Novel polymorphisms in the caprine PrP gene: a codon 142 mutation associated with scrapie incubation period

Wilfred Goldmann,1 Trevor Martin,2 James Foster,1 Steve Hughes,2 Grace Smith,1 Ken Hughes,2 Michael Dawson2 and Nora Hunter1

1 Institute for Animal Health, BBSRC and MRC Neuropathogenesis Unit, Ogston Building, West Mains Road, Edinburgh EH9 3JF, UK
2 Central Veterinary Laboratory, Weybridge, Surrey, UK

Age at disease onset and rate of progression of transmissible spongiform encephalopathies in man, sheep and mice are modulated by the host genome, in particular by the PrP gene and its allelic forms. Analysis of the caprine PrP gene revealed several different alleles. Four PrP protein variants were found, three of which were goat specific with single amino acid changes at codons 142, 143 and 240. The fourth was identical to the most common sheep PrP protein variant (Ala136-Arg144-Gln171). The di-morphism at codon 142 (Ile → Met) appeared to be associated with differing disease incubation periods in goats experimentally infected with isolates of bovine spongiform encephalopathy, sheep scrapie CH1641 or sheep-passaged ME7 scrapie.

Introduction

Transmissible spongiform encephalopathies (TSEs) are fatal, neurodegenerative diseases of humans and other mammals, which can be experimentally transmitted to a variety of mammalian species, including rodents. Because the nature of the TSE agent is still unknown, indirect evidence is relied upon for understanding host–agent interactions through the observation of host genetic control, neuropathology and other disease phenotypes. The most important host gene in disease modulation is the gene encoding prion protein (PrP), PrP<sup>C</sup> is the target of disease-specific alterations in biochemical properties, leading to accumulation as PrP<sup>Sc</sup> in brain and peripheral tissues (for review see Hope & Manson, 1991). Open reading frame (ORF) polymorphisms of the PrP gene have been associated with disease phenotypes, especially with incubation periods after experimental challenges and age at disease onset of natural TSEs of humans and sheep (for reviews see Brown, 1992; Hunter, 1993). For instance, sheep of different breeds having a PrP variant with Val at position 136 (Table 1<sub>b</sub>) are highly susceptible to experimental (Goldmann et al., 1991<sup>b</sup>) and natural (Hunter et al., 1994, 1996) scrapie. In contrast, sheep with Arg at codon 171 (Table 1<sub>b</sub>) rarely develop natural scrapie (Westaway et al., 1994; Belt et al., 1995; Hunter et al., 1996) and generally show long incubation periods after experimental challenge (Goldmann et al., 1994). Analysis of the genetics of PrP in sheep has had considerable influence on the interpretation of the aetiology of scrapie and might also shed light on scrapie in goats.

Goats develop natural scrapie at around 3–4 years of age, but the incidence is low compared to that in sheep (Wood et al., 1992). However, goats are almost completely susceptible (incidence around 99%) when experimentally challenged with sheep scrapie by intracerebral (i.c.) inoculation. Goats have shown a similar variation in the length of disease incubation period to that described in sheep. This led Pattison & Millson (1962) to conclude of goats that ‘a variable incubation period following experimental inoculation reflects a variation in susceptibility of the animals inoculated’. Segregation of different incubation period groups was also seen in CH1641 scrapie-affected goats (Foster & Dickinson, 1988), and was more recently observed in goats affected by bovine spongiform encephalopathy (BSE) (Foster et al., 1993; and this paper). We report here four different PrP coding region sequences in goats and their deduced protein sequences. Additionally, the variations in experimental scrapie and BSE incubation periods were analysed for association with these PrP polymorphisms.

Methods

- Animals. The goats, from the Institute for Animal Health, Neuropathogenesis Unit (NPU), Edinburgh, UK, were of a genetic mixture, with Anglo-Nubian, Saanen, Toggenburg and British Alpine...
ancestry. Four goats affected by natural scrapie were of unspecified breed, except for one Angora, and did not come from the NPU herd, which does not have natural scrapie.

