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# Polymorphism of keratin-associated protein (KAP) 7 gene and its association with wool traits in Rambouillet sheep

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ABSTRACT

In the present study, association of polymorphic variants of KAP 7 gene with wool traits in Rambouillet sheep was investigated by using SSCP-PCR. Genomic DNA was isolated from blood samples of 100 Rambouillet sheep. A 388 bp segment was amplified by PCR using *ovine* specific primers for KAP 7 gene. The results identified 3 genotypes, viz. AA, AB and BB with genotype frequencies of 0.340 (34), 0.270 (27) and 0.390 (39) respectively and allele frequencies of 0.475 for allele A and 0.525 for allele B. The $\chi^2$-test showed that the genotype distribution was not in agreement with Hardy-Weinberg equilibrium (HWE). KAP 7 gene has significantly higher clean wool yield and greasy fleece weight in males whereas females have significantly superior fibre diameter, higher staple length and wool count. BB genotype has significantly higher staple length followed by AA and AB genotype. BB genotype has significantly higher greasy fleece weight production followed by AB and least by AA genotype. From the study, it may be concluded that KAP 7 gene might be a potential molecular marker for genetic selection for higher staple length and greasy fleece weight in Rambouillet sheep.

**Key words**: Keratin associated protein 7, PCR-SSCP, Polymorphisms, Rambouillet sheep, Wool traits

The efficiency of wool processing is dependent on the consistency of wool fibres as 90% of the wool fibre is made up of keratins intermediate-filament proteins (KRTs) and keratin-associated proteins (KAPs) (McLaren *et al.* 1997). The KRTs form the skeletal structure of the wool fibre and are embedded in a matrix of KAPs. These proteins are connected through disulphide cross-linkages which are important for the stability and the mechanical properties of wool (Feughelman 1996, Schweizer *et al.* 2006). The KAP genes are small, between 0.6 and 1.5 kb in size and are intron less (Powell 1996). The matrix KAPs are divided into three groups based on their amino acid compositions: the high-sulphur proteins (16–30% cysteine content) KAP1.n, KAP2.n, KAP3.n; ultra-high-sulphur proteins (30% cysteine content) KAP4.n, KAP5.n, KAP10.n and high-glycine-tyrosine proteins (KAP6.n, KAP7.n, KAP8.n) (Plowman 2003, Rogers *et al.* 2006 and Barba *et al.* 2009). The high glycine-tyrosine (HGT) KAPs are predominantly present in the orthocortex of the wool fibre and are the first sub-group of KAP genes expressed in the wool follicle (Gong *et al.* 2012). The HGT-KAP 7 gene is located in chromosome 1 (McLaren *et al.* 1997).

The most important wool traits for the apparel industry are fibre diameter (FD), staple strength, colour (lack of pigmented fibre), staple length (SL) etc. These essential parameters are indicators of the spinning efficiency of the wool (Plowman *et al.* 2009). A reduction in the content of HGT-KAPs appears, at least in part, to be responsible for the felting lustre mutant found in Merino sheep (Li *et al.* 2009).

Many studies have reported the genetic variation at KAP 7 gene might play an important role in determining various wool traits in sheep (Wang *et al.* 2010a&b, Gong *et al.* 2012, Liu *et al*. 2014 and Sulaiman and Hua-chun 2015). Furthermore, there have been reports associating variation in the KAP loci with variation in fibre diameter (FD), wool length and wool yield (Wang *et al.* 2010a, Liu *et al.* 2014). Diminutive information is known about KAP 7 gene, which led our interest to investigate polymorphism of KAP 7 gene and assess the effect of polymorphism on wool traits in Rambouillet sheep.

## MATERIALS AND METHODS

*Resource populations and DNA isolation:* Blood samples were collected from 100 Rambouillet sheep, viz. male (32) and female (68), selected randomly from Sheep Breeding

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and Research Farm, Reasi (A hill area with variable climatic conditions, ranging from sub-tropical to the semi temperate), Jammu, along with their wool records, viz. clean wool yield (CWY), fibre diameter (FD), crimps per inch (CPI), staple length (SL), wool count (WC) and greasy fleece weight (GFW).

Blood samples was collected from jugular vein aseptically in sterile EDTA coated vacutainer. Genomic DNA was isolated by HiPurATM SPP Blood DNA Kit. Purity and concentration of genomic DNA was determined by using Nano-drop spectrophotometer. Genomic DNA quality was assessed by using 0.8% horizontal submarine agarose electrophoresis. Gel was then examined on UV trans-illuminator for quality assessment. The genomic DNA samples having good quality (intact bands with no smearing) were used for further analysis.

