

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

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**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 <sup>Sc</sup>	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
PrP <sub>core</sub>	Proteinase-K-digested PrP <sup>Sc</sup>
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<sup>Sc</sup>) in the CNS and lymphoid tissues; PrP<sup>Sc</sup> 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<sup>Sc</sup> 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 anastasia 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<sup>A/A</sup>; PrP<sup>A</sup> 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

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

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

<sup>a</sup> Number of infected mice/number of inoculated mice

<sup>b</sup> 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)

<sup>c</sup> Average ± standard deviation (days)

<sup>d</sup> 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<sup>Sc</sup> types and their distributions in the Ka/O- and Ka/W-affected mice were different (Fig. 1d–g). Punctate and fine granular PrP<sup>Sc</sup> 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<sup>Sc</sup> 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<sup>Sc</sup>, such as the glycoform ratio and the molecular mass of proteinase-K-digested PrP<sup>Sc</sup> (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<sup>Sc</sup> (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

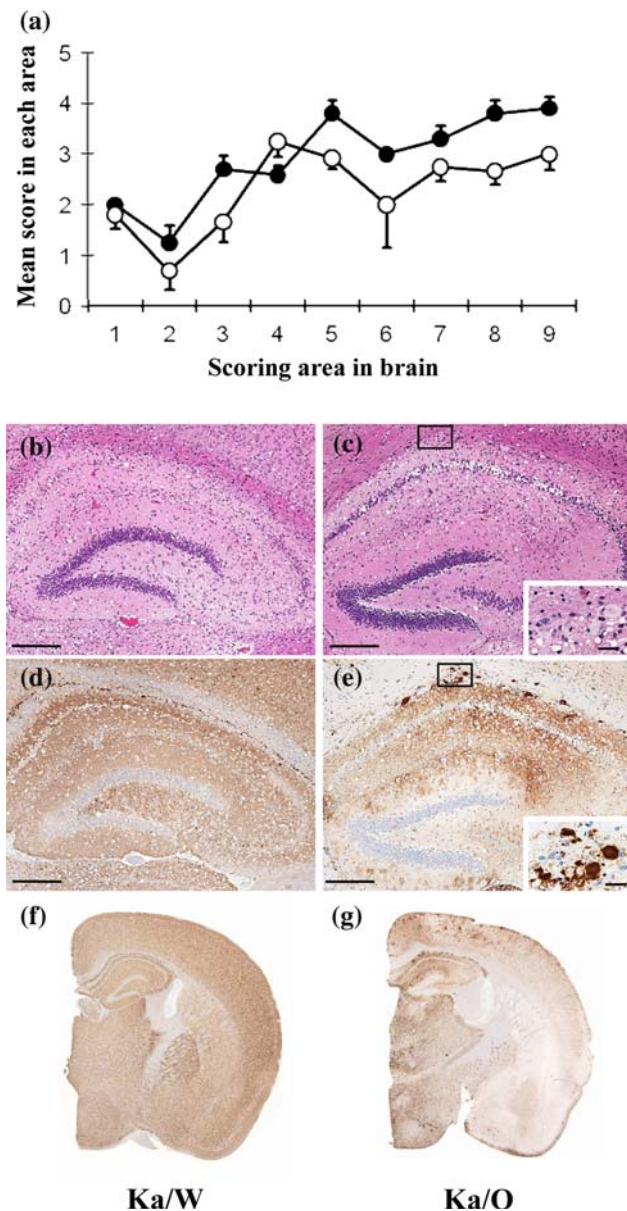
**Table 2** Body weights of the Ka/W- and Ka/O-affected wild-type ICR mice

Inoculum <sup>a</sup>	Mouse numbers	Weeks post-inoculation					
		0	12	20	28	32	36
Ka/W	6	12.3 ± 1.0 <sup>b</sup>	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)

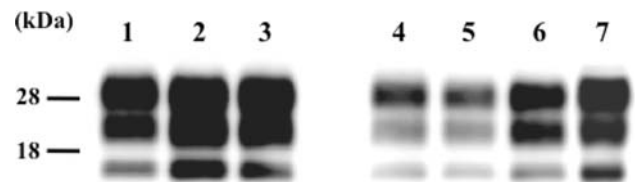
<sup>a</sup> Ka/scrapie weight-loss-type prion (Ka/W)- and Ka/scrapie obesity-type prion (Ka/O)-affected ICR mice at third passage were analyzed

<sup>b</sup> 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  $\mu\text{m}$  in **b–d** and 25  $\mu\text{m}$  in the insets of **c** and **e**

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



**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  $\mu\text{g}$  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)

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.

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