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PrP gene polymorphisms in Cyprus goats and their association with resistance or susceptibility to natural scrapie

Penelope Papasavva-Stylianou [a,*], Otto Windl [b], Ginny Saunders [b], Petroula Mavrikiou [c], Pavlos Toumazos [d], Charalambos Kakoyiannis [e]

[a] *TSE Section, Laboratory of Animal Health, State Veterinary Laboratories, Veterinary Services, 1417 Nicosia, Cyprus*
[b] *Molecular Pathogenesis and Genetics Department, Veterinary Laboratories Agency, New Haw, UK*
[c] *Department of Business Administration, School of Economic Sciences and Administration, Frederick University, P.O. Box 24729, 1303 Nicosia, Cyprus*
[d] *Animal Health and Welfare Division, Veterinary Services, 1417 Nicosia, Cyprus*
[e] *Veterinary Services, 1417 Nicosia, Cyprus*

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ABSTRACT

In contrast to scrapie in sheep, the genetic basis of susceptibility to scrapie in goats is not well understood. To study the association of prion protein (*PrP*) alleles with susceptibility to scrapie in goats in Cyprus, the coding sequence of the caprine *PrP* gene was determined in 717 goats, including 218 scrapie positive animals. Several novel polymorphisms were detected, such as a novel octarepeat variant and a stop codon mutation. Amino acids at codons 146 and 154 were associated with susceptibility to goat scrapie. Animals heterozygous for serine (S) and aspartate (D) at codon 146 were significantly under-represented in scrapie positive animals and no positive animals were found that were homozygous for these amino acids at codon 146. These results might provide the basis for genetic control of scrapie in Cypriot goats.

Introduction

Scrapie is a fatal neurodegenerative disease of sheep and goats which, together with bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt–Jakob disease (CJD) in humans, belongs to the group of transmissible spongiform encephalopathies (TSEs). The occurrence of natural scrapie is strongly influenced by alterations in the host gene that encodes the prion protein (*PrP*) (Hunter et al., 1997). Such polymorphisms might influence the conversion of PrP^{C} into the pathogenic isoform PrP^{Sc} (Bossers et al., 1997).

In sheep, several polymorphisms of *PrP* gene are associated with differences in the phenotypic expression of prion diseases, such as incubation period, pathology and clinical signs. While more than 30 polymorphisms have been described, only a few are closely associated with resistance or susceptibility to classical scrapie, in particular codons for amino acids 136 (A–V), 154 (R–H) and 171 (Q–R–H) (Hunter et al., 1989, 1994; Laplanche et al., 1993; Belt et al., 1995; Bossers et al., 1996).[1]

Although, the study of scrapie susceptibility is complicated by different *PrP* genotypes found in different breeds of sheep, almost all relevant studies suggest that the *ARR*/*ARR* genotype is the most resistant to classical scrapie. The recent finding of atypical scrapie (originally termed Nor98) in sheep, however, has shown that the genetic susceptibility can be remarkably different for other strains of scrapie (Moum et al., 2005; Saunders et al., 2006).

In goats, the association of genetic variability of *PrP* with resistance or susceptibility to classical scrapie remains uncertain. Studies in the UK revealed high variability of *PrP* in goats (Goldmann et al., 2004; Goldmann, 2008), and work in Italy suggested that the variant 222K had an association with scrapie resistance in Ionica breed goats (Vaccari et al., 2006). Moreover, the variants 143R and 154H may give some protection against natural scrapie in Greek goats (Billinis et al., 2002). A recent study in Cypriot goats suggested that the variants 146D and 146S may provide protection against scrapie (Papasavva-Stylianou et al., 2007). In the same study, the presence of the homozygous wild type (WT) allele 146N was strongly associated with susceptibility to natural scrapie.

The variant 142M has been associated with varying disease incubation periods in British goats (Goldmann et al., 1996). Furthermore, a *PrP* variant having three rather than the usual five copies of a short peptide repeat, was associated with an increased scrapie incubation period in goats (Goldmann et al., 1998). In total, 30 *PrP* amino acid polymorphisms and 16 'silent' mutations have been described so far in the *PrP* gene of goats (Table 1), which shows a similar degree of genetic variability to its ovine counterpart.

