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Comparative Sequence Analysis of Keratin Associated Protein (KAP 7.1) Gene in Two Indigenous Pashmina Goat Breeds of India

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A B S T R A C T

Keratin-associated proteins are fibrillar proteins and are the vital components of hair and wool fibres as they are part of matrix of fibres and form a cross- linked network with the keratin intermediate filaments. Variation in sequence and expression pattern of KAP may affect fibre characteristics. In this study, we used direct DNA sequencing to analyse the goat HGT-KAP 7.1gene which codes for the protein responsible for the fineness of fibre. A single transversion at 95th position was detected in the KAP 7.1 gene sequence of chegu goat. This study would lead to screening of this single nucleotide variation in larger goat population for any possible association with fibre fineness, yield or processing properties

Introduction

The main structural proteins of hair fibre are keratin-associated proteins (KAPs). In association with keratin protein their content in fibre determines its quality (Jin *et al.*, 2011). KAPs play a vital role in structural and mechanical properties of wool fibre (Liu *et al.*, 2014). Keratin proteins are encoded by two multi gene families type I (acidic) and type II (basic), so the keratin content and structure are more stable in the different species hair, but KAPs differs enormously as they are encoded by large multigene families, which are to be categorized into high sulphur KAP, ultra-high sulphur KAP and high glycine- tyrosine KAPs (Langbein *et al.*,2001; Rogers *et al.*, 2002; Yin J *et al.*, 2004. The glycine/tyrosine-rich KAPs, are smallest of the hair keratins (Mr = 6,000-9,000) and were originally separated into two groups on the basis of amino acid content and solubility (Powell and Rogers, 1997), type I (KAP7 and 8), and type II (KAP6 family). These proteins are rich in glycine, tyrosine, serine, and phenylalanine, accounting for ~50 mol % of

the amino acid content for KAP7 andKAP8 and ~77 mol % for KAP 6 proteins. KAP7 is glycine/tyrosine-rich type I component C2. The glycine/tyrosine-rich KAP group is heterogeneous. Keratin composition of hair significantly determines the quality of hair fibre, and also subject to genetic factors. Due to different expression pattern of KAP genes, the fibre characteristics significantly change in cashmere goat of the different regions and different species (Jeffery *et al.,* 2001). Therefore, from the molecular level keratin and KAP genes are the important factors for controlling fibre fineness and improving cashmere quality.

Cashmere goat generates cashmere and wool. The hair and wool of almost all animal species is mainly composed of cuticle, cortex and medulla. However cashmere commonly known as Pashmina fibre is mainly composed of cuticle and cortex with long polyhedral spindle shaped internal cortical cells (Jones, 2001), which attribute mechanical strength to the fibre. The external covering of the fibre is formed by the flattened overlapping cuticular cells (Marshal *etal.*,1990). Basically it is the secondary hair follicle which generates cashmere fibre. Histological observation of this dynamic mini organs reveals that it be divided into the outer epidermis, outer and inner root sheath, hair cuticle and cortical layer from outside to inside, while medullary layer only exists in the primary follicles (Rogers GE, 2004).The expression of keratin and their associated protein genes is prerequisite for the formation of cashmere fibre. The continuous upward movement of upper hair matrix cells from their germinal zone gives rise to mature hair shaft as the gene coding for the two structural protein families in the hair matrix, cuticular Layer and cortical layers were activated (Rogers MA, 2002). The high glycine–tyrosine (HGT) KAPs encoded by KAP6n, KAP7 and KAP8 gene families are the smallest of wool keratin. Variation in the nucleotide sequence and differential expression of these genes regulates the fibre quality in different species. The objective of this study was to identify the variation in KAP 7.1gene in two pashmina producing goat breeds 'Changthangi and Chegu' which would form the basis of a deeper study associating them with performance levels for better fibre quality of the breed. Changthangi also known as Pashmina goat is found in the Ladakh division of Jammu and Kashmir and Chegu is found in Spiti valleys of Himachal Pradesh, Chamoli, Bathwari, Ticknor, Uttarkashi, Pithoragarh, sectors of Uttrakhand (Gupta *et al.,* 2006).

