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Research Article

SEQUENCE ANALYSIS OF KERATIN ASSOCIATED PROTEIN (KAP7) GENE IN INDIGENOUS BREED OF YAK

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ABSTRACT
Keratin-associated proteins (KAPs) are structural components of wool and variation in them may affect wool characteristics. These proteins are one of the major structural proteins of the hair. In this study, we used direct DNA sequencing to analyse the yak KAP7 family which encodes glycine and tyrosine rich KAPs. Nine SNPs and five insertions were observed in the Indian yak. These mutations are novel in Yak KAP 7 gene and its GenBank accession (FJ712675) is now in public domain. This study would lead to screening of these SNPs in larger yak population for any possible association with wool yield or processing properties.

INTRODUCTION
The Keratin Associated Proteins (KAP) is characterized by an unusually high proportion of cysteine, or glycine and tyrosine residues. It is a typical representative of the fibrillar proteins, a characteristic feature of which is a high content of sulfur. Keratins are characterized by molecules that possess a complex quaternary structure. Hair is a strongly keratinized tissue formed within the hair follicle. Growth of the hair originates in matrix cells located in the bulb region. The genes encoding KAPs appear to be organised into domains. In humans, KAP genes from all of the 25 families identified are clustered and located in five chromosomal regions. Generally, each KAP gene consists of a single exon without any introns. KAP are a major component of the matrix between the KIF and may be responsible for forming the rigid hair shaft through extensive disulfide bond cross-linking with the KIF (Gillespie and Frenkel, 1976).

The glycine/tyrosine-rich KAPs, the smallest of the hair keratins (Mr = 6,000-9,000) 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 and 8 and ~77 mol % for KAP 6 proteins. KAP7 is glycine/tyrosine-rich type I component C2. The glycine/tyrosine-rich KAP group is heterogeneous.

Yak (*Bos grunniens or Poephagus grunniens*) is found in Changecgenmo valley in Ladakh and Spiti valleys of Himachal Pradesh, Jammu and Kashmir (J & K) and Arunachal Pradesh, Sikkim, and Nagaland of the north east states, of India (Nivsarkar *et al.*, 1997). The objective of this study was to identify the variation in yak KAP 7 of wool gene in Indigenous yak breed which would form the basis of a deeper study associating them with performance levels for better wool quality of the breed.

MATERIALS AND METHODS
Animals, Sample Collection and DNA Isolation
Blood samples (10 ml) were collected jugular vein puncture, using vacuum tubes treated with 0.25% EDTA. The unrelated animals were selected for the original breeding tract from N.R.C. yak. DNA extraction was performed within 24 h according to Sambrook *et al.*, (1989) with minor modifications and checked for quality and the quantity and was diluted to a final concentration of 100 ng/µl and store at 4°C for further analysis.

DNA Amplification by PCR

Polymerase chain reaction (PCR) was carried out on about 50–100 ng genomic DNA in a 25 µl reaction volume. The primers (Forward 5'- ACTTGCTCTTCACATTCTATC -3 and Reverse 5'- GGTTCCCGTAGTCATCTG -3') were designed using DNASTAR Version 4.0 (DNASTAR, Inc., USA) to amplify a 333 bp PCR product including CDS region (nucleotide 352 to nucleotide 685) of the KAP7 gene (Gene Bank X05638) according to Kuczek and Rogers (1987). The reaction mixture consisted of 200 lM each of dATP, dCTP, dGTP and dTTP, 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.75 U *Taq* DNA polymerase and 4 ng/µl of each primer (Sigma Genosys), two drops of mineral oil, using PTC-200 PCR machine (M J Research Inc., MA, USA). Following a hot start (95 ^{0}C for 5 min), 30 cycles were carried out (95^{0}C for 30s, 62^{0}C for 30s, 72 ^{0}C for 30 s), ending with a 5 min final extension at72^{0}C.

PCR Cleanup 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 kits (Biogene). The amplicons showing clear bands on agarose were further purified using Exonuclease-Shrimp Alkaline Phosphatase treatment in 96 well formats. Duplicate samples were chosen for KAP7 gene. Amplified PCR products were subjected to custom DNA sequencing from both ends (5' and 3' ends). The PCR products were sequenced by ABI 3100 (Applied Biosystem, USA).

Sequence Analysis

Nucleotide sequence alignments, translations and comparisons were carried out using the MEGALIGN software module of DNASTAR Version 4.0 (software of DNASTAR, Inc., USA). 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 developed from the nucleotide sequences of KAP 7 gene, based on neighbour-joining tree using genetic distance P was done with the MEGA 5.0 Tamura *et al.,* (2007). Randomized input order and bootstrapping with 1000 data sets were used to obtain a consensus tree.

RESULTS AND DISCUSSION

Yak (*Bos grunniens*), is one of the world's most remarkable domestic animals and has developed special regulating mechanisms in adapting to the harsh environment like high altitude Ladakh, Jammu and Kashmir (J & K) and the North East states, particularly in Arunachal Pradesh, Sikkim, and Nagaland.

