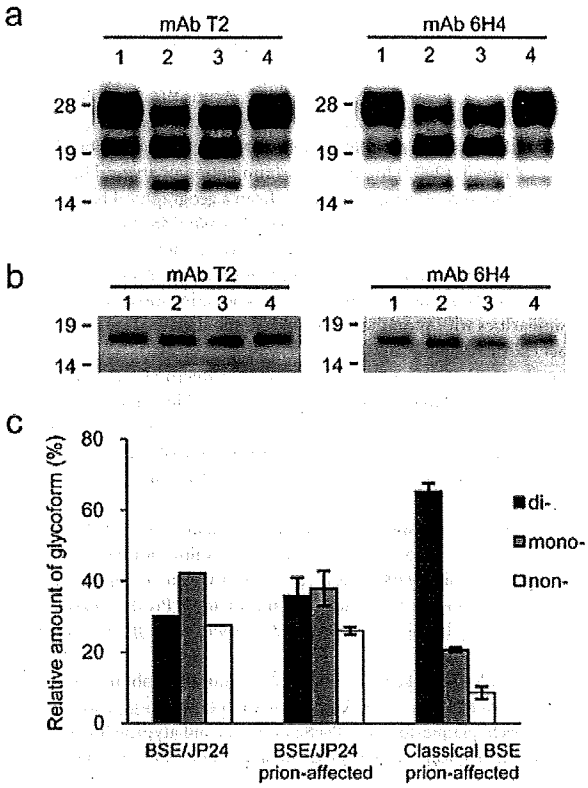

Fig. 1. Western blot analysis of PK-treated PrP^{Sc} from classical and atypical BSE cattle. (a) Western blot with mAbs T2 (left panel) and 6H4 (right panel) of PK-treated brain homogenates from classical BSE prion-affected cattle (lanes 1 and 4), BSE/JP24 isolate (lane 2) and experimentally BSE/JP24-affected cattle (lane 3); (b) samples after deglycosylation by PNGase treatment. Molecular mass standards (kDa) are indicated on the left. (c) Relative amounts of the di-, mono- and non-glycosylated forms of PK-treated PrP^{Sc}. Error bars indicate standard deviation (SD).

