

## Review

# Japanese Scrapie Cases

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(Received April 24, 2000. Accepted May 22, 2000)

**SUMMARY:** Worldwide attention has been given to scrapie, because bovine spongiform encephalopathy (BSE) could be experimentally transmitted to sheep. This ovine form of BSE was clinically identical to scrapie. In Japanese scrapie cases, a majority of the diseased sheep were from Suffolk, while 8 cases were from Corriedale. It is very likely that sheep-to-sheep transmission of scrapie has taken place in Obihiro, Hokkaido. Normal prion protein may play a role in the morphoregulatory signaling pathway, which orchestrates the specificity of a particular cellular response. Over-expression of normal prion protein in mice cause neurodegenerative disorders. Recently, *Prnd* was identified downstream of the mouse prion protein gene (*Prnp*), and encodes 179 amino acids and a prion protein (PrP)-like protein designated doppel (Dpl). Dpl was upregulated in the central nervous system of two PrP-deficient lines of mice, as well as in prionless cell lines. Dpl caused neurodegeneration similar to that caused by PrP. Linked expression of *Prnp* and *Prnd* may cause several neurodegenerative disorders.

## 1. Introduction

Prions cause degenerative disease of the central nervous system (CNS) in both humans and animals (1-4). Prion proteins are composed of the scrapie isoform of the prion protein (PrP<sup>Sc</sup>), and the normal cellular isoform of the prion protein (PrP<sup>C</sup>) (1). Investigations of prion diseases have taken on new importance in the wake of reports of an atypical, variant Creutzfeldt-Jakob diseases (5-8). All of the cases have been reported from Great Britain, Ireland, and France to date. It seems likely that bovine prions from bovine spongiform encephalopathy (BSE) were transmitted to humans through the consumption of tainted beef products. Worldwide attention has been focused on scrapie because of experimental results revealing the oral transmission of BSE to sheep. If contaminated meat and bone meal has been fed to sheep, then it is possible that an ovine form of BSE (9), clinically identical to scrapie, exists.

Postmortem neuropathological findings usually show gross spongiform degeneration in the brain (2) due to vacuolation in the dendritic and axonal processes and in the cell bodies of neurons. Proliferation of the astroglial cells is also common, along with the presence of amyloid plaques which contain fibrils of a disease-specific form of the host cell surface. On normal cells, this protein (PrP) is proteinase K sensitive (PrP<sup>C</sup>) (10), but becomes resistant to digestion (PrP<sup>Sc</sup>) during the disease process (11). The agent appears to be intimately involved with this proteinase K-resistant protein (PrP<sup>Sc</sup>). In this report, the epidemiology of Japanese scrapie is described. In the latter part, mechanisms for the pathogenesis and role of PrP<sup>C</sup> are reviewed.

## 2. Scrapie agent in sheep

Sheep scrapie was originally thought to be a genetic disorder, due to the long incubation period and vertical transfer (12). Early experiments confirmed that the disease was horizontally

transmissible among sheep by both natural means and experimentally by injection of healthy animals with scrapie brain homogenates (13, 14). The natural infection route is thought to occur by maternal transmission (and possibly, in rare case, by sire transmission), scarification on fences, or exposure to placental tissues in the field. Experiments with sheep revealed that inoculation through various routes affected the incubation period of the disease; the incubation period was longer if the animals had been inoculated subcutaneously than if they had been inoculated intracerebrally (15). Inoculation of Cheviot sheep with a short incubation strain of scrapie indicated that the incidence of scrapie in the offspring was directly influenced by the incidence of scrapie observed in the parents. If both parents were negative for scrapie, the offspring would be negative. However, if one parent was positive, the progeny had a greater than 40% chance of contracting scrapie, and if both parents were positive for scrapie, 70% of the lambs were likely die from scrapie (16). Matings between Cheviot and Suffolk sheep and subsequent experiments upon the offspring led to the conclusion that the scrapie incubation period was principally controlled by a single autosomal gene termed *Sip* (Scrapie Incubation Period) with two alleles designated sA and pA (16).

