Single strand conformation polymorphism in keratin associated protein (KAP7) gene in carpet wool breeds of indigenous sheep

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ABSTRACT

The major components of wool and goat fibre are the keratin proteins, which are largely responsible for their structural and mechanical properties. Keratin proteins are widespread in nature, being found in the nuclei and cytoplasm of almost all differentiated eukaryote cells. This study was carried out in five diverse sheep breeds; viz. Karnah and Gurej from Jammu and Kashmir, Gaddi from Himachal Pradesh (cold arid zone) and Malpura and Jaiselmeri from Rajasthan (hot arid zone). In KAP 7.0 gene PCR product amplicons developed by using forward primers (5'-ACT TGC TCT TCA CAT TCT ATC-3') and reversed primers (5'-GGT TCG CCG TAG TCA TCT G-3') of approximate size of 334 bp of complete CDs from 352 to 685 nucleotide positions in different breeds. For this gene a large variation (3-9 haplotypes) was observed in different breeds of which AA haplotype was most abundant in all breeds.

Key words: keratin associated protein, wool quality, fleece weight, genetic variation

INTRODUCTION

Wool production efficiency is mainly determined by fleece weight and wool quality. Wool quality traits are mainly fibre diameter and staple strength, and these are economically much more important for fine wools. Staple strength is more expensive to measure, but has a high correlation with the coefficient of variation of fibre diameter, which is therefore a good predictor. Wool traits have generally high levels of heritability, especially fleece weight and fibre diameter. For further improvement in wool quality traits, genetic variation need to be assessed based on genetic markers, so that the selection based on performance traits can be enhanced by combing the genetic variation in DNA based markers i.e. marker assisted selection. A limited information has been published on genes related to performance trait of sheep particularly what decides the grading of wool for the specific production type is based on few wool quality traits like staple length, medullation percentage and fibre diameter (Acharya, 1982; Basuthakur, 1987; Ahlawat *et al.*, 2009).

The wool quality traits are quantitative traits and show large variation both within breed as well as among Indian sheep breeds. The present level of diversity in sheep breeds in India has been because of selection of animals based on these parameters in developing sheep breeds for particular wool types acclimatized under particular agro-ecological niche. The opportunity exists to utilize our knowledge of major genes that influence the economically important traits in wool sheep. The important genes influencing pigmentation, wool quality and the keratin proteins, of which the latter ones are important for the morphology of the wool fibre (Purvis and Franklin, 2004). These keratin proteins are the major components of wool and fibre and are largely responsible for their structural and mechanical properties (Powell *et al*, 1997). Genetic differences at both the IF and KAP protein controlling loci may play an important role in determining phenotypes for different wool quality and production traits (Rogers *et al*, 2002). Therefore, we screened genetic variation in three important sheep breeds (Karnah, Gurej, Gaddi) from Northwestern Himalayan Temperate region and two (Malpura and Jaiselmeri) from Dry Northern-Western desert ecology using single-strand conformation polymorphism (SSCP) is a powerful method.

MATERIALS AND METHODS

Fifty animals (8 males and 42 females) from each of the five breeds from farmer's flocks were selected at random from Karnah, Gurej and Gaddi from Northwestern Himalayan Temperate region and Malpura and Jaiselmeri from Dry Northern-Western desert ecology by jugular venipuncture, using vacuum tubes treated with 0.25% EDTA. The 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.

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 software Beacon Designer 7.0 (Premier Biosoft International) to amplify a 333 bp PCR product including CDS region (nucleotide 352 to nucleotide 685) of the KAP7 gene (Gene Bank X05638). 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 MgCl2, 0.75 U Taq DNA polymerase and 4 ng/Il 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 °C for 5 min), 30 cycles were carried out (95°C for 30s, 62°C for 30s, 720C for 30s), ending with a 5 min final extension at72°C. Amplification was verified by electrophoresis on 2% (w/v) agarose gel in 1X TAE buffer using a 100 bp ladder (Invitrogen) as a molecular weight marker for confirmation of the length of the PCR products. Gels were stained with ethidium bromide (µg/ml).

Single-strand conformation polymorphism analysis: PCR products were resolved by SSCP analysis. Several factors were

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tested for each fragment in order to optimize the amount of PCR products, denaturing solution, acrylamide concentration, percentage cross linking, glycerol, voltage, running time, temperature. Each PCR product was diluted in denaturing solution (95% formamide, 10 mM NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue, 20 mM EDTA) denatured at 95°C for 5 min, chilled on ice and resolved on a polyacrylamide gel. The electrophoresis was carried out in a vertical unit (Bio-Rad Protean II xi), in 1X TBE buffer. Silver staining was as described by Sambrook *et al.* (1989). The gels were dried on cellophane paper using a gel dryer (Model 583, Bio-Rad).

RESULTS AND DISCUSSION

The major structural components of hair fiber are hair keratins (KIF) and keratin associated proteins (KAPs). The KAPs are located in the matrix around the KIF, and are therefore called matrix proteins. KAP are encoded by a large number of multigene families. Generally, each KAP gene consists of an intronless DNA sequence. KAPs are major components 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 (Powell and Rogers, 1997). These proteins are rich in glycine, tyrosine, serine, and phenylalanine, and KAP7 is glycine/tyrosine-rich type I component. We therefore, studied KAP-7 gene for genetic polymorphism in five diverse sheep breeds of Indian origin, which belongs to different agro-ecological regions that often influence their wool traits.

