). The most frequently observed mixed form is the co-occurrence of MM/MV1 and MM/MV2C, denoted as MM/MV1+2C (28).

Dura mater graft-associated CJD (dCJD) is an iatrogenic CJD caused by prion-contaminated dura mater grafts obtained from human cadavers undiagnosed as CJD. There are two distinct subgroups in dCJD that show distinct clinicopathological, biochemical and transmission properties, reflecting their different causative origins. Plaque-type dCJD is caused by the transmission of sCJD MV2K or VV2 and shows characteristic clinicopathological and biochemical features, that is, widespread PrP amyloid plaques (kuru plaques) and the accumulation of intermediate type PrP^{Sc} showing intermediate electrophoretic mobility between types 1 and 2 PrP^{Sc} (16, 18, 19, 23, 37). On the other hand, non-plaque-type dCJD has been considered to be caused by the transmission of sCJD MM/MV1 and shows clinicopathological and biochemical features indistinguishable from those of sCJD MM/MV1, that is, diffuse synaptic type PrP deposition and type 1 PrP^{Sc} accumulation.

Although sCJD MM/MV1+2C accounts for 28% of all sCJD cases in European countries where the causative dura mater grafts were manufactured (28), it has remained elusive whether this sCJD subgroup could be a causative origin of dCJD. To test this possibility, we performed experimental transmission of sCJD MM1+2C and systematic neuropathological examination of dCJD patients in the present study.

MATERIALS AND METHODS

Patients

CJD cases included in this study were patients with clinically, genetically and histopathologically proven sCJD (five cases) and dCJD (45 cases). Brain tissues were obtained at autopsy from CJD patients after receiving informed consent for research use. The diagnosis of CJD, histopathological type and PrP^{Sc} type were confirmed by PrP immunohistochemistry and Western blot analysis (13, 33). The genotype and the absence of mutations in the open reading frame of the *PRNP* gene were determined by sequence analysis as described (14). According to Parchi's classification (30), the sCJD cases were classified as follows: MM1, two cases; MM2C, one case; MM1+2C, two cases. These patients showed the typical phenotypes of each sCJD subgroup in the clinicopathological and biochemical examinations. All dCJD cases examined were 129M/M homozygotes.

Transmission experiments

The production of knock-in mice expressing human PrP with one of the codon 129 genotypes (Ki-Hu129M/M, Ki-Hu129M/V or Ki-Hu129V/V), knock-in mice expressing human PrP with lysine homozygosity at another polymorphic codon 219 (Ki-Hu219K/K), and knock-in mice expressing human/mouse chimeric PrP (Ki-

ChM) has been reported previously (2, 10, 15). The expression levels of human PrP in the brains of these knock-in mice were identical to the level observed in wild-type mouse. Intracerebral inoculation of 10% brain homogenates from the sCJD MM1+2C, MM1 or MM2C patients was performed as described (34). The inoculated mice were sacrificed at a predefined clinical endpoint, or at accidental death. One hemisphere of the brain was fixed in 10% buffered formalin for immunohistochemistry, and the other hemisphere was immediately frozen for Western blotting or serial passage. Incubation times are expressed as mean ± SEM. These experiments were approved by the Institutional Animal Care and Use Committee of Tohoku University, and performed in strict accordance with the Regulations for Animal Experiments and Related Activities at Tohoku University.

Immunohistochemistry

Formalin-fixed brain tissues were treated with formic acid (99% for human tissues or 60% for mouse tissues) for 1 h to inactivate the infectivity, and embedded in paraffin. Tissue sections were pretreated by hydrolytic autoclaving before PrP immunohistochemistry (13). The anti-PrP monoclonal antibody 3F4 (Signet, Dedham, MA, USA) was used as the primary antibody for human sections, and anti-PrP antiserum PrP-N (12) was used as the primary antibody for mouse sections. Goat-anti-mouse immunoglobulin polyclonal antibody labeled with the peroxidase-conjugated dextran polymer, EnVision+ (Dako) and anti-rabbit EnVision+ were used as the secondary antibodies.

Western blotting

PrP^{Sc} was obtained from the cerebral cortex after tissue homogenization, collagenase digestion and Sarkosyl-NaCl extraction (9). For deglycosylation of PrP^{Sc}, samples were digested with PNGaseF (New England Biolabs, Ipswich, MA, USA) (16). Protein samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting (2). PrP^{Sc} type-specific polyclonal antibodies [designated as Tohoku 1 (T1) or Tohoku 2 (T2)] (17) or anti-PrP monoclonal antibody 3F4 were used as the primary antibodies. The type 1 PrP^{Sc}-specific antibody T1 reacts with epitopes located between residues 82 and 96 of human PrP, which can be retained in type 1 PrP^{Sc} but not in type 2 PrP^{Sc} after proteinase K-digestion (17). The type 2 PrP^{Sc}-specific antibody T2 is a proteolytic cleavage site-specific antibody and can specifically react with the N-terminally cleaved type 2 PrP^{Sc} after proteinase K-digestion (17). Anti-rabbit EnVision+ and anti-mouse EnVision+ were used as the secondary antibodies. The signal intensities of the Western blots were quantified with Quantity One software using an imaging device, VersaDoc 5000 (Bio-Rad Laboratories, Hercules, CA, USA).

