Histidine at codon 154 of the prion protein gene is a risk factor for Nor98 scrapie in goats

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Prion protein gene (PRNP) polymorphisms are involved in modulating the appearance of atypical/Nor98 scrapie in sheep, with the alleles AHQ and AF141RQ strongly associated with occurrence of the disease. The presence of histidine at codon 154 has also been detected in Nor98-affected goats, but statistical analysis of the association between Nor98 and goat PRNP polymorphisms has not been reported previously. Here, a case–control study was carried out on eight Nor98-positive goats and 246 negative herdmates belonging to eight Italian Nor98 scrapie outbreaks. The results revealed that histidine at codon 154 is also strongly associated with the disease in goats.

Scrapie is a fatal, infectious, neurodegenerative disease that occurs in sheep and goats. It is characterized by accumulation in the central nervous system (CNS) of an abnormal isoform (PrPSc) of the host-encoded cellular prion protein (PrPC) (Prusiner, 1991).

An atypical form of scrapie, first detected in Norway in 1998 and named Nor98, shows distinct phenotypic characteristics when compared with classical scrapie (Benestad et al., 2003). Major differences are in PrPSc distribution in the CNS and the immunobiochemical pattern obtained by Western blot assay: (i) the intensity of the PrPSc immunostaining observed in the medulla oblongata is generally much lower in atypical scrapie than in classical cases, with the cerebellum sometimes the only area showing immunostaining; and (ii) after proteinase K treatment, the atypical scrapie isolates display a distinct multiple band pattern, with a fast-migrating band reported around 11–12 or 7–8 kDa (Benestad et al., 2008). Since the publication of the discovery of Nor98, atypical scrapie cases in sheep have been reported in several European countries (European Union, 2007), in the Falkland Islands (Epstein et al., 2005) and in the USA (Cook, 2007). Most are similar if not identical to Nor98 and have been designated ‘Nor98’, ‘Nor98-like’ or ‘atypical scrapie’. Atypical scrapie cases in goats have been reported from France, Spain, Switzerland and Italy (European Union, 2007). In Italy, all of the atypical scrapie cases found in goats have been characterized as Nor98 on the basis of their molecular features compared with reference samples kindly provided to us by S. Benestad (National Veterinary Institute, Oslo, Norway) (R. Nonno, personal communication).

The origin of the disease is still unknown, but a spontaneous, non-contagious origin, like that of human sporadic Creutzfeldt–Jakob disease, cannot be excluded. Experimental transmission to transgenic mice expressing the ovine prion protein gene (PRNP) has demonstrated that these strains are transmissible (Le Dur et al., 2005). To date, one sheep has been reported to be successfully inoculated with atypical scrapie (Simmons et al., 2007). Similar studies in goats have not yet been reported. It is unclear whether the disease can spread under natural conditions in small-ruminant populations.

Studies of genetics in sheep have revealed a strong association between classical scrapie and PRNP...
polymorphisms. The PRNP alleles VRQ (valine/arginine/glutamine) and ARQ (alanine/arginine/glutamine) at codons 136, 154 and 171 are associated with high susceptibility, whereas the ARR allele has been linked to resistance (Belt et al., 1995; Bossers et al., 1996; Hunter et al., 1996, 1997). Data about the role of genetics in resistance/susceptibility of goats to scrapie are scarce compared with those for sheep. So far, the following amino acid substitutions in the caprine PRNP gene have been described: V21A, L23P, G37V, G49S, W102G, T110N, T110P, G127S, L133Q, M137I, I142M, I142T, H143R, N146S, N146D, R151H, R154H, P168Q, T194P, R211Q, I218L, Q220H, Q222K and S240P (Goldmann et al., 1996, 1998, 2004; Wopfner et al., 1999; Billinis et al., 2002; Agrimi et al., 2003; Zhang et al., 2004; Kurosaki et al., 2005; Acutis et al., 2006, 2008; Papasavva-Stylianou et al., 2007). Association with resistance has been suggested for some polymorphisms: mutation M142 is associated with an extension of the incubation period in experimental goat classical scrapie (Goldmann et al., 1998), and increased resistance to natural scrapie is associated with K222 in Italian goats (Acutis et al., 2006; Vaccari et al., 2006), H154 in Greek and Ionica breed goats (Billinis et al., 2002; Vaccari et al., 2006) and S146 or D146 in Cyprus goats (Papasavva-Stylianou et al., 2007).

