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Sasazaki, Shinji
Nagato, Tomiyuki
Mannen, Hideyuki

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Short communication

Genetic Diversity of Prion Protein Gene in Asian Native Goat

Shinji Sasazaki, T. Nagato, H. Mannen
Graduate School of Agricultural Science, Kobe University, Kobe, Japan

Corresponding author
Shinji Sasazaki, Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan. Tel: +81-78-803-5801; Fax: +81-78-803-5801; E-mail: sasazaki@kobe-u.ac.jp

Abstract

This study seeks to investigate the genetic variability of *PRNP* in Asian goats. We sequenced the *PRNP* coding region using a total of 193 samples from seven Asian countries (Japan, Laos, Vietnam, Bhutan, Mongolia, Myanmar and Cambodia). Sequence comparison revealed five previously reported polymorphisms in the *PRNP* coding region. Two of those polymorphisms (G126A and C414T) were silent mutations, and the other three (T304G, A428G and T718C) caused amino acid changes (W102G, H143R and S240P). In the total of 193 animals, one amino acid mutation (T304G) exhibited low variability (minor allele frequency = 0.04), but the other four were high (0.31 to 0.36). In addition, allele frequencies of C414T and T718C exhibited remarkable differences among countries (p-values of 6.50E-17 and 5.49E-18). These results suggest high genetic variability of *PRNP* among these countries and are useful information for estimating genetic diversity in Asian goats.

Keywords: Prion; Scrapie; Asian goat; Genetic diversity

1. Introduction

Scrapie of sheep and goats belongs to the transmissible spongiform encephalopathy (TSE) illnesses such as bovine spongiform encephalopathy and Creutzfeldt–Jakob disease of humans. The disease is characterized by accumulation of an abnormal isoform of the cellular prion protein in the central nerve system (Prusiner, 1991). Prion protein is encoded by a single-copy autosomal gene (*PRNP*) (Prusiner, 1998).

Some studies have demonstrated the associations between *PRNP* polymorphisms of the open reading frame (ORF) and TSE infectivity. In sheep, three novel amino acid substitutions at codons 136, 154 and 171 have been reported to be associated with susceptibility/resistance to scrapie. In the combination of these three mutations, Ala-Arg-Arg haplotype was suggested to have resistance to scrapie (Belt et al., 1995; Bossers et al., 1996; Hunter et al., 1996, 1997). In caprine *PRNP*, some mutations in ORF have already been identified. An amino acid polymorphism at codon 142 has been reported to be associated with an altered disease incubation period in UK goats (Goldmann et al., 1996). Another study suggested that *PRNP* alleles carrying arginine at codon 143 and histidine at codon 154 may offer some protection against scrapie in Greek goats (Billinis et al., 2002). In addition, Actis et al. (2005) suggested a possible protective role against scrapie for the glutamine to lysine mutation at codon 222 in Italian goats.

Zhang et al. (2004) reported some amino acid polymorphisms at positions G127S, H143R, N146S, R154H, I218L, Q222K, R231R, S240P in Chinese goats, however there have been few reports on *PRNP* polymorphisms in Asian goats. To date, there has been no report of scrapie cases in Asian goats. However, it is important to investigate the genetic variability of *PRNP* in Asian goats in case of a scrapie outbreak. For this purpose, we carried out *PRNP* sequencing of 193 Asian native goats from seven countries.

2. Materials and Methods

A total of 193 samples were collected from healthy goats in Asia. All native goats living in rural area were collected randomly from several areas in each country. Genomic DNA was extracted from blood sample of 30 Japanese, 30 Laotian, 31 Vietnamese, 30 Bhutanese, 13 Mongolian, 30 Myanmar and 29 Cambodian goats according to a standard phenol and chloroform method. These individuals were unrelated or they had the least genetic relationships in three generations.

We determined the sequences of the full *PRNP* coding region in 193 Asian native goats. Forward (5'-GTA GCT GAC ACC CTC TTT ATT TTG C) and reverse primers (5'-AGC AAG AAA TGA GAC ACC ACC ACT A) were designed according to ovine *PRNP* sequence (GenBank accession no. M31313). The PCR reactions were performed using 20μl reaction volumes with 20ng genomic DNA as a template, 2.0μl reaction buffer buffer (100mM Tris-HCl, 15mM MgCl, 500mM KCl, pH8.6), 1.6μl dNTP Mix (2.5mM), 0.13μl of each primer (20nmol/ml) and 1.0U of Ex Taq polymerase Hot Start Version (Takara Shuzo Co., Tokyo, Japan). Amplification of PCR products was carried out using a standard PCR program with 5-min denaturation at 94°C, 30 cycles for 1-min at 94°C, 1-min annealing at 58°C, 1-min extension at 72°C, and final extension for 7-min at 72°C. After purification of PCR product using GENEMate DNA purification kit (ISC BioExpress, Kaysville, UT), standard double-strand DNA cycle sequencing

was performed with approximately 20ng of amplified product using ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) on ABI PRISM® 3100 Genetic Analyzer.

