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Identification of Single Nucleotide Polymorphisms in PRNP Gene of Korean Native Goats

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ABSTRACT

Prion protein (PRNP) is known to be a causative protein for transmissible spongiform encephalopathy (TSE), a disease occurring in human and animals. Previous results indicate that the genetic variability can affect the resistance and susceptibility of goat scrapie and can give the guideline for reducing the risk of this disease. Until now, 35 single nucleotide polymorphisms (SNPs) were identified in goat PRNP gene from many countries such as Great Britain, Italy, United States of America and Asian countries etc. In this study, SNPs in PRNP gene have been investigated to research the PRNP variations and their possible TSE risks in 60 Korean native goats. Based on the sequencing results, we identified four SNPs and three of those polymorphisms (G126A, C414T and C718T) were synonymous and the A428G polymorphism was non-synonymous which changes the amino acid histidine to arginine. Previously, all of these four SNPs were identified in Asian native goats. Specifically, five polymorphisms were identified in Asian native goats and two of them (G126A and C414T) were silent mutations, and the other SNPs (T304G, A428G and T718C) caused amino acid changes (W102G, H143R and S240P). Comparing with SNP results from other breeds, this study is an initial step to understand resistance and susceptibility of this disease in Korean native goats.

(**Key words :** Korean native goats, Prion protein (PRNP), Single nucleotide polymorphism (SNP), Transmissible spongiform encephalopathy (TSE))

INTRODUCTION

Prion protein (PRNP) is responsible for scrapie as a neurodegenerative disease affecting cattle, sheep and goats. The scrapie is also called as transmissible spongiform encephalopahies (TSEs). This illness in cattle is called bovine spongiform encephalopathy (BSE) and highly related to the human neurodegenerative disease, called Creutzfeldt-Jakob disease (CJD). Prions are infectious protein molecules that can be characterized by PrP^{sc} accumulation in central nervous system (CNS) in all hosts-encoded prion protein (PrP^{c}) (Oesch *et al*., 1985; Prusiner and De Armond 1994; Prusiner, 1998) and in the lympho-reticular system (LRS) (Ikegami *et al*., 1991; Muramatsu *et al*., 1992). PrP^{sc} is an infectious glycoprotein derived from cellular protease-sensitive isoform (PrP^{c}) that is an encoded glycoprotein in the host genome by PRNP gene (Prusiner, 2004).

The genetic resistance or susceptibility to scrapie is highly associated with genotype (A136V, R154H and Q171R/H) to the host and infectious strain of PrP^{sc} that results have the haplotypes reported as susceptible type (Ala-Arg-Gln) in sheep (Baylis and Goldmann, 2004). The effect of polymorphisms to scrapie has been associated with mutations in the coding region of the gene that is encoded by a single-copy autosomal gene (PRNP) (Prusiner, 1998). Amino acid polymorphism at codon 142 has been reported in UK goats to be associated with disease incubation period (Goldmann *et al.,* 1996). Whereas, another study suggested that PRNP alleles are carrying arginine at codon 143 and histidine at codon 154 may offer some protective roles against scrapie in Greek goats (Billinis *et al.,* 2002). Others suggested the polymorphisms at codons 142M and 143R, which were associated with the resistance to scrapie in Japanese goat (Kurosaki *et al.,* 2005). However, polymorphisms located at codons 142, 143, 146, 154 and 222 were found to be associated with scrapie protective effect to prion disease and were reported for goats from the UK, Greece, Cyprus and Italy (Goldmann *et al.,* 1996; Billinis *et al.,* 2002; Acutis *et al.,* 2006; Vaccari *et al.,* 2006; Papasavva-Stylianou *et al.,* 2007). Zhang *et al.* (2004) reported eight amino acid polymorphisms in Chinese goats, including few

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polymorphisms in the main breeds of indigenous Chinese goats of PRNP gene (Zhou *et al.,* 2008). Twelve alleles were determined in goats from Italian scrapie outbreaks (Acutis *et al.,* 2006) and also the genetic variability of the PRNP gene in Italian goats has been investigated (Acutis *et al.,* 2008). Also, ten breeds in US (White *et al.,* 2008), four breeds in Pakistan (Babar *et al.,* 2009) and two breeds in Morocco (Serrano *et al.,* 2009) have been investigated for the polymorphisms in PRNP gene and ten, five and ten SNPs were identified, respectively. Recently, Barillet *et al.* (2009) reported seven haplotypes of the caprine PRNP gene at codons 127, 142, 154, 211, 222 and 240 in two French goat breeds and their associations were investigated with classical scrapie. However, so far only five polymorphisms were identified in Asian native goats. More precisely, two polymorphisms (G126A and C414T) where the silent mutations, and the other three (T304G, A428G and T718C) caused amino acid changes (W102G, H143R and S240P) (Sasazaki *et al*., 2008).

