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SEQUENCE ANALYSIS OF HIGH GLYCINE-TYROSINE KERATIN-ASSOCIATED PROTEINS (KAPs) IN MAGRA SHEEP

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ABSTRACT

Keratin associated proteins (KAPs) are structural components of the wool fibre. The present study was aimed to analyse nucleotide sequence diversity in three high glycine-tyrosine KAPs gene (HGT-KAP) viz. KAP6, KAP7 and KAP8 from Magra sheep known for lustrous wool. This study was conducted to analyse single nucleotide polymorphisms (SNPs) in these three gene sequences. In open reading frame (ORF), SNPs were not located in KAP 6, however in KAP 7, 14 SNPs were located which leads to 5 synonymous and 9 non-synonymous mutations. In KAP 8 gene only one SNP was present in ORF which leads to tyrosine to asparagine amino acid change. In phylogenetic analysis, KAP 6 proteins of Magra sheep were clustered with KAP 6 amino acid sequence of cashmere goats. Most of the SNPs identified were located into 5' and 3' untranslated regions. Since 5' and 3' untranslated regions play crucial roles in post-transcriptional regulation of gene expression, these SNPs might play some role in lustrous phenotype of the wool.

Key words: Glycine, Keratin associated protein, Lustre, Magra, Single nucleotide polymorphism, Tyrosine

Keratin associated proteins (KAPs) are structural components of the wool fibre providing a semi-rigid matrix surrounding the keratin intermediate filaments (KIFs). These proteins play crucial role in defining the physico-mechanical properties of the wool fibre (Powell and Rogers, 1997). Approximately 90% of the cortical cells of wool fibre contain longitudinally arrayed KIF proteins. These filaments have an amorphous matrix surrounding them, which contains the KAPs, cross-linked with the intermediate filaments through extensive disulfide bonding (Marshall et al., 1991). A total of 29 KAP genes have been reported in sheep clustered on ovine chromosomes 1, 11, and 21 (Gong et al., 2016; Li et al., 2017). KAPs are complex class of proteins and they typically possess a high level of cysteine, or both glycine and tyrosine. Among KAP gene families KAP 1 to 3, 11, 13 to 16, and 23 to 27 are high sulphur (HS)-KAPs, families KAP 4, 5, 9, 10, 12, and 17 are ultra-high sulphur (UHS)-KAPs and families KAP 6 to 8 and KAP 18 to 22 are high glycine-tyrosine (HGT)-KAPs (Gong et al., 2016). The HGT-KAPs are predominantly found in the ortho-cortex of wool fibre. They are the first KAPs expressed after the synthesis of the KIFs in wool follicles. HGT-KAPs are indicated to have role in wool lustre (Li et al., 2009). Five family members for KAP 6 gene have been mentioned in sheep with many non-synonymous mutations (Zhou et al., 2016). Two sequence variations in KAP 7 coding region has also been reported (Gong et al., 2011). Similarly two members for KAP8 gene have been reported (Gong et al., 2014). Wide diversity in KAPs in sheep indicates its significant role in wool characteristics.

Magra sheep of India is known for producing lustrous wool (Kumar et al., 2019). Selective breeding of Magra

sheep has partly been introduced to produce lustrous fleece; however remarkable variation still exists in fleece quality of Magra sheep (Mehta et al., 2004). Therefore identifying the genetic markers responsible for lustrous wool trait can be helpful in accelerating selective breeding for improved wool trait. Additionally, quantitative polymerase chain reaction (PCR) analysis of wool follicles of Magra sheep indicated significant variations in transcript expression patterns of KAP genes between low- and high-lustrous wool producing Magra sheep (Kumar et al., 2018). In the present study single nucleotide polymorphisms (SNPs) of HGT-KAPs (KAP6, KAP7 and KAP8) from Magra sheep were analysed in order to relate with wool lustre phenotype.

MATERIALS AND METHODS

This study was conducted during November, 2017 to April, 2018. Blood samples were collected into vacutainers with the anticoagulant k_2EDTA from Magra sheep reared at Arid Region Campus, Bikaner (n=100) and from selected field flocks (n=20) of Uttarada region (Bikaner, Rajasthan). Blood samples were processed for isolation of DNA following standard phenol-chloroform method (Sambrook and Russell, 2001). Genomic DNA were used for PCR amplification of three KAP genes namely KAP 6, 7 and 8 using gene specific primers (Table 1) as described by Kumar et al. (2018). PCR reactions were carried out with initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 45 sec, 60°C (for KAP 6) and 58°C (for KAP 7 and 8) for 30 sec and 72°C for 1 min with a final extension at 72°C for 5 min. PCR products were resolved on 2% agarose gel and excised from gel on ultraviolet trans-illuminator. The amplified PCR products were purified from agarose gel using gel purification kits (Takara Bio Inc. Japan) following manufacturer instructions.

