



Polymorphism of keratin intermediate filament (kif) type I gene association of wool quality traits in Patanwadi, Marwari and Dumba breeds of sheep

A R AHLAWAT¹, P U GAJBHIYE², L L L PRINCE³, AMAR SINGH⁴ and S G GAJJAR⁵

Junagadh Agricultural University, Junagadh, Gujarat 362001 India

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ABSTRACT

For the present study, 150 blood samples (50 from each breed, viz. Dumba, Patanwadi and Marwari) were collected randomly from animals located at Sheep Breeding Farm, Morbi, Gujarat. Isolation of genomic DNA was carried out. The genotyping was done with the help of PCR-RFLP. At locus KRT 1.2, studies revealed 3 genotypes, viz. MM, MN and NN with genotype frequencies of 0.74, 0.24 and 0.02; 0.54, 0.42 and 0.04 and 0.64, 0.36 and 0.00 for Marwari, Patanwadi and Dumba breeds, respectively. The overall genotypic frequencies at KRT 1.2 locus for MM, MN and NN were 0.64, 0.34 and 0.02 respectively. The overall allele frequencies for allele M and allele N were 0.81 and 0.19 respectively. In general, the frequency of allele M was higher than that of allele N in all 3 breeds. The genotypic frequencies of MM were higher than MN and NN in all the three breeds. In *KIF* gene, all 3 breeds showed no significant deviation from Hardy Weinberg Equilibrium.

Key words: Dumba, Filament gene, Keratin intermediate wool, KRT 1.2, Marwari, Patanwadi, Restriction enzyme

India, third in the world in sheep population, has 62.5 million sheep (FAO 2011). India is endowed with wide diversity of sheep genetic resources, which forms the backbone of its rural livelihood security systems. India is the seventh largest producer of raw wool in the world, accounting for 1.8% of world production with about 4.2% of the total sheep population (Arora *et al.* 2008). It is important to exploit the inherent potential of indigenous sheep in order to improve the quality of their wool and bring it on a par with its exotic counterparts.

Genetic markers are not affected by environmental noise and would allow sheep breeders to select animals with improved wool characteristics at an early age and cull the non-desirable lambs (Theopoline 2002).

Keratin proteins, the major component of wool that are responsible for most of their structural properties, can be divided into 2 groups; the keratin intermediate (IF) proteins and the keratin-associated (KAP) proteins. The IF proteins form filaments that lie within a matrix of the KAP proteins. There are 2 families of the IF, type I and type II, and are coded for by separate gene loci that were mapped to chromosomes 11 and 3 respectively (Hediger *et al.* 1991). Linkage mapping using the AgResearch IMF flock has confirmed these physical map positions (McLaren *et al.* 1997). A few studies on molecular genetic studies of wool quality traits are available. The capacity to modify the

structural components of the wool fibre using knowledge of the genes controlling the IF/KAP protein content and balance was demonstrated in Merino sheep (Bawden *et al.* 1998). Gong *et al.* (2011) also suggested a variation in the KAP13–3 gene, which may affect gene expression, the structure and assembly of the protein, and consequently influence wool traits. Arora *et al.* (2008) evaluated genetic polymorphism of type I intermediate filament wool keratin gene in 15 native Indian sheep breeds and found that the average heterozygosity was 0.420, and none of the breeds deviated from Hardy-Weinberg equilibrium. There was predominance of the M over the N alleles. The present investigation was undertaken to study the polymorphism of *keratin intermediate filament (KIF)* gene by PCR-RFLP in Patanwadi, Marwari and Dumba sheep.

MATERIALS AND METHODS

Experimental animals: The study was designed to characterize the *Keratin Intermediate Filament Type I* gene in 150 individuals, 50 animals each of Patanwadi, Marwari and Dumba breeds from Sheep Breeding Farm, Morbi and Gujarat.

Wool samples were collected from 150 individuals and Staple length, fibre diameter and medullation % were determined.

Molecular genetic study

Blood collection and DNA isolation: Blood (8 ml) was collected from 150 individuals, by jugular vein puncture. Blood was collected in 10 ml vacutainer tube containing

Present address: ^{1,2}Cattle Breeding Farm, JAU, Junagadh (dranshuahlawat@gmail.com); ³ Scientist (ABT), CSWRI, Avikangar. ⁴ Senior Scientist (ABT), CSWRI, Avikangar. ⁵ M.V.Sc. Scholar, College of Veterinary Science and Animal Health.