* Corresponding author. Tel.: +357 22 805144; fax: +357 22 805128.
E-mail address: pstylianou@vs.moa.gov.cy (P. Papasavva-Stylianou).

[1] Amino acids are indicated by the single letter amino acid code and nucleotides are indicated with small letters throughout the manuscript.

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Table 1
Amino acid polymorphisms and 'silent' mutations described so far in the *PrP* gene of goats.

Polymorphism	First reference
W18R	Vaccari et al. (2009)
V21A	Billinis et al. (2002)
L23P	Billinis et al. (2002)
G37V	Agrimi et al. (2003)
S39R	Babar et al. (2008)
P42P[a]	Goldmann et al. (1996)
G49S	Billinis et al. (2002)
Q101R	Vaccari et al. (2009)
Q101Q	Vaccari et al. (2009)
W102G	Goldmann et al. (1998)
K107K	Billinis et al. (2002)
T110P	Agrimi et al. (2003)
V125V	Zhou et al. (2008)
G127S	Zhang et al. (2004) and Kurosaki et al. (2004)
L133Q	Acutis et al. (2006)
M137I	Acutis et al. (2006)
S138S	Goldmann et al. (1996)
I142M	Goldmann et al. (1996)
I142T	Acutis et al. (2008)
I142I	Vaccari et al. (2009)
H143R	Goldmann et al. (1996)
N146D	Papasavva-Stylianou et al. (2007)
N146S	Zhang et al. (2004) and Kurosaki et al. (2004)
R151H	Papasavva-Stylianou et al. (2007)
R154H	Billinis et al. (2002)
P168Q	Billinis et al. (2002)
V179V	Papasavva-Stylianou et al. (2007)
D181D	Papasavva-Stylianou et al. (2007)
I185F	Babar et al. (2008)
T194P	Acutis et al. (2008)
F201F	Vaccari et al. (2009)
T202T	Acutis et al. (2006)
K207K	Billinis et al. (2002)
R211Q	Wopfner et al. (1999)
R211G	Zhou et al. (2008)
I218L	Zhang et al. (2004)
T219I	Zhou et al. (2008)
T219T	Vaccari et al. (2006)
Q220H	Billinis et al. (2002)
Q222K	Agrimi et al. (2003)
Q222Q	Vaccari et al. (2009)
R231R	Zhang et al. (2004)
G232W	Vaccari et al. (2009)
G232G	Vaccari et al. (2006)
L237L	Babar et al. (2008)
S240P	Goldmann et al. (1996)

[a] Grey shading: 'silent' mutations.

Scrapie in goats in Cyprus was first recorded in 1986 in dairy goats run in mixed flocks with affected sheep (Toumazos and Alley, 1989). Since then, the disease has caused major losses in Cypriot goats. A previous study in this population found that polymorphisms at codon 146 were associated with resistance or susceptibility to natural scrapie (Papasavva-Stylianou et al., 2007). The aim of the present study was to extend the previous investigation of the *PrP* genotype of Cyprus goats in order to confirm the previous results. This was achieved by undertaking a new and larger case-control study on a different sample of animals from the previous study.

Materials and methods

Study design

The present study was carried out on 717 goats, including 218 scrapie positive, 280 scrapie negative and 219 healthy control animals, from 75 different scrapie-affected herds in Cyprus. The majority of the animals were of the Damascus breed and/or Damascus crossbred with local breeds. According to the animal registration database of the Department of Agriculture, there are estimated to be 252,000 goats in Cyprus: 15% Damascus, 1% Local Machaeras, 81.7% Damascus crossbred with other local breeds, 0.3% Saanen, 1% Local Akamas and 1% French Alpine.

