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THE ANALYSIS OF PRION PROTEIN GENE POLYMORPHISM IN SLOVAKIAN WHITE SHORTHAIRED GOATS

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ABSTRACT

Scrapie is a fatal neurodegenerative disease of sheep and goat, grouped under the transmissible spongiform encephalopathy (TSE). Age, infectious agent, as well as host factor like the PrP gene and its allelic form influence the progress of disease. The aim of our study was to detect polymorphisms in the prion protein (PrP) gene in White shorthaired goats as well as to reveal octapeptide repeats. The amplified portion of the PrP gene (Exon 3) was subjected to polymorphism detection using Denaturing Gradient Gel Electrophoresis (DGGE) and DNA sequencing. Polymorphisms in the codons 138 (agc/agt, S → S; or agt/agt; S → S), 142 (ata/atg; I → M), 168 (cca/caa; P → Q) and 179 (gtg/gtt; V → V) were found. All animals (n = 180) in this study were homozygous having 5 octapeptide repeats. This is the first time that a dimorphism in codon 179 present in allele $A_{136}R_{154}Q_{171}/A_{136}R_{154}Q_{171}$ is published. This dimorphism is a result of the silent mutation.

Key words: DGGE; PrP genotype; scrapie; Slovakia; White shorthaired goats

INTRODUCTION

Scrapie is a fatal neurodegenerative disease associated with pathological changes in the conformation of the normal prion protein (PrP^{C}), found on the surface of neurons, resulting in abnormal PrP^{Sc} prion protein. This PrP^{Sc} protein, typical for prionoses, can be accumulated in CNS of the affected animals and humans as aggregates. Accumulation of PrP^{Sc} in the brain is considered as a diagnostic marker of prion diseases (13). The occurrence of natural scrapie in sheep is influenced by alterations in the host gene that encodes the PrP protein (10). The ovine PrP gene has three exons of 52, 98, and 4028 nucleotides in length. In exon 3, the alanine (A) to valine (V) polymorphism in codon 136 and the glutamine (Q) to arginine (R) polymorphism in codon 171 are considered to be propagating factors for the development of scrapie (4, 5, 7, 15, 17).

The association between scrapie susceptibility and polymorphisms in codon 154 is still unclear. However, there is a possibility that histidine (H) in codon 154 may offer protection from scrapie in some sheep breeds. Polymorphisms in the codons 112, 127, 137, 138, 141, 143, 151, 168, 175, 176, 180, 189, 211, and 241 are rare and have not been associated with any disease phenotype in natural and experimental scrapie (2, 6). Similarly, in goat, PrP gene polymorphisms are recorded in the codons 102 (tryptophan to glycine, W → G), 110 (threonine to proline, T → P), 127 (glycine to serine, G → S), 142 (isoleucine to methionine, I → M), 143 (histidine to arginine, H → R), 154 (arginine to histidine, R → H), 168 (proline to glutamine, P → Q), 211 (arginine to glutamine, R → Q), 220 (glutamine to histidine, Q → H) and 222 (glutamine to lysine, Q → K) (2). A disease association has also been observed with dimorphisms in the codons 142, 143 and 154 (1, 3, 8). Codon 142 (I → M) is associated with an altered disease incubation period (3).

A different kind of protein variation is found in a series of glycine-rich octa- or nonapeptide sequences in the N-terminal region of the PrP protein. A PrP allele found in cattle, sheep and goat encodes five of these sequences in the following arrangement: nonapeptide P1(1×), octapeptide P2 (3×) and

nonapeptide P3 (1×) (7, 8). In goats, a PrP allelic variant polymorphism, containing only three octapeptide repeats, is supposed to be associated with an increased scrapie incubation period in goats (9). It is possible that the different profiles of PrP polymorphisms reported for sheep and goats could lead to a differential phenotypic expression of individual scrapie strains (3).

The first case of scrapie in Slovakia was diagnosed in Merino sheep in March 2003 (11). Since then, sporadic cases of sheep scrapie have occurred; 48 cases have been reported up to 2006. However, to date, no scrapie in goats has been diagnosed in Slovakia (**www.svsr.sk**). The aim of this study was to investigate the PrP genotype in the Slovakian goat population.