RESULTS

The clinical features of the two patients with sCJD MM1+2C are summarized in Table 1. Histopathological examination of the brain revealed the coexistence of MM/MV1 pathology, that is, diffuse synaptic type PrP deposition, and MM/MV2C pathology (ie, large confluent vacuoles and perivacuolar PrP deposition) (Figure 1A–C). Western blot analysis of PrP^{Sc} using a conventional anti-PrP

Table 1. Summary of clinical features of the sCJD MM1+2C cases.

	Case #1	Case #2
Gender	Male	Female
Age at onset (years)	68	66
Initial symptom	Progressive dementia	Progressive dementia
Myoclonus (months)*	1	6
Akinetic mutism (months)*	2	10
PSWCs on EEG (months)*	1	10
Duration of illness (months)	2	44

*Duration until the appearance of myoclonus, akinetic mutism or PSWCs on EEG from onset.
Abbreviations: PSWC = periodic sharp-wave complex; EEG = electroencephalogram.

antibody 3F4, type 1 PrP^{Sc}-specific antibody T1 or type 2 PrP^{Sc}-specific antibody T2 showed the coexistence of types 1 and 2 PrP^{Sc} in both patients (Figure 1D). To compare the relative amounts of the type 1 or type 2 PrP^{Sc} between the patients, the signal intensities of the T1- or T2-reactive PrP^{Sc} were normalized by those of the 3F4-reactive PrP^{Sc}. The type 1 : type 2 ratios in case #1 and case #2 were 90:10 and 55:45, respectively. We then inoculated intracerebrally the brain homogenates from the two sCJD MM1+2C cases into PrP-humanized knock-in mice. These knock-in mice expressed human PrP with either one of the codon 129 genotypes (Ki-Hu129M/M, Ki-Hu129M/V or Ki-Hu129V/V), human PrP with the 219K/K genotype (Ki-Hu219K/K) or human/mouse chimeric PrP (Ki-ChM) (2, 10, 15). The mean incubation periods and attack rates of sCJD MM1+2C prion-inoculated mice were similar to those of MM1 prion-inoculated mice (Table 2). In particular, Ki-ChM mice showed remarkably short incubation times after challenge with sCJD MM1+2C prions, similar to those after challenge with MM1 prions (34). By contrast, sCJD MM2C prions could not be transmitted to any PrP-humanized knock-in mouse lines examined, as reported previously (18). Indeed, although experimental coinfection with distinct prion strains can result in prolonged incubation periods, referred to as "prion strain interference" (3, 7), the coexisting MM2C prions did not interfere with the transmission of MM1 prions even in the mice inoculated with MM1+2C case #2 materials, which contained equal amounts of type 2 PrP^{Sc} and type 1 PrP^{Sc}. Moreover, the neuropathological features were also identical between the sCJD MM1+2C prion-inoculated mice and the MM1 prion-inoculated mice, regardless of the *PRNP* genotype of mice (Figure 2A). All MM1+2C prion-inoculated mice showed only diffuse synaptic type PrP deposition, and the neuropathological characteristics of MM2C such as large confluent vacuoles or perivacuolar PrP deposition were not observed. Moreover, Western blot analysis of PrP^{Sc} in the brain revealed that all sCJD MM1+2C prion-inoculated mice produced only type 1 PrP^{Sc} regardless of the *PRNP* genotype (Figure 2B). Even in Western blot analysis using type 2 PrP^{Sc}-specific antibody T2, no PrP^{Sc} signal was detected in the MM1+2C prion-inoculated mice and the MM1 prion-inoculated mice. The sizes of type 1 PrP^{Sc} produced in the sCJD prion-inoculated mice seemed to be slightly smaller than those in the human cases (inocula). Similar downward size shift of PrP^{Sc} after transmission has been recog-