Table 1. Preparation of reaction mixture for PCR amplification

Sr. No.	Stock solution	Final conc.	Per sample
1.	Dist. water	-	11.20 µl
2.	10× PCR buffer	1X	2.0 µl
3.	25mm MgCl ₂	1.5 mM	1.2 µl
4.	dNTPs (10mm)	200 µM	0.4 µl
5.	Taq DNA polymerase (5U/µl)	1 U	0.2 µl
6.	Primer forward	10 picomoles	1.0 µl
7.	Primer reverse	10 picomoles	1.0 µl
8.	Template DNA	100 ng	3.0 µl
Total volume		20 µl	

EDTA as anticoagulant. Blood was transported to laboratory in thermocol box on dry ice. The samples were stored at -20°C. The genomic DNA was isolated from blood by phenol-chloroform extraction method quality of DNA was checked by 0.8% agarose gel electrophoresis. Gel was then examined on UV trans- illuminator for checking of quality of DNA samples.

PCR amplification: Primers and protocol for amplification were followed from Hua *et al.* (2009). The primers used for the polymorphism study of *KIF* gene were *Ovine Type I IF*, Exon 1 (KRT 1.2 locus): F 5' CAC AAC TGT GGC TTG GTG AAC TTG 3'; R 5' CTT AGC CAT ATC TCG GAT TCC CTC 3' (Table 1).

The amplification included a initial denaturation at 94°C for 5 min, 35 cycle of denaturation at 95°C for 30 sec; annealing at 65–52°C for 30 sec; extension at 72°C for 45 sec followed by final extension at 72°C for 7 min. After the execution of PCR programme, the PCR products were stored at 4°C.

The amplicons were checked by 2% agarose gel electrophoresis at 90V for 1 h. The PCR amplified product of KRT 1.2 locus was cleaved with the *MspI* restriction enzyme in 12 µl reaction volume at 37 °C for 16 h. The RE digested products were electrophoresed in 2% agarose gel, and the genotyping patterns were visualized by ethidium bromide staining. The data on birth weight (kg), 3 month weight (kg), 6 month weight (kg) and adult body weight (kg) were collected, compiled and analysed.

Statistical analysis

Least squares model for wool quality traits data on 3 breeds of sheep

$$Y_{ijk} = \mu + b_i + f_j + e_{ijk}$$

where, μ , general mean; b_i , fixed effect of i^{th} breed on Y_{ijk} , where i , 1–3; f_j , fixed effect of j^{th} genotype on Y_{ijk} , where j , 1–3 (j_1 indicates genotype MM, j_2 indicates genotype MN and k_3 indicates genotype NN); e_{ijk} , random error.

The statistical analysis was done using software programme LSMLMW (Harvey 1990).

Frequency distribution of different alleles in 3 breeds was tested for Hardy – Weinberg Equilibrium (HWE) using following statistical (χ^2) test as will be discussed in results and discussion.

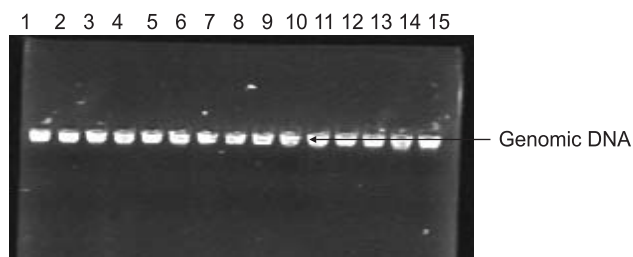


Fig. 1. DNA quality checked on 0.8% agarose gel.

RESULTS AND DISCUSSION

In this study, genetic polymorphism of KIF gene at locus KRT 1.2 was studied for the 3 breeds of sheep. DNA isolation from sheep blood and quality check: DNA quality was checked on 0.8% agarose gel electrophoresis visualized with ethidium bromide under UV illuminator (Fig. 1).

PCR amplification for KIF gene: Two regions of the keratin gene were amplified from ovine genomic DNA as expected. PCR products containing the segment of KRT 1.2 loci were 480 bp. Agarose gel electrophoretograms are visible in Figure 2. These PCR products were used for RFLP. PCR product were resolved on 2.5% agarose gel and visualized with ethidium bromide under UV transilluminator. PCR product of 480bp (lane 1 to 12, Fig. 2) and L-100bp DNA ladder is visible.

