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Review article

PrP genetics in ruminant transmissible spongiform encephalopathies

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Abstract – Scrapie, bovine spongiform encephalopathy (BSE), and chronic wasting disease (CWD) are prion diseases in ruminants with considerable impact on animal health and welfare. They can also pose a risk to human health and control is therefore an important issue. Prion protein (*PrP*) genetics may be used to control and eventually eradicate animal prion diseases. The *PrP* gene in sheep and other representatives of the order *Artiodactyles* has many polymorphisms of which several are crucial determinants of susceptibility to prion diseases, also known as transmissible spongiform encephalopathies (TSE). This review will present the current understanding of *PrP* genetics in ruminants highlighting similarity and difference between the species in the context of TSE.

scrapie / transmissible spongiform encephalopathy / *PrP* / genetics / ruminant

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1. INTRODUCTION

The *PrP (PRNP, prn-p)* gene encodes the prion protein (PrP), which plays a major role in transmissible spongiform encephalopathies (TSE) or prion diseases. It was already established that variations of the PrP protein sequence were associated with disease onset [66] when *PrP* transgenic mouse models finally proved that scrapie did not develop without PrP protein expression [16]. Further experiments in transgenic mice with different *PrP* gene dosage also suggested an inverse correlation between PrP protein level and incubation period length [17]. The PrP amino acid sequence and *PrP* expression level can therefore

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be seen as one of the key elements in this group of diseases.

PrP polymorphisms in sheep and other representatives of the order *Artiodactyles* are common. Several are crucial determinants of susceptibility to and incubation periods of TSE such as scrapie and chronic wasting disease (CWD). Clinical scrapie cases in sheep and goats have been recorded for many decades in almost all regions of the world [22]. Disease susceptibility and resistance follow genetic rules based on the *PrP* genotype. It is indeed a sign of the close association between *PrP* alleles and sheep scrapie susceptibility that the crucial amino acid variations in ovine *PrP* were discovered very early on, once the gene sequence became available [29]. Repeatedly the same polymorphisms in three codons (136, 154 and 171) appeared associated with different scrapie outbreaks in sheep, due to the relative high frequencies of these polymorphisms in most sheep populations. However, with the discovery of atypical forms of scrapie more recently [9] it has become apparent that the link between PrP genetics and disease susceptibility will have to be re-evaluated.

The degree of amino acid sequence similarity of PrP between species will often have consequences for the transmissibility of TSE between them. It is also often the case that the same or a similar polymorphism in two species will have the same or a similar effect on their TSE susceptibility. Observations regarding the genetic variation of *PrP* in related species are therefore important in the attempt to understand and control susceptibility. The *PrP* gene may be of crucial importance to disease but it is most likely not the only gene exerting influence on it. Several chromosomal regions have been associated with susceptibility [65, 84], but no other gene has yet been unambiguously associated with disease.

This review will present the current understanding of *PrP* genetics and genomics in species of the suborder *Ruminantia* (order *Artiodactyles*) highlighting similarity and difference between the species in the context of prion diseases.

2. *PRP* GENOMICS IN SHEEP, GOATS, CATTLE AND DEER

The *PrP* gene in sheep, goats and cattle has been mapped to chromosome 13 [51]. The genomic sequence available around the *PrP* gene locus is 32 kb in sheep (accession No. U67922), 78 kb in cattle (accession No. AJ298878) and 65 kb in mule deer (accession No. AY330343), oddly there is No equivalent complete sequence from the goat genome yet. Only the genomes of species in the genus *Odocoileus* (e.g. mule deer, white-tailed deer) contain a pseudogene (*PrP*ψ, accession No. AY371694) with all the features of a classical retro-element [15]. The functional length of the *PrP* gene is approximately 21 kb and it is composed of three exons. Exons I and II are small ($\leqslant$ 100 bp) and form the non-coding 5'UTR of the transcript. Exon III ($\sim$ 4 kb) contains the full open reading frame and the 3'UTR of the transcript. Introns I and II are approximately 2.5 kb and 14 kb in length, respectively.

All four genomes described here produce a messenger RNA of about 4.6 kb, but sheep and goat also generate in peripheral tissues by alternative polyadenylation a second mRNA of $\sim$ 2.1 kb [34, 45]. This short mRNA is produced at significant level and may be differently regulated compared to the long mRNA [34]. No 2.1 kb mRNA has been detected in cattle. Whether the deer transcript shows alternative polyadenylation is not known. The specific functions of exons I and II in the 5'UTR are also unknown. Differential splicing of exon II has not yet been observed, however cattle have a particular alternative splicing at exon I which creates either a 5'UTR with an additional 115 bases (exon Ib) [44] or a 5'UTR without exon I [40]. Again, their role is unidentified but one could speculate that the different mRNA are recognised in different regulatory pathways and in that way protein expression could be regulated at various cellular levels.