nized in a transmission experiment using a different PrP-humanized knock-in mouse line (22). However, we reported previously that type 1 PrP^{Sc} produced in the sCJD prion-inoculated mice retained epitopes for the type 1 PrP^{Sc}-specific antibody T1 (residues 82–96) or for another type 1 PrP^{Sc}-specific antibody POM2 (residues 83–89) (17). In addition, we confirmed that type 2 PrP^{Sc} produced in the sCJD prion-inoculated knock-in mice could be detected by the N-terminal cleavage site-specific antibody T2 similar to type 2 PrP^{Sc} of human sCJD cases (17, 18),
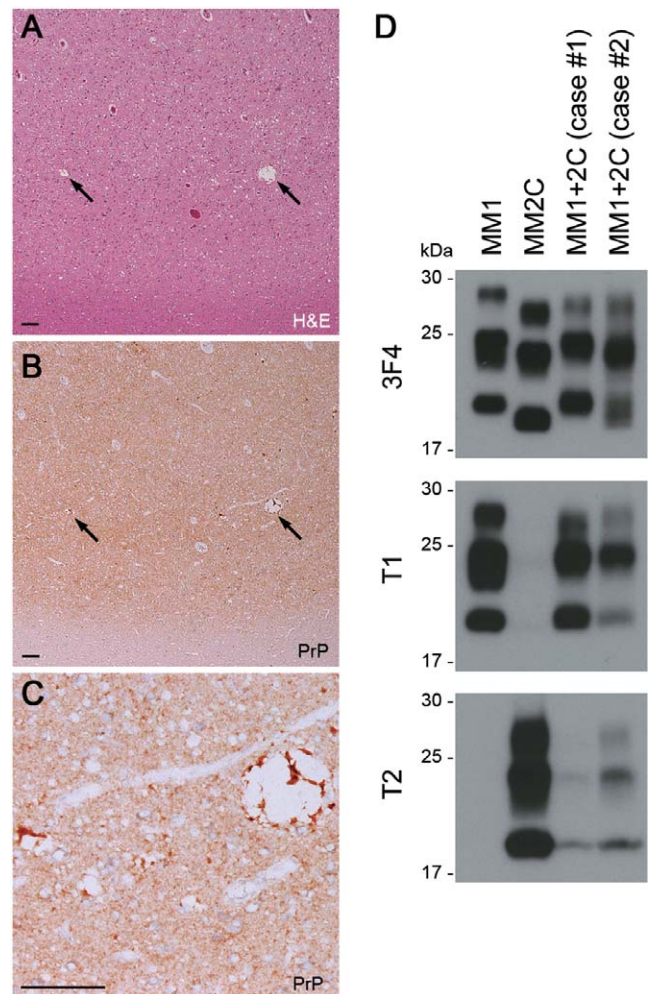

Figure 1. Neuropathological and biochemical properties of the sCJD MM1+2C cases. **A.** Histopathological analysis showed scattered large confluent vacuoles (arrows) in addition to spongiform changes in the cerebral cortex (H&E; frontal cortex of case #1). **B.** Immunohistochemical analysis of PrP revealed the coexistence of perivacuolar PrP deposition (arrows) and synaptic type PrP deposition (anti-PrP antibody 3F4; frontal cortex of case #1). **C.** Higher magnification of perivacuolar PrP deposition in panel B. Scale bars: 100 µm. **D.** Western blot analysis using a conventional anti-PrP antibody 3F4, type 1 PrP^{Sc}-specific antibody T1 or type 2 PrP^{Sc}-specific antibody T2 revealed the co-occurrence of types 1 and 2 PrP^{Sc} in the sCJD MM1+2C cases. The amounts loaded per lane were as follows: MM1, 280 µg (wet weight of brain tissue); MM2C, 100 µg; MM1+2C (case #1), 250 µg; or MM1+2C (case #2), 125 µg.

Table 2. Transmission of sCJD MM1+2C, MM1 or MM2C to PrP-humanized knock-in mice.

Inoculum (ID)	Mouse line (human *PRNP* genotypes at codons 129 and 219)				
	Ki-Hu129M/M (M/M, E/E)	Ki-Hu129M/V (M/V, E/E)	Ki-Hu129V/V (V/V, E/E)	Ki-Hu219K/K (M/M, K/K)	Ki-ChM
MM1+2C (case #1)	615 ± 8 (6/6)*	ND	717 ± 44 (6/6)	647 ± 49 (5/5)	162 ± 13 (6/6)
MM1+2C (case #2)	580 ± 8 (5/5)	595 ± 6 (4/4)	588 ± 11 (4/4)	594 ± 10 (5/5)	160 ± 9 (4/4)
MM1 (H3)†	467 ± 24 (8/8)	490 ± 26 (5/5)	774 ± 32 (6/6)	573 ± 52 (5/5)	142 ± 3 (5/5)
MM1 (NR)	531 ± 47 (5/5)	508 ± 55 (6/6)	588 ± 3 (5/5)	527 ± 31 (6/6)	154 ± 5 (6/6)
MM2C‡	— (0/7)	— (0/7)	— (0/7)	— (0/8)	— (0/6)

*Mean incubation periods (days ± SEM) and attack rates (number of mice positive for PrP accumulation in immunohistochemical analysis/number of inoculated mice).