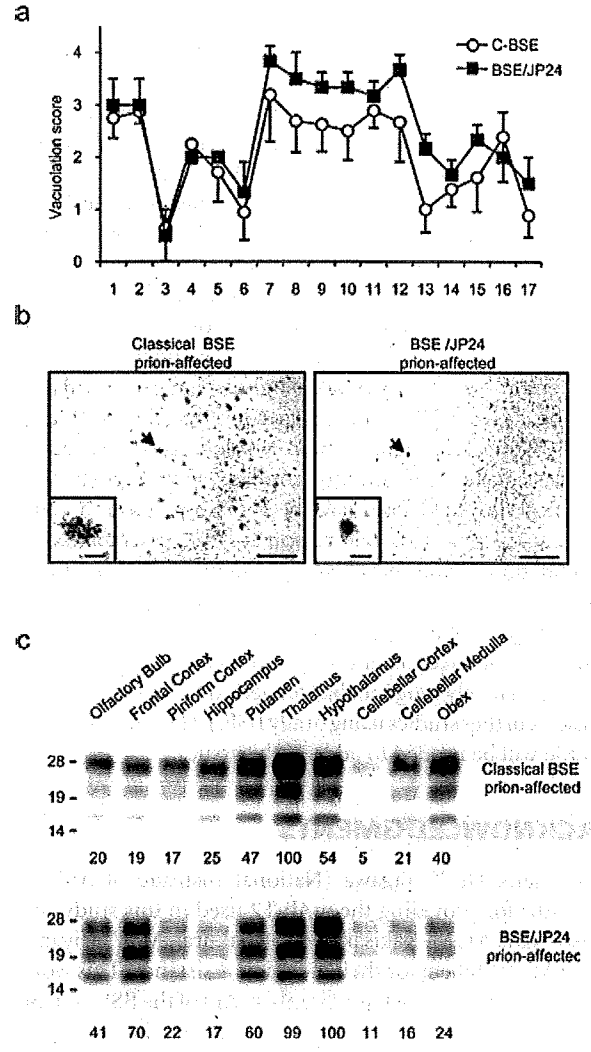

Fig. 2. Pathological and biochemical comparison between classical BSE and BSE/JP24 prion-affected cattle. (a) Lesion profile of classical BSE and BSE/JP24 prion-affected cattle. The mean scores for the classical BSE prion-affected cattle (C-BSE; open circles, $n = 3$) and BSE/JP24 prion-affected cattle (BSE/JP24; closed squares, $n = 3$) are shown. Error bars indicate SD. The neuroanatomical regions are as follows: 1, nucleus of the solitary tract; 2, nucleus of the spinal tract of V; 3, hypoglossal nucleus; 4, vestibular nuclear complex; 5, cochlear nucleus; 6, cerebellar vermis; 7, central gray matter; 8, rostral colliculus; 9, medial geniculate nucleus; 10, hypothalamus; 11, nucleus dorsomedialis thalami; 12, nucleus ventralis lateralis thalami; 13, frontal cortex; 14, septal nuclei; 15, caudate nucleus; 16, putamen; 17, claustrum. (b) PrP^{Sc} deposition in the frontal lobe of classical BSE- (left panel) and BSE/JP24 prion-affected (right panel) cattle. Stellate-type PrP^{Sc} deposit and PrP-plaque are indicated by arrows and insets, respectively. Bars in the main panels = 200 μm; bars in the insets = 20 μm. (c) Comparison of regional PrP^{Sc} deposition in the brain between classical BSE and BSE/JP24 prion-affected cattle. A representative western blot of PrP^{Sc} is shown. The levels of PrP^{Sc} relative to the thalamus (classical BSE prion-affected cattle) or hypothalamus (BSE/JP24 prion-affected cattle) are indicated below the panels.

the classical BSE and BSE/JP24 prion-affected cattle. PrP^{Sc} deposits were pronounced in the neuropil of the thalamus and midbrain, particularly in the periaqueductal gray matter of the brains from both experimentally BSE prion-affected cattle (data not shown). Consistent with this, western blot analysis also showed that there were no marked differences in the topography of PrP^{Sc} deposition, except for high PrP^{Sc} levels in the frontal cortex of BSE/JP24 prion-affected cattle (Fig. 2c). Interestingly, these immunohistochemical and neuropathological properties closely resembled those of the BASE-affected cattle (8).

In summary, we demonstrated the successful transmission of the BSE/JP24 prion to cattle. The BSE/JP24 prion-affected cattle sustained the molecular properties of PK-treated PrP^{Sc} as those of the original BSE/JP24 isolate. Although most brain regions except for the medulla oblongata of the original BSE/JP24 isolate were unable

to be investigated due to inadequate specimen collection, in comparison to experimentally BSE/JP24 prion-affected cattle, both neuropathological features, such as severe vacuolation in the medulla oblongata at the obex level and the presence of PrP^{Sc} plaques, closely resembled each other. Based on molecular properties of PK-treated PrP^{Sc} and a detailed comparison of the immunohistochemical and neuropathological properties, the BSE/JP24 prion was distinguishable from those in the classical BSE prion, and appear to be rather similar to the BASE prion (8).

Of interest, experimental transmission of the BSE/JP24 prion to cattle induced a shorter incubation period and more severe neuropathological changes compared to the classical BSE prion, suggesting that the BASE and BSE/JP24 prion might be more virulent in cattle species. However, such speculation conflicts with reports that atypical BSE field cases have been mainly found in adult and aged cattle (5). The reason for this discrepancy in incubation periods between experimentally and naturally affected cattle is unknown. These observations may imply that atypical BSE are sporadic forms of BSE. Alternatively, the route of infection and/or prion titer may be attributed to the relatively long incubation period in natural atypical cases. Further studies using orally BSE/JP24 prion-affected cattle will be needed to address this issue.