Sources of inoculum. Animals were inoculated as described previously (Foster & Dickinson, 1988; Foster et al., 1993). In short, the BSE homogenate is a pool of four infected cattle brains and SSBP/1 and CH1641 are brain homogenates of natural scrapie passaged in Cheviot sheep (Dickinson & Outram, 1986; Foster & Dickinson, 1988). These Cheviot sheep of the NPU flock have been bred into two separate lines having different responses to experimental scrapie. Scrape is linked to the host gene Sip, which has two alleles, sA and sP. The Val142 allele of PrP is associated with sA while all the other alleles (Table 1b) are associated with sP (Hunter et al., 1996). The Sip genotype is now only used when the PrP genotype cannot be verified experimentally, i.e. due to the lack of appropriate tissue samples. SSBP/1 was passaged in Cheviot sheep carrying the Sip sA allele; CH1641 was passaged in Sip pApA Cheviot sheep. The isolate spME7 is a brain homogenate from an ME7-affected mouse passaged once (Sip pApA; expt E) or twice (Sip pApA; expts F, G) in Cheviot sheep before inoculation of goats (Foster & Dickinson, 1988). Animals were observed for clinical signs of disease throughout their lives and sacrificed when disease was advanced or when indicated for animal welfare reasons according to Home Office regulations. Diagnosis was confirmed by biochemical and/or histopathological analysis of brain tissue as described previously (Hope et al., 1988; Foster et al., 1993). Brain tissue from animals free from signs of scrape but culled for animal welfare reasons was also analysed for neurohistopathology and if found to be negative the animals were classified as survivors. Tissue from four natural goat scrapie cases (aged 3–4.5 years) and three age-matched healthy goats was supplied by Veterinary Investigation (VI) Centres and the Central Veterinary Laboratory (CVL). Scrapie was confirmed by histopathology.

DNA isolation from blood and frozen tissues and DNA analysis. DNA was isolated from various goat tissues, including blood, brain, spleen and lymph node, as described previously (Hunter et al., 1993). Genomic DNA was amplified by PCR with varying pairs of PrP-specific oligonucleotides (based on the ovine PrP gene sequence as described previously; Goldmann et al., 1991b, 1994). This was followed by isolation of the generated DNA fragments from agarose gels with DEAE membrane (NA45; Schleicher & Schuell) and sequencing with Sequenase (Amersham) as recommended by the manufacturer. In some cases, DNA fragments were purified using Wizard PCR Preps (Promega) sequenced with an ABI Primer Dye Terminator Sequencing kit and run on an ABI 373A DNA sequencer (Applied Biosystems). Alternatively, membrane-isolated DNA fragments were digested with restriction enzymes SmaI or NsiI to establish their haplotype for codons 42 and 142, respectively. Genotype frequency and incubation period differences were assessed for significance using the Student’s t-test for small sample size.

DNA isolation from paraffin-embedded tissues. Formalin-fixed, paraffin-embedded brain tissue was used as the source of DNA from all CH1641-infected goats and from some ME7-infected animals. The tissues were 15–20 years old and stored at ambient temperature. Paraffin blocks were cut and processed in areas and with equipment not generally used for ruminant nucleic acid work to minimize the risk of cross-contamination. DNA was extracted as described by Kösel & Graeber (1994) with modifications, mainly a 48 h incubation with proteinase K. PCR amplifications were performed on variable amounts of DNA (0.3–3 μg) with Mg²⁺ concentration between 1.5 and 3 mM. Oligonucleotides were chosen to amplify 200 bp around codon 142 or around codon 240. The protocol used for PrP gene amplification (Goldmann et al., 1994) was modified: annealing temperatures were between 50 and 62 °C and 40 cycles were used. In some cases where the first PCR reaction was inefficient, an aliquot was amplified without purification in a second reaction with nested primers. The resulting DNA fragments were analysed as described above.