MATERIALS AND METHODS

Animals

The 180 healthy White shorthaired goats (which represent the dominant bread of goat in Slovakia) in this study originate from two farms (Farm A = 102; Farm B = 78). Genomic DNA was isolated from blood leukocytes. Blood was collected in 1.5 % EDTA. DNA extraction and purification was done according to Sambrook *et al.* (14).

Analysis of octapeptide repeats

The PrP open reading frame was amplified using primers (PRNP1 and PRNP2) as shown in Table 1, which generated 349 bp amplicons in the case of 5 octapeptide repeats. The PCR cycling conditions were as follows: initial denaturation 95 °C for 5 min, followed by 35 cycles of 95 °C for 1.0 min, 65 °C for 1.0 min, 72 °C for 1.0 min with a final extension at 72 °C for 10 min. PCR products were analysed in 1.8 % ethidium-bromide-stained agarose gel.

Table 1. Primers used for PCR amplification

Primer	Sequence 5' → 3'	Product
pTK-F1	GGCCTTGGTGGCTACATGCTG	176 bp
pTK-R1	CGCCCGCCGCGCCCCGCGCCCGCCCGC CGCCCCCGCCCG TTTTTATGTTGACACA GTCATGCAC	
pTK-F2	GTGGTAGCCTCAGTCAGTGGAACA	451 bp
pTK-R2	GAGGAGGATCACAGGAGGGGAA	
PRNP 1*	ACGTGGGCCTCTGCAAGAAGCGAC	349 bp
PRNP 2*	GCACTTCCCAGCATGTAGCCACCA	

*— primers described by Walawski and Czarnik (18)

PrP gene polymorphism study

The PrP open reading frame was amplified by using primers pTK-F1 and pTK-R1 (Table 1), which generated a 176 bp amplicon, involving codons 127 to 185. Primer pTK-R1 contains a long GC part (*GC clamp*), which will remain double stranded during DGGE. The PCR cycling conditions were as follows: initial denaturation 95 °C for 5 min, followed by 30 cycles of 95 °C for 1.0 min, 59 °C for 1.0 min, 72 °C for 1.0 min with a final extension at 72 °C for 10 min. After denaturation (94 °C.10 min^{-1}) and renaturation (30 °C.10 min^{-1}) PCR fragments were separated on 6 % polyacrylamide gel (37.5 : 1; acrylamide/bis) containing a linear gradient (20—65 %) of denaturants (urea and formamide) in 0.5× TAE buffer (40 mmol Tris-acetate, 1 mmol EDTA). Electrophoresis was done at 100 V for 16 h at 58 °C in 0.5× TAE buffer in a DGGE unit (Ingeny, The Netherlands). Gel was stained using silver staining. Goats were segregated into different groups according to the DGGE profiles.

DNA sequencing

Two or three representative samples from each DGGE profile were amplified by PCR with primer set pTK-F2 and pTK-R2 (Table 1), which generated a 451 bp fragment of the coding region of the goat PrP gene. Sequencing was done on the ABI PrismTM 377 Sequencer using Big Dye Terminator Kit (Applied Biosystems). Sequences were evaluated using the Sequence Navigator Program and aligned by Multalin **(http://prodes.toulouse.inra.fr/multalin/multalin.html**). Consequently sequences were compared with known sequences of the goat PrP gene as published in Pubmed (Genebank). Sequences of novel alleles were submitted into the Genebank under the accession numbers AY897571 and AY897572.

RESULTS AND DISCUSSION

All goats were homozygous for 5/5 octapeptide repeats showing no octapeptide variability in the examined population (Fig. 1). The results are in accordance with the previous observation in goats, sheep and other related species (3). On the other hand, Goldmann *et al.* (9) reported three octapeptide repeats in goats, which are associated with an increased scrapie incubation period.