Table 1. Primers used for PCR amplification of keratin associated proteins in Magra sheep

Gene	Primers sequence (5' 3')	Annealing temperature (^{0}C)	Amplicon size (bp)
KAP 6	ctggagcaggtcagagtttg agcgacattcaggcctttag	60	712
KAP 7	ggcaagatgtttaggcaagc gcatcaccctatccaggtgt	58	905
KAP 8	gtaaagcctgcttccagagg tccactatccacctcactgc	58	901

PCR purified products were quantified by Nano drop UV Spectrophotometer. PCR products were sequenced in both directions using Sanger dideoxy sequencing (Agri Genome Labs Pvt Ltd., Kochhi, Kerala). KAP nucleotide sequences were aligned with ovine KAP gene sequences available in NCBI GenBank and were analysed for the presence of single nucleotide polymorphisms (SNPs) in 5' UTR, coding region and 3' UTR regions of the gene. Phylogenetic relationships between sequences and mis-sense mutations in ORFs were detected by *in silico translating* nucleotide sequences using MEGA7 program. Evolutionary relationships among amino acids sequences of natural fibre producing animals available in NCBI data base have been generated from CLUSTAL program (https://www.ebi.ac.uk/ Tools/simple_phylogeny) using Neighbor-Joining method (Kumar et al., 2016).

RESULTS AND DISCUSSION

The PCR amplifications of KAP 6, 7 and 8 genes were confirmed on agarose gel electrophoresis (Plate 1). Previous study indicated wide variation in the expression pattern of KAP genes in Magra sheep wool follicles (Kumar et al., 2018). To gain insight into the presence of single nucleotide polymorphisms (SNP) in these three genes, a total 100 nucleotide sequences (50 from each group viz., lustrous and low lustrous) from animals showing variant transcript expression were analysed. Nucleotide sequence analysis revealed 4, 31 and 7 SNPs in KAP 6, 7 and 8 genes,

respectively. In KAP 6 gene frequent occurring SNPs were located in promoter (c. -329 T>C), 5'-UTR (c. -8 C>G) and 3'-UTR (c.*69 C>G). These SNPs were also confirmed from NCBI blast analysis (JX185126, KT725840, and GU319872). In KAP 7 gene, frequent occurring SNPs were located in open reading frame (c.173 G>A) which leads to an amino acids change (S58N) also confirmed from NCBI blast analysis (JN091630 and JN091631) and in 3'-UTR (c.*112 C>T; c.*136 G>T; c.*142 A>G; c.*294 G>A and c.*310 A>G). SNPs in the 3'-UTR regions of KAP 7 are not available in NCBI database search. Similarly in KAP 8 gene frequent occurring SNPs were located in 5'-UTR (c. -208 C>G; c. -65 C>T) and in open reading frame (c. 100 T>A) which leads to an amino acid change (Y34N) also confirmed from NCBI blast analysis (JN091634 and JN091635).

The results revealed that most of these SNPs were located to 5' and 3' untranslated regions (UTRs) of gene (Plate 2). The 5'- and 3' -UTRs play important roles in post-transcriptional regulation of gene expression (Pesole et al., 2001). UTRs play crucial role in post-transcriptional control of mRNA transport, sub-cellular localization, and stability and translation efficiency. These proteins are an important constituent of the amorphous KAPs matrix in wool and it has been envisaged that SNPs identified may affect its expression potential.

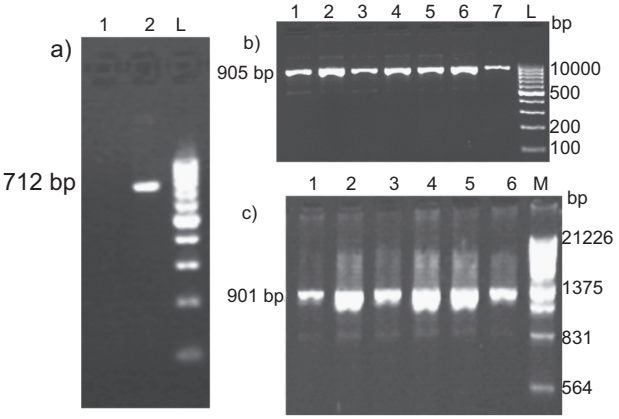

Plate 1. Amplified PCR products of a) KAP 6 (712 bp), b) KAP 7 (905 bp) and c) KAP 8 (901 bp) genes (Lane L-100 bp DNA ladder; lane M-Double digest DNA marker