There is the question whether sheep that survive after inoculation with a particular strain of scrapie are actually resistant or whether the incubation period is so long until prior to the appearance of clinical signs, and sometimes animals die without any symptoms. This extended incubation period may be important, giving that sheep are capable of passing scrapie on through maternal transmission. In the case of Dickinson's experiment (1968), the whole flock was inoculated, and "resistant" sheep have been used to produce a scrapie-resistant flock. It was Dickinson himself who questioned the idea of resistance and whether the animals were in fact infected with scrapie, but their lifespan of the animals coupled with the incubation period of the scrapie meant that the disease did not reach the clinical stage (17). Unfortunately, attempts to create scrapie-resistant flocks are also hampered by the accelerated action of other scrapie strains in some breeds. This question of resistance may also be important when

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addressing the species barrier, which will be described later.

### 3. Epidemiology of scrapie

Scrapie affects most breeds of sheep. It has a widespread distribution in the world but has been successfully eradicated in Australia, where it was established only locally and briefly, following importation (18) from New Zealand, and from some locations and flocks in Iceland. Some countries in South America and Europe are also likely free of the disease.

In Japan, since the first outbreak of scrapie in Obihiro of Hokkaido in 1984 (19, 20), postmortem brain tissues have been attained in more than 20 cases of pathologically confirmed scrapie. Since attention was drawn to the syndrome of ovine scrapie, particularly its lateral transmission in Obihiro area, more frequent diagnoses have been undertaken than before by various animal health stations in Hokkaido (21). During the period from 1984 to 1986, approximately 50 cases were reported in Hokkaido, and more than 10 cases outside Hokkaido; i.e., one scrapie-like disease incidence in Yamagata in 1983, two pathologically confirmed cases in Yamagata and Fukushima, respectively, in 1984, and additional scrapie-like disease cases in Iwate, Ibaraki, and Chiba in 1984. In 1987, three incidences were reported in Kanagawa, and the presence of scrapie was pathologically confirmed in one case by brain tissue inspection (Fig. 1-3). In regard to the duration of ovine scrapie disease, it is a 3- to 6-month period on average in Suffolk sheep. It is quite probable that sheep-to-sheep transmission of scrapie took place in Obihiro, Hokkaido, since approximately 50 cases in Suffolk and three cases in Corriedale originated from sheep in Obihiro or those transferred from

there (22).

From 1989 to 1990, four Corriedale sheep (less than 2 years old) died for no obvious reason. Based on histopathological examination, a diagnosis of scrapie was made in Hokkaido (23) as well as in Fukushima and Miyazaki (Fig. 1). In addition, a few cases of scrapie with pruritus have been reported in Suffolk and Corriedale sheep in Japan over the past 10 years (21-25) (Table 1). All of these sheep had been transferred from a farm in Hokkaido with a history of incidence of scrapie in Suffolk sheep except in one case in Yamagata in 1983, and in one case in Fukushima in 1984.

It was recognized that all of these cases with the ataxia type of scrapie were enzootic, and all of the sheep with the ataxia type of scrapie belonged to the Corriedale breed (Table 1). In 1989 Obihiro case, a 19-month-old Corriedale ewe suffering from cryptogenic dysstasia for 2 months was sacrificed and examined by histological method. The sheep was smaller than healthy sheep of the same age. No significant gross pathological lesions were found in any of the organs including CNS. Histopathologically, the majority of vacuolated nerve cells and spongiform lesions were observed mainly in the brain stem (25). Scrapie-associated fibril (SAF) proteins were also detected in the brain extract of this case by Western blot analysis (WBA). In 1991 Fukushima case, the sheep was from a farm with a history of incidence of scrapie prior to its transference to an animal facility. She was two years old and did not show any clinical symptoms, even when she was kept in the same facility with another scrapie-affected sheep that

