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Prospects for applying breeding for resistance to control scrapie in goats: The current situation in Italy

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Abstract

Genetic selection has been used to control transmissible spongiform encephalopathies (TSEs) in sheep populations based on the association between PRNP polymorphisms and resistance to scrapie. In goats even if a protective role has been suggested for several poly- morphisms (I142M, H143R, N146D/S, R154H, R211Q, Q222K) by different European studies, a similar, univocal association has not been proved so far.
The aim of this paper was to identify target polymorphisms in goats and their potential applicability in a selection plan in Italy. For this purpose, the existing genetic data on mod- ulating resistance to classical and atypical scrapie in goats in this country will be used as starting point.
Two Italian case–control studies concluded that the polymorphism at codon 222 (from glutamine to lysine Q/K) is important in influencing the susceptibility of goats to classical scrapie. Moreover, goats are susceptible to an unusual form of scrapie, named Nor98, and the H154 mutation was shown to be statistically associated with this disease in goats in an Italian case–control study. Currently, a strategy based on killing goats carrying the H154 mutation is being applied to manage atypical scrapie outbreaks in Italy.
The current situation in Italy bodes well for the applicability of breeding plans based on the K222 mutation; data from independent studies on the role of K222 as a protective or even a resistance factor and its frequency in several Italian breeds are available. In the near future, as new data on K222 will be reported, testing the application of selective culling in classical scrapie goat outbreaks may become feasible.

Introduction

Scrapie, a neurodegenerative disease affecting sheep and goats, is one of several transmissible spongiform encephalopathies (TSEs) or prion diseases. It is character- ized by the accumulation in the central nervous system (CNS) of an abnormal isoform (PrPSc) of a host-encoded cellular prion protein (PrPC) (Prusiner, 1991).
Although scrapie is an infectious disease, the sus- ceptibility of sheep is strongly influenced by poly- morphisms of the prion protein gene (PRNP). PRNP haplotypes valine/arginine/glutamine (VRQ) and ala- nine/arginine/glutamine (ARQ) at codons 136, 154, and 171, respectively, are associated with high susceptibility to scrapie, whereas the ARR haplotype has been linked to decreased susceptibility or even resistance (Belt et al., 1995; Bossers et al., 1996; Hunter et al., 1996, 1997). Accordingly, the European Union (EU) has implemented programs for the genetic control of scrapie susceptibility in sheep populations. In compliance with Regulation (EC) 999/2001, as amended, several Member States have intro- duced breeding programmes to select for resistance to TSEs in sheep populations increasing the frequency of the ARR haplotype.
Various European studies have suggested that several polymorphisms (I142M, H143R, N146D/S, R154H, R211Q, Q222K) can modulate scrapie susceptibility in goats, but no univocal association has been proved so far (Goldmann et al., 1996; Billinis et al., 2002; Acutis et al., 2006a; Vaccari et al., 2006; Papasavva-Stylianou et al., 2007; Barillet et al.,

2009).
Moreover, goats are susceptible to a different form of scrapie, known as atypical scrapie (Nor98), first reported in sheep in Norway in 1998. Distinct phenotypic features from classical scrapie (Benestad et al., 2003) characterize Nor98. The major differences reside in the PrPSc distribu- tion in the CNS and in the immunobiochemical patterns obtained by Western blot assay: the intensity of PrPSc immunostaining is mainly observed in the cerebellum, while the Western blot profile displays a fast-migrating band around 11–12 kDa (Benestad et al., 2008). Reports of atypical scrapie cases in goats have come from France, Spain, Switzerland, Norway, and Italy (EU Report, 2007).
Nor98 in sheep shows a different genetic target: the two alleles AHQ and AF141 RQ represent risk factors for atypical scrapie (Moum et al., 2005). Interestingly, the H154 muta- tion was shown to be statistically associated with Nor98, also in goats, in a recent Italian case–control study (Colussi et al., 2008).
Classical scrapie in goats was first described in Italy in 1997 (Capucchio et al., 1998); since then, 50 outbreaks have been recorded, 10 of which of atypical scrapie. According to National Institute of Statistics (ISTAT, 2007) estimate, the Italian livestock is about 920,000 goats. The rearing sys- tem in the Mediterranean area is characterized by a mixed management of sheep and goats. Given this situation, while the prevalence of scrapie in goats is usually lower than in sheep, scrapie still poses a problem for the economy and for animal welfare. In addition, goats could take effect as healthy carriers for scrapie, allowing scrapie strains to persist in sheep population as well. In the light of these cir- cumstances, a control strategy based on selection, as done for ovine species, would be desirable.
The aim of this paper was to identify target polymor- phisms in the PRNP gene and their potential applicability in a selection programme in goats in Italy, using the exist- ing genetic data on modulating resistance to classical and atypical scrapie in goats as starting point.