*PCR amplification:* A pair of primers for amplification of KAP 7 gene was used on the basis of published sequence of Wang *et al.* (2010a and b) having primer sequences: forward (5' CAG CTT GAG GTA TAA AAG GTC C 3'); reverse (5' GTC CCG TAG TCA TCT GGA G 3') primers. PCR was carried out in a final volume of 25 µl. The reaction mixture consisted of 5 µl (30ng/µl) of genomic DNA, 1 µl (20 pmol/µl) of forward and reverse primers, 12.5 µl of 2 × PCR master mix and 5.5 µl of distilled water. Amplification was performed in Eppendorf thermal cycler and was programmed for amplification of KAP 7 gene: Initial denaturation step at 94°C for 4 min followed by 35 cycles of cyclic denaturation at 94°C for 30 sec, annealing at 56°C for 45 sec, extension at 72°C for 30 sec and final extension at 72°C for 10 min were carried out. To confirm the targeted PCR amplification, 5 µl of PCR product from each tube was mixed with 1 µl of 6× loading dye buffer and loaded on 2% agarose gel containing ethidium bromide (1% solution @ 5 µl/100ml) alongwith 100 bp DNA ladder at a constant voltage of 80 V for 45 min. in 0.5 × TBE buffer.

*Single-strand conformational polymorphism (SSCP) analysis of PCR amplicons*: To explore genetic polymorphism in KAP 7 gene; amplified PCR products were subjected for SSCP through 10% polyacralamide gel electrophoresis (Acrylamide: Bisacrylamide (49:1) 10 ml; 5× TBE 6 ml; Ammonium persulfate (20%) 150 µl; TEMED 30 µl; Autoclaved TDW 13.82 ml and total volume 30 ml). The gel was given pre-run at 250 V for 30 min to remove any polar impurity. PCR product (amplicon) was mixed with formamide dye and loaded in gel with the help of long tip micropipette carefully. Electrophoresis was performed at 4°C for 8 h at 200 V. DNA bands on the gel were visualized by the silver staining technique (Bassam *et al.* 1991).

*Statistical analysis:* Based on the results of genotyping for KAP 7 gene, genotypic frequencies, allelic frequencies, Hardy-Weinberg equilibrium (HWE), population genetic indexes such as gene homozygosity (Ho), gene heterozygosity (He), effective allele numbers (ne), Shannon's Information index (I) and fixation index (FIS) were as executed in POPGENE 32 version 1.32 software (Yeh *et al.* 1999). The polymorphism information content (PIC) was calculated (Botstein *et al.* 1980). The data on CWY (%), FD (µ), CPI, SL (cm), WC, GFW (kg) were subjected to least squares analysis by using Statistical Package for Animal Breeding (SPAB2) programme (Sethi 2006). The following model was used for this purpose:

$$Y_{ijk} = \mu + G_i + S_j + e_{ijk}$$

where, $Y_{ijk}$, $k^{th}$ observation under $j^{th}$ sex and $i^{th}$ genotype; µ, overall population mean; $G_i$, effect of $i^{th}$ genotype; $S_j$, effect of $j^{th}$ sex and $e_{ijk}$, random error associated with each observation and assume to be normally and independently distributed with mean zero and variance $(0, \sigma_e^2)$.

## RESULTS AND DISCUSSION

A total of 15–20 µg of DNA was obtained from the 300 µl of the blood. DNA samples having OD values from 1.7 to 1.9 only were used for further analysis. The genomic DNA samples having good quality without smearing were used for further analysis. The amplified PCR product on 2% agarose gel revealed a single compact band of 393 bp size in Rambouillet sheep under UV transilluminator and documented by gel documentation system. The PCR product of the same bp size was also reported by Wang *et al.* (2010 a and b) in Tibetan sheep.

The PCR product of 388 bp was subjected to polyacrylamide gel electrophoresis used for the detection of SSCP variants. As two variants and three patterns of different SSCP variants were found in each case i.e., AA, AB and BB (Fig. 1). The SSCP variants with same patterns were also reported by Wang *et al.* (2010a and b) in Tibetan sheep and Sulaiman and Hua-chun (2015) in Hetian sheep for KAP 7 gene.