†Transmission data of sCJD MM1 prions (H3) have been reported previously (10, 16–18, 34).

‡None of the sCJD MM2C prion-inoculated mice had developed disease by the end of their life span. Transmission data of Ki-Hu129M/M, Ki-Hu129M/V and Ki-Hu129V/V have been reported previously (18).

Abbreviation: ND = not done.

suggesting that the PrP^{Sc} type-specific N-terminal cleavage by proteinase K is not altered in these knock-in mice. Therefore, although the sizes of PrP^{Sc} produced in the sCJD prion-inoculated mice can slightly differ from those of human counterpart, it does not affect the typing of PrP^{Sc} that was determined by differences in the N-terminal proteolytic cleavage site. Whether the size shift can relate to C-terminal cleavage of PrP^{Sc} or to posttranslational modification of PrP, for example, glycosylphosphatidylinositol anchor, should be examined in the future. Taken together, the neuropathological and biochemical analyses revealed that MM2C prions were not transmitted to the sCJD MM1+2C prion-inoculated mice.

To examine whether the transmission properties of sCJD MM/MV1+2C were identical to those of MM/MV1 in human iatrogenic CJD cases, we performed systematic neuropathological evaluation of 45 cases with dCJD, representing approximately one-third of dCJD cases in Japan. Histopathological examination throughout the whole brain has an advantage over Western blot analysis to detect the focal coexistence of MM/MV2C prions with MM/MV1 prions (28). Among 45 dCJD cases, 29 showed diffuse synaptic type PrP deposition, and 16 showed widespread PrP amyloid plaques (kuru plaques) (Figure 3A). However, none of the dCJD cases showed sCJD MM/MV1+2C-like pathology despite the fact that sCJD MM/MV1+2C accounts for 28% of all sCJD cases in European countries where the causative dura mater grafts were manufactured (Figure 3B) (28), suggesting that MM/MV2C prions are also not transmissible in human iatrogenic CJD cases.

DISCUSSION

sCJD MM1+2C prion-inoculated mice lacked the neuropathological and biochemical characteristics of MM2C prions. In addition, sCJD MM/MV1+2C-like pathology was entirely absent in the dCJD cases in the systematic evaluation in the present study. These results suggest that sCJD MM/MV1+2C prions and MM/MV1 prions are identical in transmission properties both in animal experiments and in human iatrogenic CJD cases because MM/MV2C prions lack transmissibility. As transmission experiments using PrP-humanized knock-in mice have been so far conducted on very limited numbers of sCJD MM1+2C cases, further analyses with larger series, including cases with predominant accumulation of MM2C prions, may be needed to generalize the

implications of the present study. However, the conclusion of the present study is supported by several lines of evidence as follows. First, sCJD MM1+2C prions and MM/MV1 prions showed the same transmission properties also in a transmission study using nonhuman primates (29). Second, the absence of successful transmission (22) or extremely low transmissibility (4) of sCJD MM2C prions has also been revealed by transmission studies using other lines of PrP-humanized knock-in mouse. Finally, the incidence rate of non-plaque-type dCJD (68%) among the total dCJD cases in Japan perfectly matches the sum total of the incidence rates of sCJD MM/MV1 (40%) and MM/MV1+2C (28%) among all sCJD cases in European countries (30, 37) where the causative dura mater was collected from human cadavers. As virtually all Japanese dCJD patients were of the same 129M/M genotype (25), reflecting overwhelming predominance of the 129M/M homozygosity in the general population in Japan (8), the disease phenotypes of the dCJD patients simply depended on the prion strain infected (19). Indeed, the incidence rate of plaque-type dCJD (32%) among the total dCJD cases in Japan is also close to the total sum of the incidence rates of its causative origins [ie, sCJD VV2 (15%), MV2K (8%) and MV2K+2C (3%)] in European countries (30). Taken together, the present study suggests that not only sCJD MM/MV1 but also MM/MV1+2C can be a causative origin of non-plaque-type dCJD.