2. Materials and methods

Published papers on the role of genetics in modulating susceptibil- ity/resistance to classical (Acutis et al., 2006a; Vaccari et al., 2006) and atypical scrapie in goats in Italy were analysed (Colussi et al., 2008), along with a survey on the genetic variability of PRNP gene in caprine breeds from Northern and Southern Italy (Acutis et al., 2008).

3. Results

Table 1 illustrates the main features of the studies. Fourteen amino acid substitutions and 20 deriving haplo- types were described in Italian goats (Table 2). The two case–control studies on classical scrapie showed a possi- ble role of K222 as a protective factor, with no recorded cases carrying this mutation. Colussi et al. (2008) found the K222 mutation in a Nor98 positive goat but in link- age with mutation H154; no significant association with atypical scrapie was found for this mutation either when considered as single mutation or when associated in the haplotype with H154 ($p = 0.66$; $p = 0.23$, respectively).
In the study of PRNP gene variability in goat breeds, K222 was found to be highly related to breeds from Southern Italy, with an average frequency of 15.7% (95% Confidence Interval [95% CI]: 12.0–19.5), though also at lower level (2.8%; 95% CI: 1.5–4.1) in Northern breeds.
Vaccari's study highlighted H154 as offering lower sus- ceptibility against classical scrapie. In fact, it was detected in positive animals, but especially in goats without symp- toms and

positive only at the lymphoreticular system (LRS) suggesting its role in delaying progression of the disease. Different results were obtained by Acutis et al. (2006a), who found similar frequencies between cases and controls.
In contrast, H154 was found to be highly associated with atypical scrapie.
The distribution of H154 appeared to be more common in breeds from Southern Italy (7.6%; 95% CI: 4.8–10.3), than in Northern breeds (4%; 95% CI: 2.4–5.6); and it was absent in two out of the four Northern breeds analysed.
Similarly to H154, Vaccari et al. (2006) found lower susceptibility also for the mutation R143; it was absent in positive animals with clinical symptoms while it was present in preclinical and negative animals. However, Acutis et al. (2006a), found this mutation just in South- ern goats and at similar frequency in positive and negative animals.

4. Discussion

There are several factors for considering a PRNP allelic variant as a candidate target for positive selection against scrapie: (1) statistical strength of the association between candidate mutation and resistance to the disease; (2) role of the mutation in different breeds and (3) in relation to different scrapie strains; (4) presence of healthy carriers; (5) frequency of the mutation in the population to evaluate the feasibility of a breeding programme; and (6) behaviour of homozygote animals.
Our analysis of the four studies on the genetic suscep- tibility of Italian goats showed that most of the data of interest have come from independent studies, which com- plement each other in some aspects.
The main result is that polymorphism K222 is a promis- ing candidate for selection in a breeding programme against classical scrapie in goats. This polymorphism has been found associated with resistance in two different studies and on an adequate number of animals, resulting in a good level of statistical significance. One study also anal- ysed preclinical animals (testing the LRS), and no positive animal carrying the K222 mutation was found, showing no evidence of the presence of healthy carriers. The strength of the association between K222 and scrapie resistance is con- tinuously monitored by the surveillance system through systematic genotyping of all positive goats found in Italy: no K222 positive animals have been found out of 32 cases diagnosed since 2004 (Vaccari, personal communication).
In the two case–control studies, all the animals exam- ined were K222 heterozygous. Barillet et al. (2009) reported three natural scrapie cases in K222 heterozygous goats in France, indicating the need of determining the role of K222 in homozygous animals. There could be a situation like that in sheep, where ARR/ARR animals are highly resistant, while some cases of disease are occasionally reported in ARR/XXX (Baylis and Goldmann, 2004) which are consid- ered “semi-resistant”.
As regards goat breeds, the two case–control stud- ies showed that K222 maintains its protective role in at least three different breeds (Table 1), giving promising evi- dence for a possible extensive application of a breeding programme. The presence of this substitution in breeds from both Northern and Southern Italy would facilitate the implementation of selection plans, even if the low fre- quency in some breeds should be taken into account.
Nowadays, there is no report on the role of K222 in relation to different classical scrapie strains; there are only two studies on molecular characterization by immunoblot assay of some sheep and goat scrapie Italian isolates, suggesting a low variability of the strains circulating in Italy (Nonno et al., 2003; Acutis et al., 2006b). Moreover, the transmission of goat scrapie isolates was successfully achieved in the bank vole (Myodes glareolus) (Piening et al., 2006), showing a phenotype similar to that obtained in Italian sheep