Materials and Methods

Animals, sample collection and DNA isolation

Changthangi goats were selected randomly from Changthang regions of Leh Ladakh division of Jammu and Kashmir and Chegu goats were selected from Chamoli, Uttarkashi, Bathwari and Ticknor, Pithoragarh sectors of Uttrakhand for blood sampling. In this study, blood samples (5 ml each) were collected from jugular vein puncture, using vacuum tubes treated with 0.25% EDTA and transported to laboratory at 4°C.Genomic DNA was isolated by phenol-chloroform extraction method (Sambrook and Russel, 2001) with slight modification and checked for quality and the quantity and was diluted to a final concentration of 250/ng and store at 4°C further analysis.

DNA amplification by Polymerase Chain Reaction (PCR)

PCR was carried out on about 50–100 ng genomic DNA in a reaction volume of 25 µl. 40ng each of diluted primers (Forward 5'-*TCCTTCTTGGGTAACTTGCTC*-3' Reverse R 5'-*GCCCTCAATTCTCTGTGT*) were used

to amplify a 365 bp PCR product of the KAP 7.1 gene. The reaction mixture consisted of 200µM dNTP, 1 unit of Taq DNA polymerase, 1.5mM MgCl2, 1.5mM 10X buffer, using Eppendorf PCR machine. Following a hot start (95°C for 10 min), 35 cycles were carried out (95°C for 30s, 54°C for 45s, 72 °C for 45s), ending with a 10 min final extension at72°C.

PCR clean-up and DNA Sequencing

The PCR product was visualized by electrophoresis through 1.8% (w/v) agarose gel by staining with ethidium bromide. The PCR products were purified by PCR purification kit (Thermo). Duplicate samples were chosen for KAP 7.1 gene. Amplified PCR products were subjected to custom DNA sequencing from both ends (5' and 3' ends) (). The PCR products were sequenced by Xcelris Labs India. Nucleotide sequence alignments, translations and comparisons were carried out using the Seaview software 2017. The BLAST algorithm of NCBI (National Center for Biotechnology Information) was used to search the NCBI GenBank (http://www.ncbi.nlm.hih.gov/) databases for homologous sequences. The phylogenetic tree was developed from the nucleotide sequences of KAP 7.1 gene, based on neighbour-joining method using SEAVIEW-4 software 2017.

Results and Discussion

Cashmere goat breed is a genus of world most earliest domesticated animal species called goat (*Capra hircus*) and has developed special regulating mechanisms in adapting to the harsh environment of Northern Himalayan ranges, Changthangi and Chegu breeds are mainly reared for meat and fibre purpose. The breeders are completely dependent on these awesome creatures. It is widely accepted that the keratin associated protein 7.1 gene (KAP7.1) belongs to the high glycine-tyrosine group and is the main structural gene. Looking towards the importance of KAP gene, direct DNA sequencing was done to identify the variation(s) if any in KAP7.1 gene in Changthangi and chegu which may be further helpful in genetic improvement in these goat breed. Therefore, we considered it to be very important to screen KAP7.1 gene for such kind of variations. Genetic variability in KAP7.1gene was assessed by direct DNA sequencing. A single transversion C→A at 95th position of chegu goat sequence was identified, when compared with KAP7.1 gene in Changthangi goat and with reference sequence (AY510121.1) of Capra hircus keratin associated protein 7.1 available at NCBI GenBank database (Table-1).