The reasons for the lack of transmissibility of sCJD MM/MV2C prions remain unknown. As the disease duration of sCJD MM/MV2C patients is the longest among sCJD subgroups (30), the replication of MM/MV2C prions may not proceed efficiently. This seems to be the same situation as that of self-propagating misfolded proteins that are transmissible between neighboring cells within a patient but are not transmissible between individuals, so-called "prionoid" (1). These self-propagating misfolded proteins can be efficiently transmitted to "primed" animals such as overexpressors or disease-prone mutants (6, 11, 21). Similarly, MM/MV2C prions can also be transmissible to primed animals such as transgenic mice overexpressing human PrP (20, 24), or transgenic mice overexpressing bank vole PrP (36), with a 100% attack rate. The lack of transmissibility of MM/MV2C prions to non-primed hosts suggests that CJD patients showing neuropathological and biochemical characteristics of MM/MV1+2C, MM/MV2C or MV2K+2C can be diagnosed as
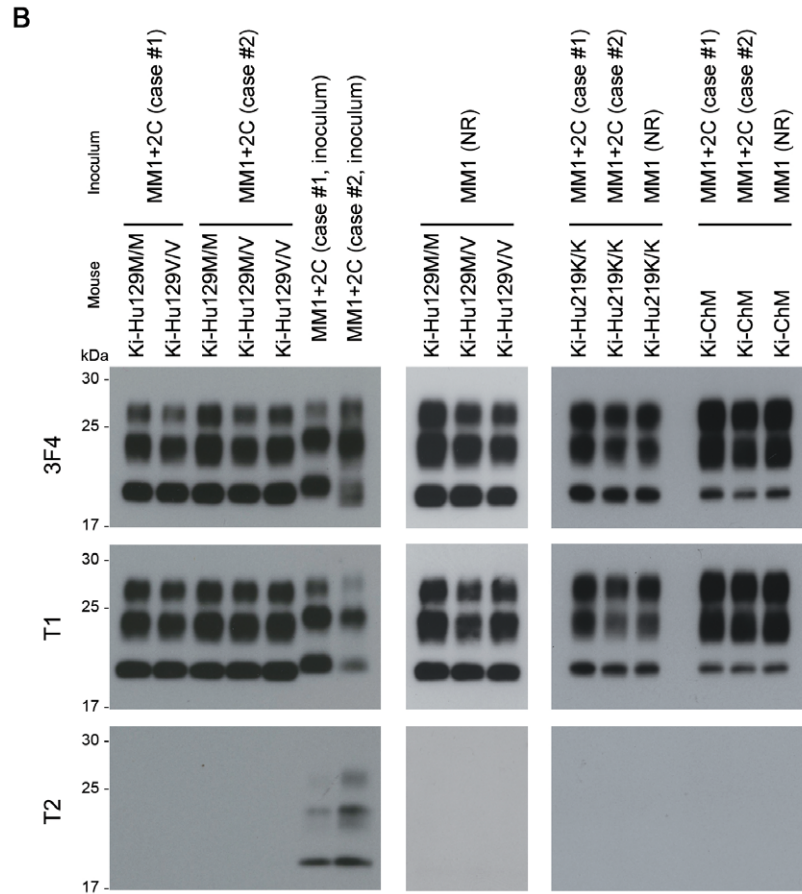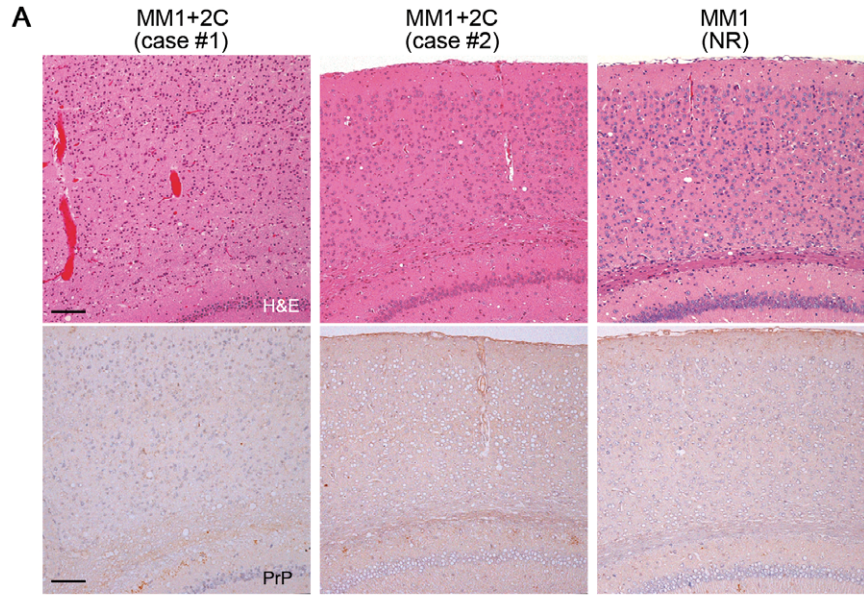
Figure 2. Neuropathological and biochemical properties of the PrP-humanized mice inoculated with sCJD MM1+2C prions. **A.** Histopathological analysis of the brain of Ki-Hu129M/M mice inoculated with sCJD MM1+2C prions (case #1 or case #2) or MM1 prions (NR). The MM1+2C prion-inoculated mice showed only spongiform changes (H&E, upper panels) and synaptic type PrP deposition (anti-PrP antibody PrP-N, lower panels), similar to the MM1 prion-inoculated mice. Large confluent vacuoles or perivacuolar PrP deposition were absent in all the MM1+2C prion-inoculated mice. Scale bars: 100 μm. **B.** Western blot analysis using a conventional anti-PrP antibody 3F4, type 1 PrP^{Sc}-specific antibody T1 or type 2 PrP^{Sc}-specific antibody T2. The MM1+2C prion-inoculated mice produced only type 1 PrP^{Sc} regardless of the *PRNP* genotype. Type 2 PrP^{Sc} was not detected even in Western blot analysis using the T2 antibody.