Nomenclature. PrP ORF differences in goat are described as haplotypes 1–4 or by the descriptions wild-type (wt), wtprp Met142 and Arg143. Table 1 gives details of the amino acids in other codons of interest for the goat variants (Table 1a) and equivalent ovine PrP variants for reference (Table 1b). Genotypes are described for a specific codon position by reference to this codon, e.g. (Ile/Met)142 or by reference to the descriptions in Table 1, e.g. (wt/wtprp).

Results

PrP ORF sequence and haplotypes

The full PrP ORF was sequenced from nine different genomic DNA samples. Four PrP haplotypes were found based on these ORF sequences and four different PrP protein variants can be inferred from these (Table 1a). Amino acid dimorphisms were observed in codons 142, 143 and 240. Additionally, silent mutations were detected in codons 42 and 138. The change in codon 42 abolishes a SmaI restriction site, whereas the transitions in codons 142 and 143 create NsiI and Mael restriction sites, respectively. The HindIII RFLP described by Hunter et al. (1989) was found in-linkage with the codon 240 change (Table 1b) but not with the other changes. The silent C → T transition in codon 138 (sequenced in 25 samples) was found in-linkage with the dimorphism in codon 240, C was on a haplotype with serine and T was linked to Pro. The SmaI dimorphism is not linked to any of the other dimorphisms (46 samples were analysed). The PrP ORFs of all goats reported in this paper have five octapeptide repeats, as have sheep and most other species that have been studied.

Frequencies of haplotypes

The frequencies of the four haplotypes (Table 1a) within the NPU herd and the seven samples supplied by the CVL/VI Centres (see Methods) are as follows. Samples from 59 healthy and scrapie-affected goats were analysed for the codon 240 dimorphism (Ser/Pro)240: 34% of the goats carried haplotype 1 (Ser240) and 66% carried haplotypes 2, 3 and 4 (Pro240), with genotype frequencies of 44% (Pro/Pro)240, 13.6% (Ser/Ser)240 and 42.4% (Pro/Ser)240. Eighty-four animals were analysed for the codon 142 dimorphism (Ile or Met) and 63 goats were analysed for the codon 143 dimorphism (His or Arg). Haplotypes 1 and 2 were detected with a frequency of 84.5%, whereas the haplotype 3 frequency was 13.1%. Haplotype 4 was rare, with a frequency of about 2.4% (n = 4). The genotype frequencies with respect to the codon 142 dimorphism were as follows: 84.5% (Ile/Ile)142, 1.2% (Met/Met)142 and 14.3% (Ile/Met)142.

BSE transmission

Fifteen out of 17 goats inoculated by different routes with the BSE isolate developed disease (Foster et al., 1993; and this
Table 1. Haplotypes of caprine (a) and ovine (b) PrP genes

Sheep variants are given for reference only (Hunter et al., 1996).

<table>
<thead>
<tr>
<th>Amino acid at codon:</th>
<th>Restriction†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smal</td>
</tr>
<tr>
<td>136</td>
<td>42</td>
</tr>
<tr>
<td>141</td>
<td>142</td>
</tr>
<tr>
<td>142</td>
<td>154</td>
</tr>
<tr>
<td>143</td>
<td>171</td>
</tr>
<tr>
<td>154</td>
<td>240</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RFLP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>* h1, 5 kb HindIII RFLP fragment; h2, 3-4 kb HindIII RFLP fragment.</td>
</tr>
<tr>
<td>† Indicated restriction enzyme cuts (+) or does not cut (−) at the codon shown.</td>
</tr>
</tbody>
</table>