ACKNOWLEDGMENTS

We thank Dr Y. Tagawa (National Institute of Animal Health) for providing the mAb T2 used in this study. We also thank Ms M. Kakisaki, Ms M. Sakurai, Ms Y. Miyama and Ms N. Tabeta for their technical assistance. This work was supported in part by a Grant-in-Aid of the BSE and the Prion Disease Control Project from the Ministry of Agriculture, Forestry, and Fisheries of Japan; a grant for BSE research from the Ministry of Health, Labor, and Welfare of Japan.

REFERENCES

1. Wells G.A., Scott A.C., Johnson C.T., Gunning R.F., Hancock R.D., Jeffrey M., Dawson M., Bradley R. (1987) A novel progressive spongiform encephalopathy in cattle. *Vet Rec* **121:** 419–20.
2. Bruce M., Chree A., McConnel I., Brown K., Fraser H. (1996) Transmission and strain typing studies of scrapie and bovine spongiform encephalopathy. In: Court L., Dodet B. eds. *Transmissible Subacute Spongiform Encephalopathies: Prion Diseases.* Paris: Elsevier, pp. 259–62.
3. Simmons M.M., Harris P., Jeffrey M., Meek S.C., Blamire I.W., Wells G.A. (1996) BSE in Great Britain: consistency of the neurohistopathological findings in two random annual samples of clinically suspect cases. *Vet Rec* **138:** 175–7.
4. Kuczius T., Haist I., Groschup M.H. (1998) Molecular analysis of bovine spongiform encephalopathy and scrapie strain variation. *J Infect Dis* **178:** 693–9.
5. Ducrot C., Arnold M., de Koeijer A., Heim D., Calavas D. (2008) Review on the epidemiology and dynamics of BSE epidemics. *Vet Res* **39:** 15.
6. Jacobs J.G., Langeveld J.P., Biacabe A.G., Acutis P.L., Polak M.P., Gavier-Widen D., Buschmann A., Caramelli M., Casalone C., Mazza M., Groschup M., Erkens J.H., Davidse A., van Zijderveld F.G., Baron T. (2007) Molecular discrimination of atypical bovine spongiform encephalopathy strains from a geographical region spanning a wide area in Europe. *J Clin Microbiol* **45:** 1821–9.
7. Casalone C., Zanusso G., Acutis P., Ferrari S., Capucci L., Tagliavini F., Monaco S., Caramelli M. (2004) Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. *Proc Natl Acad Sci USA* **101:** 3065–70.
8. Lombardi G., Casalone C., D'Angelo A., Gelmetti D., Torcoli G., Barbieri I., Corona C., Fasoli E., Farinazzo A., Fiorini M., Gelati M., Iulini B., Tagliavini F., Ferrari S., Caramelli M., Monaco S., Capucci L., Zanusso G. (2008) Intraspecies transmission of BASE induces clinical dullness and amyotrophic changes. *PLoS Pathog* **4:** e1000075.
9. Yamakawa Y., Hagiwara K., Nohtomi K., Nakamura Y., Nishijima M., Higuchi Y., Sato Y., Sata T.; Expert Committee for BSE Diagnosis, Ministry of Health, Labour and Welfare of Japan. 2003. Atypical proteinase K-resistant prion protein (PrPres) observed in an apparently healthy 23-month-old Holstein steer. *Jpn J Infect Dis* **56:** 221–2.
10. Hagiwara K., Yamakawa Y., Sato Y., Nakamura Y., Tobiume M., Shinagawa M., Sata T. 2007. Accumulation of mono-glycosylated form-rich, plaque-forming PrPSc in the second atypical bovine spongiform encephalopathy case in Japan. *Jpn J Infect Dis* **60:** 305–8.
11. Masujin K., Shu Y., Yamakawa Y., Hagiwara K., Sata T., Matsuura Y., Iwamaru Y., Imamura M., Okada H., Mohri S., Yokoyama T. (2008) Biological and biochemical characterization of L-type-like bovine spongiform encephalopathy (BSE) detected in Japanese black beef cattle. *Prion* **2:** 123–8.
12. Wells G.A., Spiropoulos J., Hawkins S.A., Ryder S.J. (2005) Pathogenesis of experimental bovine spongiform encephalopathy: preclinical infectivity in tonsil and observations on the distribution of lingual tonsil in slaughtered cattle. *Vet Rec* **156:** 401–7.
13. Buschmann A., Gretzschel A., Biacabe A.G., Schiebel K., Corona C., Hoffmann C., Eiden M., Baron T., Casalone C., Groschup M.H. (2006) Atypical BSE in Germany–proof of transmissibility and biochemical characterization. *Vet Microbiol* **117:** 103–16.
14. Iwata N., Sato Y., Higuchi Y., Nohtomi K., Nagata N., Hasegawa H., Tobiume M., Nakamura Y., Hagiwara K., Furuoka H., Horiuchi M., Yamakawa Y., Sata T. (2006) Distribution of PrP(Sc) in cattle with bovine spongiform encephalopathy slaughtered at abattoirs in Japan. *Jpn J Infect Dis* **59:** 100–7.
15. Hayashi H., Takata M., Iwamaru Y., Ushiki Y., Kimura K.M., Tagawa Y., Shinagawa M., Yokoyama T. (2004) Effect of tissue deterioration on postmortem BSE diagnosis by immunobiochemical detection of an abnormal isoform of prion protein. *J Vet Med Sci* **66:** 515–20.
16. Bencsik A.A., Debeer S.O., Baron T.G. (2005) An alternative pretreatment procedure in animal transmissible spongiform encephalopathies diagnosis using PrPsc immunohistochemistry. *J Histochem Cytochem* **53:** 1199–202.
17. Furuoka H., Yabuzoe A., Horiuchi M., Tagawa Y., Yokoyama T., Yamakawa Y., Shinagawa M., Sata T. (2007) Species-specificity of a panel of prion protein antibodies for the immunohistochemical study of animal and human prion diseases. *J Comp Pathol* **136:** 9–17.