Restriction fragment length polymorphism: The following PCR-RFLP were generated by the KRT 1.2 *MspI* RFLP polymorphisms: 159, 126, 100 and 95 bp for MM genotype, 259, 126 and 95 for NN genotype and 259, 159, 126, 100 and 95 bp for MN genotype (Fig. 3). Arora *et al.* (2008) in his study on 15 Indian native sheep breeds found a similar banding pattern for MM, MN and NN genotype. PCR products were digested with *MspI* enzymes and

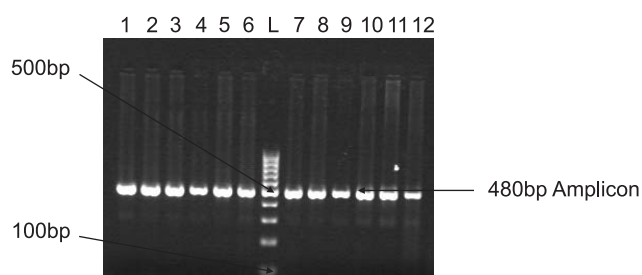


Fig. 2. Amplification of keratin gene (KRT 1.2) fragment.

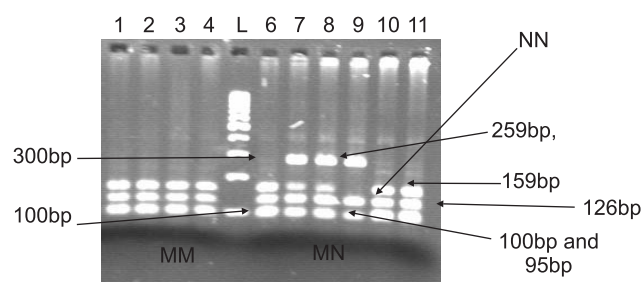


Fig. 3. Representative genotyping of KRT 1.2 gene.

Table 2. Genotype and allele frequencies of the KIF gene across three sheep breeds

Breed (sample size)	Genotype at KRT locus 1.2			Allele	
	MM	MN	NN	M	N
Marwari (50)	0.74	0.24	0.02	0.86	0.14
Patanwadi (50)	0.54	0.42	0.04	0.75	0.25
Dumba (50)	0.64	0.36	0.0	0.82	0.18

Table 3. Overall genotypic and allelotypic frequencies of KIF gene across three sheep breeds

KRT locus 1.2		
Genotype	MM	0.64
	MN	0.34
	NN	0.02
Allele	M	0.81
	N	0.19

resolved on 2.5% agarose gel, L represented 100bp DNA ladder. Respective genotypes are labelled.

Analysis of genotypic frequencies and allele frequencies of keratin intermediate filament type I gene: RFLP of KRT 1.2 locus revealed three genotypes, viz. MM, MN and NN with genotype frequencies of 0.74, 0.24 and 0.02; 0.54, 0.42 and 0.04 and 0.64, 0.36 and 0.00 for Marwari, Patanwadi and Dumba breeds, respectively (Table 2).

Genotypic frequencies of *KIF type-I* gene at locus KRT 1.2 in Marwari breed of sheep were 0.74, 0.24 and 0.02 for MM, MN and NN, respectively; while, the allelic frequencies were 0.86 and 0.14 for M and N, respectively. The chi square test for Hardy Weinberg equilibrium indicated non-significant differences ($P>0.05$) among the gene and genotypic frequencies with respect to KIF Type-I gene ($\chi^2=0.009$). Hence it was inferred that Marwari population was in Hardy Weinberg equilibrium with respect to locus KRT 1.2 (Table 2).

Genotypic frequencies of KIF Type-I gene at locus KRT 1.2 in Patanwadi breed of sheep were 0.54, 0.42 and 0.04 for MM, MN and NN, respectively; while, the allelic frequencies were 0.75 and 0.25 for M and N, respectively. The chi square test for Hardy Weinberg equilibrium indicated non-significant differences ($P>0.05$) among the gene and genotypic frequencies with respect to KIF Type-I gene ($=0.61$). Hence was inferred that Patanwadi population was in Hardy Weinberg equilibrium with respect to locus KRT 1.2 (Table 2).

Genotypic frequencies of KIF Type-I gene at locus KRT 1.2 in Dumba breed of sheep were 0.64, 0.36 and 0.00 for MM, MN and NN, respectively; while, the allelic frequencies were 0.82 and 0.18 for M and N, respectively. The chi square test for Hardy Weinberg equilibrium indicated nonsignificant differences ($P>0.05$) among the gene and genotypic frequencies with respect to *KIF Type-I* gene ($=2.25$). Hence it could be concluded that Dumba population was in Hardy Weinberg equilibrium with respect to locus KRT 1.2 (Table 2).