CH1641-scrapie transmission

Six goats inoculated i.c. with the same CH1641-scrapie isolate in two independent experiments (C and D) developed disease after 319, 359 and 675 days (Foster & Dickinson, 1988) and 414, 490 and 894 days, respectively (Table 2). Additionally, four s.c. injections (expt D) resulted in one affected goat with an incubation period of 1777 days and three survivors for 2086, 2154 and 2154 days (no material for DNA extraction was available for the latter two). PrP genotypes of eight animals were analysed for the codon 142 and 143 dimorphisms. Both animals with long incubation periods after i.c. challenge (675 and 894 days) and the 2086 day survivor were (Ile/Met)142 (His/His)143. The goat having a 490 day incubation period after i.c. challenge was (Ile/Ile)142 (His/Arg)143. The other four affected goats (i.c.-challenged, incubation periods of 319, 359 and 414 days; s.c.-challenged, incubation period of 1777 days) were of the homozygous (Ile/Ile)142 (His/His)142 genotype. Summarizing the two experiments, it is clear that goats carrying the Met142 variant survive the scrapie challenge significantly longer than the group carrying combinations of wt, Wtpr o or Arg143 alleles. The incubation period of the i.c.-challenged (Ile/Met)142 heterozygotes was about twice that of the homozygotes in both experiments, which is in agreement with the BSE transmission results.

Transmission of sheep-passaged ME7

Five goats were i.c.-challenged with sheep-passaged ME7 (spME7) (expts E and F; Table 2). Homozygous (Ile/Ile)142 (His/His)143 animals succumbed with short incubation periods.
Table 2. Association of PrP codon 142 genotypes with TSE incubation periods

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Inoculation route</th>
<th>Expt*</th>
<th>No. of goats inoculated</th>
<th>Goats affected</th>
<th>No.</th>
<th>Incubation period (days)†</th>
<th>No.</th>
<th>Incubation period (days) No.</th>
<th>Incubation period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSE</td>
<td>i.c.</td>
<td>A</td>
<td>3</td>
<td>(Ile/Ile)₁₄₂</td>
<td>3</td>
<td>528 ± 29</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>i.c.</td>
<td>B</td>
<td>6</td>
<td>(Ile/Met)₁₄₂</td>
<td>4</td>
<td>569 ± 25</td>
<td>2</td>
<td>984, 985</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>s.c.</td>
<td>B</td>
<td>5</td>
<td>(Met/Met)₁₄₂</td>
<td>4</td>
<td>799 ± 64</td>
<td>0</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td>CH1641</td>
<td>i.c.</td>
<td>B</td>
<td>3</td>
<td>(Ile/Ile)₁₄₂</td>
<td>3</td>
<td>941, 1501</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>i.c.</td>
<td>C</td>
<td>3</td>
<td>(Ile/Met)₁₄₂</td>
<td>2</td>
<td>319, 359</td>
<td>1</td>
<td>675</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>i.c.</td>
<td>D</td>
<td>3</td>
<td>(Met/Met)₁₄₂</td>
<td>2</td>
<td>414, 490</td>
<td>1</td>
<td>894</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>s.c.</td>
<td>D</td>
<td>4</td>
<td>(Ile/Ile)₁₄₂</td>
<td>1</td>
<td>1777</td>
<td>1</td>
<td>895</td>
<td>0</td>
</tr>
<tr>
<td>spME7</td>
<td>i.c.</td>
<td>E</td>
<td>3</td>
<td>(Ile/Ile)₁₄₂</td>
<td>1</td>
<td>481</td>
<td>1</td>
<td>895</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>i.c.</td>
<td>F</td>
<td>3</td>
<td>(Ile/Ile)₁₄₂</td>
<td>2</td>
<td>320, 335</td>
<td>1</td>
<td>640</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>s.c.</td>
<td>G</td>
<td>6</td>
<td>(Ile/Ile)₁₄₂</td>
<td>6</td>
<td>377 ± 40</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>SSBP/1</td>
<td>i.c.</td>
<td>H</td>
<td>3</td>
<td>(Ile/Ile)₁₄₂</td>
<td>3</td>
<td>580 ± 15</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>i.c.</td>
<td>I</td>
<td>4</td>
<td>(Ile/Ile)₁₄₂</td>
<td>4</td>
<td>620 ± 40</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
</tbody>
</table>

