Identification of an allelic variant of the goat PrP gene associated with resistance to scrapie

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The association between PrP gene variations and scrapie susceptibility was studied in a single herd of Ionica breed goats. The entire herd comprised 100 animals, 11 of which were clinically affected and showed pathological prion protein (PrPSc) deposition in both their central nervous system (CNS) and lymphoreticular system (LRS). Among asymptomatic goats, nine harboured PrPSc in both CNS and LRS, 19 showed PrPSc only at the LRS level and 61 animals had no PrPSc deposition. Genetic analysis of the PrP gene coding sequence revealed the presence of several polymorphisms, namely G37V, T110P, H143R, R154H, Q222K and P240S. Silent polymorphisms were also found at codons 42, 138, 219 and 232. The effect of PrP polymorphism on scrapie susceptibility was assessed by comparing the genotype distribution at each locus among animals with different pathogenetic and clinical disease stages. Significant differences in the distribution of genotypes were observed for codons 154 and 222, with polymorphism at codon 154 modulating susceptibility to scrapie and lysine at codon 222 being associated with scrapie resistance. The allelic variant encoding lysine at position 222 could be a valuable candidate to select in the framework of appropriate breeding programmes for scrapie resistance in goats.

INTRODUCTION

Scrapie of sheep and goats belongs to the transmissible spongiform encephalopathies (TSEs) or prion diseases, a group of slowly progressive and invariably fatal neurodegenerative conditions affecting man and animals. Bovine spongiform encephalopathy (BSE) in cattle, as well as Creutzfeldt–Jakob disease (CJD) and its ‘variant’ in man, the latter resulting from exposure to the BSE agent (Bruce et al., 1997; Hill et al., 1997), are also members of the TSE group. TSEs are characterized by accumulation in the central nervous system (CNS) of an abnormal isoform (PrPSc) of the host-encoded prion protein (PrPC) (Oesch et al., 1985; Prusiner & DeArmond, 1994; Prusiner, 1998). During the pathogenesis of sheep scrapie, PrPSc accumulates in the lymphoreticular system (LRS) at a stage preceding neuroinvasion and neurological symptoms in both natural (Ikegami et al., 1991; Muramatsu et al., 1992; Schreuder et al., 1996; Andreoletti et al., 2000) and experimental (Heggebo et al., 2003) disease.

The susceptibility of sheep to scrapie is influenced by the PrP genotype of the host, as well as by the strain of the agent associated with the infection (reviewed by Baylis & Goldmann, 2004). The ovine PrP gene is highly polymorphic; the most important variations in modulating sheep susceptibility to scrapie are those at codons 136, 154 and 171, which give rise to five different alleles, namely VRQ, ARQ, AHQ, ARH and ARR. VRQ/VRQ, VRQ/ARQ, VRQ/ARQ and ARQ/ARQ genotypes have been associated with susceptibility to scrapie, whereas the ARR/ARR genotype has been associated with the highest level of resistance to the disease. On this basis, the European Union (EU) has established management strategies for TSEs in small ruminants, to be achieved both by means of ad hoc breeding programmes of the ovine population of each member country and by means of selective culling based upon the PrP genotype.

Similarly to sheep, the R154H polymorphism has also been described in goats (Billinis et al., 2002), although no
variation at codons 136 and 171 has yet been reported in this species. A number of other polymorphisms have been documented in goats, namely V21A, L23P, G49S, W102G, T110P, G127S, I142M, H143R, N146S, P168Q, Q220H, Q222K and P240S (Goldmann et al., 1996; Billinis et al., 2002; Agrimi et al., 2003; Kurosaki et al., 2005). An allele containing only three, instead of the normal five, octapeptide repeats has also been described (Goldmann et al., 1998).