The gene promoter region regulates production of messenger RNA and consequently controls to a large extent protein expression. The amount of PrP protein, PrP tissue distribution and temporal availability are therefore

all genetically controlled. The *PrP* gene promoter is active in most tissues and *PrP* mRNA can be readily detected throughout development in most ruminants [34]. The ruminant *PrP* promoter contains several well characterised transcription factor motifs within its core (e.g. SP-1, AP-2). Upstream of the core sequence are four motifs highly conserved in sequence and position between species [94] and present in all known *PrP* promoters; they are probably involved in the regulation of transcription.

3. GENETIC VARIATION OF *PRP* GENE

The *PrP* gene open reading frame consensus for *Bovidae* and *Cervidae* has a length of 256 amino acid codons. Processing of the primary translation product leads to a mature PrP protein with 210 amino acids. With the discovery that ovine *PrP* polymorphisms influence the susceptibility to scrapie and BSE and modulate the disease progression, began a remarkable search for polymorphisms and mutations in other ruminants, but also in other phylogenetic groups. The *PrP* gene is now one of the most sequenced genes. More than a hundred species have been analysed and in some species several hundred or even thousands of individual sequences have been recorded in research programmes.

About 40 archetypal *PrP* sequences are available from the families *Cervidae* and *Bovidae* alone. All currently available species *PrP* gene sequences add up to a total of about 155. This number increases to about 300 when all allelic variants based on amino acid polymorphisms are counted. This dataset is an important source of information underpinning the search for mechanisms that link *PrP* with TSE susceptibility and resistance. Importantly, this dataset will in future also be of benefit to assess normal PrP function.

Two types of polymorphisms are found in ruminant PrP protein. The first are single nucleotide polymorphisms (SNP) in the DNA which often cause single amino acid changes. They represent the vast majority of all variants. The second are precise insertions or deletions of PrP-characteristic octapeptide repeats of the type PHGGGWGQ in the N-terminal domain.

3.1. Single amino acid polymorphisms

– *Caprinae* (sheep and goats)

At time of writing, 43 ovine alleles (*Ovis aries*) and 22 caprine alleles (*Capra hircus*) have been described for domesticated breeds in peer-reviewed publications. It has to be assumed that this number will very soon be out-of-date as new polymorphisms and allelic combinations are still discovered on a regular basis and additional polymorphisms have been deposited in gene databases (see also [49]). So far 39 out of 256 codons (15%) are polymorphic. Including the archetypal allele (here and in the literature referred to as ARQ based on amino acids in codons 136, 154 and 171), which is shared by both species, there are a total of 64 alleles (Tab. I). However some of the polymorphisms reside within the N-terminal and C-terminal signal-peptides, which are cleaved off during protein maturation. It follows that the PrP protein of 210 amino acids exists in at least 55 unique variants. All are found in domesticated animals for which the number of analysed sequences is very high. Is there a similar variability in wild sheep and goats? Available data are too limited and not peer-reviewed to answer this conclusively. *PrP* analysis of *Ovis canadensis* (Bighorn sheep) (accession No. DQ648468 to DQ648477) suggests significant genetic variation in some free-ranging populations.

For ovine *PrP* genetics it is now accepted standard to describe the alleles in reference to the three codons (136, 154 and 171) that are of particular importance for scrapie and BSE. The polymorphisms are A136V/T, R154H/L and Q171R/H/K. Ovine *PrP* alleles are therefore abbreviated as eg. $A_{136}R_{154}Q_{171}$ (ARQ), $V_{136}R_{154}Q_{171}$ (VRQ), etc. Other variants with additional polymorphisms are abbreviated as eg. $AF_{141}RQ$. Although sometimes also used for other species eg. goats and cattle to denote the archetypal allele, it is less informative regarding TSE in cattle because these three codons are not polymorphic in cattle.

A review of published polymorphisms for a total of about 15 500 ovine ARQ alleles from many breeds and many flocks in Europe, USA, China, and Japan is presented in Table II. This large number allows a good estimate

Table I. Alleles in sheep and goats.

