REVIEW

Scrapie

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Abstract

Scrapie and other transmissible spongiform encephalopathies (TSEs) are characterized by similar pathology, biochemistry and genetics. The PrP protein and its conversion to the disease-related isoform, PrP^{SC}, are crucial for the development of all TSEs. Although scrapie is more often studied in laboratory rodents, it is not a natural disease of these animals, and much can be learned from the normal hosts, sheep. Disease incidence is linked to polymorphisms and mutations of the PrP gene. The complex relationships between PrP genotype and the survival of sheep subjected to scrapie infection are now being investigated in terms of the different structure of the PrP protein molecules produced by each allele. It is these structures and their differing abilities to convert to PrP^{SC} that hold the key to understanding why TSEs occur.

Index Entries: Scrapie; CJD; PrP; prion; genotype; disease incidence.

1. Introduction

Scrapie, which affects sheep and goats, is the best studied of a group of fatal neurodegenerative diseases affecting the central nervous system (CNS) of many mammals: the transmissible spongiform encephalopathies or TSEs. Scrapie is experimentally transmissible to mice and hamsters and in these laboratory rodents, different strains of scrapie have been demonstrated and cloned *(1)*. There are many hypotheses on the etiology of the disease. The unusual physicochemical resistance of the infectious agent (for example. **ref. 2**) has led to suggestions that it may be made up mostly or totally of protein. A disease which is both the result of, and transmitted by, such a rogue protein has been variously described as a prion disease *(3)*, an infectious amyloidosis *(4)* or the result simply of a genetic mutation *(4–6)*. Others, sticking to the dogma that genetic information has to be carried in nucleic acid, believe it to be a virus *(7,8)* or a virino (mostly protein but with a small nucleic-acid component; *see* **ref. 9**). Many years of effort have gone into the search for the DNA or RNA believed to form part of such structures, so far without success. Despite what many textbooks say, the nature of the agent is not finally resolved *(10)* and indeed a full explanation of the known characteristics of the TSEs is difficult whichever theory is being proposed.

2. Clinical Signs

Clinical signs of scrapie in sheep *(11)* start with mildly impaired social behavior followed by locomotor incoordination or ataxia with trembling. Pruritis and wool loss can result from the animal attempting to relieve what seems to be an intense itching by scratching against fenceposts or by biting the affected area, and these clinical signs can last from 2 wk to 6 mo. Descriptions in the literature vary in different outbreaks of scrapie. For example, in a group of scrapie-affected sheep in Shetland between 1985–1991 *(12)*, most animals showed signs of pruritis and emaciation, others had pruritis, emaciation, and hyperaesthesia, and others showed all these signs plus ataxia. These authors have also described sheep apparently with scrapie and such a short clinical course that they are simply found dead. In a report of clinical signs in scrapie-affected sheep in Japan *(13)*, some animals (Suffolks and Corriedales) showed signs of pruritis, but others (Corriedales) had died for no obvious reason, scrapie was diagnosed after histopathological examination.

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Table 1
Normal and Disease-Related PrP

	Normal	Disease-related
Name	PrP^C	PrP^{SC}
PK	Sensitive	Partially resistant
Detergent	Soluble	Insoluble
Length	~250 amino acids	~250 amino acids
Structure	alpha helix and loops	beta sheet
Glycosylated	2 sites	2 sites
Molecular weight(-PK)	33–35 kilodaltons	33–35 kilodaltons
Molecular weight (+PK)	degraded	27-30 kilodaltons
Antigenicity	bind to same antibodies	bind to same antibodies
Found	cell surface, GPI anchored	fibrils, deposits
Expression	many tissues	brain, CNS, lymph nodes, spleen, tonsil
Expression in disease	protein levels constant	protein levels increases
Turnover	rapid	slow
Scrapie infectivity	does not co-purify	co-purifies

[a]Abbreviations: PK, proteinase K; GPI, glycosylphosphatidylinositol.