Arch Virol (2009) 154:1929–1932
DOI 10.1007/s00705-009-0534-2

BRIEF REPORT

Isolation of two distinct prion strains from a scrapie-affected sheep

Kentaro Masujin · Yujing Shu · Hiroyuki Okada · Yuichi Matsuura · Yoshifumi Iwamaru · Morikazu Imamura · Shirou Mohri · Takashi Yokoyama

Received: 27 July 2009 / Accepted: 29 September 2009 / Published online: 30 October 2009

Abstract We performed a transmission study using mice to clarify the characteristics of the most recent case of scrapie in Japan. The mice that were inoculated with the brain homogenate from a scrapie-affected sheep developed progressive neurological disease, and one of the scrapie-affected mice showed unique clinical signs during primary transmission. This mouse developed obesity, polydipsia, and polyuria. In contrast, the other affected mice exhibited weight loss and hypokinesia. In subsequent passages, the mice showed distinct characteristic scrapie phenotypes. This finding may prove that different prion strains coexist in a naturally affected sheep with scrapie.

Abbreviations

PrP^{Sc}	Abnormal prion protein
CNS	Central nervous system
Ka/O	Kanagawa/scrapie obesity-type prion
Ka/W	Kanagawa/scrapie weight-loss-type prion
mAb	Monoclonal antibody
PrP	Prion protein
PrPcore	Proteinase-K-digested PrP^{Sc}
sCJD	Sporadic Creutzfeldt–Jacob disease
TSE	Transmissible spongiform encephalopathy
WB	Western blotting

Scrapie is a transmissible spongiform encephalopathy (TSE) that affects sheep and goats. It is characterized by spongiform changes in the central nervous system (CNS) and accumulation of an abnormal prion protein (PrP^{Sc}) in the CNS and lymphoid tissues; PrP^{Sc} is the major component of prions [1]. Thus far, multiple strains of scrapie prions have been identified [2–6]. These strains can be distinguished on the basis of the incubation period, the lesion profile, and the pattern of the PrP^{Sc} accumulation in the transmission studies with mice. The characteristic phenotypes of these prion strains are conserved during serial passage within a single host [2]. However, the mechanism of emergence of prion strains is still unknown.