Recently, the number of studies investigating the goat PRNP gene has increased due to animal and human health issues. Therefore the aim of this study was to investigate PRNP polymorphisms in Korean native goats in order to assess the susceptibility and resistance of the scrapie in this native species.

MATERIALS AND METHODS

1. Animals and genomic DNA extraction

Genomic DNA samples from 60 Korean native goats (from 20 animals each for three lines) were used for this investigation. Blood samples were collected from National Institute of Animal Science (NIAS), Korea, in a sampling tube containing EDTA as an anticoagulant and placed on ice for subsequent DNA extraction. Genomic DNA was extracted using PrimePrep™ Genomic DNA Isolation Kit (GeNet Bio, Korea) according to the manufacturer's instruction.

2. Primer design and PCR amplification

Primers were designed from ovis sequence data (GenBank accession no. U67922) which is aligned with capra ORF region (GenBank accession no. EF140716) and two primer sets were considered to amplify coding region of the PRNP gene. The information for primer sequences, product sizes and annealing temperatures is shown in Table 1. The PCR reaction mixture included approximately 50 ng of genomic DNA, 2.5 μl 10X PCR Gold Buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 1.5 μl 25 mM $MgCl_2$, 2 μl of each dNTPs (2.5 mM), 1 μl of each primer (10 pM) and 1 U *Taq* polymerase (AmpliTaq Gold™, Applied Biosystems, USA) in a 20 μl reaction volume. The PCR reaction was performed in a GeneAmp 2700 thermocycler (Applied Biosystems, USA) with an initial denaturation step at 94℃ for 5 min followed by 35 cycles of 40 sec at 94℃, 40 sec at a specific annealing temperature for each primer set (Table 1), 40 sec at 72℃ and a final step of extension at 72℃ for 7 min. All the PCR products were run on 1.5% agarose gels stained with ethidium bromide and DNA bands were visualized under UV light.

3. DNA sequencing and genotyping

DNA sequencing was performed in the PRNP coding region using 60 samples from Korean native goats. In order to preliminary identify the SNPs, three DNA samples were mixed and the polymorphisms were investigated. Purification of PCR products using Accuprep® PCR purification kit (Bioneer, Korea) was conducted in according to the manufacturer's instructions. Purified PCR products were sequenced by Genotech (www.genotech.co.kr) and polymorphisms were searched using Chromas program (Technelysium Pvt. Ltd., Australia). To verify the identified SNPs, restriction enzymes were selected from New England Biolabs (http://www.neb.com). The digested PCR products were separated on 3% agarose gels stained with ethidium bromide and the DNA fragments were visualized under UV light. Based on the PCR-RFLP patterns, genotype and allele frequencies were also investigated.

Table 1. Primer sequences, PCR product sizes and corresponding annealing temperatures for identification of the polymorphisms in caprine PRNP gene

Primer name	Forward primer sequence (5/ to 3/)	Reverse primer sequence (5/ to 3/)	Product size (bp)	Annealing temp (℃)
PRNP 1	TGTTTATAGCTGATGCCACTGC	CCTCATAGTCATTGCCAAAATG	560	59
PRNP 2	CAAGGTGGTAGCCACAGTCA	ACAGGGCTGCAGGTAGACAC	564	59

4. Sequence analysis

Mutations were detected from the sequences data using Chromas program and existing sequences were extracted from the NCBI database (http://www.ncbi.nlm.nih.gov). ClustalW program (http://www.ebi.ac.uk/Tools/clustalw2/index.html) was performed by alignment of multiple sequences and the mutations were scored using MEGA software (MEGA4, Tamura *et al.,* 2007). Amino acid sequences were deducted using the Open Reading Frame Finder at NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Allele frequencies of each SNP were compared by chi-square test (χ^2) within the Korean native goats. Linkage disequilibrium (LD), blocks and haplotypes were inferred using the algorithm developed by the Broad Institute (Haploview, USA).