Table.1 Single nucleotide variation identified in KAP 7.1 gene Chegu and Changthangi goat

Position	Capra hircus	Changthangi Goat	Chegu Goat	Type of Change
95	**C**	**C**	**A**	**Transversion**

Fig.1 Amplified PCR product (365bp) of KAP7.1 gene

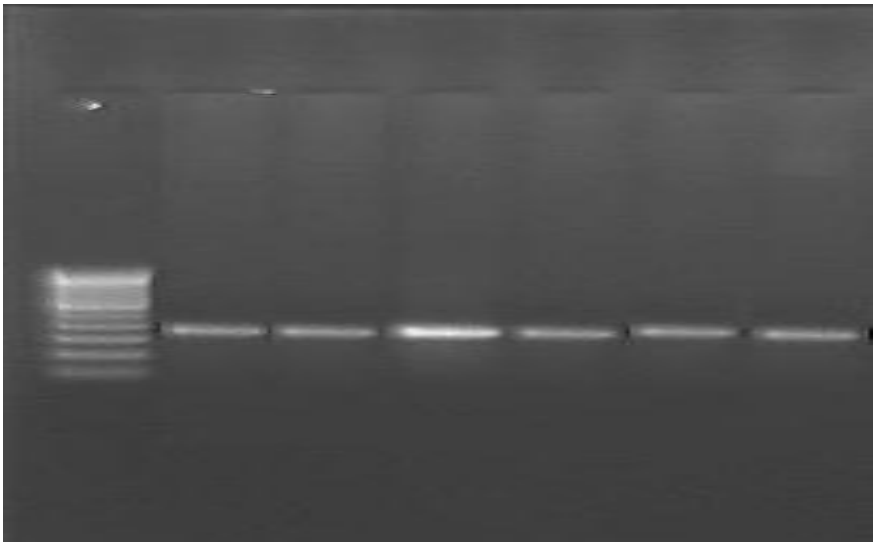
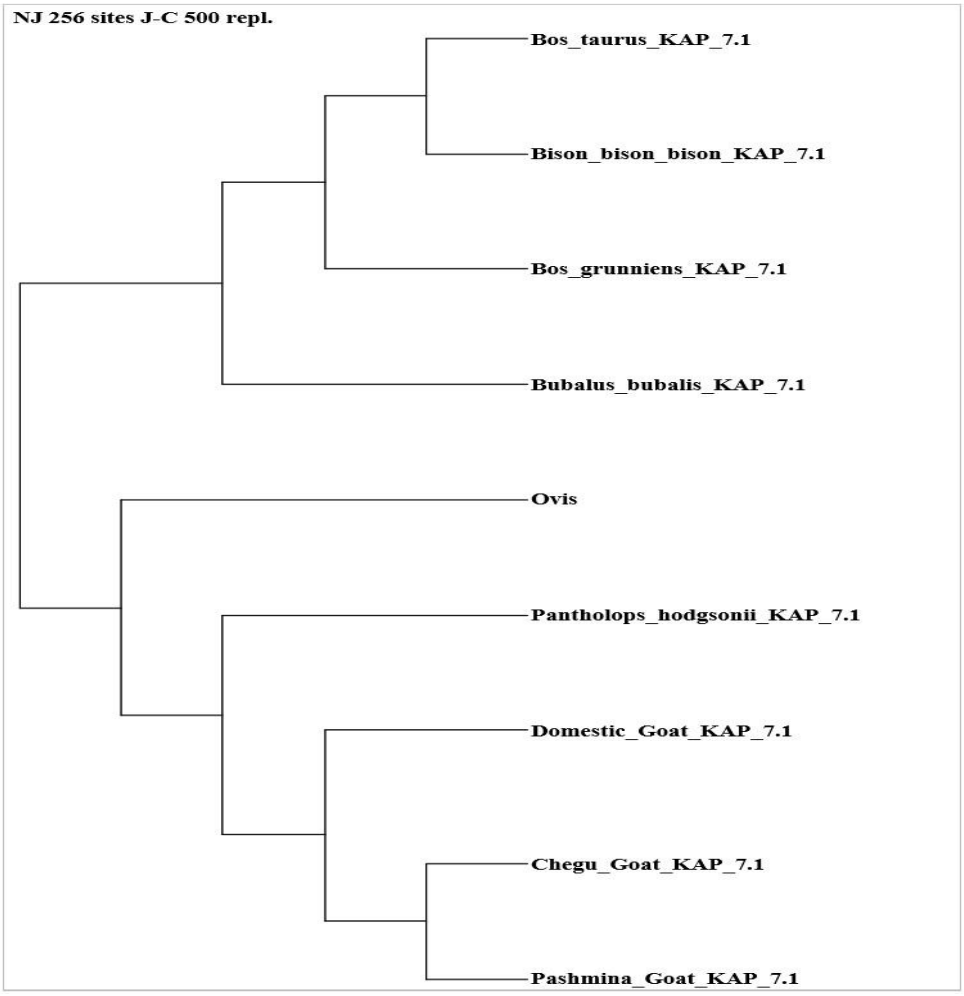