Lesions in the brain include neuronal degeneration with the formation of vacuoles, proliferation of astroglial cells, but no demyelination or other overt inflammatory responses. One study of scrapie-affected sheep in Britain described seven different patterns of vacuolation *(14)* and the variation both in symptoms and clinical phase may either be the result of sheep-breed characteristics or may indirectly indicate the presence of strains of scrapie that target and damage specific brain areas. These features develop in the later stages of the incubation period and it is very difficult to detect (by histopathology) those animals with scrapie that are not yet visibly affected by the disease.

3. The Nature of the Infectious Agent

The only consistent marker of scrapie and a hallmark of all TSEs, is an abnormal aggregated form of a glycoprotein protein called PrP. The normal form of the PrP protein (PrP^C) is found attached to neuronal cell surfaces via a glyocophosphatidylinositol (GPI) anchor. The function of PrP^C is not known, however there are several intriguing hints that it may have a role in T-cell activation *(15)*, neuronal electrophysiology *(16,17)* and sleep continuity *(18)*. The aggregated form of PrP is partially resistant to proteases and is a disease specific marker, usually represented as PrP with superscript giving the disease, e.g., PrP^{SC} in sheep, PrP^{BSE} in cattle and PrP^{CJD} in humans. PrP^{SC} is also closely associated with scrapie itself because preparations enriched for infectivity contain high concentrations of aggregated PrP protein. The main differences between the two isoforms of PrP are listed in **Table 1**.

In one of the major hypotheses on the nature of the scrapie agent, PrP^{SC} is itself the infecting entity agent or "prion." In this theory, PrP^{SC} arising from an infection or from a mutant PrP gene acts as a catalyst in the conversion of endogenous PrP^C into yet more PrP^{SC} thus either destroying the normal function of the protein or poisoning the neurons and resulting in degenerative disease *(19)*. Natural scrapie in sheep tends to be familial in appearance and has been said to result from a recessive gene, the protein product of which causes disease *(20)*. However, once scrapie occurs, it is often transmissible experimentally, not the case with proven genetic diseases; for example, thalassemia *(21)*.

In another hypothesis, PrP^C would act as a receptor for the scrapie agent *(22)*. Mutant forms of the PrP protein would have a stronger affinity for the agent than the wild-type protein, resulting in disease only in the mutant genotype. Such a

close association between PrP sequence and successful infection would fit the genetic data just as well as the "genetic disease" hypothesis. Indeed, it is almost impossible to tell the difference between a simple genetic disease and genetic control over susceptibility to a widespread infection. However, this theory could encompass both a viral etiology and a "protein-only" etiology as all it requires is some form of recognition and binding to the infecting agent.

Although some reports have demonstrated a dissociation between PrP fibrils and infectivity *(7,23)* the usual finding is a partial co-purification of PrP^{SC} and infectivity. A ratio of 100,000 PrP molecules to one infectious particle has been published *(24)*, but this author also pointed out that minor components that co-purify with PrP^{SC} and infectivity need to be eliminated before concluding that PrP is the sole component of the infectious agent. Infectious brain preparations contain nucleic acids, for example. Although these could be nonspecific host genomic degradation by-products of the extraction procedures, some workers have reported nucleic acids to be found specifically in infected brain extracts and regard them potentially as the information molecule of a scrapie virus *(25)*.

Proponents of yet another hypothesis believe an independent scrapie genome, probably a small nucleic acid, would confer scrapie strain characteristics while depending on the aggregation of PrP^{SC} for protection from host defense mechanisms *(1)*. The infectious entity is a "virino."

4. Diagnostic Tests

The presence of PrP^{SC} is used both to diagnose a TSE at postmortem and to search for infected tissues within the body of an animal. There are at least two methods for detection of PrP^{SC}. First, the PrP fibrils themselves can be visualized under the electron microscope and are referred to as scrapie associated fibrils (SAF) *(26,27)* or prion rods. Second, the protein can be detected either on blots made from electrophoresis gels or in sections of tissue by means of PrP specific antibodies (Western blots or immunocytochemistry) *(28,29)*. The presence of SAF or PrP^{SC} can be extremely helpful in difficult diagnoses, for example, sheep with no obvious clinical signs that are simply "found dead," were shown to have been affected by scrapie by demonstrating the presence of SAF in brain extracts *(27)*.