Homozygosity for isoleucine at codon 142 has been associated with a shorter incubation time in goats challenged experimentally with different TSE isolates, as well as with BSE (Goldmann et al., 1996), although I/M heterozygous and M/M homozygous animals remained susceptible to the disease. The association between scrapie and the PrP genotype of the host has been investigated in a naturally affected goat herd in Greece, suggesting that PrP alleles carrying arginine and histidine at codons 143 and 154, respectively, may offer some protection against scrapie (Billinis et al., 2002).

Although scrapie has been known in goats for a long time (Chelle, 1942) and has been described in several countries, including Italy (Capucchio et al., 1998), the genetic variability of PrP in goats and its correlation with scrapie susceptibility still remain unresolved and, to date, no significant association of a particular polymorphism, nor of a specific PrP allele, with scrapie resistance has been reported. As a consequence, the breeding programmes established in sheep populations within the EU have not been adopted in goats, but the recently reported case of BSE in a goat in France has caused public-health concern (Eloit et al., 1998), the genetic variability of PrP in goats and its correlation with scrapie susceptibility still remain unresolved and, to date, no significant association of a particular polymorphism, nor of a specific PrP allele, with scrapie resistance has been reported. As a consequence, the breeding programmes established in sheep populations within the EU have not been adopted in goats, but the recently reported case of BSE in a goat in France has caused public-health concern (Eloit et al., 1998). Circulation of the BSE agent within the caprine population may represent a problem that is hard to manage, although there are biochemical methods that are able to discriminate BSE from scrapie (Stack et al., 2002; Nonno et al., 2003; Lezmi et al., 2004; Thuring et al., 2005). The availability of genetic data in relation to TSE resistance in goats is of paramount importance in this context, providing a powerful additional tool in the control of small-ruminant TSEs.

In this study, polymorphisms of the PrP gene in relation to the prevalence of clinical scrapie and preclinical infection were investigated in 100 goats from a single Ionica breed herd involved in the vaccine-associated epidemic in 1997–1998 affecting the Italian sheep and goat populations (Capucchio et al., 1998; Agrimi et al., 1999).

METHODS

Animals. The scrapie outbreak under study was officially diagnosed in 1997 in Apulia, a southern region of Italy. It was one of several outbreaks included in the scrapie epidemic that occurred in Italy in 1997–1998 and was causally linked to the subcutaneous administration of a vaccine against ‘contagious agalactia’ (an infectious disease of sheep and goats caused by Mycoplasma agalactiae) that had been prepared with a homogenate of mammary gland, lymph nodes and brain tissue from M. agalactiae-infected sheep (Capucchio et al., 1998; Agrimi et al., 1999). Scrapie had never been detected before in the herd. After the diagnosis of scrapie was officially confirmed, animal movement restrictions were applied in and at a short time the herd underwent compulsory culling. At the time of culling, clinical examination of all animals of the herd (n = 100) was carried out. Tissue samples were collected from each animal of the herd after killing by means of intravenous injection of Tanax (Hoechst Roussel Vet).

Immunohistochemistry. CNS (obex) and LRS (tonsil, lymph nodes, spleen and third eyelid) tissues were fixed in 10% neutral-buffered formalin for 2–4 weeks. After decontamination with 98% formic acid for 1 h, all specimens were washed overnight in tap water. Tissue samples were subsequently embedded in paraffin and cut into 6 μm-thick sections. All sections were placed onto silanized slides (DakoCytomation). After heating at 60 °C for 24 h, sections were rehydrated and then pretreated with 98% formic acid for 1 min and by hydrated autoclaving at 121 °C for 30 min (with subsequent overnight cooling) for the detection of PrPSc by anti-PrP mAbs.