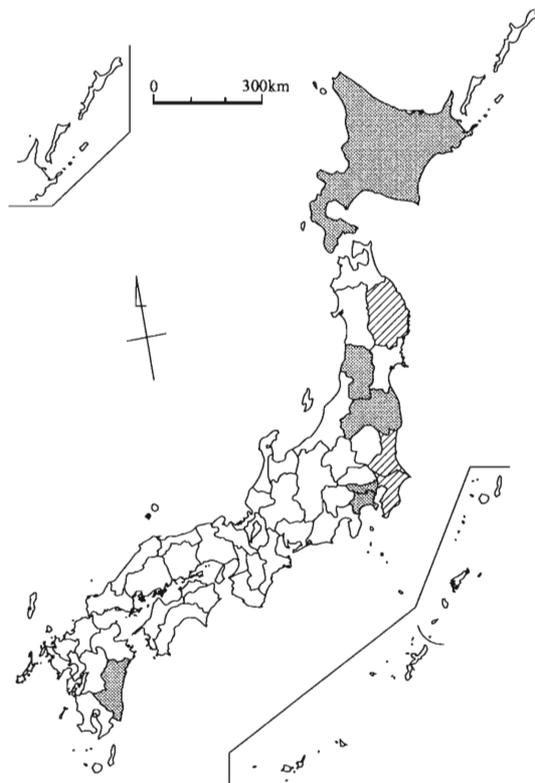


Fig. 1. Distribution of scrapie-affected sheep by prefecture, 1984 to 1999.

- : Affected sheep pathologically confirmed
- ▨: Affected sheep confirmed by personal communication



Fig. 2. Pruritus observed in the shoulder of a scrapie-affected sheep in Kanagawa.

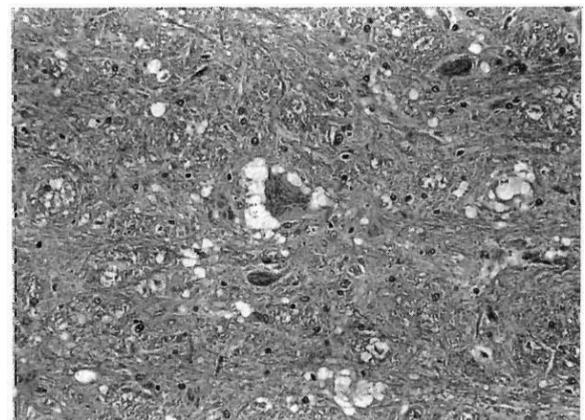


Fig. 3. Single and multiple vacuoles in nerve cells of the nucleus olivaris in a scrapie case in Hokkaido. H-E staining (×600).

Table 1. Clinical sign of scrapie in Japan

	Pruritus type	Ataxia type	Asymptomatic type
Age of sheep	3-4 years old	1-2 years old	2 years old
Number of sheep*	28	4	1
Reported in	1984-1987	1988-1999	1991
Breed of sheep	Suffolk, Corriedale	Corriedale	Corriedale
Symptoms	Pruritus, Ataxia Paralysis	Ataxia Paralysis	-
Duration of disease	2-3 months	0.5-1 month	-
Histopathology	Spongiform Encephalopathy	Spongiform Encephalopathy	Spongiform Encephalopathy

\* Number of sheep observed in the National Institute of Animal Health, Ibaraki.

was devoid of pruritus. The sheep was three months pregnant on sacrifice. Vacuolated nerve cells were found throughout the brain stem. SAF protein was detected in the brain and spleen extract by WBA (24). In most of the recent Japanese scrapie cases, scratching has not been a symptom. Therefore, veterinarians who examine sheep clinically and pathologically should consider this asymptomatic condition recently reported in Japan.

#### 4. Transmission process of infectious prion and species barrier

One model for the transmission of scrapie hypothesis that a PrP<sup>Sc</sup> molecule may combine with a PrP<sup>C</sup> molecule to give a heterodimer. The PrP<sup>C</sup> molecule is transformed to a PrP<sup>Sc</sup> molecule, which then goes on to transform other PrP<sup>C</sup> molecules (1). This model does not exclude the presence of a nucleic acid genome within the protein coat. Infectious extracts enriched with SAF and PrP<sup>Sc</sup> can be partially denatured and subjected to SDS-polyacrylamide electrophoresis without significant loss of infectivity (26), indicating that if a nucleic acid is present, it must be very tightly associated with the protein. As a further twist, there are reports that infectivity can be dissociated from the production of PrP<sup>Sc</sup> (27, 28). Not all PrP<sup>Sc</sup> appears to be infectious. There are around 10<sup>5</sup> PrP<sup>Sc</sup> molecules per unit of infectivity (29), and Rubenstein has performed experiments in which PrP<sup>Sc</sup> is degraded without a correspondingly large decrease in infectivity titers (30).