DGGE analysis segregated the samples (n = 180) into 5 groups (Table 2; Fig. 2). Further DNA sequencing revealed that all groups had either the $A_{136}R_{154}Q_{171}/A_{136}R_{154}Q_{171}$ (ARQ/ARQ) genotype, or the ARQ/ARQ allele with additional polymorphic codons (Fig. 3). DGGE profile A, showing only the ARQ/ARQ allele, without additional polymorphisms, was found in 32 (31.4 %) goats from Farm A and 16 (20.5 %) goats from Farm B.

Table 2. Occurrence of PrP alleles in Slovakian White shorthaired goats

DGGE	Genotype	Farm A		Farm B	
		n = 102	%	n = 78	%
A	ARQ/ARQ	32	31.4	16	20.5
B	ARQ/ARQ + SS_{138}	49	48.0	35	44.9
C	ARQ/ARQ + $SS_{138}PQ_{168}$	0	0	2	2.6
D	ARQ/ARQ + $SS_{138}VV_{179}$	19	18.6	21	26.9
E	ARQ/ARQ + $SS_{138}IM_{142}$	2	2.0	4	5.1

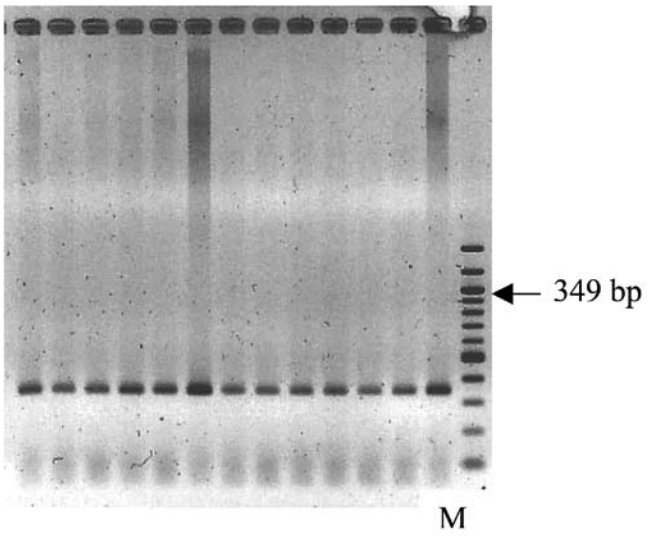

Fig. 1. Ethidium bromide stained PrP gene fragment of goat homozygous 5/5 octapeptide repeats

Legend: M: 100 bp molecular weight marker

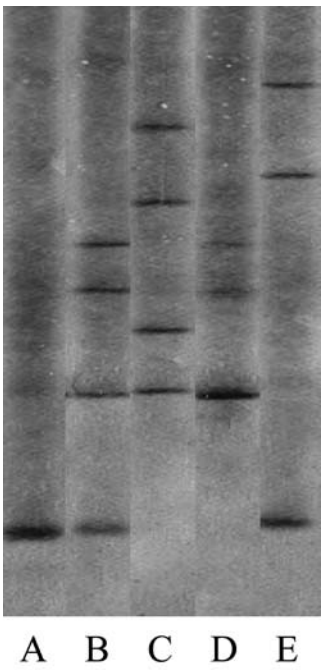

Fig. 2. Denaturing gradient gel electrophoresis of PCR-amplified DNA fragments showing different banding patterns indicating various PrP genotypes, confirmed by DNA sequencing

Legend: DGGE profile: A, ARQ/ARQ; B, ARQ/ARQ + SS_{138}; C, ARQ/ARQ + $SS_{138}PQ_{168}$; D, ARQ/ARQ + $SS_{138}VV_{179}$; E, ARQ/ARQ + $SS_{138}IM_{142}$

Among the 4 remaining clusters DGGE profile B occurred frequently, with an additional dimorphism in codon 138 (ag*c*/ag*t*). The c → t nucleotide substitution had no effect on the amino acid sequence (serine; S) in the PrP protein and is considered silent. This nucleotide substitution was also found in DGGE profiles D and E. The goats with DGGE profile C were homozygous (agt/agt) in codon 138 (Fig. 3). Earlier studies in goats (3, 8) and sheep (15, 16) have also reported a dimorphism in codon 138, however with change in the amino acid sequence (S → N, serine to asparagine). Until now this mutation has not been recorded in goats.