* A–J signify different experiments. A–D had one survivor (see Methods) each: A, 2920 days post-inoculation (p.i.); B, 1767 days p.i. (PrP sequence of this animal was established for the start codon and between nucleotide positions 320 and 440 and from nucleotide 540 to the stop codon); C, 2086 days p.i.; D, 3500 days p.i. (PrP sequence of this animal was established between nucleotide position 160 and the stop codon). In E two survivors were not genotyped, in F one affected goat (450 days) was not genotyped and in G the full PrP ORF was sequenced for three goats in this group: one (wt/wt), one (wt/wtpro) and one (wtpro/wtpro).† ± SEM. NA, Not applicable.

of 320, 335 and 461 days, whereas the goats of genotype (Ile/Met)₁₄₂ (His/His)₁₄₃ died of scrapie after 640 and 895 days (Table 2). In another experimental spME7 challenge of six goats (expt G; Table 2), five goats developed scrapie and died at 377 ± 40 days; all were (Ile/Ile)₁₄₂ (His/His)₁₄₃. The sixth animal, which was of the same genotype, survived for 3500 days post-inoculation without developing scrapie.

Transmission of SSBP/1 scrapie

Seven goats i.c.-challenged with SSBP/1 (expts H and J; Table 2) died of scrapie with incubation periods of 603 ± 8 days. Six were of the (Ile/Ile)₁₄₂ (His/His)₁₄₃ genotype and one was (Ile/Ile)₁₄₂ (His/Arg)₁₄₃ (659 days).

Combined results of experimental scrapie and BSE incubation periods

The experimental challenges presented in this study were all primary passages from either cattle (BSE) or sheep (SSBP/1, CH1641 and spME7). The incubation period for all affected (Ile/Ile)₁₄₂ goats after i.c. challenge was 487 ± 22 (SEM) days (n = 26). The disease incubation for all affected (Ile/Met)₁₄₂ goats was 846 ± 56 days (n = 6), which is significantly different from the (Ile/Ile)₁₄₂ homozygotes (P < 0.001). The two affected (His/Arg)₁₄₃ goats died of scrapie after 575 ± 85 days, which is not significantly different from all (Ile/Ile)₁₄₂ (His/His)₁₄₃ homozygotes. It is, however, different from the (Ile/Met)₁₄₂ (His/His)₁₄₃ goats (P < 0.05).

Natural scrapie cases

Natural scrapie in goats is rare and only four cases were genotyped, their ages ranging from 3 to 4.5 years. The PrP ORF of an Angora goat was sequenced in full. The genotype was (wt/pro/wt_pro) (Table 1); no new sequence change was detected. Only PrP codons 136 (codon associated with natural scrapie in sheep), 142, 143 and 240 were genotyped for the other three goats. All three were (Ala/Ala)₁₃₆ (Ile/Ile)₁₄₂ (His/His)₁₄₃, but differed in codon 240. Two being of the (Pro/Ser)₂₄₀ genotype (3 and 4.5 years old) and one of the (Pro/Pro)₂₄₀ genotype (3 years old). Five healthy goats were also sequenced in full. Their genotypes were as follows: two (wt/pro/wt_pro), one (wt/wt) one (met₁₄₂/met₁₄₂) and one (wt/wtpro) pro₁₄₃).