Allele	Wt-aa*	Species	Reference	Allele	Wt-aa*	Species	Reference
ARQ archetype	–	Sheep, goat	[29, 32]	A-S146-RQ-P240	N - S	Goat	[74]
A21-ARQ-P240	V - S	Goat	[11]	A-D146-RQ	N	Goat	[74]
P23-ARQ	L	Goat	[11]	A-G151-RQ	R	Sheep	[1]
V37-ARQ	G	Goat	[3, 92]	A-C151-RQ	R	Sheep	[86, 88]
S49-ARQ	G	Goat	[11]	A-H151-RQ	R	Goat	[1]
R85-ARQ	G	Sheep	[61]	A-F152-RQ	Y	Sheep	[95]
R101-ARQ	Q	Sheep	[1]	AHQ		Sheep, goat	[11, 31]
3rep-G102-ARQ	5rep- W	Goat	[33]	ALQ		Sheep	[4]
P110-ARQ	N	Goat	[3, 92]	AHR		Sheep	[60]
T112-ARQ	M	Sheep	[62]	AR-S167-Q	R	Sheep	[21]
I 112-ARQ	M	Sheep	[95]	AR-L168-Q	P	Sheep	[37]
P116-ARQ	A	Sheep	[80]	AR-Q168-Q-P240	P - S	Goat	[74]
A127-ARQ	G	Sheep	[42]	AR-D172-Q	Y	Sheep	[1]
V127-ARQ	G	Sheep	[38]	ARH		Sheep	[8]
S127-ARQ	G	Sheep	[38, 95]	ARR		Sheep	[29]
S127-ARQ-P240	G - S	Goat	[35]	6rep-ARR	5rep	Sheep	[78]
S127-ARH-176D	G - N	Sheep	[63]	ARK		Sheep	[21]
Q133-ARQ-P240	L	Goat	[3]	ARQ-E175	Q	Sheep	[1]
VRQ		Sheep	[31]	ARQ-K176	N	Sheep	[91]
VRR		Sheep	[60]	ARQ-Y180	H	Sheep	[2, 42]
VHQ		Sheep	[21]	ARQ-R189	Q	Sheep	[38]
TRQ		Sheep	[12]	ARQ-L189	Q	Sheep	[38, 95]
A-T137-RQ	M	Sheep	[13]	ARQ-S195	T	Sheep	[21]
A-I137-RQ-P240	M - S	Goat	[3]	ARQ-S196	T	Sheep	[21]
A-R138-RQ	S	Sheep	[21]	ARQ-Q211	R	Sheep	[8]
A-N138-RQ	S	Sheep	[86, 88]	ARQ-Q211	R	Goat	[35]
A-F141-RQ	L	Sheep	[13]	ARQ-L218	I	Goat	[95]
A-M142-RQ-P240	I - S	Goat	[32]	ARQ-H220-P240	Q –S	Goat	[11]
A-K142-RQ	I	Sheep	[93]	ARQ-K222	Q	Goat	[35, 95]
A-R143-RQ	H	Sheep	[21, 42]	ARQ-P240	S	Goat	[32]
A-R143-RQ-P240	H - S	Goat	[11]	ARQ-S241	P	Sheep	[42]
A-S146-RQ	N	Sheep	[95]				

Only peer-reviewed journal data. * WT-aa: amino acid of archetype (wildtype).

of the genetic variability of *PrP*. A surprisingly high percentage of 24% (~ 3 800 ARQ alleles) was reported to have an additional polymorphism (Tab. II). Most of these alleles have low frequencies. Some would currently be classified as rare, but this could obviously change when more flocks are analysed. Allele frequency differences can clearly be seen from the published data between breeds and countries, which may become important when breeding strategies have to be tailored to individual needs.

A similar review of published data for goats revealed that from a total of about 1 900 caprine ARQ alleles, 29.6% (556 alleles) were polymorphic at another codon (Tab. III). Again, these observations cover many breeds and populations from Europe, China and Japan. A high level of *PrP* variation is therefore not unique to sheep.

– *Bovinae* (cattle), *Cervinae* and *Capriolinae* (deer)

In cattle (species *Bos taurus*, *Bos indicus, Bos javanicus* and *Bos gruniens*) there are only eight *PrP* alleles based on single amino acid changes described to date. That results in eight unique mature PrP variants. In bison and buffalo the number is 14 alleles, with 11 unique mature PrP variants. Altogether there are 22 alleles expressing 19

Table II. Number of published observations for additional polymorphisms in the ovine ARQ allele.

Polymorphism	*n* observations	Reference	Polymorphism	*n* observations	Reference
R85	1	[61]	C151	35	[36]
R101	23	[36, 61]	H151	4	[36]
T112	692	[36, 38, 61, 93]	F152	7	[95]
I 112	65	[95]	S167	16	[36]
P116	5	[36]	L168	50	[36]
A127	2	[36]	D172	3	[36]
V127	27	[38, 61, 93]	E175	46	[36]
S127	172	[38, 61, 95]	K176	17	[36, 93]
T137	177	[36, 78, 93]	Y180	21	[2, 36]
R138	15	[36]	R189	3	[38]
N138	43	[36]	L189	89	[38, 61, 95]
F141	1 604	[2, 36, 61, 78, 93]	S195	4	[36]
K142	1	[93]	S196	2	[36]
R143	103	[2, 36]	Q211	1	[8]
S146	2	[61, 95]	S241	565	[36, 78, 95]
G151	5	[36]			

ARQ wildtype, n = 12 088 [2, 36–38, 61, 78, 93, 95].