PrP^{SC} has also great potential in the development of a preclinical diagnostic test. In a study of sheep of genotypes expected to develop natural scrapie (*see* **Subheading 5.3.**) PrP^{SC} was found in tonsil biopsies at times less than halfway through the expected incubation period and about 1 yr before the expected onset of clinical disease *(30)*. Tonsil biopsies can, relatively easily, be taken from live sheep and cattle and this approach may at last offer a means to detect infected but healthy animals prior to slaughter.

Attempts have been made to identify other TSE-specific substances present in fluids or tissues amenable to biopsy procedures. One of these is a brain protein called 14-3-3 found in cerebrospinal fluid (CSF) of CJD patients, however as 14-3-3 is also elevated in CSF following a stroke, it may only be useful when CJD is already suspected for clinical reasons *(31)*.

The alternative method of detecting infection, by mouse bioassay, is both expensive and time-consuming because of the long incubation periods involved. However, it can be a more sensitive assay than protein determination. For example, PrP^{SC} was detected in brain but not in spleen or placenta of a scrapie-affected pregnant sheep, however, infectivity was transmitted from both the brain and placental homogenates to laboratory mice *(32)*.

5. Genetics of Scrapie Susceptibility

5.1. Scrapie in Mice

The major gene controlling scrapie susceptibility in mice is called *Sinc* (or *Prn-i*) and a wealth of evidence from both traditional genetics and from transgenic studies suggests that this gene is actually the PrP gene (e.g., *33,34*). Mouse lines lacking a functional PrP gene (PrP-null mice) are resistant to scrapie *(35)* and mice differing in PrP gene sequence at amino acid codons 108 and 189 also differ in patterns of incubation period following challenge with certain scrapie strains. These

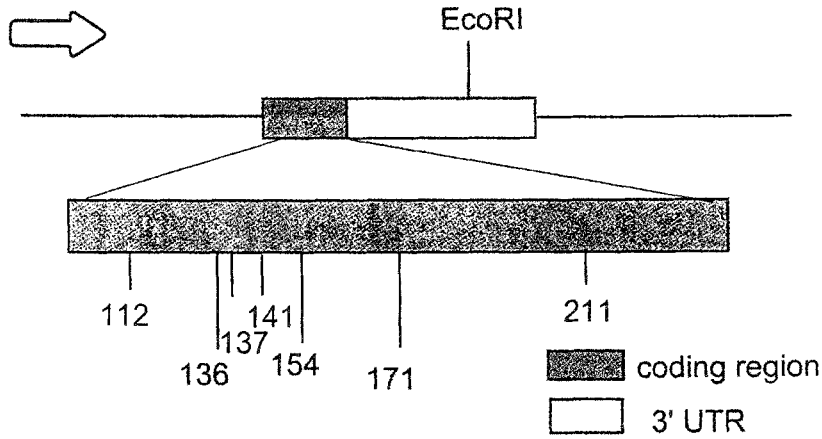

Fig. 1. Map of the sheep PrP gene. Within the rectangle, the shaded area represents the protein coding region and the numbers indicate the polymorphic codons some of which are detailed in **Table 1**. The clear area represents the downstream (3') untranslated region which contains a polymorphic *Eco*RI restriction site. The arrow indicates the direction of transcription.

Table 2
Sheep PrP Codon 136, 154, and 171 Variants

PrP codon		
136	154	171
V[a]	R	Q
A	R	Q
A	H	Q
A	R	H
A	R	R

[a]Single letter amino-acid code: A; alanine; H; histidine; Q, glutamine; R, arginine; V, valine.

patterns are reliably reproducible and are very characteristic. For example, BSE, when transmitted to mouse lines, gives both a distinct incubation period and lesion profile (graph of degree of pathology damage vs brain area). This feature allowed the recent confirmation that BSE in cattle and the new-variant CJD (found in young humans) were caused by the same infectious agent *(36)*.