The scrapie positive goats were 2–10 year-old animals with clinical signs consistent with scrapie that were confirmed positive after histological and biochemical examination of the brain (rapid or discriminatory testing). The scrapie negative goats were animals with clinical signs similar to scrapie but confirmed negative after histological examination and/or rapid testing of the brain. The healthy control goats had no clinical signs. These and the scrapie negative animals were herd matched and, whenever possible, age, breed and sex matched with scrapie positive animals. From each outbreak, we attempted to include an equal number of scrapie positive, scrapie negative and healthy control animals but, for the healthy control animals, this was not feasible in every case, since some of the farmers had sold their animals in the meantime. The maximum number of animals from one outbreak was 51 animals comprising of scrapie positive animals ($n = 10$), scrapie negative animals ($n = 24$) and healthy controls ($n = 17$).

The histological examination and the rapid testing of the brains were performed using the protocol of the OIE Manual and the TeSeE BioRad test, respectively. The discriminatory test was performed at the EU Community Reference Laboratory (Veterinary Laboratories Agency, UK; VLA) using the VLA hybrid method (Stack et al., 2002).

Of the 218 scrapie positive cases in our study, all were tested by histology, 193 by rapid test and 23 (index cases) by the VLA hybrid method according to the Regulations (EC) 999/2001 and (EC) 36/2005; a BSE-specific pattern was excluded for all of them. Of the 280 scrapie negative animals, all were tested by histology, 277 by rapid test and 3 by the VLA hybrid method.

The five heterozygous S146 and D146 scrapie positive cases in our study were determined to be positive either by histology and rapid testing (two ND cases and one NS case), by histology and discriminatory testing (one NS case) or by histology only (one ND case).

Molecular analyses

All 717 samples were analysed by DNA sequencing. Genomic DNA was isolated from ethylene diamine tetraacetic acid-treated blood with the Nexttec Genomic DNA Isolation Kit. PCR amplification of the entire coding region of the *PrP* gene was performed using the primers ov*PrP* ex3 for (5′-actgctatacagtcattcattatgct-3′; base pairs, bp 22,183–22,208) and ov*PrP* ex3 rev (5′-cgccaagggtattattatacagg-3′; bp 23,182–23,160) (GenBank U67922).

Both strands of the PCR products were sequenced by dye terminator cycle sequencing using the ABI3100 capillary sequencer (Agrobiogen Laboratory). The amplification reactions for the DNA sequencing were performed using the primers PYellow for (5′-gatttttacgtgggcatttga-3′; bp 22,222–22,242), PGreen for (5′-cccagtaagccaaaaaccaa-3′; bp 22,590–22,609), PRed rev (5′-acagggctgcaggtagacac-3′; bp 23,123–23,104) and PBlue rev (5′-tggttggggtaacggtacat-3′; bp 22,765–22,746).

Statistical analysis

All cases and controls were divided into four groups of ages (Supplementary Table 1) and data were analysed statistically using the χ^2 goodness-of-fit test. Also, genotype frequencies among scrapie positive, scrapie negative and healthy control goats were analyzed statistically using the chi-square test of association. The terms 'significant' or 'highly significant' refer to P values <0.05 and <0.01, respectively. To quantify the risk of susceptibility to scrapie, the odds ratios (ORs) for codons 102, 146 and 154 were calculated using as 'case' the scrapie positive animals and as 'risk factor' the genotypes WW_{102}, NN_{146} and RR_{154}.

Results

A total of 218 confirmed caprine scrapie cases were examined in parallel with 280 scrapie negative and 219 healthy control goats. There was no statistical difference between the ages of scrapie positive and scrapie negative animals ($\chi^2 = 5.50$, 3 degrees of freedom [df], $P > 0.10$). In comparison, there was a statistical difference between the ages of scrapie positive and healthy control animals ($\chi^2 = 60.47$, 3 df, $P < 0.01$) (Supplementary Table 1).

The entire *PrP* gene coding sequence of all animals was sequenced. Twelve amino acid polymorphisms and seven 'silent' mutations of the caprine *PrP* gene were identified. Ten amino acid polymorphisms have previously been described: codon 102, W → G; codon 110, T → P; codon 142, I → M; codon 143, H → R; codon 146, N → D or S; codon 151, R → H; codon 154, R → H; codon 168, P → Q; and codon 240, P → S. Two amino acid polymorphisms were novel: in codon 163, a caa → taa substitution