"sporadic" CJD even if they have a medical history of known risk factors for iatrogenic CJD such as dural grafting or treatment with growth hormone. An alternative explanation is that the PrP-humanized knock-in mice, humans or nonhuman primates (29)

were much more susceptible to MM/MV1 prions than to MM/MV2C prions and this prevented the appearance of histopathological or biochemical features related to MM/MV2C prions despite the existence of undetectable levels of MM/MV2C
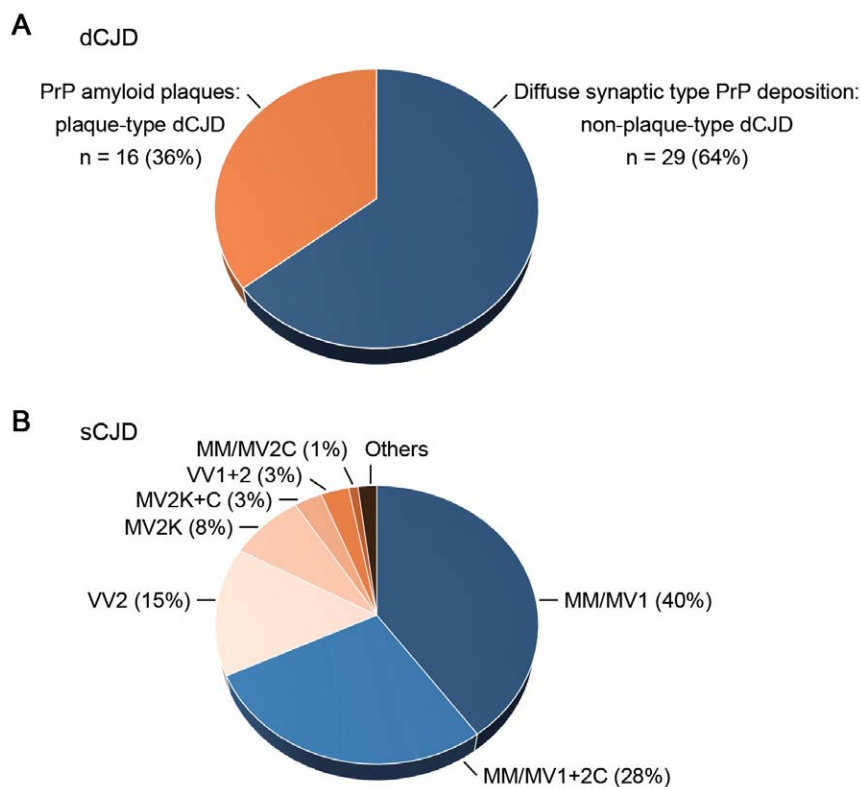
A dCJD

B sCJD

Figure 3. Neuropathological evaluation of 45 cases with dCJD. **A.** Histopathological examination throughout the whole brain of the dCJD patients revealed that 29 cases (64%) showed the diffuse synaptic type PrP deposition and 16 cases (36%) showed widespread PrP amyloid plaques. None of the dCJD patients had large confluent vacuoles or perivacuolar PrP deposition. **B.** Frequency of each sCJD subgroup in European countries (30).

prions in the brain. To test this possibility, serial passages from MM1+2C prion-inoculated mice to animals that are not permissive to MM1 prions but susceptible to MM2C prions will be needed in the future.

The clinical features of the sCJD MM/MV1+2C patients can differ from those of MM/MV1 patients depending on the extent of the MM/MV2C pathology (5, 28). The disease duration became significantly longer and the cerebellar signs less frequent with increasing MM/MV2C pathology (28). In contrast, the transmissibility of sCJD MM/MV1+2C prions was identical to that of MM/MV1 prions regardless of the abundance of MM/MV2C prions in the present study, as MM/MV2C prions lacked transmissibility. This would be an important finding from the viewpoint of public health. As the current decontamination/disinfection procedures against CJD prions were developed before genotyping and molecular classification become available, the effectiveness of the current procedures against sCJD MM/MV1+2C prions has not been tested. However, the present study suggests that the current procedures, considered to be effective against MM/MV1 prions, are sufficient for the decontamination/disinfection of MM/MV1+2C prions as well.