The lack of variation in the PrP gene of laboratory mouse strains does not reflect the situation in sheep *(37)* and humans *(38)* where many PrP genotypes are found and have been linked to incidence of TSE in sometimes quite complex ways. However, the mouse studies have been (and continue to be) immensely informative about the pathogenesis of TSEs, pointing the way to issues that may be of importance in the etiology of the natural diseases. For example, the use of severe combined immunodeficient (SCID) mice which show resistance to peripheral infection with scrapie, have strongly implicated the immune system as a primary target for replication of infectivity before it is delivered to the CNS and attacks the brain *(39)*. Susceptibility can be regenerated by reconstitution of the SCID mice with wild-type bone marrow *(40)*. Because natural scrapie in sheep is thought to gain entry to the animal via peripheral routes, a more detailed examination of the role of cells of the immune system in early scrapie infection may be warranted in ruminants.

5.2. Experimental Scrapie in Sheep

Polymorphic variants of the PrP gene have been associated with the incidence of both experimental and natural TSEs in sheep *(41,42)* (**Fig. 1** and **Table 2**) and goats *(43)*.

In the Neuropathogenesis Unit (NPU), Cheviot sheep-flock valine at codon 136 (V_{136}) on both PrP alleles (VV_{136}), is linked to short incubation periods (167 d, +/- 5) following subcutaneous (sc) inoculation with a source of scrapie known as SSBP/1. Sheep with longer incubation periods (322 d +/- 16) are heterozygotes with one V_{136} allele and one encoding alanine (VA_{136}) (44). Alanine encoded on both alleles (AA_{136}) is linked with resistance to sc challenge with SSBP/1. Inci-

Table 3
PrP Genotypes of NPU Cheviots and Occurrence of Natural Scrapie

PrP genotype	n	Age at death (scrapie) mean (range)[a]	Lifespan (no scrapie) mean (range)[a]
$VV_{136}RR_{154}QQ_{171}$	22[b]	804 (497-971)	–
$VA_{136}RR_{154}QQ_{171}$	4[b]	1208 (1107-1287)	–
$VA_{136}RR_{154}RQ_{171}$	34	–	1961(1125-3435)
$VA_{136}HR_{154}QQ_{171}$	10	–	1647(1180-2610)
AA_{136}	[c]	[c]	[c]

[a]Days.
[b]Cases occurring since 1990.
[c]This genotype never gets natural scrapie in this flock.

dence of scrapie in NPU Cheviots following challenge with BSE or the scrapie source CH1641 however, does not associate primarily with codon 136 variation. Instead, animals encoding glutamine at codon 171 (Q_{171}) on both PrP alleles (QQ_{171}) succumb to intracerebral (ic) inoculation, whereas those having one allele with arginine (R_{171}) have a much longer incubation period *(44)*. This linkage of SSBP/1-induced experimental disease with the V_{136} allele was confirmed in USA Cheviots *(45)*, so is not specific to the NPU flock.

Although SSBP/1 targets animals carrying V_{136}, a minor influence of codon 171 is seen because VA_{136} animals that are also RQ_{171} have a longer incubation period (364 d +/– 17) than those which are QQ_{171} (260 d +/– 15). Similarly with CH1641 and BSE, although the major effect on incubation period depends on the codon 171 genotype (QQ_{171} being the most susceptible genotype), animals encoding V_{136} (VV_{136} or VA_{136}) have longer incubation periods than AA_{136} sheep *(44)*.

In Suffolk sheep, codon 136 has limited polymorphism, and the PrP V_{136} allele, although it can be found *(46,47)*, is rare. Suffolk sheep do, however, succumb to scrapie and one study in U.S. Suffolk sheep demonstrated that orally administered infectious material would cause disease in a proportion of animals *(48)*. The inoculum was pooled brain and spleen from scrapie-affected Suffolk sheep. Inoculated animals succumbed to disease in 622 d +/- 240 and were all $AA_{136}QQ_{171}$ genotype. Not all $AA_{136}QQ_{171}$ sheep succumbed, but the oral route is known to be less efficient than other routes. No $AA_{136}RQ_{171}$ or $AA_{136}RR_{171}$ animal developed scrapie.

