

Genetic variation at ASIP and KAP1.1 gene loci in indigenous sheep

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ABSTRACT

The study reports the polymorphism in the ovine Agouti Signalling Peptide (ASIP) and the keratin associated protein (KAP1.1) gene loci in four indigenous sheep breeds namely Marwari, Jaisalmeri, Malpura and Kheri. At the ASIP gene locus two alleles were observed in all sheep breeds. The frequency of the predominant allele ranged from 0.819 to 0.986 with an overall average of 0.934 across the four sheep breeds. The KAP1.1 gene locus across the four sheep breeds revealed the presence of 4 alleles. All tested animals were observed to be heterozygous. These preliminary results contribute to the assessment of genetic diversity available within genes associated with wool quality traits in indigenous sheep genetic resources.

Keywords: ASIP, KAP1.1, genetic polymorphism, Indian sheep

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INTRODUCTION

The biodiversity available in the sheep breeds of India is a virtual gold mine of ovine germplasm which still remains unexploited. India possesses 39 registered sheep breeds and several lesser known populations hitherto uncharacterized phenotypically as well as genetically. Each breed/population is unique in its adaptation to its agricultural production system and agro-ecological environment and thereby possesses genes and traits of excellence. The genetic diversity of these breeds represents a unique resource to respond to the present and future needs of meat and wool industry both in developed and developing countries. These sheep are generally reared by the resource poor farmers and pastoralists. These native breeds tend to flourish under low input conditions but with the displacement of traditional agricultural systems by mechanization / industrialization, non availability of grazing land, dilution of breeds by uncontrolled intermixing and infusion of exotic germplasm and absence of any planned strategies for conservation of indigenous breeds, many of these breeds have been threatened or endangered (Bhatia and Arora, 2005). These breeds are primarily maintained for different types of major products namely apparel wool, carpet wool, meat and meat-carpet wool. India is the 7th largest producer of

raw wool in the world producing 43.30 million kg of raw wool (http://texmin.nic.in/sector/Note_Woolen_Sector_wwt_skbabbar.pdf), 85% of which is carpet wool produced mainly by sheep from Rajasthan. Considering the importance of native sheep in carpet wool production, there is a need to identify genetic markers for wool quality traits such as staple strength, fibre diameter, luster etc. The genetic diversity available in the Indian sheep breeds can be exploited to improve the wool quality. Although India ranks third in the world sheep population, it is the third largest importer of raw wool from Australia. The need of the hour is to first characterize our own breeds and utilize their genetic potential for improvement. Although most of the indigenous sheep breeds have been described phenotypically (Acharya, 1982) as well as by neutral markers (Arora et al., 2011), information on available diversity at functional genes for wool quality is scarce (Arora et al., 2008).

DNA polymorphism is useful for identifying variation in known genes of potential importance. Animals with the desired genes /trait can be identified and selected to increase productivity and profitability. In sheep, genes of particular importance are those that influence prolificacy, pigmentation, wool quality and keratin proteins. The Indian sheep breeds of North

western arid and semiarid (NWSA) region are typically carpet wool type. Wool characteristics are not only due to multiple genes, but are also influenced by environmental and management factors. Wool fibre is mainly made up of the keratin, which consists of the keratin intermediate filament (IF) proteins (KRT) and the keratin IF-associated proteins (KAP) (Purvis and Franklin, 2005). Polymorphism at the keratin gene loci is responsible for the observed variation in wool characteristics and impacts on wool quality. Hence, this genetic variation may be used in marker-assisted selection for superior wool producing animals. The colour of wool is also important because superior colour (bright and white) can be dyed to the maximum range of shades and consequently is worth more than poor coloured wool. One of the most important locus that influences the pigmentation of fleece and hair is Agouti, which codes for melanocortin receptor binding protein. In the absence of this protein, production of eumelanin is stimulated causing the expression of colour in fleece and hair (Parsons et al., 1999; Smit et al., 2002).

In the present study genetic variation was assessed at the Agouti Signaling Peptide (ASIP) and the keratin associated protein (KAP1.1) gene loci. The agouti gene influences coat colour in sheep. The genomic organization of the ASIP gene is generally highly conserved in mammalian species, including mouse, human, horse, pig, cow and dog (Rieder et al., 2001; Girardot et al., 2005; Kerns et al., 2004). The Agouti gene locus is known to be implicated in control of production of eumelanin and pheomelanin responsible for pigmentation of fleece and hair. Coat color is an important breed characteristic and production trait. The contribution of ASIP to coat color pattern of domestic sheep has been investigated by Norris and Whan (2009). The KRT proteins forming wool filament are embedded in a matrix of keratin associated proteins (KAP) in an organized fashion.

KAPs form the major component of wool fibre characterized by high contents of cysteine, tyrosine or glycine residues (Powell and Rogers, 1997; Plowman, 2003). KAP1-1 is characterized by having a high percentage of cysteine residues available to form cross-links.

