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Characterization and polymorphism of Keratin Associated Protein 1.4 gene in goats

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ABSTRACT

Keratin-associated proteins (KAPs) are among the main structural components of the animal fibers and form semi-rigid matrix wherein the keratin intermediate filaments (KIFs) are embedded. Variation in the KAP genes has been reported to affect the structure of KAPs and hence fiber characteristics. As no information is available on this gene in *Capra hircus* therefore, present work was undertaken to characterize and explore the different polymorphic variants of KAP1.4 gene at DNA level in different breeds/genetic groups of goats of Kashmir. Cashmere (Changthangi, 30 animals) and non-Cashmere (Bakerwal and Kargil goats, 20 animals each) goats formed the experimental animals for the study. Single strand conformation polymorphism technique was employed for exploring variability at gene level. On exploring the size variability in KAP1.4 gene between Ovine and Caprine, it was concluded that sheep KAP1.4 gene has a deletion of 30 nucleotides. In comparison to published nucleotide sequences of sheep, goat sequences explored are differing at positions 174, 462 and 568 and at these positions "G", "T" and "T" nucleotides are present in sheep, but are replaced by "A", "C" and "C" respectively, in goats. By SSC studies, two genotypes were observed in each genetic group and in Bakerwal goats the genotypes were designated as A1A1 (0.40) and A1A2 (0.60) and were formed by two alleles A1 (0.70) andA2 (0.30). The different SSC patterns observed in Kargil goats were designated as B1B1 (0.35) and B1B2 (0.65) genotypes with frequencies of B1 and B2 alleles as 0.675 and 0.325, respectively. Similarly, two genotypes C1C1 (0.60) and C1C2 (0.40) were observed in Changthangi goats and the frequencies of C1 and C2 alleles were 0.80 and 0.20, respectively. These alleles were later confirmed by sequencing. The sequences of these alleles are available in NCBI under Acc. No's. JN012101.1, JN012102.1, JN000317.1, JN000318.1, JQ436929 and JQ627657. It was concluded that all the alleles observed in a breed were unique to the breed. The designated A1 and A2 alleles of Bakerwal goats differ from each other at positions 245 and the nucleotides observed were "C" or "A" and at position 605 of the nucleotide sequence "T" or "C", were observed. The designated B1 and B2 alleles of Kargil goats differed from each other at positions 224, 374, 375 and 521. The nucleotides observed in two SSC pattern were C→G, A→G, G→A and T→C, respectively. The designated C1 and C2 alleles of Changthangi goats differed from each other at one position 440 with the change of "A"→"C".

Only two mutations C224G and G375A in Kargil goats resulted in change of the Cysteine (C)→Serine (S) and Alanine (A)→Threonine (T), respectively. The nucleotide sequences of KAP 1.4 gene in Bakerwal, Kargil and Changthangi goats showed 99.7% similarity with each other and 96.7% with sheep and 74.4% with mice. Average guard fiber length and diameter were 81.02 ± 0.16 mm and 67.53 ± 0.97 μm, respectively, and average down fiber length and diameter was 48.38 ± 0.70 mm and 13.32 ± 0.29 μm, respectively for Changthangi goats. Average guard fiber length and diameter were 63.51 ± 4.52 mm and 105.31 ± 4.48 μm, respectively for Bakerwal goats and 62.60 ± 5.03 mm and 107.18 ± 2.30 μm, respectively for Kargil goats. The effects of the observed genotypes on Cashmere fiber diameter, Cashmere fiber length in Changthangi goats and guard fiber length and guard fiber diameters in Changthangi, Kargil and Bakerwal goats were found to be non-significant ($P > 0.05$).The nonsignificant association between the polymorphism and fiber attributes reported herein may be due to small sample size.

Abbreviations: DNA, Deoxyribose Nucleic Acid; EDTA, Ethylenediaminetetraacetate; KAP, Keratin Associated Protein; MFD, Mean Fiber Diameter; MFL, Mean Fiber Length; NCBI, National Centre of Biotechnology Information; PCR, Polymerase Chain Reaction; SSCP, Single Strand Conformation Polymorphism.

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1. Introduction

In India Cashmere is harvested from the animals mainly by combing and to complete the harvesting of Cashmere of an animal it takes a few days to a week depending upon the shedding status of secondary fibers by the animal. Variability does exist in production potentials of the Cashmere goats and some animals produce up to 800 g of Cashmere annually thereby indicating that if proper selection procedures are followed both the quality and quantity of Cashmere can be increased. But in view of the adverse climatic conditions, great fluctuation in the weather conditions and difficulty in accessibility to the rearing areas, the recording of the animal's fiber production, and measurements of the fiber quality traits such as diameter, length, fineness, strength etc. are difficult and time consuming and therefore selections based on phenotype are comparatively inaccurate and least effective. Cashmere fibers like other animal fibers are composed of proteins called Keratins. These proteins are responsible for the structural and chemical properties of the fiber (Li et al., 2009). Keratin proteins are of two types namely Keratin Associated Proteins (KAP) and Keratin Intermediate Filament Proteins (KIF). During the growth of fiber from its follicle control is exercised by several multigene families to produce a coordinated synthesis of the Keratin proteins. The relative proportions of Keratin proteins of these families in the fiber vary between species and within species and these ratios are known to fluctuate in response to dietary, chemical and hormonal changes (Gillespie and Marshall, 1980). Consequently, variations in family of Keratin genes play an important role in quality and production of the animal fiber. Identification of these genes controlling the cashmere quality in terms of fiber diameter and length will offer an opportunity to improve production efficiency, product quality and product diversity, through utilizing them in the breeding programs for developing specific lines. Therefore the present study was planned to initiate the work on gene based marker selection for enhancing Cashmere production by characterization of the KAP 1.4 gene and explore its polymorphism in Cashmere and non-Cashmere goats.