Incubation with primary mAbs, avidin–biotin complex (ABC) treatment and detection of PrPSc were carried out by means of a Dako Autostainer (DakoCytomation) at room temperature. Sections were treated with 0.3% normal goat serum (Vector) in PBS for 30 min. PrP immunohistochemistry was carried out with a mixture of two anti-PrP mAbs, I89 and F99 (kindly provided by K. O’Rourke, USDA, Pullman, WA, USA), recognizing the 142–145 and 220–225 peptide epitopes, respectively, of the PrP sequence. Both mAbs were diluted at 1:3000 in PBS added with 3% normal goat serum; the mAb mixture was applied to the sections for 45 min. After washing with PBS, sections were submitted to ABC (Vector) and amino-ethylcarbazole (DakoCytomation) chromogen treatment for 45 and 5 min, respectively. Sections were finally counterstained with Mayer’s haematoxylin and mounted with Eukitt aqueous solution.

In each run, control sections of the obex and palatine tonsils from scrapie-positive and healthy control sheep and goat were also included.

Western blot. Frozen brain tissues from the obex region were analysed according to a Western blot protocol, which allows discrimination of BSE from scrapie by measuring the molecular mass of the unglycosylated proteinase-resistant PrPSc fragment and by discriminatory binding of the antibody P4, whose epitope is spared by proteinase K treatment only in scrapie samples. This method is officially approved and used in Italy in the framework of the current EU legislation (Regulation EC 36/2005) for scrapie/BSE discrimination. Brieﬂy, brain homogenates [10% (w/v) in 100 mM Tris/HCl (pH 7.4) and 2% Sarcoyl] were digested for 1 h at 37 °C with proteinase K (200 μg ml−1; Sigma-Aldrich). Proteinase treatment was stopped with 3 mM PMSF (Sigma-Aldrich). Treated homogenates were then denatured for 10 min at 90 °C with NU-PAGE sample buffer (Invitrogen). Ten microlitres of each sample was loaded onto precast 12% bis-Tris polyacrylamide gels (Invitrogen). Electrophoresis was carried out at 200 V for 60 min and Western blotting was performed on PVDF membranes (Millipore). PrPSc was detected with either mAb SAF84 (0.8 μg ml−1, epitope at aa 163–173; SPI-BIO) or mAb P4 (0.2 μg ml−1, epitope at aa 93–98; R-Biopharm). Horseradish peroxidase-conjugated anti-mouse immunoglobulin (diluted 1:80000 for 1 h; Pierce) was used as secondary antibody. Membranes were developed with an enhanced chemiluminescence method (SuperSignal Femto; Pierce) and detected by the VersaDoc imaging system (Bio-Rad). Brain tissue from a Cheviot sheep (AHQ/AHQ) infected experimentally with BSE was used as a control.

For analysis of PrPSc from LRS, tissues (400–600 mg) were homogenized (10%, w/v) in 100 mM Tris/HCl (pH 7.4) with Preprons (Prionics). Homogenates (900 μl) were added to 100 μl 100 mM Tris/HCl (pH 7.4) containing 20% Sarcoyl (Sigma-Aldrich) and...
incubated for 20 min at room temperature before proteinase K (50 µg ml⁻¹) digestion for 60 min at 37 °C. Proteinase treatment was stopped with 3 mM PMSF (Sigma-Aldrich) and the treated homogenates were centrifuged at 20,000 g for 60 min. Pellets were dissolved in 50 µl NU-PAGE sample buffer (Invitrogen), heated at 95 °C for 10 min and centrifuged in a microcentrifuge at 12,000 r.p.m. for 5 min. Supernatants (20 µl) were loaded onto 12% bis-Tris polyacrylamide gels (Invitrogen). Electrophoresis and Western blotting were performed as described above; PrPSc was detected with mAh P4 (0-4 µg ml⁻¹).

**Genetic analysis.** Before the goats were killed, a sample of approximately 5 ml blood was collected into EDTA Vacutainer tubes and stored at −20 °C. DNA was extracted from blood with phenol/chloroform/isooamyl alcohol (25:24:1), precipitated with ethanol and sodium acetate and resuspended in distilled water to adjust the final concentration to 50 ng µl⁻¹.