A 60-month-old Suffolk ewe developed ananastasia and eventually died in Kanagawa prefecture, Japan, and it was diagnosed as scrapie (Ka/scrapie). To clarify the biological properties of prions in this most recent case of scrapie in Japan, we examined the transmissibility of scrapie prions in wild-type ICR mice (PrP allotype $PrP^{A/A}$; PrP^{A} encodes PrP with leucine at codon 108 and threonine at codon 189; Japan SLC, Inc., Japan) by using previously described methods [7]. All of the mice that were inoculated with the brain homogenate of scrapie-affected sheep developed progressive neurological diseases; one of the disease-affected mice exhibited unique clinical signs during primary transmission (Table 1). After an incubation period of 469 days, this mouse developed obesity, polydipsia, and polyuria followed by slowness of movement; the prion responsible for these symptoms is designated as the Ka/scrapie obesity-type (Ka/O) prion. In contrast, after an incubation period of 457 ± 21.1 days, the other disease-affected mice (15) exhibited weight loss, hypokinesia, and uncoordinated hind-limb movements; the prion responsible for these symptoms was designated as the Ka/scrapie weight-loss-type (Ka/W) prion. To further investigate the

K. Masujin and Y. Shu contributed equally to this work.

K. Masujin · Y. Shu · H. Okada · Y. Matsuura · Y. Iwamaru · M. Imamura · S. Mohri · T. Yokoyama (✉)
Prion Disease Research Center, National Institute of Animal Health, 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan
e-mail: tyoko@affrc.go.jp

Table 1 Transmission of Ka/scrapie in wild-type ICR mice

First passage (16/16[a])		Second passage		Third passage	
15/16[b]	457 ± 21.1[c]	→ 5/5	255.8 ± 28.8	→ 10/10	151.5 ± 5.6
1/16[d]	469	→ 5/5	287.0 ± 6.5	→ 10/10	272.3 ± 29.0

[a] Number of infected mice/number of inoculated mice

[b] Mice exhibiting weight loss and hind-limb ataxia. All of these mice showed the same clinical signs and same neuropathological phenotype. The brain homogenate of 1 of the 15 mice was used for the second passage (incubation period, 463 days)

[c] Average ± standard deviation (days)

[d] A single mouse exhibiting polydipsia, polyuria, and obesity

properties of these prions, brain homogenates from the Ka/O- and Ka/W-affected mice were inoculated into wild-type mice, and these mice were subjected to neuropathological and biochemical examinations. The mice that were inoculated with the brain homogenate of the Ka/O-affected mouse developed obesity, polydipsia, and polyuria after an incubation period of 287.0 ± 6.5 days. Conversely, those inoculated with the brain homogenate of the Ka/W-affected mice exhibited weight loss and hind-limb ataxia after an incubation period of 255.8 ± 28.2 days. Moreover, mice in the subsequent Ka/O and Ka/W passage lines showed different clinical signs, and the incubation periods of the third passage lines in the Ka/O- and Ka/W-affected mice were 272.3 ± 29.0 and 151 ± 5.6 days, respectively. The body weights of the Ka/O- and Ka/W-affected mice at the third passage are shown in Table 2.

Neuropathological examinations of these mice were performed by using previously described methods [7]. Spongiform changes were detected throughout the brains of both the Ka/W- and Ka/O-affected mice. The degree of vacuolation in the brains of the Ka/W-affected mice was more severe than that in the Ka/O-affected mice (Fig. 1a–c). Immunohistochemical analyses were performed by using previously described methods [7, 8]. The PrP^{Sc} types and their distributions in the Ka/O- and Ka/W-affected mice were different (Fig. 1d–g). Punctate and fine granular PrP^{Sc} were predominantly and uniformly distributed throughout the brains of the Ka/W-affected mice (Fig. 1d, f). In contrast, in the Ka/O-affected mice, coarse granular PrP^{Sc} was predominantly distributed in the thalamus, the brain stem, and the cerebral cortex (Fig. 1e, g), while PrP plaques were observed in the corpus callosum, the thalamus, and the cerebral cortex (inset of Fig. 1e).