Discussion

Goat PrP is expressed from a single gene and is translated from a 4.5 kb mRNA in brain (Hunter et al., 1989; Goldmann et al., 1990). Based on partial gene sequences of goat PrP exons and the promoter region, it is likely that the gene structure is homologous to that of the sheep PrP gene (data not shown) (Westaway et al., 1994). A PrP protein sequence of 256 amino
Table 3. Alignment of PrP protein sequences

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Species (haplotype or breed)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>130</td>
<td>Goats (wt, wt&lt;sub&gt;PrP&lt;/sub&gt;); sheep (wt)</td>
<td>This paper; Goldmann et al. (1991 a, b)</td>
</tr>
<tr>
<td></td>
<td>Goats (Met&lt;sub&gt;PrP&lt;/sub&gt;)</td>
<td>This paper</td>
</tr>
<tr>
<td>145</td>
<td>Goats (Arg&lt;sub&gt;PrP&lt;/sub&gt;)</td>
<td>This paper</td>
</tr>
<tr>
<td></td>
<td>Sheep (Val&lt;sub&gt;136&lt;/sub&gt;)</td>
<td>Goldmann et al. (1991 a)</td>
</tr>
<tr>
<td></td>
<td>Sheep (Phe&lt;sub&gt;141&lt;/sub&gt;)</td>
<td>Hunter et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Kretzschmar et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>Goldfarb et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>Hamster (Syrian)</td>
<td>Locht et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Hamster (Chinese, Armenian)</td>
<td>Oesch et al. (1985)</td>
</tr>
<tr>
<td></td>
<td>Possum</td>
<td>Windl et al. (1995)</td>
</tr>
</tbody>
</table>

Acids was deduced from the ORF of the gene sequence. Characteristic features of PrP, i.e. N-glycosylation sites, are conserved and the degree of sequence similarity (> 99%) to sheep PrP protein is more reminiscent of allelic variation than genetic diversity between species. However, despite the fact that the amino acid changes associated with scrapie susceptibility in sheep, i.e. Val at codon 136, were not found in TSE-affected goats, there appears to be a modulation of scrapie susceptibility associated with a different codon: 142 (Ile or Met).

The PrP variant encoded on haplotype 1 (wt; Table 1a) is identical to a common PrP variant in sheep (wt; Table 1b) (Goldmann et al., 1990, 1991b; Hunter et al., 1993); another (haplotype 2; wt<sub>PrP</sub>) shows only a Ser → Pro substitution in codon 240, which has not been found in other ruminants but is present in mink and ferret PrP (Bartz et al., 1994). No significant association of this codon 240 dimorphism with different scrapie incubation periods was observed (data not shown). Biochemical studies of rodent PrP have shown that the C-terminal region of PrP, including codon 240, is cut off during post-translational processing (Stahl et al., 1990). Goat and sheep PrPs are likely to be similarly C-terminal-processed. This protein modification could explain why there was no apparent association of codon 240 with disease. Our study showed that goats with PrP combinations (wt/wt), (wt/wt<sub>PrP</sub>) and (wt<sub>PrP</sub>/wt<sub>PrP</sub>) succumb to Cl1641 scrapie and BSE with very similar incubation periods to Cheviot sheep of the equivalent genotypes (see Table 1b) and experimentally challenged in the same way (Goldmann et al., 1994). There is little difference in the response of goats and sheep of the equivalent PrP genotype to spME7 challenge (W. Goldmann, J. Foster & N. Hunter, unpublished results). Our SSBP/1 results are also in agreement with previous data on SSBP/1-affected goats, which succumbed 420–680 days post-inoculation (Pattison et al., 1959; Pattison & Millson, 1960).

Changing from i.c. to peripheral inoculations (s.c. or oral) significantly lengthens incubation periods in goats and leads to an increased number of survivors. Both phenomena probably reflect the lower effective challenge dose of these routes. However, the possibility remains that as yet undetected PrP polymorphisms are associated with the few survivors of experimental scrapie inoculation. These results also imply that scrapie isolates differ in their effectiveness in inducing disease with peripheral routes of inoculation. Hence, investigations into peripheral pathogenesis in ruminants and its host control will be essential to the understanding of the transmission of natural scrapie. Our results confirm the principal role of the PrP gene in control of disease and suggest that other genes which may differ between sheep and goats have only limited influence when infectivity is administered i.c.