The structure of PrP has received much attention due to the fact that only structural differences have been substantiated between PrP<sup>C</sup> and PrP<sup>Sc</sup>. The secondary structure and conformational differences have been studied by Fourier transform infrared, fluorescence, and circular dichroism spectroscopy (31-33). The most powerful way to elucidate the tertiary structure of a protein at high resolution is by X-ray crystallography, but the PrP protein has not yet been crystallized due to difficulties in obtaining sufficient material and overcoming noncrystalline aggregation effects. At this moment X-ray crystallography does not provide the detailed structure of prion protein. A three-dimensional (3D) model structure of PrP has been hypothesized by Huang et al. (34). It has been observed spectroscopically that PrP<sup>C</sup> is mostly  $\alpha$ -helix, whereas PrP<sup>Sc</sup> has a high  $\beta$ -sheet component (35).

The most vexing problems in the hypothesis for prion infectivity are the presence of at least 20 well characterized scrapie strains and the ability of these strains to be passaged through different animal species without alteration of the incubation period or pathology. The hypothesis for prion infectivity suggests that strain variation is induced by different

conformational and posttranslational changes. However, it is hard to imagine 20 or more stable pathogenic conformational changes in one protein which has the same amino acid sequences. Since some strains can be passaged unchanged between two species, each has its own PrP primary structure. Bessen and Marsh (36) suggested that there were differences in conformation on the basis of different rates of digestion of PrP<sup>Sc</sup> between two strains of transmissible mink encephalopathy (TME) by proteinase K, and that after digestion, the PrP<sup>Sc</sup> from one strain was 10 amino acid residues larger than the PrP<sup>Sc</sup> from the other strain. This differential digestion of PrP<sup>Sc</sup> was noted more than a decade ago in studies on SAFs from different scrapie strains (37). Differences have also been observed in PrP<sup>Sc</sup> between fatal familial insomnia (FFI) and familial Creutzfeldt-Jakob disease (CJD) cases. In this case of FFI, the PrP isoforms differed in relative abundance of the glycosylated forms and in the protease cleavage site (38). Monari et al. concluded that because FFI and CJD PrP protein differ only at codon 129, that difference alters the conformation of the protein (38). These FFI and CJD models rely on their respective protein conformations as the basis for differential targeting to different populations of neurons within the brain, to produce the characteristic brain lesions associated with the individual strain of scrapie (39). These FFI and CJD results are interesting, but do not necessarily answer all of the questions posed by strain determination.

When the scrapie agent is passaged in a species of animal different from the one in which it has been replacing, the incubation period is prolonged at the first passage, setting a new but predictable length of time on subsequent passages. This phenomenon is known as the "species barrier" effect (40). Diseases do not always occur after inoculation with scrapie-infected brain homogenates; some scrapie agent strains do not produce clinical signs in certain animal species and other strains appear to be specific to a particular species. Attempts to modify the species barrier by sensitizing the new host by exposure to tissues of the old host before inoculation met with only slight effects (40). The presence of tissue from a different species might cause immunological effects upon inoculation. This immunological effect might be observed, if a post protein formed a part of the infectious agent. The more closely related the two species, the smaller the species barrier. The effect would be specific and would only affect the first passage within a different species (41).

#### 5. Role of PrP<sup>C</sup> and PrP-like protein

Recently, we have established two distinct systems of cell lines, one derived from the brain of mice homozygous for

