PRION PROTEIN GENE (PRP) POLYMORPHISMS IN SCRAPIE AFFECTED INDIGENOUS GREEK GOATS *(Capra prisca)*

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ABSTRACT

Scrapie is a neurodegenerative disease, belonging to transmissible spongiform encephalopathies group. In goats, unlike to sheep, no strong association between certain prion protein (PrP) polymorphisms and scrapie has been determined. In our study, 33 affected goats from scrapie outbreaks in Greece were genotypically analyzed. The main detected polymorphisms are referred to codons 17, 22, 28, 30, 127, 143, 154 and 196. No polymorphisms have been found in codons 142, 154 and 236. The predominant genotype was $MM_{17}GG_{22}PP_{28}PP_{30}GG_{127}II_{142}$ $HH_{143}RR_{154}TT_{196}II_{236}$ (15/33 goats). The usual five octapeptide repeat in codons 54–102 revealed either polymorphisms or partial deletion.

Keywords: scrapie, goats, polymorphisms

INTRODUCTION

Scrapie is a neurodegenerative disease of sheep and goats that belongs to the transmissible spongiform encephalopathies (TSEs) group, linked to genetic background. In goats, unlike sheep, no strong association between certain prion protein (PrP) polymorphisms and scrapie has been determined. This absence of polymorphisms associated to the sensitivity/resistance renders difficult the establishment of breeding programs for scrapie resistance in goats, compared to sheep (European Union Decision 2003/100/EC).

The polymorphisms (amino acid substitutions) described so far in caprine PrP are V21A, L23P, G37V, G49S, W102G, T110N, T110P, G127S, I142M, H143R, N146S, R151H, R154H, P168Q, 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; Papasavva-Stylianou et al., 2006). The W102G polymorphism has been found only in combination with a PrP variant containing three instead of the usual five octapeptide repeats (Goldmann et al., 1998). Silent mutations have been also described at codons 42 (a→g), 107 (g→a), 138 (c→t), 207 (g→a) and 231 (a→c) (Goldmann et al., 1996; Billinis et al., 2002; Zhang et al., 2004).

The aim of the present study was to determine and describe the PrP genotype of scrapie affected indigenous Greek goats (*Capra prisca*), in order to further proceed to genetic association studies.

MATERIALS AND METHODS

In our study, 33 scrapie affected goats (National Surveillance TSEs Programme 2003–2006, Greek Ministry of Rural Development and Food) were genotypically analyzed. Genomic DNA was isolated from frozen brain tissues by using manual PUREGENE^{TM}, DNA Purification system (Cell & Tissue Kit, D-5500A, GENTRA systems). DNA products were evaluated for their quantity and quality, using electrophoresis (Sambrook et al., 1989). PCR amplification of the entire Open Reading Frame (ORF) of PrP gene was performed in two steps using two subsequent PCR reactions: first PCR reaction uses the universal primers M13F and M13R, that anneal about 24bp further of the extreme 5' and 3' regions of the PrP-coding sequence, respectively and second "nested" PCR reaction using primers G1-G2 (Billinis et al., 2002) to specifically amplify the 768bp ORF fragment (Table 1).

Amplification reactions were performed in a PTC-200 Cycler and products were visualized by staining with ethidium bromide after the electrophoresis of an 8 μl reaction mixture on 1% agarose gels. PrP polymorphisms were detected twice by DNA sequencing on both strands of the M13F-M13R and G1G2 PCR products. In the present study, genotypes are described by the single letter amino acid code.

RESULTS

The main polymorphisms detected are related to codons 17, 22, 28, 30, 127, 142, 143, 154, 196 and 236 (Table 2). The predominant genotype was $MM_{17}GG_{22}PP_{28}PP_{30}GG_{127}II_{142}HH_{143}RR_{154}TT_{196}II_{236}$ (15/33 goats or 45.45%), which is considered as the "wild type" (reference to *C. hircus* sequence of Barbieri and Capucci, BLAST GenBank, EF139167, GI: 119489905). Sequencing results revealed amino acid alterations before codon 37, and specifically a dimorphism in codon 17 (L/R17M), a polymorphism in codon 22 (C22G) and in codon 30 (T30P).

In 8 affected goats, the usual five octapeptide repeat in codons 54–102 (Goldmann et al., 1998) revealed either polymorphisms or was partly deleted (1 goat). The W102G polymorphism has been found in two cases without the combined presence of three instead of the usual five octapeptide repeats (Goldmann et al., 1998).

