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Short communication

The polymorphism of a novel 30 bp-deletion mutation at *KAP9.2* locus in the cashmere goat

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ABSTRACT

The keratins and keratin-associated proteins (KAPs) are a large heterogeneous group of proteins that make up about 90% of the cashmere fiber. Keratin-associated proteins 9.2 gene (*KAP9.2*) is one of the ultra high sulfur *KAP*s, which might play an important role in the bundling of intermediate filaments. In this study, the deletion/insertion mutation of *KAP9.2* gene in 997 cashmere goat samples was firstly detected, at the same time, parts of these samples were sequenced. The results showed that two alleles were detected at this *KAP9.2P1* locus, named allele W and D. The frequencies of the *KAP9.2*-W allele in Inner Mongolia White cashmere ($n = 785$) and Shaanbei White cashmere goat breeds ($n = 212$) were 0.878 and 0.790, respectively. The χ^2-test showed that the genotype distributions in these two cashmere goat breeds were not in agreement with Hardy–Weinberg equilibrium. According to the classification of polymorphism information content (PIC), Shaanbei White cashmere goat was more polymorphic at this locus. Moreover a 30 bp-deletion mutation was described at *KAP9.2P2* locus for the first time and no deletion/insertion was described at *KAP9.2P1* locus. The results possibly revealed that the size polymorphism existed in the two Chinese cashmere goat and the 30 bp-deletion mutation was possibly caused by variations in the number of the decapeptide repeat structures.

1. Introduction

The cashmere fiber consists of two main structures, the cuticle and the cortex. In human, the cuticle forms the hair surface, the cortex is the major site of keratinisation and, thus, essential for shaft rigidity (Langbein et al., 1999, 2001). The keratins and keratin-associated proteins (KAPs) are a large heterogeneous group of proteins that make up about 90% of the wool fiber (Powell and Rogers, 1994). The hair keratins represent the type I (acidic) and type II (basic) two multigene families. They form the 8–10 nm intermediate filaments (KIF) of trichocytes by co-polymerization of type I and type II members, which are differentially expressed during hair fiber development (Langbein et al., 1999, 2001). The KIF embedded in a matrix, which is an amorphous space in the cortex region. The hair KAPs were originally identified as being the major part of the matrix mass surrounding the 10 nm hair KIF in the cortex of the hair follicle (Rogers and Schweizer, 2005).

KAPs are encoded by a large number of multigene families. KAP genes are small in size (less than 1 kb), generally contain a single exon, and these exons are separated by

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2.5–38 kb of intervening sequences. Based on amino acid composition, the KAPs have been divided into three categories, the high sulfur KAPs (<30 mol% cysteine content), the ultra high sulfur KAPs (<30 mol% cysteine content), and the high tyrosine/glycine KAPs (Powell and Rogers, 1996). Rogers and Powell (1993) introduced a species-independent nomenclature using the abbreviations KAP1.n through KAP8.n for the eight members known at that time with "n" referring to a number identifying individual members fitting into a given family on the basis of sequence homology and the nature of repeat structures often present in these proteins. Up to now, 23 KAP gene families are found (*KAP*1.n–*KAP*23.n), of these, *KAP*1–3,10–16 and 23 belong to high sulfur *KAP*s, *KAP*4,5,9,17 are ultra high sulfur *KAP*s, *KAP*6,8,18,22 constitute high glycine/tyrosine *KAP*s.

KAP9.2 gene is one of the ultra high sulfur *KAP*s which are important for the hair formation. The ultra high sulfur proteins are predominantly found in the cuticle with some also found in the cortex. In 1989, Alistair et al. reported that the ultra high sulfur and the high sulfur keratins provided the cysteines for the cross-linkage of the hair proteins to form intracellularly the physical and chemical inert structure of the hair. In human, the presence of a high cysteine content in the ultra high sulfur *KAP*s has led to the assumption that these residues might play an important role in the bundling of KIFs viacysteine cross-links, thus the ultra high sulfur *KAP*s probably are significant in this KIF bundling. Furthermore, parts of the human ultra high sulfur *KAP*s were identified with highly keratinized portions of the hair cortex, which was consistent with the hypothesis mentioned (Rogers et al., 2001).

China has a centuries-old history of breeding cashmere goat and abundant cashmere breeds resources, and it is one of the biggest cashmere-produced countries. The Inner Mongolia White cashmere goat is one of the most famous cashmere goat breeds in the world. While the Shaanbei White cashmere goat is a new breed which came from Liaoning cashmere goat and Shaanbei indigenous goat and was named by Ministry of Agriculture of the People's Republic of China in 2002, but its multiple cashmere patterns have already been known. This type of cashmere has a long thin rather than crude long-haired cashmere. It appears as spherical, but not tangles, and hair braids can be differentiated clearly (Zhao et al., 2008).