Two amino acid dimorphisms in codons 142 and 143 were found with low frequency in this goat genotype study. The codon 143 dimorphism was also recently described in African dwarf goats (Obermaier et al., 1995). Despite the fact that individual experiments included only a few carriers for these alleles, all experiments without exception showed that the change from Ile to Met at codon 142 is associated with increased disease incubation periods. It is not yet possible to determine whether the codon 143 dimorphism is associated with disease as there were only two affected goats carrying the Arg<sub>142</sub> allele in this study. These two cases seem to indicate that a major phenotype similar to that of the Met<sub>142</sub> allele is unlikely.

An association of PrP variants with long incubation period is not uncommon; e.g. Arg<sub>171</sub> in ovine PrP is linked to survival in experimental and natural scrapie (Goldmann et al., 1994; Hunter et al., 1994, 1996; Westaway et al., 1994; Clouscard et al., 1995; Belt et al., 1995). Additionally, some scrapie mouse models (Bruce et al., 1991) as well as cases of iatrogenic Creutzfeldt–Jakob disease (Palmer et al., 1991) show that
survival advantage can also be associated with PrP heterozygosity. It appears that long incubation periods are, at least in BSE-affected goats, not only associated with heterozygosity but also with homozygosity at codon 142. Why then are prolonged incubation times of widely different scrapie sources linked to the Met142 allele? Recent research suggests that two quite different mechanisms may lead to long incubation times. In the first model, based on PrP protein dosage, the length of incubation period correlates with the PrP protein concentration, as has been demonstrated using different strains of scrapie in mice with only one functional PrP gene copy (PrP<sup>−/−</sup>; Manson et al., 1994; Büeler et al., 1994). Hence, an extended incubation period in goats could be the consequence of a much lower Met<sub>142</sub> PrP concentration than wt PrP. In the other model, based on the conversion of PrP<sup>WT</sup> into protease-resistant PrP<sup>Sc</sup>, differences in the conversion kinetics between wt and mutant PrP may result in slower disease progression: e.g. it has been shown that PrP<sup>conversion</sup> is sensitive to sequence differences between PrP<sup>WT</sup> and PrP<sup>Sc</sup> (Priola et al., 1994; Kocsisko et al., 1995). Even a single amino acid change appears to significantly reduce the conversion to protease-resistant PrP. Priola & Chesebro (1995) demonstrated in a cell culture-based assay that formation of protease-resistant PrP is inhibited by Met at codon 139 of Syrian hamster PrP (or 138 of mouse PrP).

In the context of this study, it is of great interest that codon 139 of hamster PrP is in a position homologous to codon 142 of goat PrP (Table 3). It remains to be established whether one or both of these models or yet another mechanism can explain the observed phenotype.

Our preliminary analysis of four natural scrapie goats showed no carriers of haplotypes 3 (Met<sub>142</sub>) and 4 (Arg<sub>113</sub>) and no homozygous (Ser/Ser)<sub>138</sub> animals. The age at scrapie onset of the four animals was typical for goats (Wood et al., 1992). Various studies of natural sheep scrapie in various breeds have demonstrated the association with a codon 136 mutation (Val) in ovine PrP, but there is also a wealth of evidence that (wt/wt) homozygous sheep, i.e. of the Suffolk breed, are at risk of developing scrapie (Hunter et al., 1994; Westaway et al., 1994). Only further analysis of the full ORF of a larger number of animals will establish whether a PrP gene mutation associated with natural disease does exist in goats.

An increasing number of polymorphisms detected in ruminants and their subsequent association (or non-association) with disease, combined with similar studies in rodents and man, have already profoundly influenced the concepts of PrP and its key role in scrapie. More may be learned about these processes by analysing not only pathogenic and susceptibility-enhancing PrP mutations, but also mutations that lead to enhanced scrapie survival.

The authors would like to thank the British Veterinary Investigation Centres staff for samples, information and histopathology. This work was supported in part by the Ministry for Agriculture, Fisheries and Food of the UK.

References


Received 1 April 1996; Accepted 13 June 1996