### Transacting effect of prion gene complex

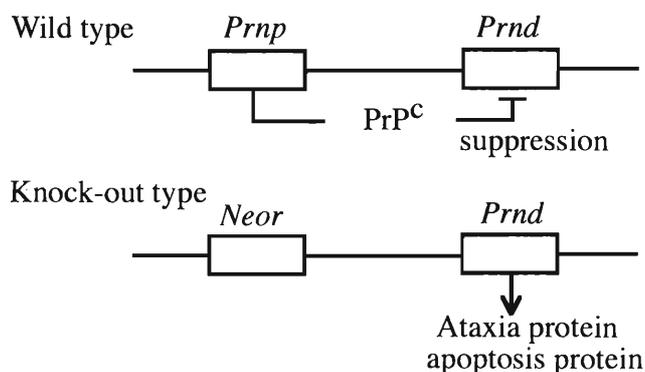


Fig. 4. Structure and relationship of the *Prnp* and *Prnd* genes.

disrupted *Prnp* gene and the other from the brain of wild-type mice. Cells were immortalized by recombinant retrovirus vector-mediated transduction of the SV40 T-antigen gene. The prionless cell line shows a short neurite extension in comparison with wild type cell line after treatment with phorbol 12-myristate 13-acetate (PMA). The wild type cell line survived in serum-free medium with or without PMA, whereas the prionless cell line dramatically died within 24 h, showing apoptosis. This cell death is prevented by transfection of *Prnp* (42). Our results support the idea that the loss of PrP<sup>C</sup> function plays some pathogenic role in prion disease. PrP<sup>C</sup> might thus support a receptor, signal transduction, or iron channel function (43). If so, the role of PrP<sup>C</sup> will control the survival or death of neuronal cells in vivo. Since many neurons are activated for a long time survive and may undergo cell death in vivo.

In a previous report, the novel locus *Prnd* is identified at 16 kb downstream of the mouse *Prnp* (Fig. 4) and encodes a 179 residue of the PrP-like protein designated doppel (Dpl)(44). *Prnd* generates major transcripts of 1.7 and 2.7 kb as well as some unusual chimeric transcripts generated by intergenic splicing with *Prnp*. Dpl is expressed in the CNS of two PrP-deficient (*Prnp*<sup>0/0</sup>) lines of mice, both of which develop late-onset ataxia, suggesting that Dpl may provoke neurodegeneration. The wild type (*Prnp*<sup>+/+</sup>) line showed Dpl in the CNS only in the embryonic stage. Since Dpl-caused neurodegeneration is similar to PrP, the linked expression of the *Prnp* and *Prnd* genes may play a previously unrecognized role in the pathogenesis of several neurodegenerative disorders.

### 6. Pathology

In recent cases reported in Great Britain, 79 exhibited pruritus and emaciation; 36 showed pruritus, emaciation, and hyperaesthesia; and 18 displayed these three signs in addition to ataxia. However, 26% of dead sheep diagnosed as scrapie based on histopathological examination were without clinical signs. It was concluded that scrapie cannot be consistently diagnosed based on clinical signs alone, even by experienced veterinary surgeons (45, 46). Even in our cases, scrapie in sheep has not been easily diagnosed based on clinical signs including persistent pruritus, weight loss, nervousness, and/or lack of motor coordination. The forms of natural scrapie are more diverse than usually described in the veterinary literature (47).

Histologically, vacuolation in the cytoplasm of neurons is

conspicuous in the medulla oblongata. In Japanese cases, it is also evident in the neurons of the thalamus (19). Proliferation of astrocytes is also a conspicuous feature. By immunohistochemistry of tissue sections for PrP<sup>Sc</sup>, positive staining was regularly observed in the hippocampus, thalamus, and medulla oblongata in our cases (19).

### 7. Preclinical diagnosis of scrapie

In Japan and Great Britain, WBA is applied to biopsies of lymph nodes (48, 49). Sometimes this is successful for detecting experimentally or naturally scrapie-affected animals. In Europe, PrP<sup>Sc</sup> has been immunohistochemically detected in tissue sections of the tonsil from affected sheep (50).

Our findings indicate that scrapie agents can be detected from the placentae of naturally infected animals through inoculation into mice intracerebrally (24). It takes approximately 6 months to detect histological lesions in the mice brain. Therefore, it may become possible to diagnose scrapie infection in a large number of sheep by the isolation of these transmissible agents from the placentae during the preclinical stage without requiring any surgical procedure (51). Recently, capillary electrophoresis was used to detect PrP<sup>Sc</sup> in the blood from naturally infected sheep, for preclinical diagnosis (52).

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