The coding sequence (CDS) of the goat PrP gene was analysed as described previously (Vaccari et al., 2001). Briefly, two overlapping regions representing the entire CDS were amplified separately with the following two sets of −21M13 or M13 reverse-tailed primers [nucleotides are numbered, in parentheses, according to Goldmann et al. (1990)]; P1f–21M13, 5’-TGTTAACGCGCTGTTTACGT-GGCCATTGATGC-3’ (21–39)/P1rM13rev, 5’-CAGGAAACAGCTATGACCGGCTGCAGGTAG-3’ (522–504); and P2f–21M13, 5’-TGTTAACGCGCGGTGTTGCGCTACATGCGG-3’ (455–472)/P2rM13rev, 5’-CAGGAAACAGCTATGACCGGCTGCAGGTAG-3’ (913–895).

Reactions were set up in a 100 µl reaction volume containing 1 µg genomic DNA, 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 200 µM dNTPs, 0.5 µM each primer and 5 units AmpliTaq Gold. Reactions were carried out as described above with a heat step of 10 min at 96 °C, 30 cycles of 30 s at 96 °C, 15 s at 57 °C and 45 s at 72 °C, and an elongation step of 10 min at 72 °C. Primers P1f–21M13 and P2rM13rev were used to generate an 843 bp fragment. The PCR product was purified with Montage PCR centrifugal filter devices (Millipore). The fragment was subsequently cloned by using a TOPO TA cloning kit (Invitrogen). Transformation was performed according to the manufacturer’s instructions and utilizing *Escherichia coli* TOP10 One Shot competent cells (Invitrogen). After kanamycin selection, microbial colonies were tested by PCR for the presence of the 843 bp insert. Plasmids were then purified with a Qiagen Plasmid Mini purification kit and sequenced to determine the PrP gene allelic variant.

Sequencing reactions were carried out by using BigDye Terminator Cycle Sequencing Ready Reaction kits and detected with an ABI Prism 310 apparatus.

**RESULTS**

**Diagnosis and PrPSc detection at the CNS and LRS levels**

In total, 11 scrapie-affected goats with clinical signs (group 1) were found in the herd. Immunohistochemical investigations revealed the presence of PrPSc in the obex of these goats and in at least one of the LRS districts under study (Fig. 1). An additional nine animals with no clinical signs (group 2) showed PrPSc deposition at both the CNS and LRS levels. Moreover, 19 goats (group 3) exhibited positive PrPSc immunolabelling in at least one of the LRS districts examined, but were negative in the CNS. The remaining 61 animals (group 4) showed no PrPSc immunostaining at the CNS or LRS levels (Table 1).

Western blot analysis confirmed the presence of PrPSc in the CNS and/or LRS of goats in groups 1, 2 and 3 (Fig. 2 and data not shown). Analysis of proteinase-resistant PrPSc from CNS was performed by using a Western blot method that was able to discriminate BSE from scrapie; brain tissues from a sheep experimentally infected with BSE and an
Italian natural sheep scrapie case were used as controls. This analysis showed that all goats in group 1 (Fig. 2, lanes 1–4) and group 2 (lanes 6–8) had a similar PrPSc molecular pattern, which was identical to sheep scrapie (lane 9) and clearly distinguishable from BSE (lane 5), based on the higher molecular mass of the unglycosylated fragment and on the differential binding of the P4 mAb.

In animals from group 1, the amount of PrPSc detected by Western blot at the obex level was generally higher than in goats from group 2 (Fig. 2).

All animals that had PrPSc deposition at the CNS level also showed PrPSc at the LRS tissue level.

Interestingly, the prevalence of infection in this herd was very high, with 39% of goats harbouring PrPSc. The age of goats was 4.6 ± 1.9 years (mean ± SD) in group 1, 5.1 ± 1.4 years in group 2, 4.6 ± 1.6 years in group 3 and 4.5 ± 1.6 years in group 4. Statistical analysis yielded no significant differences in the age distribution among the four groups.