Table III. Number of published observations for polymorphisms in caprine PrP.

Polymorphism	*n* observations	Reference	Polymorphism	*n* observations	Reference
A21	6	[11]	R143	142	[3, 11, 32, 59, 92, 95]
P23	1	[11]	S146	26	[74]
V37	43	[3, 92]	D146	21	[74]
S49	2	[11]	H151	9	[74]
G102	6	[33, 59]	H154	100	[3, 11, 74, 92, 95]
P110	9	[3, 92]	Q168	11	[74]
S127	20	[3, 35, 59, 95]	Q211	35	[35, 59]
Q133	2	[3]	L218	19	[95]
I 137	10	[3]	H220	4	[11, 74]
M142	44	[3, 30, 35, 59]	K222	47	[3, 35, 92, 95]

Wildtype (equivalent to sheep ARQ), n = 1323 [3, 11, 32, 33, 35, 59, 74, 92, 95].
The polymorphism in codon 240 (Ser - Pro) has not been considered because of ascertainment bias.

unique mature proteins published for *Bovinae*. Some of the polymorphisms that have been described (position relative to five-repeat allele): W84R, G100S, K113R, V115M, H143R, S146N, and N177S [42, 95] may have relevance for TSE susceptibility, but this is as yet based on comparison with sheep, goats, and deer rather than TSE exposure studies. From a total of about 1 250 published cattle *PrP* alleles, 16% (~ 200 alleles) were variants [42, 43, 52, 76, 81, 85, 95]. Although this number is quite similar to sheep and goats, it is important to note that the modern breeding regimes applied to *Bos taurus* have resulted in a very much reduced genetic variability in European and American farmed cattle, apparently in contrast to the Asian *Bos* species.

In deer of the subfamily *Cervinae* (species *Cervus elaphus, Cervus canadensis, Cervus nippon*, and *Cervus dama*) data are available for seven unique PrP variants. Potentially important polymorphisms[1] are G59S, T98A, S100G, M132L, M208A, and E226Q [42, 57, 65]. Publications for deer of the subfamily *Capriolinae* (species *Odocoileus* spp.,

[1] Peletto S., Colussi S., Acutis P.L., unpublished observations.

Rangifer tarandus and *Alces* spp.) have revealed so far ten unique PrP variants.

Polymorphisms of interest are G65E, Q95H, G96S, A116G, G129S, S138N, V169M, N176D, M209I, S225F, and Q226K [42, 50, 54, 66]. Taking both deer subfamilies together the number of unique PrP proteins is 17. From almost 1 800 alleles that have been published for mule deer and white-tailed deer at least 25% had an additional mutation. Of the 562 wapiti *PrP* sequences 18% carried the only known polymorphism in this species (132L) [42, 53, 55, 66].

3.2. Octapeptide polymorphisms

The previous chapter has described the large variety of PrP sequences generated by single amino acid changes. There is a different type of variation unique to the PrP protein. The N-terminus of PrP contains normally three copies of the octapeptide PHGGGWGQ enclosed by two nonapeptides P(Q/H)GGGGWGQ. Within this arrangement the number of octapeptides is polymorphic. The glycine-rich peptides are also often simply referred to as repeats. All *genera* discussed here have alleles with three octapeptides (five repeats in total), cattle have alleles with two to five octapeptides (four to seven repeats in total) [30, 79]. Sheep have one additional allele with four octapeptides (six repeats in total) [78], and goats have one additional allele with only one octapeptide (three repeats in total) [33]. It has been shown that a total number of repeats above eight is associated with increased risk to Creutzfeldt-Jakob disease, a human form of TSE [28, 66]. In contrast the relevance of a repeat variation between three and seven, found in ruminants, to TSE susceptibility has not yet been fully resolved.

3.3. Species differences in *PrP* sequence

Scrapie and BSE have been found in domesticated animals or animals living in captivity from the subfamilies *Bovinae* (*Bos taurus* (cattle), *Tragelaphus strepsiceros* (Greater Kudu) and *Tragelaphus angasii* (Nyala)), *Caprinae* (*Capra hircus* (goat)) and *Hippotraginae* (*Oryx leucoryx* (arabian Oryx)) [58]. CWD is well established in captive and free-ranging deer of the subfamilies *Cervinae (Cervus canadensis)* and *Capriolinae (Odocoileus heminonus*, *Odocoileus virginianus*, *Alces alces*) and its spread in the wild is proof that TSE can become established at natural population density [67].

