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Molecular cloning and polymorphism analysis of the prion protein gene in Tan sheep of Ningxia, China

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ABSTRACT

The resistance or susceptibility of sheep to scrapie is associated with polymorphisms of the prion protein gene (*PRNP*), particularly, single nucleotide polymorphisms (SNPs) in amino acid positions 136, 154 and 171. The prion protein (PrP) gene sequence and the deduced amino acid alignment of prion protein in Tan sheep, a local Chinese sheep breed traditionally raised in Ningxia, northwestern China, were determined and variability of the PrP amino acids sequence was analyzed in this study. The PrP nucleic acids and amino acids sequences of 112 Tan sheep were highly homogenous, although polymorphism of the PrP gene was detected at several sites, particularly codons 106, 154, and 171. The analysis of both sequences revealed that the most predominant allele at codons 136, 154 and 171 in Tan sheep was ARQ, which was known to be associated with high susceptibility to scrapie in sheep. The result suggests that Tan sheep is potentially susceptible to scrapie. Our findings provide valuable information for future breeding projects to scrapie resistance in Tan sheep.

1. Introduction

Scrapie is a fatal neurodegenerative disease of sheep and goats, and is one of several prion diseases, also known as transmissible spongiform encephalopathies (TSE) of humans and a variety of mammalian animals, including Creutzfeldt–Jakob disease (CJD) and Kuru disease in humans and bovine spongiform encephalopathy (BSE) in cattle. The characteristic symptoms of TSE include various neurological disorders followed by progressive wasting and death. The transmission of the disease depends on the exposure to the infectious agent, the animal breed and the genotype of the host (Silveira et al., 2005). TSE results from normal cellular prion protein (PrP^{c}) converting to its abnormal form (PrP^{sc}), which then becomes the infective agent (Prusiner, 1998). The PrP^{c} and PrP^{sc} are encoded by the same prion protein gene (*PRNP*) with exactly the same amino acid sequences, but differ in the tertiary structure (Westergard et al., 2007).

It is well established that the resistance or susceptibility of sheep to scrapie is associated with polymorphisms in the *PRNP*, particularly, single nucleotide polymorphisms (SNPs) at amino acid positions 136, 154 and 171 (Goldmann et al., 1990). It was found that five alleles, namely $A_{136}R_{154}Q_{171}$(ARQ), $A_{136}R_{154}R_{171}$(ARR), $A_{136}H_{154}Q_{171}$(AHQ), $A_{136}R_{154}H_{171}$(ARH), and $V_{136}R_{154}Q_{171}$(VRQ), could be formed at codons 136, 154 and 171 and were associated with susceptibility or resistance to scrapie in sheep (Hunter, 1997). Among these alleles, the VRQ and ARQ variants are associated with high susceptibility, whereas the ARR variant is correlated with the greatest resistance to the disease (Belt et al., 1995; Clouscard et al., 1995; Drögemüller et al., 2001). Polymorphisms at codon 154 are of minor importance. Little is known about the association of AHQ and ARH with susceptibility to scrapie (Bossers et al., 2000). Apart from the above polymorphisms, many other polymorphisms have been identified in the ovine PrP gene at codons 83, 101, 112, 116, 127, 138, 141,157,172, 175, 176, 180, 189, 195, 196, 211, 220,223,231, 237 and 241 (Thorgeirsdottir et al., 2002; Tranulis et al., 1999; ün et al., 2008; Vaccari et al., 2001; Wang et al., 2008), but the clinical significance of these variations is unknown.

Tan sheep is an autochthonous sheep breed raised mainly in the Ningxia Hui Autonomous Region, northwest of China. It is renowned for its curled, long, soft and light wool and lamb fur, and also for its high quality meat, which is exported to Middle Eastern countries. The Tan sheep industry is of significant economic importance for a large area of northwestern China.