Considering a time zero of infection resulting from the simultaneous administration of the vaccine to all animals of the herd, the four groups under study represented susceptible animals with short (group 1) or prolonged (groups 2 and 3) incubation times, with group 4 including animals characterized by a very long incubation time, as well as goats carrying a resistant genotype.

**PrP gene polymorphisms in Ionica breed goats**

Several PrP polymorphisms were detected in the goats under study. In particular, whilst a number of polymorphisms were observed that had been described previously in other caprine breeds (H143R, R154H and P240S), some additional ones were found for the first time in the Ionica breed (Agrimi et al., 2003), namely G37V, T110P and Q222K. In this respect, the codon 37 variation was a G to T transition in the second position (GGG to GTG), the codon 110 variation was an A to C transition in the first position (ACC to CCC) and the codon 222 variation was a C to A transition in the first position (CAG to AAC).

No variation was detected at codon 142, with all animals exclusively encoding the amino acid isoleucine. Some previously reported silent polymorphisms at codons 42 and 138, as well as two new silent polymorphisms at codons 219 (ACC to ACT) and 232 (GGG to GGA), were also observed.

**PrP polymorphisms and scrapie susceptibility**

Statistical analyses were performed to evaluate the effect of each PrP polymorphism on the susceptibility of goats to scrapie (Table 2).

An initial analysis was carried out by comparing the genotype distribution at each PrP polymorphic codon between scrapie-infected animals (pooled groups 1, 2 and 3) and PrPSc-negative goats (group 4). A significant difference was found only between animals carrying the Q/Q or Q/K genotype at codon 222 (P = 0.001), with Q/K heterozygotes observed only among healthy control goats (group 4). No statistically significant difference was observed between the age of Q/K heterozygote and Q/Q homozygote animals, suggesting a protective effect of lysine at codon 222 rather than a sampling bias. No animal with the 222 K/K genotype was present in the herd.

Pairwise comparison of genotype distribution at each PrP polymorphic codon was carried out between infected animals (groups 1, 2 and 3). A significant difference in the distribution of genotypes between groups 1 and 2 was observed for codon 154 (P=0.002). The main difference between these groups is the presence of R/H heterozygous goats exclusively found in group 2, which comprises animals without clinical signs of disease, but showing PrPSc at the CNS level. Interestingly, group 3—although not statistically different from groups 1 and 2—includes several R/H heterozygous animals, supporting the effects of heterozygosity at codon 154 in delaying the progression of the disease.

### Table 1. Ionica breed goats included in the study, grouped in relation to the presence of clinical signs and to the detection of PrPSc at the level of CNS and LRS

<table>
<thead>
<tr>
<th>Group</th>
<th>No. goats</th>
<th>Clinically affected goats</th>
<th>Goats with PrPSc in:</th>
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<td></td>
<td></td>
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</tr>
<tr>
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<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td></td>
<td>No</td>
</tr>
</tbody>
</table>

### Fig. 2. Western blot of proteinase K-treated PrPSc from the obex of goats of group 1 (lanes 1–4) and group 2 (lanes 6–8) and from the obex of sheep with natural scrapie (lane 9) or experimental BSE (lane 5). PrPSc was detected by mAbs SAF84 (a) or P4 (b). In each gel, molecular markers were loaded (lane M): top to bottom, bands correspond to 32, 25 and 20 kDa. PrPSc of sheep BSE shows a lower molecular mass than that of sheep and goat scrapie cases and is discriminated well by mAb P4.
Determination of the PrP allele carrying K at codon 222

PrP genotypes resulting from the combination of all polymorphic codons are reported in Table 3. The presence of several animals with double or triple heterozygous loci prevented the identification of the allelic phase by direct sequence analysis. The wild-type PrP allele GTHRQP, representing amino acids at codons 37, 110, 143, 154, 222 and 240, respectively, was present in homozygosity in animals from all of the groups (first line of Table 3). The other main genotype, G/G T/T H/H R/R Q/Q P/P, was present only in groups 2, 3 and 4, probably reflecting the influence of heterozygosity at codon 154 on progression of the disease. The G/G T/T H/R R/R Q/Q P/P genotype, which differs from the wild type by the presence of H/R heterozygosity at codon 143, was found only in groups 3 and 4, suggesting a possible protective effect of that genotype.