Fig.2 Neighbour- joining tree obtained from KAP 7.1 gene sequence data of different species



BLAST analysis of KAP7.1 gene of chegu pashmina goat revealed homology of 100% with Changthangi pashmina goat an domestic goat (*Capra hircus*), 99% with *Ovis Aries* and *Panthlops*,95% with *Bos taurus* and *Bos grunniens*, 94% with *Bison bison*, Phylogenetic analysis of KAP7.1 gene following Neighbour- joining algorithm revealed that Chegu, Changthangi and domestic goat were found in same group, small ruminants were found in one cluster and *Bos taurus* and *Bos grunniens*, *Bison bison*, *Bubalus bubalis* were found in distinct cluster (Fig. 2).

In conclusion, the significance of this Sequential variation in keratin associated protein (KAP7.1) in any of the Indian goat breeds has not been reported and it would be meaningful if found to be related with some production traits in these breeds. Further validation of the identified variation needs to be done..

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References

Gupta, N.P., A.K. Pokharana and P.C. Patni, 2006. Prospects of production, processing and marketing of sheep and goat fibres workshop-cum- seminar on commercial goat and sheep farming and marketing Central institute for research on goats. Mathura (India), pp: 79-86

Jeffrey, E.P., Warren GB 2000. Application of proteomics for determining protein markers for wool quality traits. Electrophoresis 21:1899-1906 doi: 10.1002/ (SICI) 15222683(20000501)21:9\1899:AIDELP S1899 [3.0.CO; R

Jin, M., Wang, L., Li, S., Xing, M.X. and Zhang, X. 2010. Characterization and expression analysis of KAP 7.1 and 8.2 gene in Liaoning new-breeding Cashmere goat hair follicle. Molecular Biology Reports 38(5): 3023-3028.

Langbein, L., Rogers MA, Winter H *et al.*, 2001. The catalog of human hair keratins. J BiolChem 276:35123–35132

Powell, B.C., 1997. Molecular genetics. In: *The Genetics of Sheep*, edited by Piper L and Ruvinsky A. Oxon, UK: CAB International. pp 149-181.

Rogers, G.E., 2004. Hair follicle differentiation and regulation. International Journal of Developmental Biology 48:163170.

Rogers, M. A., L. Langbein, H. Winter, C. Eemann, S. Praetzel and J. Schweizer. 2002. Characterization of a first domain of human high glycine-tyrosine and high sulfur keratin-associated protein (KAP) genes on chromosome 21q22.1. J. Biol. Chem. 277:48993-49002.

Sambrook and Rusell, 2001. Molecular cloning. *A Laboratory Manual*. 3rd edition. Vol 1.Von Bergen, W. 1963 *Wool Handbook*, Vol I. 3rd Ed., Inter Science Publishers, London. 352-359

Yin, J., Hu TM, Li JQ 2004. Cloning and analysis of six full- length cDNA similar to sheep KAP6-1 from Cashmere goat. Acta Genet Sin 31:502–507.

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