The codon 168 (c*c*a/c*a*a) polymorphism, detected only in 2.6 % (n = 2) of the White shorthaired goats from Farm B, consisted of a c → a nucleotide substitution (DGGE profile C, Figs. 2 and 3) causing change in the amino acid sequence of the PrP protein (P → Q; proline to glutamine). A similar mutation was observed by Billinis *et al.* (3) in goat. In this study, natural goat scrapie was strongly associated with PrP genotype $G_{49}H_{143}H_{154}P_{168}Q_{200}$ and except one, all scrapie cases were homozygous for P_{168}. The goats with DGGE profile C were also different from others because they are homozygous in codon 138 (ag*t*/ag*t*; S).

Goats with DGGE profiles D and E (Figs. 2 and 3) had a dimorphism in codon 138 as well as additional mutations in the codons 179 and 142 respectively. The mutation in codon 179 (gt*g*/gt*t*) (Fig. 3) was a silent one (valine, V). The occurrence of such a mutation has not been recorded before. In this study we found 19 (18.6 %) goats from Farm A and 21 (26.9 %) goats from Farm B with this nucleotide substitution. Amino acid change from isoleucine (I) to methionine (M) was due to a dimorphism in codon 142 (at*a*/at*g*) in goats grouped under DGGE profile E. A similar dimorphism in this codon was recorded by Goldmann *et al.* (8), where they have further described its influence on the scrapie incubation period in experimentally infected goat with BSE prion, sheep scrapie CH 1641 and ME7 strains. Studies have shown that change from isoleucine to methionine in codon 142 (genotype IM_{142}) is associated with the increased incubation period of scrapie in experimentally infected animals (8, 12). In our study we found only 2 (2.0 %) goats from Farm A and 4 (5.1 %) goats from Farm B with this genotype (DGGE profile E, Figs. 2 and 3).

The polymorphism in $A_{136}R_{154}Q_{171}$ codons is linked with the resistance or susceptibility to scrapie in sheep. The ARQ/ARQ genotype belongs to risk group R3, associated with partial susceptibility to scrapie in sheep (www.defra.gov.uk). Billinis *et al.* (3) showed that polymorphisms in the codons 143 (H/R) and 154 (R/H) can protect against natural scrapie in goats. In their study the animals with natural scrapie had genotype HH_{143} or RR_{154}, whereas, animals either heterozygotes (HR_{143}, RH_{154}) or homozygotes (RR_{143}, $HH_{15}4$) were clinically healthy as well as without any histopathological signs of scrapie. Though no polymorphism was found in codon 143 in our study, all the goats were homozygous (HH_{143} or RR_{154}) indicating their possible susceptibility to natural scrapie.

New mutations presented in the PrP gene are silent mutations, which have no effect on the primary structure