The present study highlighted a putative role of lysine at codon 222 in modulating the resistance of Ionica breed goats to scrapie. As shown in Table 3, lysine at codon 222 was present in five sets of animals; the largest of these sets contained eight goats with the G/G T/T H/H R/R Q/K P/S genotype. Due to the double heterozygosity at codons 222 and 240, it was impossible to predict the PrP alleles by direct sequence analysis. To address this question, the entire PrP gene CDS from all of the above eight goats was cloned and sequenced, yielding evidence of lysine at codon 222 of the GTHRKS allele in all cases (GenBank accession no. DQ013244). This allele was also present in a goat with the G/G T/T H/H R/R Q/K S/S genotype and could also be present in two other sets (G/G T/T H/R R/R Q/K P/S and G/V T/T H/R R/R Q/K S/S) of animals. In contrast, in the goat with the V/V T/T H/R R/R Q/K P/S genotype, K at codon 222 is linked to valine at codon 37. It is impossible to predict the effect of such a rare allele on the susceptibility to scrapie.

**DISCUSSION**

In the present study, the genetic susceptibility of Ionica breed goats to scrapie has been investigated. The herd in which the work was conducted revealed a very high prevalence of scrapie infection, along with the presence of both clinically affected and asymptomatic scrapie-infected animals, the latter harbouring PrP\textsubscript{Sc} either at both the CNS and LRS levels, or only in LRS tissues. The high prevalence of infection was probably related to the use of a contaminated vaccine administered to all animals of the herd (Agrimi et al., 1999). As emphasized elsewhere (Vaccari et al., 2001), the study of such outbreaks could provide information in a similar manner to that which can be achieved through experimental-transmission studies. Following peripheral infection, such as by the intraperitoneal or oral routes, both infectivity and PrP\textsubscript{Sc} are generally detectable in lymphoid organs well before CNS involvement (Glatzel & Aguzzi, 2000). In the goats under investigation, the high number of animals that showed PrP\textsubscript{Sc} accumulation at the LRS but not at the CNS level, along with the constant involvement of lymphoid tissues in goats that had PrP\textsubscript{Sc} at the CNS level, suggest that the pathogenesis model proposing the presence of a lymphotrophic phase preceding neuroinvasion is likely to be valid in goat scrapie as well as in sheep and mice.

Molecular analysis of proteinase-resistant PrP\textsubscript{Sc} revealed that the outbreak studied was not related to the BSE strain. Furthermore, the molecular features observed were very similar to those reported in a number of other Italian sheep scrapie cases (Nonno et al., 2003). Strain typing by inoculation of a panel of mouse lines also confirmed the similarity of the scrapie strain involved in this outbreak to those in other Italian sheep and goat outbreaks (unpublished data).
Table 3. Distribution of PrP genotypes of Ionica breed goats in relation to their clinical and pathogenetic status (groups 1–4)

