

Scrapie-free Merino and Poll Dorset sheep from Australia and New Zealand have normal frequencies of scrapie-susceptible PrP genotypes

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As natural scrapie occurs only in sheep of specific PrP genotypes, one proposed aetiology was that scrapie is simply a genetic disease. However, Cheviot and Suffolk sheep of scrapie-susceptible genotypes are found in Australia and New Zealand, both generally accepted to be scrapie-free countries. A study of more common Australia and New Zealand sheep breeds (Merinos and Poll Dorsets) was carried out in order to obtain more generally applicable estimates of Australia and New Zealand sheep PrP genotype frequencies. We have confirmed that animals of highly susceptible PrP genotypes are found in Australia and New Zealand. Interestingly, the Poll Dorset sheep, although born in New Zealand, were brought to the UK as young adult animals and subsequently remained free of clinical scrapie despite 21% of the sheep having scrapie-susceptible genotypes. These results have implications for the genetic control of occurrence of the equivalent human diseases.

Scrapie in sheep and goats is a transmissible neurodegenerative disease with characteristic brain pathology including vacuolated neurones. It is one of a group of similar diseases, collectively known as transmissible spongiform encephalopathies (TSEs), affecting various mammals including cattle (bovine spongiform encephalopathy or BSE) and humans (Creutzfeldt–Jakob disease or CJD). A host protein, PrP or prion protein, is the central factor in all TSEs. An abnormally aggregated form of PrP (PrP^{Sc}) is a marker for infectivity, either because it is itself the infectious agent (the prion hypothesis) (Prusiner *et al.*, 1990) or because it forms part of the agent, acting to protect a small infectious unit of unknown composition, possibly a nucleic acid (the virino hypothesis) (Somerville, 1991).

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Mutations and polymorphisms of the PrP gene are associated with the incidence of many TSEs including those in sheep and goats. In sheep, amino acid codon numbers 136, 154 and 171 have been shown to be of particular importance in studies from several countries and many sheep breeds (e.g. Belt *et al.*, 1995; Clouscard *et al.*, 1995; Hunter *et al.*, 1996). These codons were originally shown to be associated with differing incubation periods, following experimental challenge of sheep with TSEs (Goldmann *et al.*, 1991, 1994). The most resistant genotype is AA₁₃₆RR₁₅₄RR₁₇₁. Out of hundreds of scrapie-affected sheep worldwide, only one animal of this genotype has been reported with scrapie – a Japanese Suffolk sheep (Ikeda *et al.*, 1995). This genotype is also resistant to experimental challenge with both scrapie and BSE (Goldmann *et al.*, 1994). Other genotypes encoding QQ₁₇₁ are more susceptible. For example, in Suffolk sheep the genotype AA₁₃₆RR₁₅₄QQ₁₇₁ is most susceptible, although not all animals of this genotype succumb to disease and it is quite a common genotype amongst healthy animals (Hunter *et al.*, 1997c; Westaway *et al.*, 1994). The PrP genetic variation in Suffolk sheep is much less than in some other breeds, partly because in Suffolk sheep the PrP allele encoding valine at codon 136 (V₁₃₆) is vanishingly rare (Ikeda *et al.*, 1995; Hunter *et al.*, 1997b). Breeds such as Cheviots, Swaledales and Shetlands (the so-called 'valine breeds') do encode the PrP V₁₃₆ allele and the genotype VV₁₃₆RR₁₅₄QQ₁₇₁ appears to be exquisitely susceptible to scrapie (Hunter *et al.*, 1996, 1994). In some valine breed sheep flocks affected by scrapie there is survival advantage if genotypes encode certain PrP alleles, such as A₁₃₆H₁₅₄Q₁₇₁ and A₁₃₆R₁₅₄R₁₇₁, so that despite having the high risk allele V₁₃₆R₁₅₄Q₁₇₁, animals are unlikely to develop scrapie if their genotypes are VA₁₃₆HR₁₅₄QQ₁₇₁ or VA₁₃₆RR₁₅₄RQ₁₇₁ (Hunter *et al.*, 1996). However, this has not been found to be the case in all outbreaks. VV₁₃₆RR₁₅₄QQ₁₇₁ is a rare genotype and, when it does occur in Britain and Europe, is almost always in scrapie-affected sheep and so it has been suggested that scrapie may be simply a genetic disease (Ridley & Baker, 1995). However, a study of healthy Cheviot and Suffolk sheep born and raised in scrapie-free countries (Australia and New Zealand) has shown that animals of highly susceptible genotypes are present at relatively high frequencies

Table 1. *PrP* genotypes and ages of Merino sheep in UK and Australia

Percentages have been rounded up.

