Prion protein gene (PRNP) polymorphisms in Xinjiang local sheep breeds in China

Brief Report

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Summary. Amino acid polymorphisms of the prion protein (PrP) gene (PRNP), particularly those occurring at codons 136, 154, and 171 have a significant influence on scrapie pathogenesis in many sheep breeds. We isolated blood samples from 222 sheep representing the eight main local sheep breeds in the Xinjiang Autonomous Region, the territory with the most abundant local sheep breeds in China, to identify the PRNP polymorphisms and to determine whether these breeds were at risk for developing scrapie. A new PRNP polymorphism encoding either glycine (G) or arginine (R) at codon 85 as well as eight previously reported polymorphisms at codons 101, 112, 127, 141, 146, 154, 171, and 189 in other sheep breeds were detected. Interestingly, the alanine (A)/V polymorphism at codon 136 was not observed in this study, all sheep being homozygous for A at this position. While the previously identified polymorphism of argine (R) or histidine (H) at codon 154 was detected, the H polymorphism was rare (2.25%). Four polymorphisms at codon 171 encoding glutamine (Q), R, H, or lysine (K) were detected. The predominant ARQ allele occurred at a high frequency of 77.48%, suggesting an increased risk of scrapie in Xinjiang Autonomous Region.

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Transmissible Spongiform Encephalopathies (TSEs), or prion diseases, are neurodegenerative disorders of mammals, including scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in deer and elk, and Creutzfeldt Jakob disease, kuru, and related diseases in humans. Prion diseases are characterized by the accumulation of an abnormal, protease-resistant isoform of PrP primarily in the central nervous system (CNS) and lymphoreticular systems of affected individuals and are characterized as infectious, sporadic, or genetic disorders. In several animal species and in humans, polymorphisms within the PRNP open reading frame (ORF) have been shown to influence disease susceptibility and pathologies \[12, 19, 20, 24\]. In particular, the polymorphic amino acid variations of A, V, or T at residue 136, R or H at residue 154, and Q, R, H, or K at residue 171 have been shown to significantly influence susceptibility to scrapie for many sheep breeds \[10, 25, 27, 29\]. The aim of this study was to identify the PRNP polymorphisms of sheep in Xinjiang Autonomous Region, the territory with the most abundant local sheep breeds in China, with the primary purpose of assessing the potential for scrapie susceptibility. The results showed that sheep of all eight breeds were homozygous for A at residue 136, while R was present at residue 154 in the vast majority of PRNP alleles, and all four previously identified polymorphisms at residue 171 were detected. We also report on one novel PRNP polymorphism at residue 85.

In this study, 222 blood samples from eight main local sheep breeds, including Hazake sheep (37), Markit sheep (30), Bayanbulak sheep (30), Hotan sheep (30), Xinjiang fine wool sheep (30), Qira black (30), Kuqa sheep (25), and Altay fat rumped sheep (10) were collected across Xinjiang Autonomous Region. All of the sheep were healthy and less than 3 years of age.

Genomic DNA was isolated from the EDTA-treated blood by using a genomic DNA purification kit for mammalian blood (Promega). PCR primers that amplify a 1049-bp fragment of the ovine PrP gene (GenBank accession no. U67922) including the 771-bp open reading frame (ORF) were designed using Primer premier 5.0. The forward primer was 5′-TAAGAGTTGTTACTACCTTACCTAT-3′ and the reverse primer was 5′-GCTTGTCATTTCCCAGTGCT-3′. PCR was performed in a 50-µl reaction volume, containing 10× PCR buffer, 5 µl of each primer (50 pmol/µl), 1 µl of dNTPs (10 mM), 4 µl of Takara DNA Taq polymerase, and 0.4 µl of (0.2 to 0.5 µg) sheep genomic DNA. The PCR procedure consisted of denaturation for 5 min at 95 °C followed by 30 cycles consisting of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min. A final extension at 72 °C for 10 min was employed after the cycling.

After purification, the PCR products were sequenced on both strands using dye-terminator cycle sequencing on an ABI PRISM™377 × L DNA Sequencer. All of the sequences have been submitted to GeneBank, and we have received the accession numbers from DQ149332 to DQ149502 and from DQ272606 to DQ272656.