Species within *Ruminantia* carry a minimum of 105 alleles, which encode 85 distinctive mature PrP protein variants. This variability may represent an important barrier in the cross-species transmission of TSE. The number of possible genotypes in all these species is considerable, potentially several hundred. On the other hand it has to be noted that the allele frequencies vary significantly, some of the variants have so far only been found in one or two animals. Of course that does not exclude the possibility that they are or will become numerous in specific breeds or populations. Remarkably, none of these polymorphisms has been found exclusively in TSE affected animals, adding further circumstantial evidence that ruminant TSE are not genetic diseases in contrast to some human TSE such as Gerstmann-Sträussler-Scheinker syndrome [66].

Eight positions (codons 98, 100, 146, 158, 177, 189, 208, 226) show no consensus between the various ruminant species which are reviewed here. Some of these differences can be regarded as common types of replacements, eg. Ser_{100}/Gly_{100}, which may be expected to have a relative minor effect on protein structure, although they could still impact on PrP interactions and function. Others are of more interest as they are within the neighbourhood of polymorphisms that have been associated with TSE susceptibility. Codon 158 (Tyr or His) is close to 154 which modulates scrapie in sheep and goats. Codon 177 (Asn or Thr) is in the neighbourhood of codon 171 which again is associated with scrapie. Codon 226 (Gln or Glu) is adjacent to codon 225, which has been associated with CWD susceptibility [53] and close to codon 222, which is proposed to enhance resistance to scrapie in goats [3, 92]. It remains to be seen whether these differences can give some protection for the transmission of TSE between species.

3.4. *PrP* promoter polymorphisms

Recent studies of the control regions of bovine *PrP* gene have revealed 46 polymorphisms within a 5.4 kb fragment ranging from promoter I to exon II, based on the analysis of about 140 chromosomes [43, 76]. Two prominent insertion/deletion (indel) polymorphisms were detected in the *PrP* promoter and intron I: a 23 bp indel (position 47836) and a 12 bp indel (position 49729, reference sequence AJ298878). Both sequences are transcription factor binding motifs [77].

Analysis of the ovine *PRNP* promoter for polymorphisms revealed three polymorphisms (C/A-5354, T/C-5382 and C/G-5622, reference sequence U67922) [70, 71]. The variants A-5354 and G-5622 created consensus sequences for STAT and SP1 transcription factors, respectively, and C-5382 was within conserved motif 1. We have evidence from protein-DNA binding studies that the polymorphisms in 5382 and 5622 significantly change the binding characteristics of transcription factors, but there is as yet no confirmation that this regulates *PrP* mRNA expression or that these polymorphisms are associated with sheep scrapie (Goldmann, personal observation).

4. ASSOCIATION OF *PRP* GENETICS WITH TSE

4.1. Classical scrapie in sheep

The *PrP* genetics of classical scrapie in sheep are the best investigated. Although the underlying mechanisms are poorly understood, there are specific rules that relate the *PrP* genotype to scrapie susceptibility and incubation period. In the majority of scrapie outbreaks and the majority of breeds the standard model of the three-codon *PrP* genotype applies. The association of these three codons, 136, 154, and 171 with scrapie was first established in ovine experimental scrapie models [6] but it was soon shown that they could be effectively transferred to natural disease. Based on the variation found in the UK sheep population originally 15 three-codon genotypes were defined [19]. In this standard model, codon 136 has two possible amino acids, A and V, codon 154 has also two, R and H. Codon 171 has three possible amino acids, Q, R and H. These polymorphisms are combined to form five alleles (ARQ, VRQ, AHQ, ARR, ARH) and those can be arranged in 15 genotypes, eg. ARR/ARR, VRQ/ARQ, or ARH/AHQ. Even after more than fifteen years of *PrP* genotype analysis these are still the only alleles (based on the three codons) with significant frequencies in the world. Novel combinations such as VRR and AHR were described for breeds in Germany and USA [21, 61], but they appear to be very rare indeed, the same is true for new mutations such as TRQ, ARK, and ALQ [1, 4, 12, 21]. A five-group risk classification system has been developed based on these fifteen genotypes which was modified to be applied in breeding and eradication programmes like the National Scrapie Plan (NSP) of Great Britain[2] and equivalent plans in other member states of the EU [19, 22].