<table>
<thead>
<tr>
<th>Codon</th>
<th>Total</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
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<td>5</td>
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<tr>
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<td>8</td>
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Genetic analysis of Ionica breed goats revealed a high variability of the PrP gene. Such variability, however, did not include the presence of polymorphisms at codon 136 or 171, which have been clearly shown to modulate susceptibility/resistance to scrapie in sheep. Statistical analysis revealed that there are at least two main PrP polymorphisms that can influence goat scrapie susceptibility, namely those at codons 154 and 222. Within these polymorphisms, that at codon 154 does not appear to confer scrapie resistance. However, considering the existence of a time zero of infection and assuming that the peripheral pathogenesis of scrapie in goats implies, as it seems, an early LRS tissue involvement followed by neuroinvasion and subsequent appearance of clinical signs, the data obtained in the present study suggest that the polymorphism at codon 154 plays a role in the elongation of scrapie incubation time in goats. A similar effect has been previously reported in Greek goats, where a lower susceptibility of 154 R/H heterozygous animals compared with R/R homozygous was observed (Billinis et al., 2002). An analogous effect for H/R heterozygosity at codon 143 has also been described (Billinis et al., 2002). In our study, differences in the genotype distribution at codon 143 only approach statistical significance ($P=0.014$) when groups 1 and 3 are compared; however, with the exception of a single H/R heterozygous goat in group 2, this genotype is found only in groups 3 and 4, suggesting a moderately protective effect of the H/R compared with the H/H genotype.

As for the Q222K polymorphism, a significant difference in the distribution of genotypes between PrP$^{Sc}$-positive and PrP$^{Sc}$-negative goats was found. Q/K heterozygous animals were observed only in the PrP$^{Sc}$-negative goats (group 4), suggesting a link between lysine at this codon and scrapie resistance in Ionica breed goats.

It is noteworthy that codon 222 in the PrP gene of goats is homologous to codon 219 in man, which is also polymorphic (E219K). The presence of lysine at codon 219 has been associated with increased resistance to sporadic CJD (Shibuya et al., 1998); however, this mutation does not exhibit a dominant effect in all human prion diseases. Lysine at codon 219 is regarded as permissive for PrP$^{Sc}$ formation in both Gerstmann–Straussler syndrome (GSS) cases with the 102L mutation (Muramoto et al., 2000) and in familial CJD patients with the 200K mutation (Seno et al., 2000). Additional studies on the mouse PrP codon 218, the homologous codon of 222 in goats, have suggested that it forms a discontinuous epitope, together with residues 167, 171 and 215, which is probably involved in the PrP$^{Sc}$ to PrP$^{C}$ conversion process through the binding of the putative protein X (Telling et al., 1995; Kaneko et al., 1997). Furthermore, transgenic mice expressing mutant PrP
with the Q218K mutation were resistant to the RML mouse-adapted scrapie strain (Perrier et al., 2002). These observations reinforce the potential role of codon 222 in affecting the susceptibility of Ionica breed goats to scrapie.

Another interesting finding is related to the presence of K at codon 222 within the GTHRKS PrP allele. With the exception of the Q222K substitution, this allele is homologous to the wild-type ovine ARQ allele. This finding, together with a recent report indicating the role of phenylalanine at codon 141 in conferring susceptibility to the Nor98 scrapie strain in sheep (Moum et al., 2005), demonstrates that other polymorphisms outside codons 136, 154 and 171 are able to influence the susceptibility/resistance of small ruminants to scrapie.

The frequency of Q222K polymorphisms in other goat breeds is unknown. However, the GTHRKS PrP allele detected in this study has previously been reported in a goat herd from the UK, albeit at a much lower frequency (Goldmann et al., 2004).

As the biological basis and molecular determinants of the genetic susceptibility/resistance of goats to scrapie are understood far less well than in sheep, additional work is needed to elucidate the role of PrP gene polymorphisms in the goat. In particular, studies involving different goat breeds and possibly considering the effects of different strains on the susceptibility of this species to scrapie are needed. This is made even more urgent by the recent report of the first confirmed case of BSE in a French goat (Eloit et al., 2005), which has put further pressure on the scientific community to provide additional tools and strategies aimed at managing TSEs in small ruminants and, more specifically, goat TSEs.

In this respect, the putative role of lysine at PrP codon 222 in conferring resistance to scrapie in Ionica breed goats, together with the presence of the same PrP allele in a different goat breed (Goldmann et al., 2004), provide a preliminary basis for considering this allele as the goat counterpart of the ovine ARQ allele in breeding programmes for scrapie resistance in goats.

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