<i>PrP</i> codon			<i>PrP</i> genotype frequencies (%) and ages (months)			
136	154	171	UK/M1 (%)	Age [mean, (SD), range]	Aus/M1 (%)	Age [mean, (SD), range]
VA	RR	QQ	0	—	5	36 and 60
AA	RR	QQ	49	24 (17) 12–72	46	54 (18) 36–96
AA	HR	QQ	0	—	8	66 (29) 36–96
AA	RR	RQ	40	32 (23) 12–84	28	64 (16) 36–84
AA	HR	RQ	0	—	8	56 (14) 48–72
AA	RR	RR	12	36 (8) 24–48	5	Both 72
Number of animals			43	29 (19) 12–84	39	59 (18) 36–96

Table 2. *PrP* genotypes and ages of PD1 scrapie-free sheep

Percentages have been rounded up.

<i>PrP</i> codon			<i>PrP</i> genotype frequencies		Mean age (months) and SD		
136	154	171	Total no.	% Alive in June 1996	Age brought to UK (in 1991) of all sheep	Age at death of culled sheep	Age of sheep alive in June 1996
VV	RR	QQ	3	3	18 (6)	61 (4)	75 (7)
VA	RR	QQ	7	6	24 (15)	70 (15)	78 (17)
VA	HR	QQ	0·3	0	15	59	0
VA	RR	RQ	13	16	21 (11)	65 (9)	76 (12)
AA	RR	QQ	11	11	25 (13)	69 (13)	80 (15)
AA	RR	RQ	39	38	22 (11)	66 (11)	78 (12)
AA	RR	RR	27	26	25 (11)	70 (10)	80 (12)
Number of animals			293	133	293	160	133

and that VV₁₃₆RR₁₅₄QQ₁₇₁ sheep (the most susceptible genotype known) can live up to 8 years of age, well past the usual age-at-death from scrapie (2–4 years) (Hunter *et al.*, 1997*a*, 1996).

It has been suggested that the frequencies of susceptible *PrP* genotypes in Cheviots and Suffolks (Hunter *et al.*, 1997*a*) are not relevant to the genetic disease debate because these sheep breeds are not common in Australia and New Zealand. Although this argument seems to miss the point – susceptible animals do exist in the absence of scrapie whether they are common breeds or not – a study was made of a sample of Australian Merinos and New Zealand Poll Dorsets, which are much more common breeds.

A Merino flock in Australia (Aus/M1), sampled in 1993, was compared with a UK Merino (UK/M1) flock (Table 1)

sampled in 1995. Neither flock has shown any signs of scrapie. Despite the small sample numbers, it was not difficult to find in these Merinos, *PrP* genotypes known to be susceptible to scrapie in a wide range of other breeds. The *PrP* genotype frequencies of the two samples were not significantly different ($P > 0\cdot05$), but the highly susceptible genotype VA₁₃₆RR₁₅₄QQ₁₇₁ was found in one Aus/M1 animal and AA₁₃₆RR₁₅₄QQ₁₇₁ (also at high risk of disease) was common in both flocks (46% and 49% in Aus/M1 and UK/M1 respectively). The ages of the Aus/M1 sheep were on average greater than in the UK/M1 flock but both flocks had examples of potentially susceptible sheep living to ages greater than expected ages-at-death for scrapie.

PrP genotypes were also determined for a flock of healthy Poll Dorset sheep (PD1) which had been born in New Zealand

Table 3. Survival times in UK of PD1 scrapie-free sheep

PrP genotype			Time in UK in months				
			Culled sheep			Sheep alive June 1996	
			n	Mean (SD)	Range	n	Time
136	154	171					
VV	RR	QQ	4	46 (4)	44–52	4	54
VA	RR	QQ	12	46 (3)	44–54	8	54
VA	HR	QQ	1	44		0	–
VA	RR	RQ	18	46 (2)	44–50	21	54
AA	RR	QQ	19	46 (1)	44–47	14	54
AA	RR	RQ	62	46 (2)	44–51	51	54
AA	RR	RR	44	46 (1)	43–49	35	54
Total			160			133	

and brought to the UK in 1991, at between 15 and 51 months of age. There has been no sign of scrapie in this flock, neither clinical signs nor in limited pathological examination. The imported New Zealand sheep and their offspring were kept under quarantine facilities on a farm that had no other sheep on its or adjacent property. Prior to the arrival of the first New Zealand sheep on the property, certain areas had been occupied by UK sheep. All buildings which were to hold these New Zealand sheep were cleaned and disinfected with sodium hypochlorite (2–3% available chlorine) and pastures grazed by UK sheep were ploughed and reseeded. For genotyping purposes, DNA was taken from blood samples and genotypes assigned at codons 136, 154 and 171 by previously published methods (Goldmann *et al.*, 1991, 1994; Hunter *et al.*, 1997*b*, 1994).