Arora *et al.* (2008) reported similar findings in native Indian sheep breeds. In his study he observed that the allelic frequencies differences for both alleles across the Indian breeds were insignificant. These findings corroborate with the results of the present investigation. The overall genotypic frequencies at KRT 1.2 locus for MM, MN and NN were 0.64, 0.34 and 0.02 respectively for the pooled population of the 3 breeds.

The overall allele frequencies for allele M and allele N were 0.81 and 0.19 respectively. In general, the frequency of allele M was higher than that of allele N in all 3 breeds. Three genotypes viz. MM, MN and NN were observed in the study (Table 3).

Least squares means for staple length, fibre diameter and medullation % were 6.13 cm, 42.74 μ and 80.48%, respectively (Table 4). Variation due to breed in all the wool quality traits was highly significant ($P<0.05$) as per DNMR (Table 4). Marwari breed showed highest staple length, lowest fibre diameter and lowest medullation %. The finding in our present investigation are in close agreement with results reported by Singh *et al.* (2008). The observed heterozygosity for KIF gene was 0.36, 0.42 and 0.24 for

Table 4. Least squares means and standard errors for staple length, fibre diameter and medullation %

Particulars	Staple length (cm)	Fibre diameter (μ)	Medullation (%)
Overall mean	6.13 $\pm$ 0.34 (150)	42.74 $\pm$ 1.58 (150)	80.48 $\pm$ 3.30 (150)
<i>Breed</i>			
Marwari	7.12 $\pm$ 0.37 (50)	36.77 $\pm$ 1.74 (50)	63.90 $\pm$ 3.65 (50)
Patanwadi	5.23 $\pm$ 0.35 (50)	45.32 $\pm$ 1.63 (50)	89.40 $\pm$ 3.43 (50)
Dumba	6.03 $\pm$ 0.38 (50)	46.15 $\pm$ 1.74 (50)	88.14 $\pm$ 3.65 (50)
<i>KRT locus 1.2</i>			
MM	5.93 $\pm$ 0.29 (96)	42.43 $\pm$ 1.33 (96)	77.48 $\pm$ 2.78 (96)
MN	6.20 $\pm$ 0.27 (51)	43.03 $\pm$ 1.23 (51)	75.75 $\pm$ 2.58 (51)
NN	6.25 $\pm$ 0.74 (3)	42.77 $\pm$ 3.43 (3)	88.21 $\pm$ 7.19 (3)

Table 5. Heterozygosity statistics for all loci in Marwari, Patanwadi and Dumba breeds of sheep

Locus	Sample size	Obs Hom	Obs Het	Exp Hom*	Exp Het*	Nei**	P
Marwari	100	0.76	0.24	0.7568	0.2432	0.2408	0.9226
Patanwadi	100	0.58	0.42	0.6212	0.3788	0.375	0.4390
Dumba	100	0.64	0.36	0.7018	0.2982	0.2952	0.1330

*Expected homozygosity and heterozygosity were computed using Levene (1949); ** Nei's (1973) expected heterozygosity.

Dumba, Patanwadi and Marwari breeds of sheep, respectively (Table 5). The average heterozygosity for KRT gene was 0.3037. All the 3 breeds showed significant ($P \leq 0.01$) deviation from HWE. Significantly high level of heterozygosity exists with respect to KRT gene in these 3 breeds of sheep. And so, a substantial level of genetic variation exists at KRT 1.2 locus of these breeds. Moreover, all the breeds were found to be in Hardy Weinberg equilibrium with respect to this locus. This indicated that no selection was exerted in all the 3 populations with respect to wool quality traits particularly staple length, which is closely associated with KRT 1.2 locus.

The molecular genetic analysis of KIF gene revealed that the restriction fragments generated for KRT 1.2 locus were of 159, 126 and 100 bp for MM genotype, 259, 159 and 100 bp for MN genotype and 259 and 126 bp for NN genotype. Greater frequencies of allele M and genotype MM and MN showed that selection for higher wool quality favoured MM homozygotes and MN heterozygotes. The genotypic and allelic frequencies of KIF gene showed that all 3 breeds were in Hardy Weinberg equilibrium.

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