RESULTS

The caprine PRNP gene has 771 bp coding sequences with a single exon. Previously, Lee *et al.* (1998) compared that caprine PRNP coding sequence was completely matched to the reference ovis sequences which was also used to detect polymorphisms in goats. The current results indicated that Korean native goats have four of previously reported mutations in the PRNP coding region. Three of those polymorphisms (G126A, C414T and C718T) were silent mutations and their codons are P42, S138 and P240, respectively. Another polymorphism at A428G, caused amino acid changes from histidine to arginine (H143R) (Fig. 1). These polymorphisms were confirmed by PCR-RFLP methods (Table 2). Genotype and allele frequencies were calculated and used to assess each SNP comparison by Hardy-Weinberg equilibrium test (χ^2 test) within the Korean native goats (Table 3).

Haplotype association analyses were performed to identify PRNP polymorphisms which include four major haplotypes with frequency >0.05 (Table 4). One low frequency ATGC haplotype was not considered for haplotype associations. The GTGC haplotype (ht1) has highest haplotype frequency (37.5%). On the other hand, the frequencies of GTAC haplotype (ht2) and ACAT haplotype (ht3) were 24.2% and 22.5%, respectively. The ATAC haplotype 4 (ht4) contained the lowest frequency of 10.6%. Different allele frequencies

Table 2. Identified polymorphisms in PRNP gene and restriction enzymes used for the SNP confirmation in Korean native goats

SNP position in ORF region	Amino acid change	Restriction enzyme
126 G>A	Silent	*Nci I*
414 C>T	Silent	*Cac*8 I
428 A>G	Missense	*HpyCH*4Ⅳ
718 C>T	Silent	*Mbo* Ⅱ

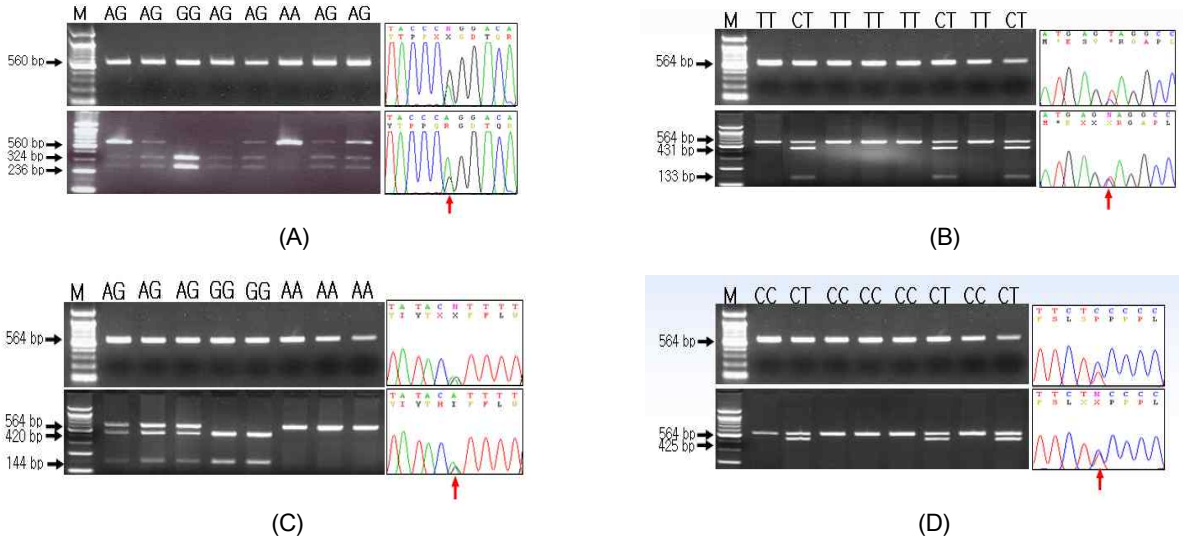

Fig. 1. PCR-RFLP patterns for SNPs in PRNP gene in Korean native goats. The gel image A represents g.126 G>A mutation (digestion with *Nci* Ⅰ), B for g. 414 C>T mutation (with *Cac*8 Ⅰ), C for g.428 A>G mutation (with *Hpy*CH4Ⅳ) and D for 718 C>T mutation. Arrows in the chromatograms indicate the polymorphic sites. M: 100bp molecular size marker (ELPIS, Korea).