In conclusion, sCJD MM/MV1+2C prions and MM/MV1 prions showed identical transmission properties. With respect to public health issues, the present study suggests that sCJD MM/MV1+2C prions and MM/MV1 prions can be considered as the same entity, apart from the classification of sCJD patients.

ACKNOWLEDGMENTS

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Aguzzi A (2009) Cell biology: beyond the prion principle. *Nature* **459**:924–925.
2. Asano M, Mohri S, Ironside JW, Ito M, Tamaoki N, Kitamoto T (2006) vCJD prion acquires altered virulence through trans-species infection. *Biochem Biophys Res Commun* **342**:293–299.
3. Bartz JC, Kramer ML, Sheehan MH, Hutter JAL, Ayers JI, Bessen RA, Kincaid AE (2007) Prion interference is due to a reduction in strain-specific PrP^Sc levels. *J Virol* **81**:689–697.
4. Bishop MT, Will RG, Manson JC (2010) Defining sporadic Creutzfeldt-Jakob disease strains and their transmission properties. *Proc Natl Acad Sci U S A* **107**:12005–12010.
5. Cali I, Castellani R, Alshekhlee A, Cohen Y, Blevins J, Yuan J *et al* (2009) Co-existence of scrapie prion protein types 1 and 2 in

sporadic Creutzfeldt-Jakob disease: its effect on the phenotype and prion-type characteristics. *Brain* **132**:2643–2658.

6. Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A *et al* (2009) Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol* **11**:909–913.

7. Dickinson AG, Fraser H, Meikle VM, Outram GW (1972) Competition between different scrapie agents in mice. *Nat New Biol* **237**:244–245.

8. Doh-ura K, Kitamoto T, Sakaki Y, Tateishi J (1991) CJD discrepancy. *Nature* **353**:801–802.

9. Grathwohl KUD, Horiuchi M, Ishiguro N, Shinagawa M (1996) Improvement of PrPSc-detection in mouse spleen early at the preclinical stage of scrapie with collagenase-completed tissue homogenization and Sarkosyl-NaCl extraction of PrP^Sc^. *Arch Virol* **141**:1863–1874.

10. Hizume M, Kobayashi A, Teruya K, Ohashi H, Ironside JW, Mohri S, Kitamoto T (2009) Human prion protein (PrP) 219K is converted to PrP^Sc^ but shows heterozygous inhibition in variant Creutzfeldt-Jakob disease infection. *J Biol Chem* **284**: 3603–3609.

11. Kane MD, Lipinski WJ, Callahan MJ, Bian F, Durham RA, Schwarz RD *et al* (2000) Evidence for seeding of beta-amyloid by intracerebral infusion of Alzheimer brain extracts in beta-amyloid precursor protein-transgenic mice. *J Neurosci* **20**:3606–3611.

12. Kitamoto T, Muramoto T, Hilbich C, Beyreuther K, Tateishi J (1991) N-terminal sequence of prion protein is also integrated into kuru plaques in patients with Gerstmann-Sträussler syndrome. *Brain Res* **545**:319–321.

13. Kitamoto T, Shin R-W, Doh-ura K, Tomokane N, Miyazono M, Muramoto T, Tateishi J (1992) Abnormal isoform of prion proteins accumulates in the synaptic structures of the central nervous system in patients with Creutzfeldt-Jakob disease. *Am J Pathol* **140**:1285–1294.

14. Kitamoto T, Ohta M, Doh-ura K, Hitoshi S, Terao Y, Tateishi J (1993) Novel missense variants of prion protein in Creutzfeldt-Jakob disease or Gerstmann-Sträussler syndrome. *Biochem Biophys Res Commun* **191**:709–714.

15. Kitamoto T, Mohri S, Ironside JW, Miyoshi I, Tanaka T, Kitamoto N *et al* (2002) Follicular dendritic cell of the knock-in mouse provides a new bioassay for human prions. *Biochem Biophys Res Commun* **294**:280–286.

16. Kobayashi A, Asano M, Mohri S, Kitamoto T (2007) Cross-sequence transmission of sporadic Creutzfeldt-Jakob disease creates a new prion strain. *J Biol Chem* **282**:30022–30028.

17. Kobayashi A, Sakuma N, Matsuura Y, Mohri S, Aguzzi A, Kitamoto T (2010) Experimental verification of a traceback phenomenon in prion infection. *J Virol* **84**: 3230–3238.

18. Kobayashi A, Iwasaki Y, Otsuka H, Yamada M, Yoshida M, Matsuura Y (2013) Deciphering the pathogenesis of sporadic Creutzfeldt-Jakob disease with codon 129 M/V and Type 2 abnormal prion protein. *Acta Neuropathol Commun* **1**:74.

