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Single Nucleotide polymorphism of KAP6.0 gene in Indian yak

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ABSTRACT

Keratin-associated proteins (KAPs) are structural components of wool and variation in them may affect wool characteristics. Variation in the Indian yak breed at KAP6.1 gene was investigated using PCR- DNA sequencing. Indian yak is the coarse carpet wool breed of India. Eight novel SNPs have been identified at the positions 1390T→C; 1406A→G; 1415G→A; 1433; T→C; 1441 G→C; 1476 A→G; 1488C→T and 1510G→A (all are transitions) and three insertions have been found at the position in between 1313-1316, respectively when compare with the reference exotic merino sheep sequence (Accession No. M95719). This study would lead to screening of these SNPs in larger sheep population for any possible association with wool yield or processing properties.

Key words: DNA sequencing, Indian yak, KAP 6 gene, SNPs

INTRODUCTION

The KAP6.1 is an important gene from group of high glycine /tyrosine which are smallest among the keratin proteins having single exon of less than 1000 bp and is located in the type II keratin gene cluster on sheep chromosome 1 (Powell *et al.*1996). These proteins are rich in glycine, tyrosine, serine, and phenylalanine. The HGT KAP varies in abundance from less than 3% in human hair and Lincoln sheep wool to 13% in Merino wool and up to 30-40 % in Echidna quill. Genetic variations in wool keratin and keratin associated proteins (KAP) are responsible in the variation of wool and fibre in different species/breeds (Powell 1997).

Yak (Poephagus grunniens) is a unique bovine species of economical and cultural importance to the tribal population living in the difficult terrains in the foothills of Himalayas. In India, there are around 71,000 yaks found in Kargil and Leh districts in Ladakh division and Doda districts of Jammu and Kashmir (47,000), West Kameng and Tawang districts of Arunachal Pradesh (13, 000), North and East districts of Sikkim (7 000) and Chamba, Lahaul-Spiti and Kinnaur districts of Himachal Pradesh (4 000) and also in few numbers in Kumaon and Garhwal region of Uttarakhand. In India, yaks are mainly distributed in the Himalayan states of Himachal Pradesh, Jammu and Kashmir, Sikkim and Arunachal Pradesh in an area of approximately 14 000, 23 000, 2 000 and 1 500 sq km, respectively (Ramesha *et al.* 2008). The present study was carried out an investigation on SNP variants of KAP 6.1 gene using PCR-DNA sequencing in random samples of yak breed from its natural habitat.

MATERIALS AND METHODS

Animals, sample collection and DNA isolation-Blood samples (5–6 ml) were obtained by jugular vein puncture in

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vaccutainer tubes (BD, India) pretreated with 0.25% ethylene di amine-tetra acetic acid (EDTA). The unrelated animals were selected at randomly from their respective breeding tract. DNA extraction was performed within 24 h of blood collection, according to Sambrook *et al.* (1989) with minor modifications. After checking the quality and quantity, DNA was diluted to a final concentration of 50 ng/ml in water and stored at 4 °C for immediate use while for long term use store at −20 °C.

DNA amplification by PCR- DNA amplification by polymerase chain reaction (PCR) was carried out on about 50–100 ng genomic DNA in a 25 µl reaction volume. The primers (Forward 5'-GGC TAT GGC TCC TGC TAC -3 and Reverse 5'-ACT GGT GAA TCC TGG TGT C-3') were designed using DNASTAR Version 4.0 software to amplify a 252 bp PCR product including CDS region (nucleotide 1115 to nucleotide 1367) of the KAP6.1 gene (Gene Bank M95719). PCR amplification was performed in a total volume of 25 µl containing ~ 100ng of genomic DNA, 10pmol of each primer, 200µM of each dNTP, 2.5 µl of 10X buffer with 1.5mM $MgCl_2$ and 1U Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India), two drops of mineral oil using PTC-200 PCR machine (MJ Research Inc., MA, USA). Following a hotstart (95 ◦C for 5 min), 30 cycles were carried out (95 ◦C for 30 s, 62 ◦C for 30 s, 72 ◦C for 30 s), ending with a 5 min final extension at 72 ◦C. Amplification was verified by electrophoresis of product on 2% (w/v) agarose gel in 1× TBE buffer (2mM of EDTA, 90mM of Tris-Borate, pH 8.3), using a 100bp ladder (Invitrogen) as a molecular weight marker for confirmation of the length of the PCR products. Gels were stained with ethidium bromide (1µg/ml).