isolates (Di Bari et al., 2008). Colussi et al.'s (2008) study showed that K222 might not confer resistance to atypical scrapie; in fact, it was found in a positive goat in linkage with the H154 mutation. This could be due to an opposing effect in which mutations resistant to classical scrapie become a target for atypical scrapie, as for the ARR haplotype in sheep (Le Dur et al., 2005), or due to the pres- ence of H154 alone, as a necessary and sufficient condition for determining atypical scrapie susceptibility.
Therefore, the role of K222, especially when detected not in linkage with H154, should be further explored to demonstrate the possible existence of an inverted genetic predisposition in which alleles associated with classical scrapie resistance become targets for atypical scrapie.
H154 does not seem to be a good target for selection, because it probably prolongs the incubation time rather than protecting animals against infection and it is a risk factor for atypical scrapie. Furthermore, while K222 was present in all the examined breeds, not all the breeds from Northern Italy carried the H154 mutation, making breed- ing plans based on this mutation less feasible, because of its uneven distribution in the breeds, and less desirable, because animals susceptible to atypical scrapie would be selected. Currently, a strategy based on killing sheep and goats carrying the H154 mutation are being applied to man- age atypical scrapie outbreaks in Italy.
Analogous considerations hold for mutation R143: the lower susceptibility it confers, appears to be related only to an extended incubation period. Bearing in mind that this mutation is completely absent in the Northern breeds anal- ysed, the implementation of a breeding programme based on this codon would be even less feasible than for H154.

5. Conclusion

The current situation in Italy bodes well for the appli- cability of breeding programmes based on the K222 mutation; data from independent studies on the role of K222 as a protective or even a resistance factor and its frequency in several Italian breeds are available.
An efficient selection strategy should start with an adequate management of goats by means of keeping studbooks, as done for Saanen, Camosciata delle Alpi, Sarda, Maltese, Orobica, Ionica, Girgentana and Garganica breeds (ASSONAPA, 2005), and by accurate individual identification of the animals. Procedures such as artificial insemination and genotyping, not only of the bucks but also of the female line to increase the frequency of K222 through services per conception, should be considered. The choice of a timescale for the progression scheme should take into account: (1) the starting frequency of the tar- geted mutation; (2) the genetic structure and (3) the size of the populations of interest, in order to maintain local breeds biodiversity and productive performance. The current breeding strategy applied for sheep could represent a good model to follow also for goats.
Nevertheless, more details about K222 are essential: experimental inocula with different strains of classical scrapie, atypical scrapie and BSE in goats, or other ani- mal models carrying the mutation of interest, are needed to clarify whether the resistance seen with K222 is related only to some strains. Moreover, the behaviour of homozygote K/K animals with respect to scrapie sus- ceptibility should be investigated to exclude the possible phenomenon of overdominance, which has already been observed in the susceptibility of mice to several scrapie strains (Dickinson & Fraser, 1979).
Experiments along this line of research and in vitro con- version assays are under way at European level (Bossers, 2006).
The role of other codons of interest in Europe, such as codon 211, whose protective role has recently been sug- gested by Barillet et al. (2009), needs to be elucidated.

Although only one case of natural scrapie in a heterozygous goat has been reported in Italy (Acutis, personal com- munication), no data about the association between this mutation and the disease is available yet.
In the near future, as new data on K222 will be reported, testing the application of selective culling in classical scrapie goat outbreaks may become feasible.