Yak breed is mainly reared for coarse carpet wool i.e. used as felt. The animals are kept in small numbers by farmers, where pedigree records are impractical to maintain. It is widely accepted that the Keratin associated gene 7 (KAP7) belongs to the high glycine-tyrosine group and is the main structural gene. Looking towards the importance of KAP gene, identification of SNPs in KAP7 gene in Indian yak is done with the help of direct DNA sequencing. This can further be helpful in genetic improvement in the yak breed. Therefore, we considered it to be very important to screen the KAP7 gene for the single nucleotide polymorphism.

Genetic variability in Keratin associated gene 7 was assessed by direct DNA sequencing, which allows the detection of changes in the nucleotide sequence and a PCR-product is affected by single base substitution. A total five addition and nine polymorphisms were identified in KAP7 gene as compared with that of exotic Sheep (AF078545). Six transitions (239T→C, 392C→T, 402G→A, 458C→T, 489 T→C and 498 G→A) and three transversions (387G→T, 398A→T, (heterozygous), and 439G→T) were observed in CDS of KAP7 gene in Indian yak (Table 1). The higher level of genetic polymorphism observed in this breed could be because the samples were collected at random from the entire breeding area where little selection is practiced.

The mega-align using DNASTAR 4.0 software, revealed complete homology with the deposited sequence of exotic merino sheep with Accession No. X05638 from beginning to end, excepting SNPs at specific

nucleotide. The direct DNA sequencing analysis complete cds region revealed one distinct sequences (GenBank Accessions FJ712675). The general nucleotide profile corresponded to the sequence of GenBank (Accession No. X05638).

Table 1: SNPs identified in KAP 7 gene Indian yaks (Bos grunniens)

Position	Exotic Sheep AF078545	Indian Sheep EF446168	Indian Yak	Type of Change
239	T	T	C	Transition
344-349			G,A,A,T,T,A	Insertions
387	G	G	T	Transversion
392	C	C	T	Transition
398	A	A	T	Transversion
402	G	G	A	Transition
439	G	G	T	Transversion
458	C	C	T	Transition
489	T	T	C	Transition
498	G	G	A	Transition

BLAST analysis of KAP7 gene revealed homology of 96% with *Rangifer tarandus,* 99% with *Bos Taurus,* 94% with *Ovis Aries,* 94% with *Capra hircus*, 93% homology with Indian *Ovis aries* (Malpura) and 89% with *Homo sapiens*. Phylogenetic analysis of KAP7 gene following Neighbour- joining algorithm revealed that Cattle and Indian yak were found in same group, small ruminants were found in one cluster and human found to distinct cluster (Figure 1).

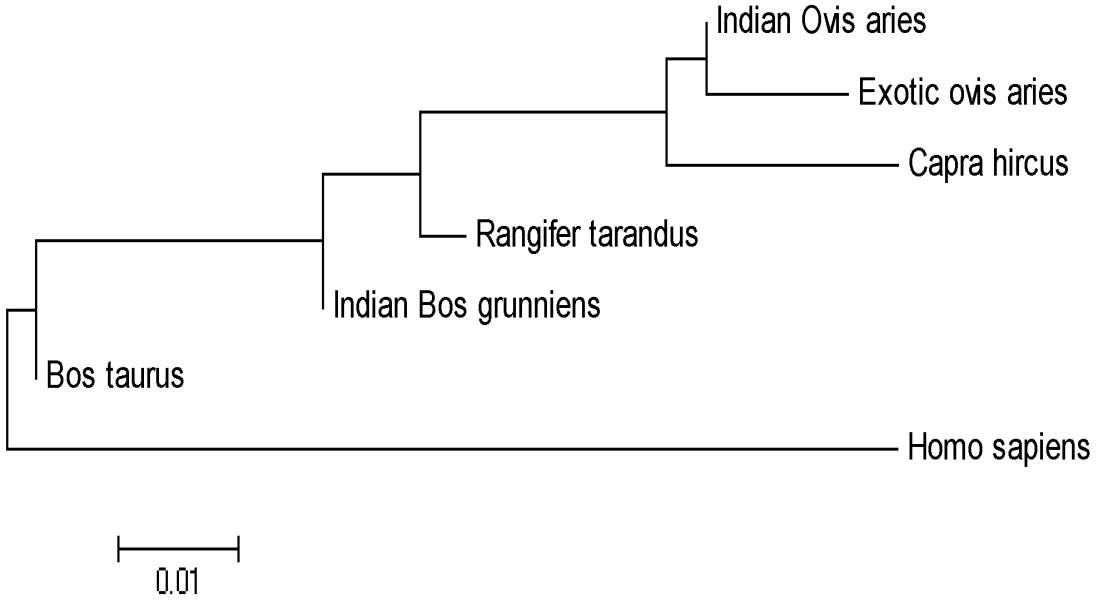

Figure 1: Neighbour- joining tree obtained from KAP 7 gene sequence data of different species

Conclusion

The significance of these SNPs in keratin associated protein in any of the Indian yak breeds has not been reported and it would be meaningful if those found are related with some production traits in these breeds.

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