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 gel were further purified using Exonuclease-Shrimp Alkaline Phosphatase treatment in 96 well formats. Duplicate samples were chosen for KAP6 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).

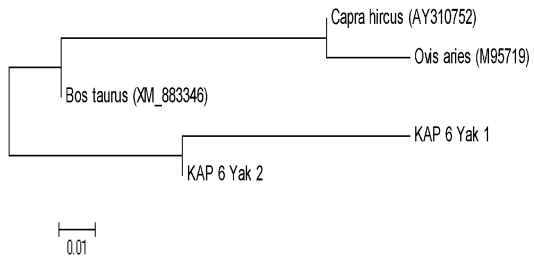
Sequence analysis- The BLAST algorithm was used to search the NCBI Genbank (http://www.ncbi.nlm.hih.gov/) databases for homologous sequences. The sequence analysis was carried out using various modules of DNASTAR Version 4.0, Inc., USA. Protein translations by EDITSEQ, sequence alignments and contigs comparisons by MEGALIGN and chromatograph evaluation by SEQMAN. Construction of a DNA nucleotide sequence based 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

Understanding the genetic basis of keratin protein in yaks will provide an opportunity for genetic improvement of the wool traits. SNP analysis is a well-established tool for the identification of genes associated with traits of economic interest in livestock populations (He *et al.* 2009; Lai *et al.* 2009). Polymorphism has been described previously in a number of KAP genes (Rogers *et al.* 1994; Gong *et al.* 2010). The sequence information obtained by directing sequencing of the PCR products was used to detect SNPs in complete CDS of KAP 6 gene in yak. Eight novel SNPs had been identified at the positions 1390T→C; 1406A→G; 1415G→A; 1433; T→C; 1441 G→C; 1476 A→G; 1488C→T and 1510G→A

(all are transitions) and three insertions have been found at the position in between 1313-1316, respectively when compare with the reference exotic merino sheep sequence (Accession No. M95719). SNPs at KAP6.1 gene results into synonymous mutation, which are not altered protein with the different amino acid sequence with difference in structural and functional characteristics (Komar 2007).

Fig 1: *Neighbour- joining tree obtained from KAP 6 gene sequence data of different species.*



in the hair shaft cortical keratinocytes at considerable distance (200 m) above the proliferative zone of the follicle bulb. This is a relatively late stage in the differentiation of the hair shaft keratinocytes. An intriguing feature of KAP6.1 gene expression in hair follicle differentiation is the variation in spatial expression and KAP6.1 expression might be believed to be responsible for the crimp or curliness of wool fibers (Powell *et al.* 1992; Fratini *et al.* 1993). The follicular expression of this group varies considerably in different species. In the present study attempts are made to detect polymorphism in KAP6.1 gene in Indian yak.

The CDS sequence of Indian yak was further subjected to basic local alignment search to know the sequence homology with the corresponding region of other species. The BLAST results showed more than 99% similarity with *Bos taurus*, 96% with *Ovis aries* and 94% with *Capra hircus.* The phylogenetic tree constructed based on Neighbour-Joining method of this region also revealed the clustering Indian yak and cattle together and small ruminants like sheep and goat formed an altogether different group.

Table 1: *SNPs identified in KAP 6 gene Indian yak (Bos grunniens)*

Position	Exotic Sheep AF078545	Arunachali yak	Type of Change
1390	T	C	Transition
1313-1316		C,T,G	Insertion
1406	A	G	Transition
1415	G	A	Transition
1433	T	C	Transition
1441 1476	G A	C G	Transition Transition
1488	C	T	Transition
1510	G	A	Transition

In future, the present authors would argue that KAP6.1 gene should be detected in more wool breed and the association between genotypes and fibre diameter ought to be further studied.

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