Fig.1 shows nine different PCR-SSCP band patterns encountered in different sheep breeds. PCR-SSCP products were generated by forward primer (5,ACTTGCTCTTCACATTCTATC-3') and reversed primer (5'-GGTTCCCGTAGTCATCTG-3'). Seven haplotypes A, B, C, D, E and F were observed in Karnah sheep breed from Kashmir. Of these A-type was preponderant (53.19%), followed by B-type (27.65 %). Other variants were present in small frequencies (Table 1). Gurej breed from the same region, however showed only three genotypes, A, B and C, of which B was most abundant (69.56%) followed by A types (28.26 %). Among these two breeds from North-western Himalayan Temperate region, Karnah breed produces finer wool of around 30 micron diameter with little modulation (Chaudhry and Chaudhary, 1995), while Gurej produces medium to coarse fleece with, low crimp having average staple length of 10 to 12 cm with 45's to 50's counts and higher medullation (Ahlawat *et al.*, 2009). Gaddi, another sheep breed from the same ecological niche from Himachal Pradesh, showed four PCR-SSCP variants of which B type had highest frequency (55.31%) followed by A types (31.91%) like Gurej breed. The wool characteristics of Gaddi are more or less similar to Gurej (Basuthakur, 1987).

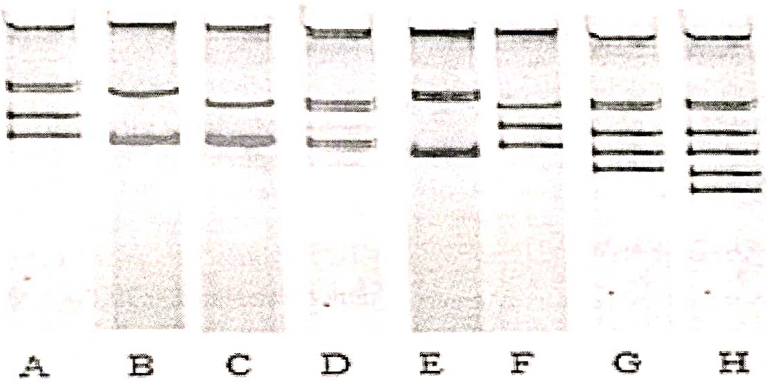

Fig. 1. Nine PCR-SSCP band patterns in KAP-7 gene of different sheep breeds of India

The PCR-SSCP pattern was most variable in Malpura sheep breed from Dry Northern desert ecology. All nine PCR-SSCP patterns were present in KAP 7 gene CDs region (Table 1) of which A type had highest frequency (53.33%) followed by E type (13.33 %) while other variants were present in smaller numbers. Another sheep breed from Northwestern desert region, Jaiselmeri, showed 4 variants of which B type was most abundant (51.06 %) while D type was having genotypic frequency of 40.42 %. Within the same environment, the open fleece was having fibre diameters of 42 micron and 39.1 micron in Malpura and Jaiselmeri respectively (Chaudhry and Chaudhary, 1995). The medullation percentage in these breeds was 71.8 and 64.7, respectively.

Table 1: Genotypic frequency of PCR-SSCP haplotypes of KAP-7 gene in different sheep breeds

Variant	PCR-SSCP Genotype frequency (%)				
	Karnah	Gurej	Gaddi	Malpura	Jaiselmeri
A	53.19	28.26	31.91	53.33	2.12
B	27.65	69.56	55.31	8.88	51.06
C	2.12	2.17	6.38	4.44	6.38
D	4.25	-	6.38	6.66	40.42
E	4.25	-	-	13.33	-
F	4.25	-	-	4.44	-
G	4.25	-	-	2.22	-
H	-	-	-	2.22	-
I	-	-	-	4.44	-

Genetic variation at both KRT and KAP loci may play an important role in determining various wool traits in sheep (Rogers, 1994; Parson *et al.*, 1994; Powel *et al.*, 1997). Whether the KAP7 gene product is itself entirely responsible for the fibre fineness (both fibre diameter and medullation percentage) is not yet clear, but the breed variation in wool traits within the same environment, could be because of genetic variability within wool genes. Parsons *et al.* (1994) described a link between the HGT loci for KAP genes clustered on ovine chromosome 1 with wool fibre diameter in Australian Merino sheep and Rogers (1994) reported variance in staple strength. These reports suggest, either the HGT loci themselves, or a gene within this region which segregates with the KAP genes, may be involved in determination of wool fibre diameter and medullation percentage

Since, single strand conformation polymorphism (SSCP) is based on the differential migration of single stranded molecules through polyacrylamide gels based on the effect of sequence variation on intra stranded loop formation, for the identification of variation at particular gene amplified product of DNA and has been used for the detection of genetic mutations in different candidate genes in different species (Orita *et al.*, 1989; Pravenec *et al.*, 1992; Morohoshi *et al.*, 1992). Such polymorphism has not been described earlier in Indian sheep or other exotic sheep breeds, so our results cannot be compared with published data. The significance of these SNPs in keratin-associated protein in any of the sheep breeds has not been reported and it would be meaningful if the variants detected in this study are related with some production traits in these breeds. Moreover, these variants may yield significant type of SNPs in KAP-7 gene after sequencing and it would indeed be interesting to see the haplotypes in other Indian breeds as well as in sheep breeds worldwide

ACKNOWLEDGEMENTS

Authors thank the Director, National Bureau of Animal Genetic Resources for the facilities provided. Funding of the project under NATP (World Bank Project) is duly acknowledged.

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