5.3. Natural Scrapie in Sheep

The NPU Cheviot sheep flock is undergoing an outbreak of natural scrapie and PrP genotype variation at amino-acid codons 136, 154, and 171 has been shown to be related to disease incidence. The primary factor in this particular natural scrapie outbreak is the PrP genotype at codon 136 (**Table 3**). The haplotype $V_{136}R_{154}Q_{171}$ was found to be an absolute requirement for scrapie to occur and all homozygous $VV_{136}RR_{154}QQ_{171}$ sheep are currently at high risk of developing scrapie *(49)*. No scrapie cases have occurred in sheep of any of the AA_{136} genotypes. $VA_{136}RR_{154}QQ_{171}$ animals also succumbed to natural scrapie with longer survival times than the homozygotes but not all VA_{136} animals were susceptible. The $VA_{136}RR_{154}$ RQ_{171} and $VA_{136}HR_{154}QQ_{171}$ subgroups are resistant, thus, any direct effect of the $V_{136}R_{154}Q_{171}$ allele was not completely dominant (**Table 3**). The association of R_{171} or H_{154} encoding alleles with reduced susceptibility has also been reported in Ile-de-France sheep (France) and in a flock of Flemish and Swifter sheep (The Netherlands), other breeds in which susceptibility is strongly associated with V_{136} *(50,51)*.

In breeds such as Suffolks and Lacuanes *(52)*, codon 136 has limited polymorphism (*see* **Subheading 5.2.**), and the PrP V_{136} allele is relatively rare. As with experimentally challenged Suffolks *(48)*, the PrP genetics appears to be much simpler than in breeds that do encode V_{136} *(46,53,54)*. In a study of U.S. Suffolk sheep affected by scrapie *(53)*, 31 scrapie sheep from 23 flocks of origin were all (100%) QQ_{171}, significantly different

Table 4
PrP Genotypes and Occurrence of Natural Scrapie in a Flock of Suffolk Sheep[a]

PrP genotype	Healthy sheep number	%	Scrapie sheep number	%
$AA_{136}RR_{154}QQ_{171}$	71	17	62	97
$AA_{136}RR_{154}RQ_{171}$	214	50	2	3
$AA_{136}RR_{154}QH_{171}$	16	4	0	0
$AA_{136}RR_{154}RH_{171}$	10	2	0	0
$AA_{136}RR_{154}RR_{171}$	119	28	0	0
Total sheep	430		64	

[a]ESCA Suffolk flock; from **ref. *54***.

from the healthy sample of 69 animals from 3 scrapie-free flocks which were 51% QQ_{171}, 43% RQ_{171}, and 6% RR_{171}. QQ_{171}, therefore, seemed to control susceptibility in this case. However, in other studies of Suffolk sheep ***(54,55)***, scrapie cases have been found in some RQ_{171} Suffolks (**Table 4**). It is not yet clear what distinguishes these animals from healthy RQ_{171} Suffolks.

Most studies so far have agreed on the protective effect associated with R_{171}, especially when homozygous ***(49,50,52,53,56)*** and PrP genotyping is now available commercially for sheep breeders intent on producing scrapie-resistant sheep. However there is a single report from Japan of a scrapie-affected Suffolk sheep of RR_{171} genotype ***(55)***. It may be that Japanese scrapie targets PrP genotypes differently from European and U.S. Scrapie, but the sheep may also be different genetically. The implications of this finding are not clear at the moment.

6. Is Scrapie a Genetic Disease?

Parry ***(20)*** believed that natural scrapie was caused by a recessive gene. Most sheep PrP geneticists would probably now say that Parry's recessive gene is likely to be PrP, which, in the Suffolk breed that he studied, is linked to scrapie incidence in a recessive manner, PrP QQ_{171} animals are at greatest risk of scrapie. Some of the strongest evidence against the genetic disease hypothesis has come from a study of Cheviot and Suffolk sheep from Australia and New Zealand. Despite both these countries being scrapie-free, it was relatively easy to demonstrate the presence of sheep of highly susceptible PrP genotypes ***(57)*** (**Table 5**). The costly procedures, involving many years of quarantine for imported animals, which Australia and New Zealand adopt to prevent scrapie infection in their sheep flocks ***(58)*** have therefore been well worth the effort as these countries do have susceptible sheep which would succumb to an introduced infection.