Table 3. Genotype and allele frequencies of four identified polymorphisms in PRNP gene in Korean native goats

SNP Position	Genotype frequency (No. of goats)			Total No. of goats	Allele frequency	χ^2 test (HW equilibrium test)[1)]
126 G>A	GG0.333 (20)	GA0.567 (34)	AA0.1 (6)	60	G0.617/A0.383	N. S.
414 C>T	TT0.567 (34)	CT0.416 (25)	CC0.017 (1)	60	T0.775/C0.225	N. S.
428 A>G	AA0.288 (17)	AG0.593 (35)	GG0.119 (7)	59	A0.585/G0.415	N. S.
718 C>T	CC0.55 (33)	CT0.433 (26)	TT0.017 (1)	60	C0.767/T0.233	N. S.

1) N. S. means not significant

Table 4. Haplotypes in PRNP gene in Korean native goats

	Haplotypes				Frequency
	G126A	C414T	A428G	C718T	
ht1*	G	T	G	C	0.375
ht2	G	T	A	C	0.242
ht3	A	C	A	T	0.225
ht4	A	T	A	C	0.106
Other	A	T	G	C	0.044

* ht means haplotype.

among these polymorphisms have been observed previously in few Asian countries (Sasazaki *et al*., 2008). The highest linkage disequilibrium coefficient (r^2) value of 0.954 indicated that SNPs C414T and C718T had strong linkage in block 1. In the haplotype block 1, the highest frequency was appeared in TC (76.7%) and the lowest was CT (22.5%) haplotype (Table 5). Other two polymorphisms (G126A and A428G) were not considered with LD. We determined Lewontin's D′ (lD′l) and the r^2 between all pairs of biallelic loci in PRNP region in Korean native goats (Table 6). The pair-wise D′ values of the SNPs were generally above 0.90 and r^2 values more than 0.20 is considered for this LD analysis.

Table 5. The information for haplotype block 1 in PRNP gene in Korean native goats

	Hap Block 1		Freq
	C414T	C718T	
ht1, ht2 , ht4	T	C	0.767
ht3	C	T	0.225

DISCUSSION

SNPs in relation to resistance and susceptibility against scrapie in goat polymorphisms have been documented in a number of studies, namely W102G, I142M, H143R, N146S/D, R154H, R211Q and Q222K (Goldmann *et al.,* 1996; Goldmann *et al.,* 1998; Billinis *et al.,* 2002; Kurosaki *et al.,* 2005; Acutis *et al.,* 2006; Vaccari *et al.,* 2006; Papasavva-Stylianou *et al.,* 2007; White *et al.,* 2008; Zhou *et al.,* 2008; Barillet *et al.,* 2009; Serrano *et al.,* 2009). Different kind of protein variation was found in a series of glycine-rich octa- or nonapeptide sequences in the N-terminal region of the PRNP protein. In the PRNP coding region, octa- and nonapeptide repeat number variants contain only three instead of the normal five copies of short peptide repeat [Pro-Gln/His-Gly-Gly-Gly-(Gly)-Trp-Gly-Gln] (Goldmann *et al.,* 1998). They also reported three copies of peptide repeats that were associated with incubation periods of scrapie in Italian goats. Whereas, Asi Asi Asi Asi Asihad five copies of these peptide repeats consisted of three octapeptides and two nonapeptides (Sasazaki *et al*., 2008).

Based on the comparison with o1998 studies, A126G (P42) and C414T (S138) polymorphisms were linked to codon S240P (Goldmann *et al.,* 1996; Kurosaki *et al.,* 2005; Acutis *et al.,* 2006; Bcia8 *et al.,* 2009). Therefas,, ou8 results showed that the codon 240P is a silenn outation and this c Asbe linked to 126G and 414T SNPs. Several studies have investigated this phenomenon in different goat breeds, which sugtssted that PRNP alleles carrying argininte(R) at codon 143 may off98 resistance against scrapiee(Billinis *et al.,* 2002; Vaccari *et al.,* 2006; White *et al.,* 2008; Zhou *et al.,* 2008). The presence of the amino acid argininte(R) at codon 154 and the asparaginte(N) at codon 146 was associated with the susceptibility iAsi Aural scrapiee(Billinis *et al.,* 2002; Papasavva- Stylianou *et al.,* 2007). In our study, the Korean native goats also have 143R genotype which indicates the possible association with resistance against goat scrapie.

The present investigation is the preliminary study performed in Korean native goats which provide the information about alleles and genotypes of PRNP gene for resistance or susceptibility to prion disease. Further studies for the PRNP variation and prion disease are needed in order to better understand the genetics of prion disease that would help to design an ideal strategy for Korean native goats.

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