The aim of this study was to investigate polymorphisms of *PRNP* in Tan sheep, and to analyze whether this traditionally raised sheep breed carries *PRNP* associated with scrapie-resistant or susceptible phenotypes. The study provides valuable information on Tan sheep genetics that can be used for future scrapie-resistant breeding projects.

Abbreviations: TSE, transmissible spongiform encephalopathies; CJD, Creutzfeldt–Jakob disease; BSE, bovine spongiform encephalopathy; PrP, prion protein; PrP^{c}, cellular prion protein; *PRNP*, prion protein gene; SNPs, single nucleotide polymorphisms; ARQ, $A_{136}R_{154}Q_{171}$; ARR, $A_{136}R_{154}R_{171}$; AHQ, $A_{136}H_{154}Q_{171}$; ARH, $A_{136}R_{154}H_{171}$; VRQ, $V_{136}R_{154}Q_{171}$; PCR, polymerase chain reaction.

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2. Materials and methods

2.1. Sampling

Blood samples were collected by venipuncture into heparinized vacuum tubes from 112 healthy, 2- to 3-year-old randomly selected Tan sheep from 4 unrelated flocks in the Tan sheep breeding zone, located in Yanchi, Ningxia Hui Minority Autonomous Region, China. All blood samples were frozen at −20 °C until use.

2.2. DNA preparation and amplification

Genomic DNA of each blood sample was extracted using a Genomic DNA Rapid Isolation Kit (BioDev-Tech, China) and amplified by PCR. PCR primer pairs which cover the whole open reading frame of the PrP gene were designed using Primer Premier 5.0 based on the *PRNP* sequence deposited in the GenBank (Accession number AY585240). The forward and reverse primers, 5′-TAGCTGATGCCAC TGCTATG-3′ and 5′-AAGGTTGCC CCTATCCTACT-3′, respectively, were synthesized and stored at −20 °C. A 770 bp nucleotide of the ovine *PRNP* was amplified in a 50 μL reaction mixture containing 15 pmol each of the forward primer and the reverse primer, 50–100 ng of genomic DNA, 10 mM of each dNTP, 2.0 mM $MgCl_2$, 3 U of Taq plus DNA Polymerase (Takara, Japan) and 19 μL purified water (ELGA Labwater, UK). PCR reaction was performed in the Alpha Unit Block Assembly for DNA Engine Systems (Bio-Rad, USA) as follows: denaturation at 94 °C for 5 min, 35 amplification cycles including denaturation at 94 °C for 30 s, annealing at 58 °C for 40 s and extension at 72 °C for 40 s, followed by a final 10-min extension at 72 °C. The amplified DNAs were confirmed by electrophoresis on a 1% agarose gel and then purified using a DNA fragment gel purification and extraction kit (BioDev-Tech, China).

2.3. Sequencing and genotyping

The purified DNAs were sequenced directly, or were ligated into the pEASY-T1 vector (TransGen, China) according to the manufacturer's