In UK Poll Dorsets, scrapie has been found in VV₁₃₆RR₁₅₄QQ₁₇₁, VA₁₃₆RR₁₅₄QQ₁₇₁ and AA₁₃₆RR₁₅₄QQ₁₇₁ genotypes at 24–36 months of age (Hunter *et al.*, 1997*b*, 1994). All of the susceptible genotypes were represented amongst the healthy PD1 sheep which, given the flock-to-flock variation in sheep genotype frequencies, did not look unusual in genetic frequency (Table 2). For example, the most susceptible genotype, VV₁₃₆RR₁₅₄QQ₁₇₁, was found in 3% of the flock (eight animals in total, four of which were still alive and healthy in June 1996 at between 69 and 81 months of age). In comparison with other valine breeds, the frequency of this genotype is well within the ranges reported in other flocks, ranging from < 0.06% to 9% in UK flocks and 4% and < 0.02% in an Australian and New Zealand Cheviot flock respectively (Hunter *et al.*, 1997*a*; and unpublished observations). The frequency of the V₁₃₆R₁₅₄Q₁₇₁ allele was also very variable. In PD1 sheep it formed 13% of the total sample and occurred in 14% of live sheep compared with 0.1–35% in UK flocks and 18% and 17% in Australian and New Zealand

Cheviots respectively (Hunter *et al.*, 1997*a*; and unpublished observations). The other susceptible genotypes (VA₁₃₆RR₁₅₄QQ₁₇₁ and AA₁₃₆RR₁₅₄QQ₁₇₁) were also found at frequencies of 7% and 11% respectively in PD1 sheep, giving a total frequency of potentially susceptible genotypes in this flock of 21%.

The ages of PD1 animals were also analysed (Table 3). The total group of 293 animals was brought to the UK at a mean age of 23 months (SD 12, median 33, mode 15, range 15–51). Some animals ($n = 160$) were culled for flock management reasons at a mean age of 68 months (SD 11, median 74, mode 60, range 59–100) and were free of clinical signs of scrapie at this time. The other sheep ($n = 133$) were still alive in June 1996 at a mean age of 78 months (SD 12, median 66, mode 69, range 59–105). There were no differences attributable to genotype (analysis not shown). The animals had lived between 43 and 52 months in the UK before being culled, or were still alive after having lived for 54 months in the UK. Therefore, animals of New Zealand origin with susceptible PrP genotypes have not developed scrapie at the age expected had they been born in an infected flock in the UK (24–48 months) and, indeed, PD1 sheep of the most susceptible genotypes have survived in the UK for more than 48 months. Animals from the PD1 flock are currently being tested to confirm their scrapie susceptibility by experimental injection with scrapie, although in a previous study which exposed 20 New Zealand Suffolk sheep to scrapie in the USA, two sheep developed scrapie and were clearly susceptible (Hourigan *et al.*, 1979).

The perinatal period may be the time of highest risk of infection. For example, Cheviot VV₁₃₆RR₁₅₄QQ₁₇₁ lambs in a flock with a high incidence of natural scrapie and subjected to extremely hygienic husbandry in the perinatal period remained healthy well past their expected age of death from scrapie (Foster *et al.*, 1996; Hunter *et al.*, 1996) (animals at least 55 months of age at the time of writing and expected age of death from scrapie is 23–30 months in this flock). It is therefore of interest that many of the sheep in flock PD1 produced lambs during their time in the UK (not shown) implying that lambing in the UK in the presence of susceptible genotypes does not itself seem to be a risk factor for scrapie development.

The data presented in this paper, following on from our previous study of Cheviot and Suffolk sheep, now effectively rule out the idea of scrapie being entirely genetic in origin and arising spontaneously from susceptible PrP genotypes. Susceptible sheep do exist in scrapie-free countries – the sample sizes needed to demonstrate this finding did not need to be large. In addition, it is possible to bring scrapie-susceptible sheep to the UK and keep them alive, healthy and free from scrapie in a clean environment. These results have implications for familial CJD in humans, also proposed to be a genetic disease. Linkage in sheep between PrP genotype and disease is very strong, as it is in some CJD-affected human families (N. Hunter, unpublished data; Poulter *et al.*, 1992). It may be that in humans, as in sheep, spontaneous TSE does not occur,

instead needing both the correct *PrP* genotype and exposure to an infecting agent before TSE disease symptoms will develop.

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