There is, however, very strong evidence that some of the human TSEs are genetic in origin; for example, the amino-acid codon 102 proline to leucine change that occurs in Gerstmann-Straussler-Scheinker syndrome ***(59)*** and is linked to disease in a dominant manner. Variation at codon 129 (methionine or valine) in the human PrP gene, on the other hand, appears simply to predispose to disease ***(60)***. The TSEs may therefore have several different etiologies linked by the pathological end product: aggregated PrP protein.

7. Interaction of PrP Protein Allotypes with Disease

The conversion of the normal host protein PrP^C to the aggregated form PrP^{SC} is fundamental to the disease process; however, the means by which the change occurs in the folding of the PrP molecule and the relationship to disease development is not fully understood. It is possible that the rate of PrP^C to PrP^{SC} conversion (which can be persuaded to occur in vitro; ***61***) is allele-dependent. In sheep terms, the protein produced by the $V_{136}R_{154}Q_{171}$ allele, linked to the highest incidence of scrapie seems to convert easily and quickly in vitro to the PrP^{SC} form ***(62,63)***. The other alleles, associated less strongly with disease or associated with longer survival times, seem in the in vitro system to produce PrP protein which converts less easily. In this hypothesis, the more easily PrP^{SC} aggregates in the brain of an affected animal, the more likely that symptoms would occur and, indeed, PrP^{SC} is believed to be toxic to neurons ***(64)***. The disease would then be passed on to another animal via the PrP^{SC} in the inoculum, which would act as a seed for the aggregation of PrP^{SC} protein in the new host. This idea has support from recent experiments in yeast where heritable information may to be passed on

Table 5
PrP Genotypes in Cheviot Sheep from UK, Australia, and New Zealand

PrP genotype	UK Number	UK %	Australia Number	Australia %	New Zealand Number	New Zealand %
$VV_{136}RR_{154}QQ_{171}$	0	0	2	4	0	0
$VA_{136}RR_{154}QQ_{171}$	14	8	5	9	17	17
$VA_{136}HR_{154}QQ_{171}$	0	0	1	2	0	0
$VV_{136}RR_{154}RQ_{171}$	6	4	6	11	18	18
$AA_{136}RR_{154}QQ_{171}$	29	17	11	20	10	10
$AA_{136}HR_{154}QQ_{171}$	23	14	9	17	0	0
$AA_{136}RR_{154}RQ_{171}$	47	28	13	24	34	33
$AA_{136}HR_{154}RQ_{171}$	14	8	2	4	2	2
$AA_{136}RR_{154}RR_{171}$	32	19	5	9	21	21
Total	168		54		102	

to daughter cells via an autocatalytic alteration in the conformation of a normal protein *(65)*. However chaperone molecules, which assist in protein folding, may also be important. The binding of what might be a chaperone—called, alarmingly, protein X—may be necessary for the seeding process, which starts the conversion of PrP^{C} to PrP^{SC} *(66)*.

8. Conclusions

The number of published papers relating to TSEs has been increasing exponentially over the last 10 yr. The genetics and pathology of the diseases are now well worked out, although it remains to be explained how natural scrapie in sheep is transmitted. Most of the current biochemical interest lies in the elucidation of the structures of the normal PrP^{C} and the disease-related aggregated form PrP^{SC}, and how these relate to infectivity. As yet, no one has produced pure PrP^{SC} in vitro and demonstrated that, at the same time, this synthesis of PrP^{SC} has also synthesized infectivity. In addition, the underlying mechanisms by which PrP protein allotypes control susceptibility are still a mystery, and interest is focused on how the single amino-acid variations might affect PrP secondary and tertiary structure. It is hoped that a complete understanding of the folding characteristics of this potentially dangerous protein will, in the end, lead to therapy or even cure for these extremely unpleasant diseases.

Note Added in Proof

Since this review was written, one antibody now recognizes the difference between PrP^{C} and PrP^{SC} (*see* **Table 1**) as described by Korth et al. *(67)*.

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