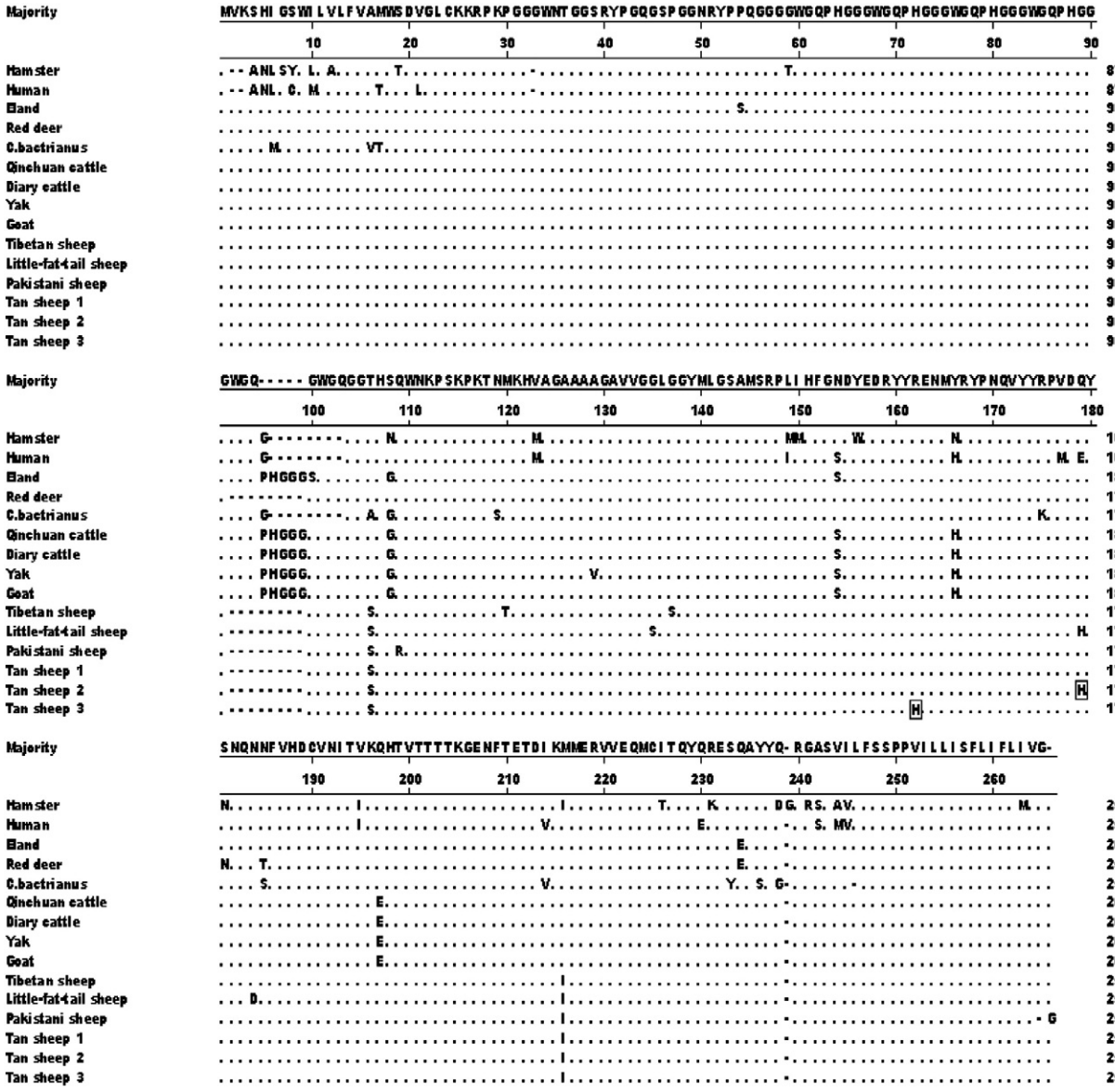

Fig. 1. Amino acid alignment of 15 mammalian prion proteins, including hamster (*Mesocricetus auratus*, M14054), human (*Homo sapiens*, NG_009087.1), eland (*Tragelaphus oryx*, EF165082), red deer (*Cervus nippon*, AY679695), Bactrian camel (*C. bactrianus*, HQ204566.1), Qinchuan cattle (*Bos taurus*, AY367638), dairy cattle (*Bos taurus*, AY367641), yak (*Bos taurus*, AY367635), goat (*Capra hircus*,EU032305.1), Tibetan sheep (*Ovis aries*,Y723287), Chinese little-fat-tail sheep (*Ovis aries*, AY029774), Pakistani sheep (*Ovis aries*, DQ346682), and 3 Tan sheep (*Ovis aries*, HM803994, HQ197668, HQ197671). Sites identical to the consensus sequence are denoted by dots, site deletion by dashes, and polymorphism by boxes.