2. Materials and methods

2.1. Experimental animals

The study was designed to characterize the KAP 1.4 gene on molecular basis in the animals of Cashmere (Changthangi), 30 animals from Leh district and non-Cashmere (Bakerwal) 20 animals from Sheep Research Station, Srinagar and (Local Kargil goats) 20 animals from Kargil district.

2.2. Morphometric study of fibers of goats

2.2.1. Collection of Cashmere fiber and guard hair

The Cashmere and Guard hair samples were collected from the mid-side region of the Cashmere goats and only guard hair from non-Cashmere producing goats for estimation of mean fiber length using $MFL = A + (\sum fd / \sum f \times Z)$ and mean fiber diameter using $MFD = A + (Z \times \sum fd / n)$. Where, A = arbitrary mean, Z = group interval, d = deviation from arbitrary mean, fd = product of frequency and deviation, and n = number of observations.

Table 1
Primers (forward and reverse) along with properties used for amplification of KAP 1.4 genes in sheep and goats.

Primer	Primer sequence (5′–3′)	M. wt (g/mol)	Tm (°C)	Length (bases)	GC (%age)	Size (bp)
Forward	GCATTACAATTCTCAGCCCAAC	6623.37	51.0	22	45.50	625
Reverse	AGAGATACTGTGTCTTGGGCA	6501.30	49.6	21	47.60	

Table 2
Reaction mixture used for digestion of the PCR products.

Reaction component	Quantity
HPLC	12 μl
10× R-buffer with BSA	2 μl
Restriction enzyme *M1S1*(*Msc1*)10 U/μl	0.1 μl
PCR product	10 μl
Total	20 μl

2.3. Molecular genetic study

2.3.1. Blood collection and DNA isolation

Ten ml of blood was collected from the jugular vein of each animal (goat) both male and female in a 15 ml sterile graduated polypropylene tube containing a few drops of EDTA (Ethylene Diamine Tetra Acetate, 0.5 M, pH = 8) as anticoagulant. The blood was mixed gently with anticoagulant and kept on ice in iceboxes and stored at −20 °C until the isolation of genomic DNA. The genomic DNA was extracted from the frozen blood samples using the standard protocol of Sambrook and Russel (2001) by phenol-choloroform extraction procedure.

2.3.2. PCR and restriction enzyme digestion analysis

For the amplification of KAP 1.4 gene in goats the primer pair forward 5′-GCATTACAATTCTCAGCCCAAC-3′ and reverse 5′-AGAGATAC TGTGTCTTGGGCA-3′ was designed from the sequence of sheep available with NCBI (Acc. no. GQ507748) utilizing "fast PCR" software (Table 1). The primers for the amplification of this gene were designed from the conserved area of the gene after identifying them through multiple sequence alignment software. This was required as there was no information available on this gene of goats in the electronic or print literature. PCR amplifications were carried out in Eppendrof Master Cycler in reaction volume of 25 μl in a 200 μl thin walled sterilized PCR tubes.

As KAP 1.4 gene is of larger size (655 bp) and for its efficient analysis in SSCP, it was fractioned into two smaller fragments using restriction enzyme *MISI* (5′…TGG↓CCA…3′). All the components required for restriction enzyme digestion were prepared on ice. HPLC, R-buffer and restriction enzyme were added in the order for required number of reaction in a 1.5 ml micro-centrifuge tube to make a master mix (Table 2). Master mix was dispensed into labelled 200 μl PCR tubes and PCR products were added to the corresponding tubes. The tubes for restriction enzyme digestion were spun shortly and incubated at 37 °C for 16 h. Digestion was stopped by heating the mixture at 60 °C for 30 min and the samples were stored at 4 °C. The bands of digested fragments in the gel study were not resolved completely and thus further analysis of the digested fragments for SSCP studies was not carried out.

Primers for the 352 bp and 303 bp DNA fragments of KAP 1.4 gene of goats were designed utilising "Fast PCR" software. The primers were designed from the reference sequence obtained after sequencing of the PCR amplicons of KAP 1.4 gene of 655 bp in goats. The primers utilized for amplification of 352 bp (forward 5′-GCA TTACAATTCTCAGCCCAAC-3′) and (reverse 5′-TAGCCAATGCTGCCACC AATG-3′) and for amplification of 303 bp (forward 5′-TTGGTGGCAG CATTGGCTATG-3′) and (reverse 5′-AGAGATACTGTGTCTTGGGCA-3′)

Table 3
Primers used for amplification of 352 bp and 303 bp fragments of KAP 1.4 genes.

Primer	Primer sequence (5′–3′)	M. wt (g/mol)	Tm (°C)	Length (bases)	GC (%age)	Size (bp)
Forward	GCATTACAATTCTCAGCCCAAC	6623.37	51.0	22	45.50	352
Reverse	TAGCCAATGCTGCCACCAATG	6375.0	59.8	21	52.40	
Forward	TTGGTGGCAGCATTGGCTATG	6508.0	59.8	21	52.40	303
Reverse	AGAGATACTGTGTCTTGGGCA	6501.0	49.6	21	47.60	