The highest risk group (R5) is reserved for animals that are at greatest risk to develop scrapie [6, 7]. In the UK and in many outbreaks outside of the UK, such as in France, Ireland and Norway [25, 69, 88] the top group are VRQ/VRQ animals. The risk in this group is so high that scrapie was for a long time regarded as a genetic disease. It has however been shown conclusively that VRQ/VRQ sheep can survive into old age when their environment is free of scrapie infection [26, 48]. The other three genotypes in R5 are VRQ/ARQ, VRQ/ARH and VRQ/AHQ. Whereas the risk estimates are similar to the VRQ/VRQ homozygote in the first two genotypes, the risk for the VRQ/AHQ is surprisingly low [7]. It suggests that the AHQ allele has a clear resistance effect compared to the ARQ allele when it is combined with VRQ. R4 animals are classified as being at lower risk to be affected with scrapie than R5 but the risk to their progeny is still significant, especially as R5 offspring can be produced from their breeding. The VRQ/ARR genotype represents this group. Risk estimates

[2] Department for Environment Food and Rural Affairs, 2008, http://www.defra.gov.uk/nsp [consulted 24 January 2008].

indicate a very significant susceptibility reduction through the presence of the ARR allele.

R3 animals have average resistance to scrapie and the same is true for their offspring, which will always belong to the R3 group. The six genotypes in this group include the ARQ/ARQ as well as the less common ARH/ARH genotype. It is of particular interest to find that an ARQ/ARQ homozygote in the UK has only average susceptibility whereas outbreaks in other countries have convincingly shown that ARQ/ARQ sheep can have similar risk to VRQ carriers. Scrapie-affected ARQ/ARQ sheep can be found in populations where VRQ carriers and ARQ/ARQ animals are similarly exposed to the agent or in populations where only ARQ/ARQ are exposed because of the absence of the VRQ allele altogether (mostly due to breed). For example, in the French "Langlade" flock, ARQ/ARQ animals were affected in parallel to VRQ carriers [25]. In a selection of Spanish flocks of the Rasa breed 95% of scrapie affected animals were ARQ/ARQ, but the VRQ/ARQ flock-mates were not affected [1]. In an Irish study the combined risk for ARQ/ARQ and ARQ/ARH animals was similar to the risk of VRQ/ARQ animals [69]. Equally, scrapie in Germany, Spain and Greece appears to involve ARQ/ARQ animals in a high percentage of cases [1, 12, 64]. Like in scrapie attacking the R5 and R4 groups, in the R3 group a risk reduction (partial resistance) of about three -times can be found in ARQ/AHQ animals [12]. R2 animals (genotypes ARR/ARQ, ARR/AHQ and ARR/ARH) are quite resistant to scrapie but their offspring can be of a higher risk classification (R3). Occasionally R2 animals are found with scrapie [7] which up to recently distinguished them from R1 animals (ARR/ARR) which were regarded as fully resistant to natural scrapie, although not to experimental exposure to BSE [46]. It has now been shown for two ARR/ARR animals from two different populations that they can carry classical scrapie, though both were subclinical cases [39].

Although there has not been a reason to fundamentally change the risk classification system for classical scrapie as developed by Dawson et al. [19], it is now clear that breeds from different countries carry different *PrP* variants and that there is as yet no proper adjustment of the risk group assignment to novel *PrP* genotypes. The additional alleles in codons 136, 154 and 171, TRQ, ALQ, ARK, VRR, AHR [4, 21, 60] alone could lead to 55 additional genotype combinations. Although some of the alleles are low in frequency and as consequence the frequency of certain genotype combinations will be very low, it should not be forgotten that at the level of individual flocks their frequency could become high through simple founder effects, such as the introduction of a specific ram.

With the genetic association to classical scrapie as described above it becomes very relevant to know the allele frequencies of the major variants in different populations and huge effort has gone into genotyping as many breeds (common and rare) as possible around the world. A survey conducted for this review of more than 20 studies [1, 2, 4, 14, 20, 21, 23, 24, 27, 38, 54, 64, 68, 69, 82, 83, 86–88, 90] reporting on sheep populations from 15 different countries in Europe, America and Asia revealed that so far all but one (Iceland) presented significant frequencies of all five major alleles (ARQ; ARR; AHQ; VRQ; ARH) in their sheep. Iceland appears not to have any ARR animals [86]. The allele frequency averaged over all animals in these studies were 56% ARQ, 30% ARR, 6% AHQ, 5% VRQ and 3% ARH. The ARK and ARH allele had average frequencies of 0.4% and 0.02%, respectively. Regional differences are apparent, such as a high frequency (~ 9%) of the ARH allele combined with a low frequency (~ 1.5%) of the AHQ allele in Asian breeds. There is a lower than average frequency (~ 22%) of ARR in the Mediterranean countries and higher than average frequency (~ 11%) of VRQ in Norway. The variation is however huge and individual flocks or breeds – especially rare breeds – may deviate significantly from these averages. Of course these figures are due to change over the next decades when genotyping and breeding programmes are taking effect.