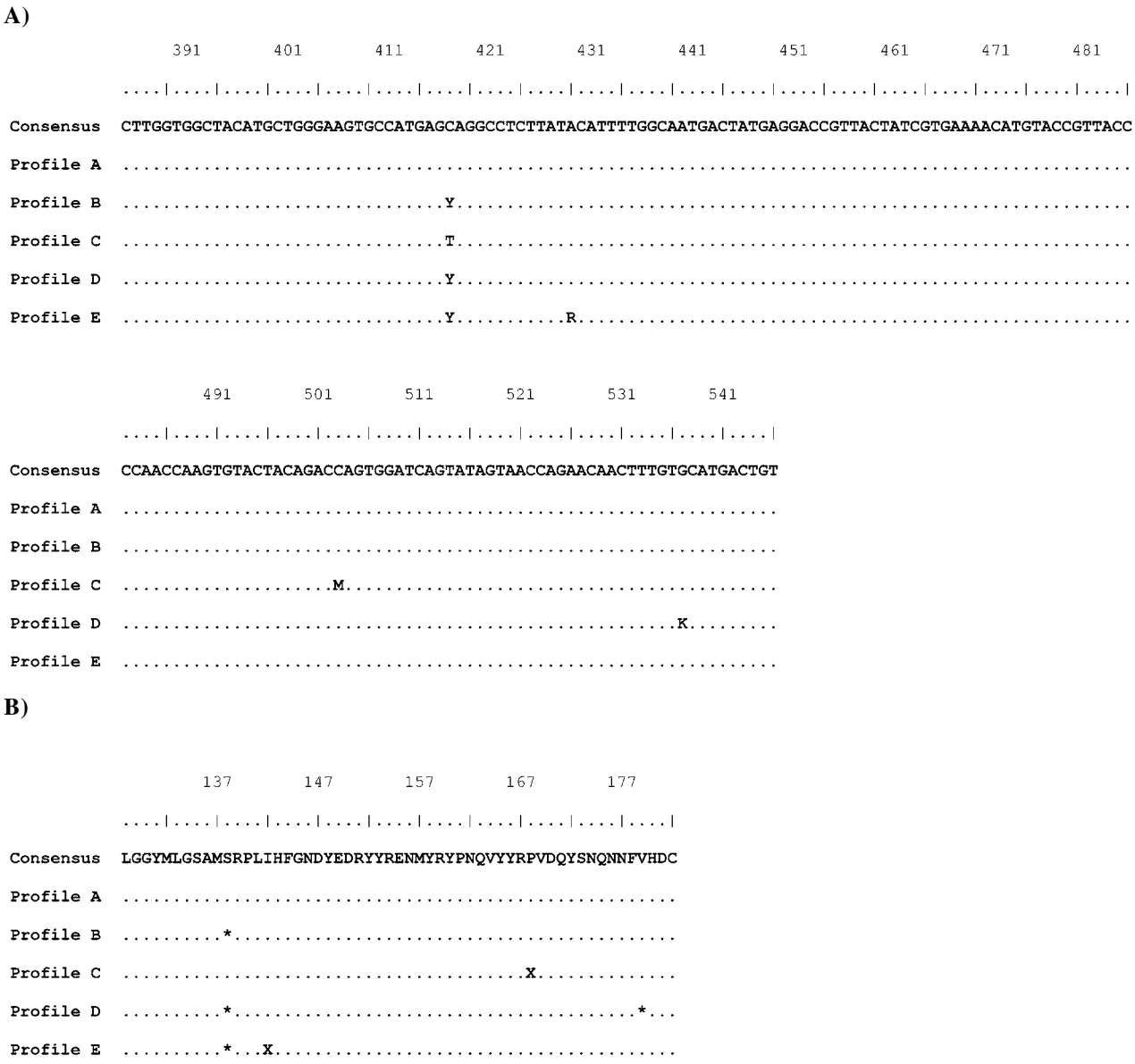

Fig. 3. Nucleotide and amino acid sequence alignment of goat PrP gene

Legend:

A) Nucleotide sequences corresponding to DGGE profiles — A, ARQ/ARQ; B, ARQ/ARQ + SS_{138}; C, ARQ/ARQ + $SS_{138}PQ_{168}$; D, ARQ/ARQ + $SS_{138}VV_{179}$; E, ARQ/ARQ + $SS_{138}IM_{142}$. The sequences are complementary to the cds part of the goat PrP gene X74758. Symbols: Y(T/C), R(G/A), M(A/C), K(G/T)

B) Amino acid sequences corresponding to DGGE profiles — A, ARQ/ARQ; B, ARQ/ARQ + SS_{138}; C, ARQ/ARQ + $SS_{138}PQ_{168}$; D, ARQ/ARQ + $SS_{138}VV_{179}$; E, ARQ/ARQ + $SS_{138}IM_{142}$. Symbols: x — mutation in amino acid; * — silent mutation

of PrP proteins, indicating no association with change in resistance or susceptibility to natural scrapie.

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