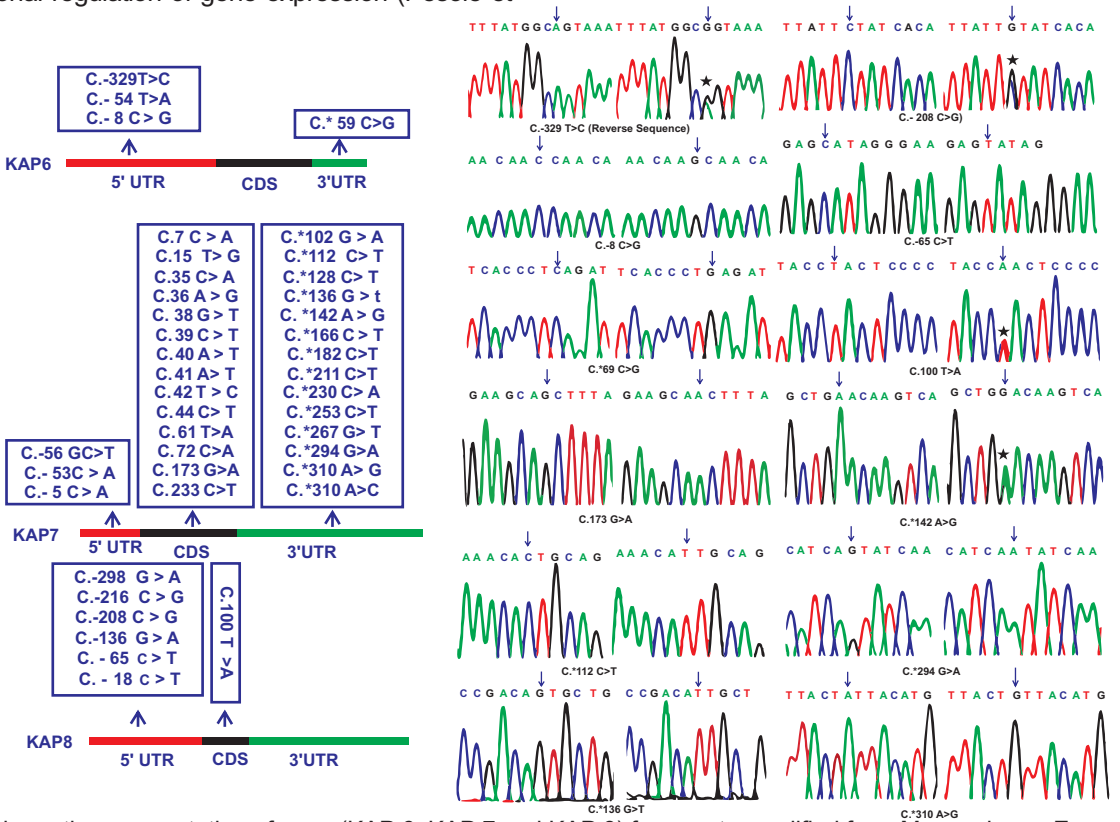

Plate 2: Schematic representation of gene (KAP 6, KAP 7 and KAP 8) fragments amplified from Magra sheep. Examples of mutations observed in 5'-UTR, ORF and 3'-UTR regions have been shown. Chromatograms of SNPs with minor allele frequency (MAF) have been shown. An asterisk (*) indicates chromatogram from heterozygous allele

Further, to understand the sequence diversity of these KAPs in lustrous Magra sheep, variant sequences were compared with sequences of common fibre producing animals available in GenBank database. Phylogenetic tree analysis revealed wide diversity in gene sequences in which Magra sheep sequences were distributed in different clusters (Plate 3). KAP 6 protein of Magra sheep has been clustered with KAP 6 amino acid sequence of Cashmere goat (AAP74769.1) of China as evident from Plate 3. Similarly, in KAP 7 and 8, amino acid sequences were clustered with sheep KAPs protein (Plate 3).