The genotypic frequency in Rambouillet sheep for KAP 7 gene was 0.34 for AA genotype; 0.27 for AB genotype and 0.39 for BB genotype whereas, the gene frequencies were 0.475 for A allele and 0.525 for B allele respectively in Rambouillet sheep. The $\chi^2$-test values for KAP 7 gene showed highly significant ($P<0.01$) $\chi^2$-test value and therefore, suggests that the population was not in Hardy Weinberg equilibrium (HWE) as in Table 1. The results obtained in the present study were in agreement with Sulaiman and Hua-chun, 2015 in Hetian sheep whereas, different results were reported by Wang *et al.* (2010a and b) in Tibetan sheep for KAP 7 gene.

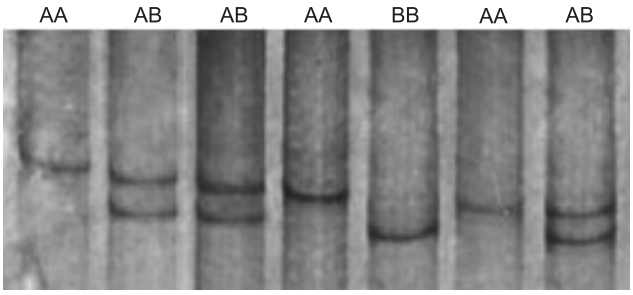


Fig. 1. The 10% PAGE electrophoresis patterns of 388 bp PCR products of KAP 7 gene in Rambouillet sheep.

Table 1. Frequency of genotypes and alleles along with population genetic indexes for KAP 7 gene in Rambouillet sheep

| Genotypes | | | Gene/Allele | | $\chi^2$-test (HWE) | Population genetic indexes | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AA | AB | BB | A | B | | Ho | He | ne | I | Fis | SE | PIC |
| 0.340 (34) | 0.270 (27) | 0.390 (39) | 0.475 | 0.525 | 21.501** | 0.54 | 0.46 | 1.85 | 0.65 | –0.03 | 0.03 | 0.35 |

**(P<0.01); HWE, Hardy-Weinberg equilibrium, figures in parentheses are number of observations;Ho, gene homozygosity; He, gene heterozygosity; ne, effective allele numbers; I, Shannon's Information index; Fis, fixation index; SE, standard error; and PIC, polymorphism information content.

*Population indices:* Expected heterozygosity (He) value was 0.50. Our results were not in agreement with those reported by Wang *et al.* (2010b) for Tibetan sheep (0.28). Effective number of alleles (ne) was 2.00. Our results were not in agreement with those reported by Wang *et al.* (2010b) for Tibetan sheep (1.38). Shannon indices (I) was found to be 0.69. Our results were not in agreement with those reported by Wang *et al.* (2010b) for Tibetan sheep (0.45). Fixation index of individual with sub-population ($F_{IS}$) was 0.46. The estimated polymorphic information content (PIC) values were with median polymorphism as 0.37. Thus, 37% of the offspring should be informative. PIC compares the polymorphism levels across markers and is used to determine the usefulness of markers for specific studies. The results obtained in the present study were not in agreement with those reported by Wang *et al.* (2010b) for Tibetan sheep (0.24) and Sulaiman and Hua-chun (2015) in Hetian sheep. Deviation from the reported studies at KAP 7 genes for (He), (ne), (I) and PIC values as shown in Table 1 may be due to breed differences, evolutionary pressure, selective breeding practices and availability of lesser number of breedable adults.

*Association of KAP 7 gene with wool traits:* Least squares analysis of variance showed non-significant effect of sex on CPI, whereas significant (P<0.05) effect of sex on CWY, FD and WC and highly significant (P<0.01) effect on SL and GFW. The effect of genotypes for CWY, FD, CPI and WC were non-significant, whereas highly significant (P<0.01) effect of genotypes were obtained for GFW and SL in Rambouillet sheep.