The objective of the present study was to investigate genetic polymorphism at known mutations in genes influencing wool quality and wool color traits in four Indian sheep breeds from Rajasthan of the NWSA region, using PCR-SSCP and/or SNP analyses.

MATERIALS AND METHOD

Animals : One hundred and forty four genomic DNA samples from four indigenous sheep breeds were investigated in the present study for elucidation of genetic variants at KAP1.1 and ASIP gene loci. Thirty six samples from each of the four breeds namely Marwari, Jaisalmeri, Malpura and Kheri sheep were analyzed. These sheep are distributed in NWSA agro-ecological region of the country and reared primarily for carpet wool and/or mutton. Genomic DNA was isolated from blood samples of unrelated individuals following standard procedure (Sambrook et al., 1989).

PCR amplification: The sequences of the primers used for amplification of the two loci are given in Table 1. PCR amplifications were performed in 25µl reaction volume with approximately 100ng genomic DNA, 1U Taq DNA polymerase, 10X Taq DNA polymerase buffer, 1.5mM, MgCl₂, 200µM each dNTPs and 0.25 µM of each primer. PCR cycling consisted of initial denaturation at 95°C for 5 minutes, followed by 35 cycles each with denaturation at 94°C for 30 seconds, annealing at specific temperature for 30 seconds, extension at 72°C for 30 seconds and a final extension at 72°C for 10 minutes.

PCR-SSCP : The PCR-single strand conformational polymorphism (PCR-SSCP) approach was used for

Table 1: Detail of the primer sequence, annealing temperature (At) and analysis technique for the functional loci investigated

Locus	Primer sequence	At (°C)	Analysis technique	Reference
ASIP	F-5'-CTTACTCCTGGCTACCTTGCTGGT-3'	63	PCR-SSCP	Smit et al., 2002
	R-5'-GATAGAGACAGAAGGGAAATCCAA-3'			
KAP1.1	F-5'-GAACCCTCCTCTCAACCCAACTCC-3'	61	SNP	Itenge-Musa et al., (2008)
	R-5'-CGCTGCTACCCACCTGGCCATA-3'			

analysis of the ASIP gene locus. Two μ l of the amplified PCR product was mixed with 10 μ l of loading dye (98% formamide, 10mM EDTA, 0.025% bromophenol blue and 0.025% xylene cyanol). The samples were denatured at 95°C for 5 minutes; immediately cooled in crushed ice and loaded onto native polyacrylamide gels. SSCP gels were electrophoresed for 16 hours at 140V at 10°C temperature in 0.5X TBE buffer. The gels were silver stained according to Bassam et al., (1991).

Statistical Analysis: Genotypes were identified on the basis of different patterns observed on the SSCP gels. Genotypic frequencies of different SSCP patterns were estimated and gene frequencies were calculated from genotypic frequencies.

Sequence analysis: Fifty microlitres of the PCR amplified products for the gene loci were purified using QIAquick PCR Purification Kit (QIAGEN). The purified PCR amplicons were sequenced on ABI 3100 sequencer in both forward and reverse direction. The sequences were analysed using DNAMAN ver 5.2.10 (Lynnon BioSoft, Vaudreuil, Quebec, Canada).

RESULTS AND DISCUSSION

ASIP gene locus, which codes for melanocortin receptor binding protein is one of the most important loci that influences the pigmentation of fleece and hair. In the present investigation a 142bp DNA fragment of the exon 2 region of ASIP gene was amplified across the

four Indian sheep breeds using PCR-SSCP. Two alleles namely N and D were observed in most of the breeds (Fig. 1). The genotypes NN and ND were detected in all the breeds, however, the homozygous DD genotype could be observed only in Jaisalmeri sheep but in very low frequency (Table 2). The frequency of the N allele ranged from 0.819 to 0.986 with an overall average of 0.934 across the four sheep breeds investigated. The D allele on the other hand was highly infrequent with an overall frequency of 0.066. All the sheep breeds investigated revealed a predominance of N allele irrespective of their face/fleece colour patterns at this locus. The allele frequency profile revealed a similar trend for all the investigated breeds as the allele frequency for N allele was observed to be higher than D allele (Fig. 2). The genotypes observed in all studied individuals of indigenous sheep were either homozygous NN or heterozygous ND genotypes however, homozygous DD genotype was absent across all the investigated breeds except Jaisalmeri. Polymorphism in the agouti gene has been reported in goats (Tang et al., 2008) and pigs (Leeb et al., 2000). In cattle, no SSCP variants were found in any of the three coding exons (Royo et al., 2005).