In recent studies, prion strains have been distinguished on the basis of the biochemical properties of the PrP^{Sc}, such as the glycoform ratio and the molecular mass of proteinase-K-digested PrP^{Sc} (PrPcore) [9–13]. We characterized the PrPcore molecules that had been extracted from the brains of the Ka/O- and Ka/W-affected mice by using a previously described method [14]. Western blotting (WB) analysis revealed that the PrPcore obtained from the Ka/O- and Ka/W-affected mice had similar glycoform patterns and molecular mass (Fig. 2). These results indicate that two strains of prions with distinct properties were isolated from a single source, i.e., the brain of the scrapie-affected sheep.

Different types of PrP^{Sc} (types 1 and 2) were reported to co-exist in a case of sporadic Creutzfeldt–Jacob disease (sCJD) [15]. Scrapie in sheep is also proposed to be caused by mixed populations of different prion strains [16, 17]. In the present study, different prion strains were isolated from the brain of a scrapie-affected sheep during the primary transmission studies. In the previously reported CJD case, the PrPcore sizes of the two strains were different. In contrast, in the case reported in this study, although their PrPcore sizes were not different, the prions of the two strains showed distinct biological characteristics. In addition, this result showed that a transmission study using experimental animals is a useful approach for the isolation and characterization of prion strains. New TSE strains are believed to emerge due to mutations caused by differences in the primary PrP sequences of the host and the inoculum [17]. We observed that 3 out of 31 mice showed the characteristic clinical signs of the Ka/O strain in repeat trials of Ka/scrapie transmission (data not shown). This data indicates that the Ka/O strain shows a constant occurrence rate of 6–9%. Therefore, our findings may indicate that mixed prion populations exist in a

Table 2 Body weights of the Ka/W- and Ka/O-affected wild-type ICR mice

Inoculum[a]	Mouse numbers	Weeks post-inoculation					
		0	12	20	28	32	36
Ka/W	6	12.3 ± 1.0[b]	39.5 ± 7.8	36.2 ± 6.9*			
Ka/O	6	12.5 ± 0.9	44.1 ± 3.8	56.8 ± 5.5*	68.8 ± 3.7**	68.6 ± 7.5*	61.4 ± 9.4
Control	6	12.6 ± 0.7	40.1 ± 6.4	47.5 ± 9.0	49.0 ± 9.0	46.2 ± 6.9	47.8 ± 8.9

The asterisks indicate statistically significant differences between the scrapie-affected mice and the age-matched control mice (Student's t test: * $p < 0.05$; ** $p < 0.001$)

[a] Ka/scrapie weight-loss-type prion (Ka/W)- and Ka/scrapie obesity-type prion (Ka/O)-affected ICR mice at third passage were analyzed

[b] Average ± standard deviation (gram)