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Reference	Breeds	Total no. of	No. of positives	Age of positives	Age of negatives	Study design	Technical approach	Statistical approach	Results
Acutis et al. (2006a)	Maltese, Camosciata cross-breeds	177	25 CNS	6.7 ±2.3	5.5 ±4.5	Case–control scrapie	Immunohistochemistry Western blot Sequencing analysis of Open Reading Frame of the PRNP gene and cloning for haplotype determination	Chi-square Test–Fisher's exact test and mixed logistic model	K222 resistant to scrapie (p = 0.029)
Vaccari et al. (2006)	Ionica	100	11 with symptoms and positive 9 without symptoms 19 without symptoms, negative	4.6 ±1.9 5.1 ±1.4 4.6 ±1.6	4.5 ±1.6	Case–control scrapie	Immunohistochemistry Western blot Sequencing analysis of Open Reading Frame of the PRNP gene and cloning for haplotype determination	Fisher's exact test and Fisher–Freeman–Halton test	K222 resistant to scrapie (p = 0.001) H154 delay in progression (p = 0.002) R143 delay in disease progression (p = 0.014)
Colussi et al. (2008)	Not reported	254	8 CNS	6.3 ±2.7	5.2 ±1.8	Case–control scrapie	Immunohistochemistry Western blot Sequencing analysis of Open Reading Frame of the PRNP gene and cloning for haplotype determination	Chi-square Test and Fisher's exact test	H154 risk Nor98 (p = 0.002) considered in the haplotype (p = 0.00012) considered as single
Acutis et	Northern Saanen, Valdostana, Camosciata, Roccaverano Southern Garganica, Cilento, Ionica, Mediterranean, Girgentana	300 178	–	–	–	Survey	Sequencing analysis of Open Reading Frame of the PRNP gene and cloning for haplotype determination.	Chi-square Test and Fisher's exact test. F-statistics and Chord distances calculations	R143 (***p < 0.001), H154 (**p < 0.01) K222 (***p < 0.001) associated Southern breeds M142 associated Northern breeds (***p < 0.001)

Table 1
Summary of the main features of each reference considered.

Amino acid position														Reference papers
37	110	127	133	137	142	143	151	154	168	194	211	222	240	
G	T	G	L	M	I	H	R	R	P	T	R	Q	S	All four papers
–	–	–	–	–	–	–	–	–	–	–	–	–	P	All four papers
V	–	–	–	–	–	–	–	–	–	–	–	–	–	All four papers
V	–	–	–	–	–	–	–	–	–	–	–	–	P	Vaccari et al. (2006)
–	P	–	–	–	–	–	–	–	–	–	–	–	–	All four papers
–	P	–	–	–	–	–	–	–	–	–	–	–	P	Vaccari et al. (2006)
–	–	S	–	–	–	–	–	–	–	–	–	–	P	Acutis et al. (2006a, 2008), Colussi et al. (2008)
–	–	–	Q	–	–	–	–	–	–	–	–	–	P	Acutis et al. (2006a), Colussi et al. (2008)
–	–	–	–	I	–	–	–	–	–	–	–	–	P	Acutis et al. (2006a, 2008)
–	–	–	–	–	M	–	–	–	–	–	–	–	P	Acutis et al. (2006a, 2008), Colussi et al. (2008)
–	–	–	–	–	T	–	–	–	–	–	–	–	–	Acutis et al. (2008), Colussi et al. (2008)
–	–	–	–	–	–	R	–	–	–	–	–	–	P	All four papers
–	–	–	–	–	–	–	H	–	–	–	–	–	P	Colussi et al. (2008)
–	–	–	–	–	–	–	–	H	–	–	–	–	–	All four papers
–	–	–	–	–	–	–	–	H	–	–	–	–	P	Vaccari et al. (2006)
–	–	–	–	–	–	–	–	H	–	–	–	K	–	Colussi et al. (2008)
–	–	–	–	–	–	–	–	–	Q	–	–	–	P	Acutis et al. (2006a, 2008), Colussi et al. (2008)
–	–	–	–	–	–	–	–	–	–	P	–	–	–	Acutis et al. (2008)
–	–	–	–	–	–	–	–	–	–	–	Q	–	–	Acutis et al. (2008), Colussi et al. (2008)
–	–	–	–	–	–	–	–	–	–	–	–	K	–	All four papers

Table 2
Polymorphisms (columns) and relative haplotypes (rows) described in Italian goats.