A clear association between genetics and atypical/Nor98 scrapie in sheep was established by Moum et al. (2005). Genetic analysis of 38 Nor98 cases led to the conclusion that the AHQ allele and the allele characterized by the L141F substitution (designated AF141RQ) were strongly associated with the disease. This association was confirmed by recent studies from France (Arsac et al., 2007), the UK (Saunders et al., 2006) and Germany (Lühken et al., 2007). Furthermore, atypical/Nor98 cases have also frequently been reported in sheep carrying the ARR allele; these sheep then appeared not to be resistant to these forms of scrapie. Nor98-affected goats diagnosed in France (Le Dur et al., 2005) and Switzerland (Seuberlich et al., 2007) carried the H154 mutation, but statistical analysis of the association between Nor98 and goat PRNP polymorphisms has not been reported previously. To fill this gap, we carried out a case–control study on goats from Italian Nor98 outbreaks. Eight Nor98-positive goats and 246 negative herdmates belonging to eight Italian Nor98 scrapie outbreaks were studied. They corresponded to all of the goat atypical scrapie outbreaks detected in Italy from 2005 to September 2007 and came from central to southern Italy, where the main Italian goat population is present. All of the cases were found through active surveillance, tested with the Bio-Rad TeSeE rapid test and Western blotting was carried out on the obex region and found to be negative. These were therefore included as controls in the study. The herd size range was 3–125, with a mean of 31.5. The mean age (+ SD) of the animals was 62.4 ± 25.5 months and the mean age of the cases was 76.5 ± 33.1 months. Genomic DNA was isolated from blood using a Thermo LabSystems KingFisher instrument (Thermo Electron Corporation) and a ChargeSwitch Sheep Blood kit (Invitrogen). PCR amplification of the open reading frame of the caprine PRNP gene was performed according to a previously described protocol (Acutis et al., 2006) using primers p8(+) and p9(−) (Bossers et al., 1996). Polymorphisms were detected by direct DNA sequencing of both strands of the PCR products using Big Dye Terminator v3.1 chemistry (Applied Biosystems) and a four-capillary ABI Prism 3130 Genetic Analyzer (Applied Biosystems). Sequencing primers were p8(+), p61(+), p60(−) and p9(−) (Belt et al., 1995; Bossers et al., 1996). SeqScape software v2.5 (Applied Biosystems) was used for sequence alignment.

In the positive cases, three polymorphisms (in bold) were identified, giving rise to four haplotypes: \(R_{154}Q_{222}S_{240}\) corresponding to the ovine wild-type; \(R_{154}Q_{222}P_{240}\); \(H_{154}Q_{222}S_{240}\) corresponding to the sheep AHQ allele; and \(H_{154}K_{222}S_{240}\), which is described here for the first time. To confirm the presence of the novel haplotype, a cloning strategy based on chemically competent cells was applied (TOPO TA Cloning kit for Sequencing; Invitrogen) according to the manufacturer’s instructions and using the sequencing primers p61(+) and p9(−). PCR products from eight to 12 colonies were sequenced using Big Dye Terminator v3.1 chemistry. In the controls, 13 goats presented mutations H154 and K222 in their genotype; they were also cloned to look for the presence of the new haplotype, which was subsequently found in seven animals.

The genotypes of the cases are shown in Table 1: the \(H_{154}Q_{222}S_{240}\) allele was present in heterozygosis in six positive goats and in homozygosis in one animal; one positive goat was heterozygous for the novel allele with a double mutation at codons 154 and 222.

Genotyping of the controls revealed the following mutations at the following frequencies: V37 (1.4%), P110 (2.5%), S127 (2.2%), Q133 (0.2%), M142 (2.2%), T142 (1%), R143 (0.4%), H151 (1.2%), H154 (17.3%), Q168 (1%), Q211 (5.5%), K222 (7.9%) and P240 (43.1%).
Based on these results, only the mutations and the alleles found in the positive cases were considered in the statistical analysis.