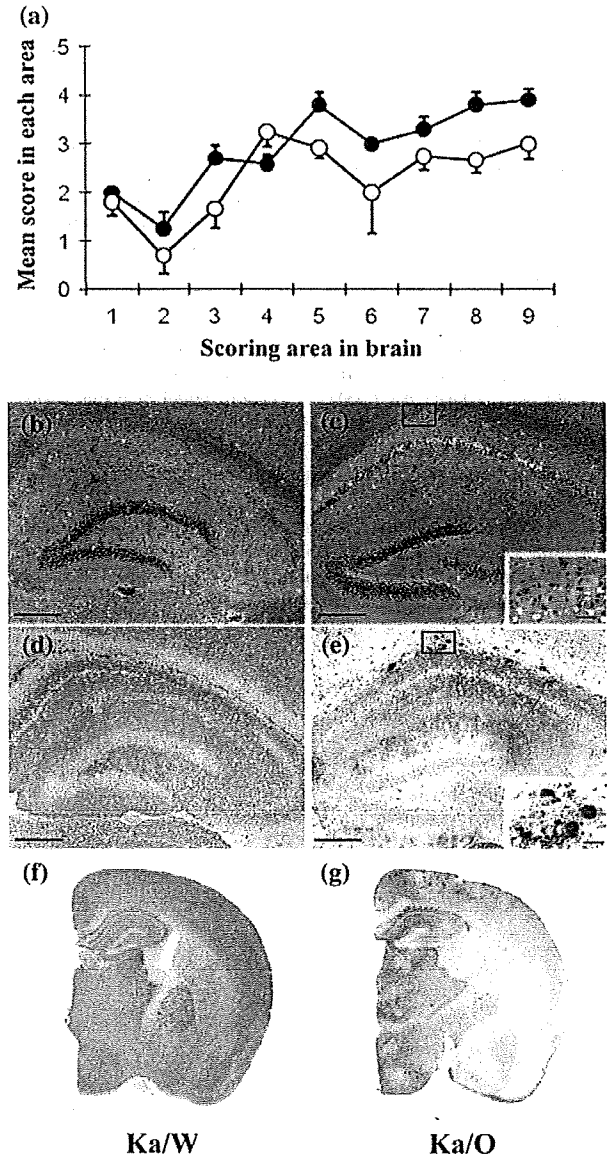

Fig. 1 Neuropathological analysis of the Ka/scrapie weight-loss-type prion (*Ka/W*)- and Ka/scrapie obesity-type prion (*Ka/O*)-affected mice. **a** Lesion profile of the affected mice. The vacuolation in the following brain regions was scored on a scale of 0–5 (mean values): *1* dorsal medulla, *2* cerebellar cortex, *3* superior cortex, *4* hypothalamus, *5* thalamus, *6* hippocampus, *7* septal nuclei of the paraterminal body, *8* cerebral cortex at the levels of the hypothalamus and the thalamus, and *9* cerebral cortex at the level of the septal nuclei of the paraterminal body [18]. *Filled circle* Ka/W ($n = 5$), *open circle* Ka/O ($n = 5$). A section of the hippocampus of the affected mice was stained with hematoxylin and eosin (**b**, **c**), and immunostaining was performed by using the monoclonal antibody (mAb) SAF84 (**d**–**g**). The coronal sections at the level of the hippocampus are shown (**f**, **g**). The *insets* in the lower right corners (**c**, **e**) are enlarged images of the *small boxes* in the corresponding panels. The *bar* represents 200 μm in **b**–**d** and 25 μm in the *insets* of **c** and **e**

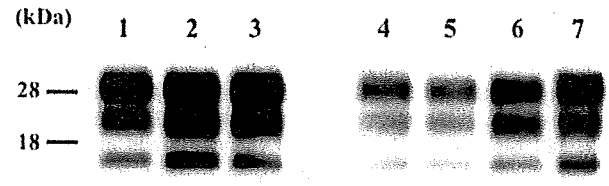

Fig. 2 Western blotting (WB) analysis for detecting proteinase-K-digested prion protein (PrPcore) in the brains of Ka/scrapie weight-loss-type prion (Ka/W)- and Ka/scrapie obesity-type prion (Ka/O)-affected mice. *Lanes 1–3* Ka/W-affected mice, *lanes 4–6* Ka/O-affected mice, *lane 7* Ka/scrapie. *Lanes 1* and *4*: mice in the first passage; *lanes 2* and *5*: mice in the second passage; and *lanes 3* and *6*: mice in the third passage. The equivalent of 0.25 μg of brain tissue was loaded into each lane. PrPcore was detected using the monoclonal antibody (mAb) T2 [19]. The molecular markers are shown on the *left* (kDa)

scrapie-affected sheep and that one of these strains becomes dominant during prion propagation in mice. Our results suggested that the Ka/W strain was the dominant strain in the brain of Ka/scrapie-affected sheep, while the Ka/O strain seemed to be the less dominant strain, which may have been inefficiently selected during interspecies transmission.

In this study, we examined the biological characteristics of prions in the most recent case of scrapie in Japan. On the basis of our results, we conclude that multiple prion strains coexist in a scrapie-affected sheep. To elucidate the molecular epidemiology of prion diseases, further studies should be conducted to clarify the mechanism underlying the emergence of new prion strains.

Acknowledgments All animal experiments were reviewed by the Committee of the Ethics on Animal Experiment of the National Institute of Animal Health. We are thankful to Naoko Tabeta and Mutsumi Sakurai for their technical assistance. This study was supported by a grant-in-aid from the BSE and other Prion Disease Control Project of the Ministry of Agriculture, Forestry, and Fisheries, Japan.