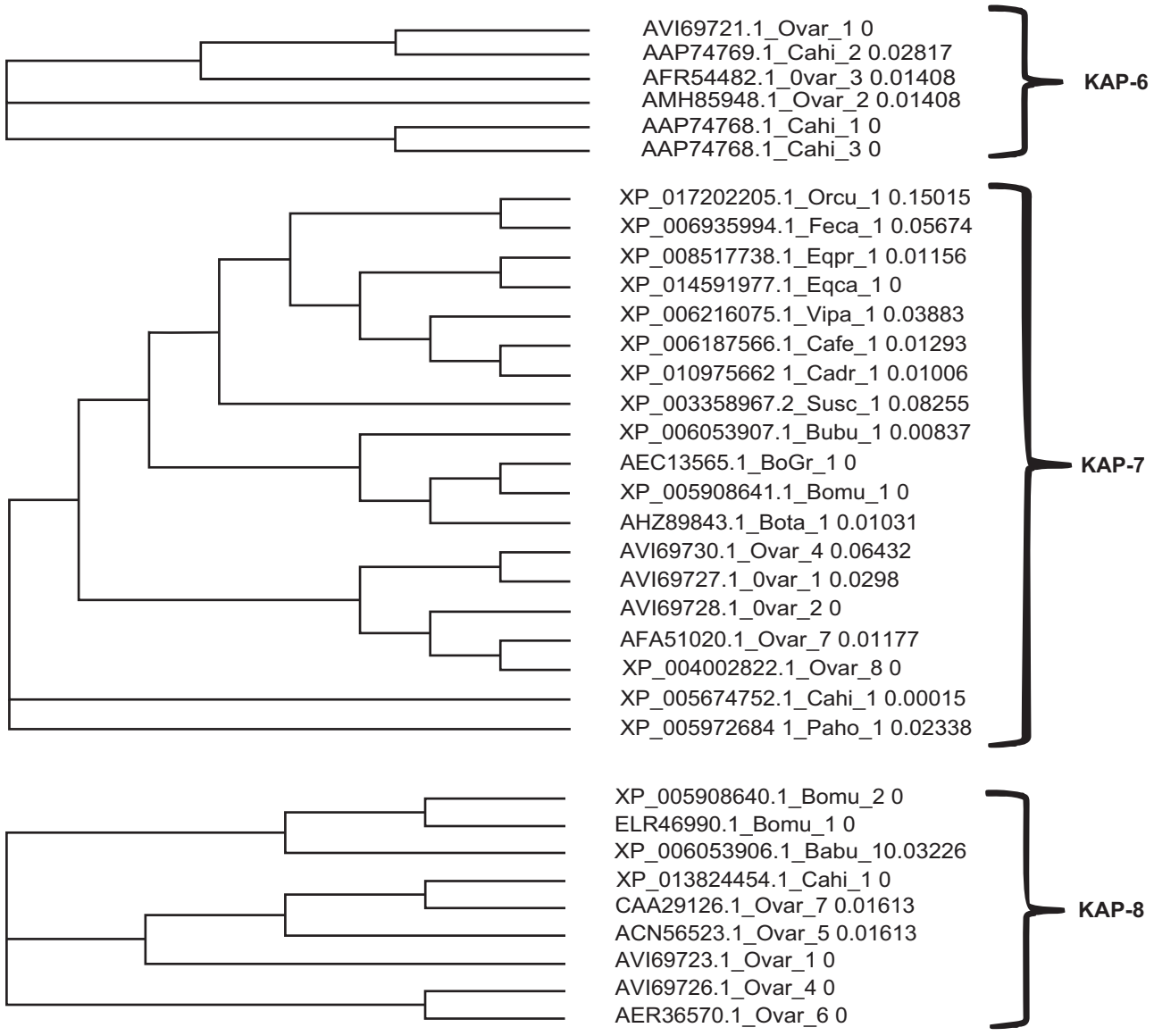

Plate 3. Phylogenetic tree showing relationship among related species based on KAP-6, -7 and -8 complete ORF amino acid sequences comparison. Ovar: *Ovis aries*; Cahi: *Capra hircus*; Orcu: *Oryctolagus cuniculus*; Feca: *Felis catus*; Eqpr: *Equus przewalskii*; Eqca: *Equus cabalus*; Vipa: *Vicugna pacos*; Cafe: *Camelus ferus*; Cadr: *Camelus dromedarious*; Susc: *Sus scrofa*; Bubu: *Bubalus bubalis*; Bogr: *Bos grunniens*; Bomu: *Bos mutus*; Bota: *Bos taurus*; Paho: *Pantholops hodgsonni.*

Some of the mis-sense mutations reported in the present study are found novel to Magra sheep breed and were not reported earlier. SNPs identified suggested further investigation to confirm the link with wool quality traits in Magra sheep.

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