Statistical analysis was carried out using STATA 9.2 software (Stata Corporation; www.stata.com): a χ² test for independence considering Yates’ correction or Fisher’s exact test was performed to look for associations between each allele and Nor98 scrapie status (Table 2), comparing the frequencies of genotypes with and without a given allele between cases and controls. The same analysis was carried out considering the mutations individually (Table 3). A significant association was found between Nor98 in goats and the H154Q222S240 allele, which was present in seven cases out of eight (87.5%) compared with 43.8% in the controls. The only case without H154Q222S240 carried the new allele with the mutations H154 and K222, but this allele was not significantly associated with Nor98. In the analysis of the single mutations, H154 was again significantly associated with Nor98, being present in 100% of the cases compared with 45.6% of the controls. The frequency of distribution of K222 did not differ between cases and controls. Direct standardization was applied (STATA 9.2) to verify the presence of potential confounders, calculating the expected number of cases in a standard population that resumes all the characteristics of the studied populations: the variables of age and geographical origin of the goats did not distort the association between the H154Q222S240 allele and the appearance of an atypical scrapie case.

In conclusion, our case–control study demonstrated that the H154Q222S240 allele is a risk factor for Nor98 in goats. This allele is homologous to the ovine AHQ allele, which is associated with high susceptibility to Nor98 scrapie in sheep, suggesting that the agent–host interaction is similar in the two different species. Mutation L141F, associated with very high risk of atypical scrapie in sheep, or any other mutation at codon 141, has not been found in the caprine PRNP gene so far.

Interestingly, only one of the studied Nor98-positive goats did not carry the H154Q222S240 allele; it still presented histidine at codon 154 but linked with lysine at codon 222 in a novel haplotype. No association of this new allele with Nor98 was found, perhaps because of the low number of animals carrying it. Even so, the significant association of mutation H154 could suggest that this polymorphism also played a role in the occurrence of the disease in this haplotype. The newly reported allele is of note for two reasons. Firstly, it shows that assortment of PRNP mutations in haplotypes can vary, which could be relevant for a case–control study in which an uncorrected allele identification could give misleading results. Secondly, this new allele presented mutation H154, associated here with susceptibility to Nor98 in goats, but also mutation K222, associated with resistance to classical scrapie in goats in Italy (Acutis et al., 2006; Vaccari et al., 2006). This finding deserves attention, as this opposing effect is reminiscent of sheep polymorphisms such as ARR, which is associated with resistance to classical scrapie and susceptibility to atypical scrapie (Le Dur et al., 2005). Therefore, the role of K222, especially when not in linkage with H154, should be explored further to demonstrate in goats, as in sheep, the existence of an inverted genetic predisposition in which alleles associated with classical scrapie resistance become targets for atypical scrapie.

The current sheep breeding programme implemented by several European countries aims to increase genetic resistance to classical scrapie, but no direct action is being taken to control atypical scrapie by genetics. In their opinion on the breeding programme for TSE resistance in sheep (EFSA, 2006), the European Food Safety Authority stated that the current selection may be expected to reduce the occurrence of atypical scrapie by also targeting, although not specifically, the AHQ and AF141RQ alleles, and that selective culling in ovine Nor98 outbreaks of susceptible animals carrying these alleles could be an effective measure to decrease the risk of atypical scrapie without causing a heavy loss of animals. In light of the data reported in this study, a control strategy targeting mutation H154 may also be suggested for goat Nor98 scrapie outbreaks.

**Table 2. Statistical analysis by allele for Nor98 case–control status**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Occurrence</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R154Q222S240</td>
<td>Present</td>
<td>2</td>
<td>85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>6</td>
<td>161</td>
<td>0.03</td>
<td>0.44</td>
</tr>
<tr>
<td>R154Q222P240</td>
<td>Present</td>
<td>5</td>
<td>146</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>3</td>
<td>100</td>
<td>0.04</td>
<td>0.58</td>
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<tr>
<td>H154Q222S240</td>
<td>Present</td>
<td>7</td>
<td>75</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>1</td>
<td>171</td>
<td>9.06</td>
<td>0.0002</td>
</tr>
<tr>
<td>H154K222S240</td>
<td>Present</td>
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<td>7</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Absent</td>
<td>7</td>
<td>239</td>
<td>0.26</td>
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**Table 3. Statistical analysis by single mutation for Nor98 case–control status**

<table>
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<th>Mutation</th>
<th>Occurrence</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>χ²</th>
<th>P value</th>
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<td>P240</td>
<td>Present</td>
<td>5</td>
<td>165</td>
<td></td>
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</tr>
<tr>
<td></td>
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<td>81</td>
<td>0.01</td>
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<td>H154</td>
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<tr>
<td></td>
<td>Absent</td>
<td>0</td>
<td>169</td>
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<tr>
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<td>37</td>
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<td></td>
<td>Absent</td>
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<td>209</td>
<td>0.09</td>
<td>0.66</td>
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References