Allele frequencies in each country were calculated from sequencing result and Pearson's Chi-squared test was performed to examine the difference of allele frequencies among countries.

3. Results and Discussion

We sequenced the *PRNP* coding region using genomic DNA in Asian goat to determine the caprine *PRNP* coding sequence and to identify the polymorphisms. Sequencing results revealed that the 771bp full-length sequence of the *PRNP*-coding region completely corresponded to the reference sequence (accession number U67922) determined by Lee et al. (1998)

In the *PRNP* coding region, a short octa- or nonapeptide repeat [Pro-Gin/His-Gly-Gly-Gly-(Gly)-Trp-Gln-Gln] has been identified in humans, cattle and sheep. In repeat number variants, three or five copies were identified in goat (Goldmann et al., 1998). They reported three copies of peptides repeat were associated with incubation periods of scrapie in Italian goats. In this study, five copies of these peptide repeats consisted of three octapeptides and two nonapeptides and were only identified in Asian native goats.

Comparing the sequences from a total of 193 animals in Asian goats revealed five previously reported polymorphisms in the *PRNP* coding region. Two of those polymorphisms (G126A and C414T) were silent mutations, and the other three (T304G, A428G and T718C) caused amino acid changes (W102G, H143R and S240P). No polymorphisms were detected at codons 136 (Ala), 154 (Arg) or 171 (Gln) that have been reported to be associated with susceptibility/resistance to scrapie in sheep. This result suggested that all Asian goats have the haplotypes reported as susceptibility type (Ala-Arg-Gln) in sheep.

The three amino acid changes (W102G, H143R and S240P) detected in this study have been previously reported on the association between mutations and scrapie in other populations. Goldmann et al. (1998) reported Trp to Gln substitution in codon 102 and suggested the association with modulation of the Cu^{2+}-binding function of the prion protein. Billinis et al. (2002) reported the association between H143R and R154H and resistance against scrapie in Greek goats. All 51 scrapie-affected animals carried the HH143 RR154 genotype, with the exception of two goats (HR143), both of which had detectable protease resistance. In addition, they also suggested that no significant association of codon 240 with disease was observed because the C-terminal region of the prion protein, including codon 240, is removed during the post-translational attachment of a glycoinositol phospholipid tail. These results suggested that three amino acid changes detected in Asian goats might be candidate mutations associated with scrapie.

Table 1 lists allelic frequencies of *PRNP* polymorphisms for each country in Asian goats. These frequencies were derived based on the reference sequence. In a total of 193 animals, one amino acid mutation (T304G) exhibited low variability (minor allele frequency = 0.04), but the other four were high (0.31 to 0.36). A428G mutation has been previously reported in sheep by O'Rourke (2000). They reported that the G allele is very rare in sheep, but the current study on goats revealed high variability (G allele

frequency of 0.18 to 0.55) in each country. Allele frequencies of C414T and T718C exhibited remarkable differences among countries (p-values of 6.50E-17 and 5.49E-18). Minor allele frequencies in Cambodia (0.81 and 0.79) were higher than that in other countries (0.10 to 0.48).

In this study, we determined the caprine *PRNP* coding sequence and revealed high genetic variability among the countries in Asia. These results are primary information for understanding the genetic diversity in Asian goats.

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Table 1. Allele frequencies of *PRNP* polymorphisms in Asian native goats.

Reference[1]	(n=)	G	T	C	A	T
DNA sequence		G126A	T304G	C414T	A428G	T718C
Amino acid		P42P	W102G	S138S	H143R	S240P
Vietnam	31	0.90	1.00	0.10	0.45	0.10
Japan	30	0.87	0.98	0.13	0.78	0.12
Laos	30	0.70	0.98	0.30	0.67	0.18
Mongol	13	0.62	0.77	0.31	0.54	0.31
Bhutan	30	0.55	0.95	0.40	0.68	0.38
Myanmar	30	0.68	0.95	0.48	0.82	0.43
Cambodia	29	0.35	0.97	0.81	0.81	0.79
Total	193	0.67	0.96	0.36	0.69	0.33
p-value [2]		9.78E-11	8.32E-05	6.50E-17	3.52E-05	5.49E-18

[1] Reference sequence (Accession number; M31313) was determined in sheep by Goldmann et al. (1990).

[2] Pearson's Chi-squared test was performed to examine the difference of allele frequencies among countries.