Taken together, the objective of this study was to identify sequence variation of the cashmere goat *KAP*9.2 gene in two cashmere goat breeds (Inner Mongolia and Shaanbei White cashmere goat) by methods of electrophoresis and DNA sequencing.

2. Materials and methods

2.1. Goats and DNA sources

The edge tissues of ear samples from these two cashmere goat breeds were obtained from the centers of cashmere goat reproduction in Inner Mongolia Autonomous Region (Inner Mongolia White cashmere goat, $n = 785$) and Shaanxi province (Shaanbei White cashmere goat, $n = 212$), respectively. Genomic DNA was extracted from these specimens using phenol/chloroform as described by Sambrook and Russell (2001).

2.2. PCR amplification

Zhao et al. (2008) described a 24 bp-deletion mutation at *KAP6.2* (HGT-KAP) locus in Shaanbei cashmere goat. In order to detect whether there was another deletion mutation at *KAP9.2* (ultra high sulfur KAPs) locus or not, two pairs of primers were designed from a published gene sequence (GenBank Accession No. AY510124). The sequences of these primers were: P1: forward 5′ ACCCTCCACCACTGACGC 3′ and reverse 5′ ACAGGGGCACAGTAAACC 3′; P2: forward 5′ ACCTGCCACAAGCCTACT 3′ and reverse 5′ CACATGGTTCTATGACAGG 3′. The size of the PCR products was 426 bp and 335 bp, respectively, containing the whole CDS region. Each PCR was performed in a 25 μL reaction volume containing: 50 ng genomic DNA, 10 pM of each primer, 1× buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs and 0.625 units of *Taq* DNA polymerase (MBI). The cycling protocol for P1 was 5 min at 95 °C, 33 cycles of 94 °C for 30 s, 63.5 °C annealing for 35 s, 72 °C for 45 s, with a final extension at 72 °C for 10 min. The cycling protocol for P2 was 5 min at 95 °C, 33 cycles of 94 °C for 30 s, 61.2 °C annealing for 35 s, 72 °C for 45 s, with a final extension at 72 °C for 10 min.

2.3. Detection variation in the KAP9.2 gene and DNA sequencing

PCR products were electrophoresed on 3% agarose gels with 1× TBE buffer (89 mM Tris, 89 mM boric acid, and 2 mM Na_2EDTA), containing 200 ng/mL ethidium bromide. A 7 μL aliquot of PCR products was added to 1.5 μL of loading dye (0.025% bromophenol blue, 0.025% xylene cyanol, 40%, w/v sucrose) and the gels were run at a constant voltage (100 V) for 1–1.5 h.

Aliquots of 5 μL PCR products were loaded on 10% PAGE gel (80 mm × 73 mm × 0.75 mm) in 1× TBE buffer and constant voltage 120 V for 2.5 h. The gel was stained with 0.1% silver nitrate solution.

The PCR fragments from different patterns in the two breeds were amplified by the pair of primers, then sequenced in both directions by ABI PRIZM 377 DNA sequencer (PerkinElmer).

2.4. Statistical analysis

Differences in genotypic and allelic frequencies at *KAP9.2* locus between Shaanbei White and Inner Mongolia cashmere populations were analyzed using a χ^2-test, which was performed by SPSS software (Version16.0). Population genetic indexes (gene heterozygosity, gene homozygosity and effective allele numbers) were calculated by Nei methods (Nei and Roychoudhury, 1974; Nei and Li, 1979). The polymorphism information content (PIC) was calculated by Botstein methods (Botstein et al., 1980) using the software PIC-Calc 0.6. The formulas were as follows:

$$H_o = 1 - \sum_{i=1}^{n} P_i^2, \qquad H_e = \sum_{i=1}^{n} P_i^2$$

3. Results and discussions

Few deletion mutations were detected at *KAP9.2*P2 locus (Fig. 1), however, in this paper, the polymorphisms (named as WW, WD and DD) were firstly detected at *KAP9.2P1* locus, the partial CDS region involved (Figs. 2 and 3). Frequencies of allele *KAP9.2*-W in Shaanbei White cashmere

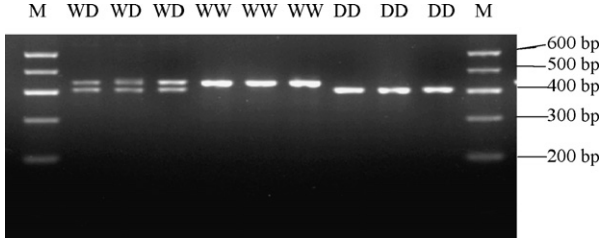

Fig. 1. The 10% PAGE electrophoresis patterns of *KAP9.2*P1 gene in goat (M: marker).