References

1. Prusiner SB (1991) Molecular biology of prion diseases. Science 252:1515–1522
2. Kimberlin RH, Walker CA, Fraser H (1989) The genomic identity of different strains of mouse scrapie is expressed in hamsters and preserved on reisolation in mice. J Gen Virol 70:2017–2025
3. Bruce ME (1993) Scrapie strain variation and mutation. Br Med Bull 49:822–838
4. Shinagawa M, Takahashi K, Sasaki S, Doi S, Goto H, Sato G (1985) Characterization of scrapie agent isolated from sheep in Japan. Microbiol Immunol 29:543–551
5. Horiuchi M, Nemoto T, Ishiguro N, Furuoka H, Mohri S, Shinagawa M (2002) Biological and biochemical characterization of sheep scrapie in Japan. J Clin Microbiol 40:3421–3426

6. Hirogari Y, Kubo M, Kimura KM, Haritani M, Yokoyama T (2003) Two different scrapie prions isolated in Japanese sheep flocks. Microbiol Immunol 47:871–876
7. Masujin K, Shu Y, Yamakawa Y, Hagiwara K, Sata T, Matsuura Y, Iwamaru Y, Imamura M, Okada H, Mohri S, Yokoyama T (2008) Biological and biochemical characterization of L-type-like bovine spongiform encephalopathy (BSE) detected in Japanese black beef cattle. Prion 2:123–128
8. Furuoka H, Yabuzoe A, Horiuchi M, Tagawa Y, Yokoyama T, Yamakawa Y, Shinagawa M, Sata T (2007) Species-specificity of a panel of prion protein antibodies for the immunohistochemical study of animal and human prion diseases. J Comp Pathol 136:7–9
9. Bessen RA, Marsh RF (1992) Biochemical and physical properties of the prion protein from two strains of the transmissible mink encephalopathy agent. J Virol 66:2096–2101
10. Collinge J, Sidle KC, Meads J, Ironside J, Hill AF (1996) Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. Nature 383:685–690
11. Parchi P, Capellari S, Chen SG, Petersen RB, Gambetti P, Kopp N, Brown P, Kitamoto T, Tateishi J, Giese A, Kretzschmar H (1997) Typing prion isoforms. Nature 386:232–234
12. Somerville RA, Chong A, Mulqueen OU, Birkett CR, Wood SC, Hope J (1997) Biochemical typing of scrapie strains. Nature 386:564
13. Kuczius T, Groschup MH (1999) Differences in proteinase K resistance and neuronal deposition of abnormal prion proteins characterize bovine spongiform encephalopathy (BSE) and scrapie strains. Mol Med 5:406–418
14. Yokoyama T, Kimura KM, Ushiki Y, Yamada S, Morooka A, Nakashiba T, Sassa T, Itohara S (2001) In vivo conversion of cellular prion protein to pathogenic isoforms, as monitored by conformation-specific antibodies. J Biol Chem 276:11265–11271
15. Puoti G, Giaccone G, Rossi G, Canciani B, Bugiani O, Tagliavini F (1999) Sporadic Creutzfeldt–Jacob disease: co-occurrence of different types of PrP^{Sc} in the same brain. Neurology 53:2173–2176
16. Kimberlin RH, Walker CA (1978) Evidence that the transmission of one source of scrapie agent to hamsters involves separation of agent strains from a mixture. J Gen Virol 39:487–496
17. Collinge J, Clarke AR (2007) A general model of prion strains and their pathogenicity. Science 318:930–936
18. Fraser H, Dickinson AG (1968) The sequential development of the brain lesion of scrapie in three strains of mice. J Comp Pathol 78:301–311
19. Hayashi HK, Yokoyama T, Takata M, Iwamaru Y, Imamura M, Ushiki YK, Shinagawa M (2005) The N-terminal cleavage site of PrP^{Sc} from BSE differs from that of PrP^{Sc} from scrapie. Biochem Biophys Res Commun 328:1024–1027