The KAP1-1 (previously known as B2A), encoded by the KRTAP1-1 gene, is designated as a high-sulphur protein belonging to the KAP1-1.n family (Powell and

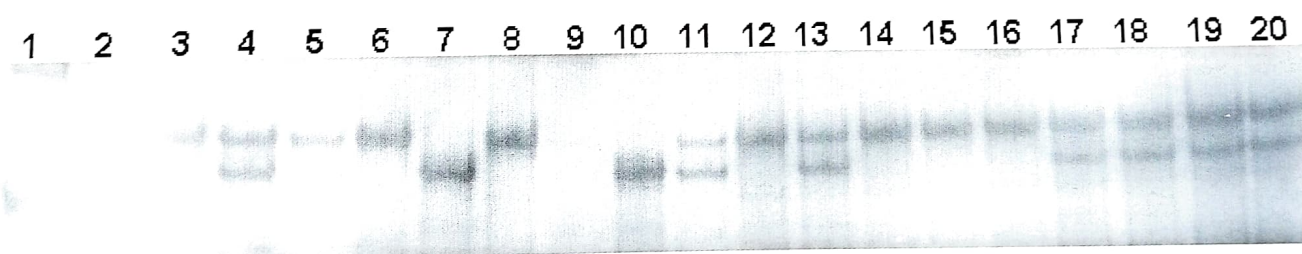


Fig.1. SSCP patterns of ASIP gene in four NWASA sheep breeds. Lane1: DNA ladder; lane 2, 3, 5, 6, 8, 12, 14, 15 and 16: NN genotype; lane 4, 9, 11, 13, 17, 18, 19 and 20: ND genotype; lane 7 and 10: DD genotype.

Table 2: Alleles and genotype frequencies of ASIP gene determined by PCR-SSCP in Marwari, Jaisalmeri, Malpura and Kheri sheep breeds/populations

Breed/population	Face colour	Allele frequency		Genotype frequency		
		N	D	NN	ND	DD
Marwari	Black	0.986	0.014	0.973	0.027	0.0
Jaisalmeri	Black	0.819	0.181	0.722	0.194	0.084
Malpura	Light brown	0.945	0.055	0.889	0.111	0.0
Kheri	Dark brown	0.986	0.014	0.973	0.027	0.0
Mean		0.934	0.066	0.889	0.089	0.021

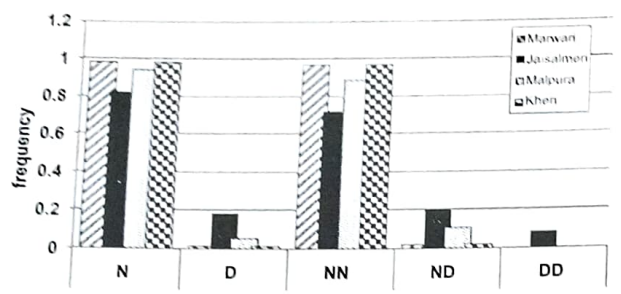


Fig. 2. Pattern of allele and genotype frequencies at ASIP gene locus determined in Marwari, Jaisalmeri, Malpura and Kheri sheep breeds/populations.

Rogers, 1997; Plowman 2003). This protein family comprises of four members: KAP1-1, KAP1-2 (B2B), KAP1-3 (B2C) and KAP1-4 (B2D) (171, 156, 151 and 181 amino acids respectively) (Elleman and Dopheide, 1972; Powell et al., 1983), each differing in the number of consensus decapeptide repeats (QTSCCQPTSI). Polymorphism in the ovine keratin and KAP genes has been reported by many workers (Beh et al., 2001; McLaren et al., 1997; Rogers and Powell, 1993; Rogers et al., 1994). In the present study, a 311bp region of KAP1.1 gene locus was amplified and sequenced across the four sheep breeds. Sequence alignment revealed the presence of 4 variations in the KAP1.1 gene with respect to the reference sequence HQ110109 (GenBank). These were 142C>G, 144C>T, 154C>G and 228C>G. All these variations were observed to be heterozygous. Itenge-Mweza et al., (2007) have reported three alleles at this locus in Merino sheep. The KAP genes that code for the structural proteins of the wool fibre may be candidate genes with major effects on wool production variations. The KAP genes have also been associated with variation in wool traits including especially staple strength (Rogers et al., 1994) and fibre diameter (Beh et al., 2001). Therefore, association of variations in KAP1.1 gene with specific wool traits may provide genetic markers useful for production of wool fibre with desired characteristics.

The study, thus, reveals the presence of genetic polymorphism in the ovine ASIP and KAP1.1 gene loci across four indigenous sheep breeds namely Marwari, Jaisalmeri, Malpura and Kheri. These are, however, preliminary results and further analysis of more breeds is required to determine the total diversity existing at these loci. Hence, gene loci associated with wool quality traits need to be evaluated further in other indigenous

breeds as well, to get a comprehensive view of available polymorphism, which can then be exploited to develop gene marker systems for selection of animals/breeds with desired traits.

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