Table II shows that almost 25% of all ARQ alleles have additional polymorphisms and TSE genetics research is only at the beginning to verify their disease association, risk classification and utilisation in breeding programmes. Indeed, the question whether there are alleles other than ARR and AHQ associated with resistance to classical scrapie have been addressed frequently. In Icelandic scrapie outbreaks carriers of the $AC_{151}RQ$ allele were absent from affected animals [86]. Experimental challenges with BSE of $ARP_{168}Q/ARL_{168}Q$ sheep resulted in survivors [37]. Survivors were also found after challenges with BSE/scrapie of genotypes carrying the alleles $AT_{137}RQ$, $AK_{142}RQ$ or $ARQK_{176}$[94]. Often however these studies suffer from low statistical significance because of low allele frequency, or it is unclear how the experimental results on susceptibility translate into risk assessments to the natural exposure. Nevertheless these investigations will have to continue in sheep and transgenic mouse models to enhance our ability to enforce the most appropriate eradication and breeding programmes.

4.2. Atypical scrapie in sheep

The details of *PrP* genetics in atypical scrapie are presented in the special issue ("Prion diseases in animals") by Benestad et al. [10]. However, it is important to highlight here some of the genetic differences between classical and atypical scrapie which are the topic of current TSE research. Whereas there have been many thousands of animals with classical scrapie worldwide, the number of atypical cases stands currently at only several hundred. Most cases of atypical scrapie are found in animals of *PrP* genotype risk groups R1-R3 which are the groups that show low susceptibility to classical scrapie, Studies of atypical scrapie from Norway [68], Germany [64], France [5] and the UK [78] all come to the same conclusion, namely that susceptibility is associated with *PrP* codons 141 and 154. Sheep of AHQ/AHQ and AHQ/ARQ genotypes were overrepresented in cases versus controls, and this may also apply to goat breeds that carry the AHQ allele [5]. The $AF_{141}RQ$ allele conferred higher susceptibility than the $AL_{141}RQ$ allele. Indeed, the $AF_{141}RQ$ allele may represent a higher risk than the AHQ allele. Surprisingly, ARR/ARR animals are also susceptible to atypical scrapie [16]. In light of these findings a review of the $AF_{141}RQ$ allele frequency in a total of 15 777 ARQ alleles from flocks in various countries revealed 1 833 $AF_{141}RQ$ alleles (11.6%) [36, 38, 61, 64, 68, 78]. There appears to be quite a difference in the frequency of $AF_{141}RQ$ in the UK flocks (21%) compared to the rest of the world (5%). Whether this difference is due to the selection of the flocks in the studies or reflects a true difference remains to be established. $AF_{141}RQ$ is certainly not a rare allele and homozygous $AF_{141}RQ/AF_{141}RQ$ sheep are found regularly. When comparing averages from different breeds in different countries the AHQ allele frequency range appears to be 0.2% (USA) [21] to 12.5% (Norway) [68, 88]. The mean of the AHQ frequency for the 20 studies (see above) is 6.3% and regional frequencies are 2.5% for Italy and Spain [1, 2, 4, 27], 3.8% for Iceland [86], 9.6% for Greece [12, 24] and 7.1% for New Zealand [14]. These data suggest that susceptible genotypes are available in most populations and also that genetic screening could be used for susceptibility control.

4.3. Scrapie in goats

Goat TSE genetic studies also have their origin in experimental challenges. Intracerebral inoculations of goats with scrapie resulted always in disease in all animals, their incubation periods were often relatively short and tightly grouped [75]. However, in many countries the prevalence of natural goat scrapie is much lower compared with sheep. Whether this is due to different *PrP* genetics or different agent strains remains to be established. Additionally, future epidemiological studies will show whether mixed sheep/goat holdings, a common practice in Mediterranean countries but rare in Northern Europe such as the UK, are a risk factor.

The first polymorphism that could be associated with experimental scrapie was I142M, however the $AM_{142}RQ$ allele did not alter susceptibility. IM_{142} heterozygotes had a

lengthened incubation period after experimental inoculation with BSE and scrapie [32]. A PrP variant with three repeats and an additional W102G polymorphism was also associated with longer incubation periods after experimental challenges [33]. Polymorphisms with some resistance (low risk) association to scrapie are H143R, N146S/D, R154H and Q222K [3, 11, 74, 92], but as yet numbers are still small to conclude whether these polymorphisms result in complete resistance to all classical scrapie strains. Heterozygote HR_{143}, RH_{154} or QK_{222} animals all seem to be more protected compared to HH_{143}, RR_{154} or QQ_{222} homozygote animals. Codons 136 and 171 have not been found polymorphic in goat *PrP*, so that all goat alleles are equivalent to the ARQ or AHQ alleles of sheep. The equivalence for sheep and goats in low risk association between RH_{154} genotype and classical scrapie but high risk association between RH_{154} and atypical scrapie [5] implies that the mechanisms underlying the interactions between PrP and agent are the same in different species, therefore highlighting the fact that it ought to be possible to establish general rules for *PrP* genetics. We may therefore deduce for example that R143 in sheep will also have a low risk association.