19. Kobayashi A, Matsuura Y, Mohri S, Kitamoto T (2014) Distinct origins of dura mater graft-associated Creutzfeldt-Jakob disease: past and future problems. *Acta Neuropathol Commun* **2**:32.

20. Korth C, Kaneko K, Groth D, Heye N, Telling G, Mastrianni J *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* **100**:4784–4789.

21. Luk KC, Kehm VM, Zhang B, O'Brien P, Trojanowski JQ, Lee VM (2012) Intracerebral inoculation of pathological α-synuclein initiates a rapidly progressive neurodegenerative α-synucleinopathy in mice. *J Exp Med* **209**:975–986.

22. Moda F, Suardi S, Di Fede G, Indaco A, Limido L, Vimercati C *et al* (2012) MM2-thalamic Creutzfeldt-Jakob disease: neuropathological, biochemical and transmission studies identify a distinctive prion strain. *Brain Pathol* **22**:662–669.

23. Noguchi-Shinohara M, Hamaguchi T, Kitamoto T, Sato T, Nakamura Y, Mizusawa H, Yamada M (2007) Clinical features and diagnosis of dura mater graft associated Creutzfeldt-Jakob disease. *Neurology* **69**:360–367.

24. Notari S, Xiao X, Espinosa JC, Cohen Y, Qing L, Aguilar-Calvo P *et al* (2014) Transmission characteristics of variably protease-sensitive prionopathy. *Emerg Infect Dis* **20**:2006–2014.

25. Nozaki I, Hamaguchi T, Sanjo N, Noguchi-Shinohara M, Sakai K, Nakamura Y *et al* (2010) Prospective 10-year surveillance of human prion diseases in Japan. *Brain* **133**:3043–3057.

26. Parchi P, Giese A, Capellari S, Brown P, Schulz-Schaeffer W, Windl O *et al* (1999) Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann Neurol* **46**:224–233.

27. Parchi P, Zou W, Wang W, Brown P, Capellari S, Ghetti B *et al* (2000) Genetic influence on the structural variations of the abnormal prion protein. *Proc Natl Acad Sci U S A* **97**:10168–10172.

28. Parchi P, Strammiello R, Notari S, Giese A, Langeveld JP, Ladogana A (2009) Incidence and spectrum of sporadic Creutzfeldt-Jakob disease variants with mixed phenotype and co-occurrence of PrP^Sc^ types: an updated classification. *Acta Neuropathol* **118**:659–671.

29. Parchi P, Cescatti M, Notari S, Schulz-Schaeffer WJ, Capellari S, Giese A *et al* (2010) Agent strain variation in human prion disease: insights from a molecular and pathological review of the National Institutes of Health series of experimentally transmitted disease. *Brain* **133**:3030–3042.

30. Parchi P, Strammiello R, Giese A, Kretzschmar H (2011) Phenotypic variability of sporadic human prion disease and its molecular basis: past, present, and future. *Acta Neuropathol* **121**:91–112.

31. Prusiner SB, Scott MR, DeArmond SJ, Cohen FE (1998) Prion protein biology. *Cell* **93**:337–348.

32. Puoti G, Giaccone G, Rossi G, Canciani B, Bugiani O, Tagliavini F (1999) Sporadic Creutzfeldt-Jakob disease: co-occurrence of different types of PrP^Sc^ in the same brain. *Neurology* **53**: 2173–2176.

33. Schoch G, Seeger H, Bogousslavsky J, Tolnay M, Janzer RC, Aguzzi A, Glatzel M (2006) Analysis of prion strains by PrP^Sc^ profiling in sporadic Creutzfeldt-Jakob disease. *PLoS ONE* **3**:e14.

34. Taguchi Y, Mohri S, Ironside JW, Muramoto T, Kitamoto T (2003) Humanized knock-in mice expressing chimeric prion protein showed varied susceptibility to different human prions. *Am J Pathol* **163**:2585–2593.

35. Uro-Coste E, Cassard H, Simon S, Lugan S, Bilheude JM, Perret-Liaudet A *et al* (2008) Beyond PrP^pres^ type 1 / type 2 dichotomy in Creutzfeldt-Jakob disease. *PLoS Pathog* **4**:e1000029.

36. Watts JC, Giles K, Patel S, Oehler A, DeArmond SJ, Prusiner SB (2014) Evidence that bank vole PrP is a universal acceptor for prions. *PLoS Pathog* **10**:e1003990.

37. Yamada M, Noguchi-Shinohara M, Hamaguchi T, Nozaki I, Kitamoto T, Sato T (2009) Dura mater graft-associated Creutzfeldt-Jakob disease in Japan: clinicopathological and molecular characterization of the two distinct subtypes. *Neuropathology* **29**:609–618.