For 142, 154 and 236 codon polymorphisms, which are widely correlated to natural scrapie, the following were detected:

- in codon 142, the only detected substitution was I (Isoleucine)
- in codon 154, R (Arginine) was predominant, except for two cases where L (Leucine) was present and
- for codon 236, 54.55% of the animals did not possess the "protective" amino acid I (Isoleucine). P (Proline) was present in 12.12% of the goats, in combination with a new polymorphism concerning M (Methionine) in 6.06% of the goats.

CONCLUSIONS

The predominant genotype $MM_{17}GG_{22}PP_{28}PP_{30}GG_{127}II_{142}HH_{143}RR_{154}TT_{196}II_{236}$ has no similarity to previous reported variants in *Capra prisca* (Billinis et al., 2002) except for the observation that almost half of the animals tested (45.45%) carried the genotype $HH_{143}RR_{154.}$ previous findings relating the genotype $GG_{127}II_{142}HH_{143}$ (Acutis et al., 2006) to potential protection to scrapie, was not confirmed here.

No polymorphisms were found in codons 146 or 151 (Papasavva-Stylianou et al., 2006) and usual five-octapeptide repeat was present in 25 scrapie affected goats. Previously, unreported polymorphisms were detected in codons 17, 22 and 30, but more research needs to be carried out in the healthy *Capra prisca* population to detect genetic association(s) to the disease. Further data also need to be collected for verification of the "protective" effect of polymorphism in codon 236.

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Table 1. PCR conditons.

Target gene	Target codons	Primers (5'→3')	Fragment size (bp)	Master mix (50 µl / reaction)	Cycler coditions
PrP gene (Open Reading Frame)	*–9 to 256+8*	**M13 F**: – CAG GAA ACA GCT ATG ACC GAT AAT GAA AAC AGC AAG GTT GCC **M13 R**: – TGT AAA ACG ACG GCC AGT CCC TCT TTA TTT TGC AGA GAA GTC	779	0.5–1 µg genomic DNA, 0.8 mM dNTPs, 1.5 mM $MgCl_2$, 3.5 units Taq-polymerase (Invitrogen), 20 pmol of each primer.	94°C/4'- 94°C/30"x40 **59°C/1'x40** 72°C/30"x40 -72°C / 5'
	1 to 256	**G1**:– ATG GTG AAA AGC CAC ATA GGC AGT– **G2**:– CTA TCC TAC TAT GAG AAA AAT GAG–	768	1µl M13 PCR product, 0.8 mM dNTPs, 1.5 mM $MgCl_2$, 3.5 units Taq-polymerase (Invitrogen), 20 pmol of each primer.	94°C/4'- 94°C/1'x40 **59°C/1'x40** 72°C/1'x40- 72°C/5'

Table 2. PrP polymorphism in scrapie affected indigenous goats (*Capra prisca*) [single letter symbolises heterozygous alteration, while two letters (e.g. M/M) symbolises homozygous alteration].

Allele	**Codon**											**Allele frequency (%)**
	17	22	28	30	127	142	143	154	196	236	five-repeat in 54-102	
1	M	G	P	P	G	I	H	R	T	I	+	45.45
2	–	–	–	–	–	–	–	L	–	–	+	6.06
3	L/R	–	S/L	–	–	–	–	–	P	P	+	3.03
4	L	–	–	–	–	–	–	–	–	–	+	3.03
5	L	–	–	–	–	–	–	–	–	P	N94G, A96G	3.03
6	R	–	–	–	–	–	–	–	–	P	+	3.03
7	L	–	–	–	–	–	–	–	–	S	D90G, G102W	3.03
8	–	C	–	–	–	–	–	–	–	–	+	3.03
9	–	C	–	–	–	–	–	–	–	T	+	3.03
10	–	–	L	–	–	–	–	–	–	P	V56G, N58G, S59G, I100S	3.03
11	–	–	–	T	–	–	–	–	P	–	W60S	3.03
12	–	–	–	T	–	–	–	–	–	S	G102W	3.03
13	–	–	–	–	–	–	–	–	–	–	Q80H	3.03
14	–	–	–	–	–	–	–	–	–	S	+	3.03
15	–	–	–	–	–	–	–	–	P	M/M	+	3.03
16	–	–	–	–	–	–	–	–	–	M	+	3.03
17	–	–	–	–	–	–	–	–	–	–	Q80H, S81G,C83G	3.03
18	–	–	–	–	–	–	–	–	–	–	84–97 deleted	3.03