4.4. Chronic wasting disease in deer

Genetic susceptibility to natural disease is investigated by comparing the proportion of affected and unaffected animals in relation to their genotype. This model assumes uniform exposure of the population to the TSE agent, which may be a special problem when the exposure of free-ranging populations is investigated. Even so, a statistical significant bias in the *PrP* genotypes between CWD-affected and healthy white-tailed deer has been found in captive [73] and free-ranging populations [55]. The data suggested that the polymorphisms Q95H and G96S reduce the risk of infection. Homozygote SS_{96} as well as heterozygote GS_{96} and GH_{95} deer are underrepresented in CWD-affected populations [55, 73]. CWD in mule deer was also shown to have a genetic component with the polymorphism S225F. Heterozygote SF_{225} are significantly less common in CWD-affected deer both in Wyoming and Colorado [53]. None of the genotypes seem to confer full resistance to CWD.

Free-ranging and farmed CWD-affected wapiti (Rocky Mountain elk) were reported to have an association with polymorphism M132L, in which again heterozygote ML_{132}have some protection from CWD [72]. This view has been challenged by a recent study of Colorado wapiti CWD, in which ML_{132} cases are not uncommon (Perucchini, Miller, Goldmann personal observations).

4.5. BSE in cattle

No association between BSE susceptibility and cattle *PrP* alleles with six or five repeats has been found [46]. There is also as yet no other PrP protein polymorphism in cattle that can be associated with BSE. However the recently discovered *PrP* promoter polymorphisms may be associated with susceptibility to BSE in British and German herds [41, 56]. The main effect is with the 12 bp deletion allele (12del). Homozygous 12del/12del and heterozygous 12del/12ins animals appear more at risk to develop BSE than homozygous 12ins/12ins animals. The highest risk is associated with the deletion of both, motifs in the following genotype: 23del-12del/23del-12del. A model that links *PrP* expression with disease may be developed, in which interaction between transcription factors binding differentially to the polymorphic motifs regulates *PrP* expression in tissues that are important for BSE. Although there are still many questions to be answered, there may be at last a genetic association to BSE susceptibility.

5. CONCLUSION

This review has shown that the control of TSE in ruminants should be possible by using our knowledge of *PrP* genetics and yet the occurrence of atypical scrapie puts exactly this ability to control scrapie into question. It very much appears as if all the effort made to breed for classical scrapie resistance could ultimately lead to an increased risk for the population to have carriers of atypical scrapie. One

of the hot issues in the coming years will therefore be the search for an explanation for why the susceptibility for both forms of scrapie apparently resides at the opposite spectrum of the known *PrP* genetics. It may reflect the selection by two different agent strains or involve novel *PrP* or non-*PrP* genetics.

There is no equivalent in ruminants to the human disease-linked *PrP* mutations [66]. *PrP* polymorphisms are more likely to be found in survivors than affected animals, with one noticeable exception, the VRQ allele. Although VRQ/VRQ animals are at extreme high disease risk when in infected populations, the heterozygote genotypes VRQ/ARR and VRQ/AHQ are of very low risk to both types of scrapie. This may explain why the VRQ allele has been maintained in many sheep population. It is of interest to ask why there are so many PrP variants in ruminants and especially sheep. Is the maintenance of a large number of alleles (balancing selection) within these species contributing to a TSE survival advantage? It is certainly seen in many TSE models that heterozygous allele combinations exhibit a lengthening of the incubation period or reduced susceptibility. Understanding the genetic variability of *PrP* in various species and the selection forces acting on the gene may therefore help us to decide breeding strategies in TSE affected species.

Up to very recently it was believed that there could be an absolute resistance in the presence of *PrP* expression as opposed to the *PrP* gene knock-out effect seen in mice. The ARR/ ARR genotype was the model, it produced apparent resistance in sheep and it was hoped that similar genotypes would eventually be found in the other ruminant species. Having the proof that all PrP protein variants including ARR can be converted to abnormal isoforms, it is now likely that only relative resistance can be achieved as long as PrP protein is expressed in the organism. Only by using genetic manipulation technologies will it be shown whether the elimination of *PrP* expression can produce absolute resistance linked to normal livestock capable of withstanding the stress of the farm environment and normal social animal interactions.

Not all observed disease modulation can be followed back to the *PrP* genotype. This has been clear for a while and direct evidence exists for sheep that point to a few loci in the genome that have significant additional influence. It has been difficult however to specify the gene or genes that reside within these loci. New technologies and pooled scrapie databases may enhance the chance to finally reveal these other genes. Out of this could emerge an additional genetic approach to control and eradicate TSE.

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