Table 2 shows nonsignificant differences between the least squares means of sex for CPI, whereas significant (P<0.05) difference on CWY, FD and WC and highly significant (P<0.01) differences on SL and GFW. Males had higher level of production on CWY (56.76±0.24%) and GFW (2.18±0.10 kg); whereas, female were superior in FD (21.83±0.10µ); SL (5.97±0.12 cm) and WC (62.44±0.26). Nonsignificant differences between the least squares means of various genotypes were observed for CWY, FD, CPI and WC; whereas the least squares means of various genotypes for SL and GFW differed highly significant (P<0.01). Higher scale of SL was recorded for BB genotype (5.73±0.16 cm) followed by AA genotype (5.19±0.19 cm) and AB genotype (4.98±0.20 cm) showed in (Fig 2). High GFW was recorded for BB genotype (2.09±0.09 kg) followed by AB genotype (1.81±0.11 kg) and least by AA genotypes (1.49±0.10 kg) (Fig 3) in Rambouillet sheep. The

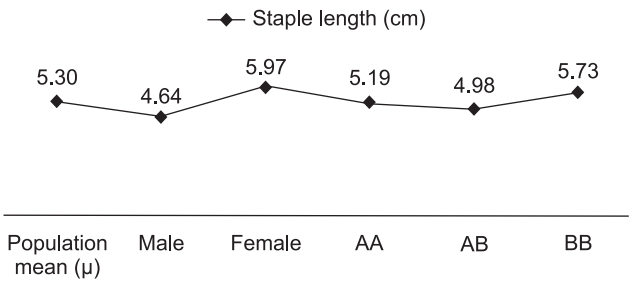


Fig. 2. Least squares means of sex and genotypes at KAP 7 gene for staple length (cm) in Rambouillet sheep.

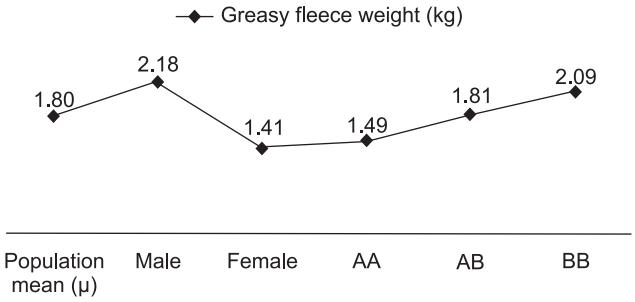


Fig. 3. Least squares means of sex and genotypes at KAP 7 gene for greasy fleece weight (kg) in Rambouillet sheep.

results of the present study were not in agreement with those reported by Wang *et al.* (2010a) for wool length. Deviation in the results obtained in the present study may be due to breed differences, gene × environment interaction, level of expression of gene, breeding polices followed within the population and difference in the managemental practices.

KAP 7 gene was found polymorphic with AA, AB and BB genotypes as revealed by PCR-SSCP analysis. BB genotype had significantly (P<0.01) higher staple length followed by AA and AB genotype. BB genotype had significantly (P<0.01) higher greasy fleece weight production followed by AB and least by AA genotype. The polymorphisms in the KAP 7 gene might be a potential molecular marker for GFW in Rambouillet sheep.

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Table 2. Least squares means and standard error for wool traits for KAP 7 gene in Rambouillet sheep

| Particular | Clean wool yield (%) | Fibre diameter (μ) | Crimps per inch | Staple length (cm) | Wool count | Greasy fleece weight (kg) |
|---|---|---|---|---|---|---|
| Mean (100) | 56.47±0.15 | 22.03±0.08 | 13.28±0.17 | 5.30±0.11 | 61.90±0.23 | 1.80±0.06 |
| *Sex* | * | * | NS | ** | * | ** |
| Male (32) | 56.76±0.24$^{b}$ | 22.24±0.14$^{b}$ | 13.26±0.29 | 4.64±0.18$^{a}$ | 61.36±0.38$^{a}$ | 2.18±0.10$^{b}$ |
| Female (68) | 56.17±0.17$^{a}$ | 21.83±0.10$^{a}$ | 13.30±0.19 | 5.97±0.12$^{b}$ | 62.44±0.26$^{b}$ | 1.41±0.07$^{a}$ |
| *Genotype* | NS | NS | NS | * | NS | ** |
| AA (34) | 56.37±0.25 | 21.82±0.14 | 13.61±0.29 | 5.19±0.19$^{a}$ | 62.42±0.38 | 1.49±0.10$^{a}$ |
| AB (27) | 56.10±0.26 | 22.06±0.15 | 13.14±0.31 | 4.98±0.20$^{a}$ | 61.64±0.40 | 1.81±0.11$^{b}$ |
| BB (39) | 56.93±0.22 | 22.21±0.12 | 13.10±0.26 | 5.73±0.16$^{b}$ | 61.65±0.33 | 2.09±0.09$^{c}$ |

**(P<0.01), *(P<0.05), non-significant (NS) values bearing same superscripts in a column under different subgroup did not differ significantly. Figures in parentheses are number of observations.

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