A Study on Keratin-Associated Protein (KAP) 3.2 Gene and Its Polymorphism in Sandyno Breed of Sheep

R. Bharathesree1*, R. Saravanan2, M. Jeyakumar2 and N. Murali2

1Veterinary Dispensary, Palayajeyamkondam, Kulithalai Division, Karur District, 639102, India.
2Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal, 637001, India.

Authors’ contributions

This work was carried out in collaboration among all authors. Author RB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RS, MJ and NM managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

ABSTRACT

The current study investigates the polymorphic patterns of keratin-associated protein (KAP) 3.2 gene in Sandyno breed of sheep. Genomic DNA was isolated from blood samples of 51 numbers of Sandyno breed. Ovine specific primer associated PCR amplification of KAP 3.2 gene revealed product at 393 bp and genotyped by PCR-SSCP (Single Strand Conformation Polymorphism) method and visualized under silver staining technique. KAP 3.2 gene locus revealed 3 genotypes, viz. AA, AB and BB with a frequency of 0.84, 0.16 and 0 in Sandyno breed with allele frequencies of A(0.92) and B(0.08). Regarding population genetic indices, the effective number of alleles (Ne) for KAP 3.2 in Sandyno breed of sheep was found to be 1.1716. The PIC values was 0.1356 and FIS values was negative (–0.0864) in this breed. The result revealed that the selected population of Sandyno breed of sheep was in Hardy-Weinberg equilibrium without any significant deviation from the population mean and was monomorphic for KAP 3.2 gene.
Keywords: Keratin Associated Protein (KAP) 3.2; Sandyno; PCR-SSCP; silver stain; monomorphic.

1. INTRODUCTION
Keratin Associated Protein (KAP) was one of the major genes that influence the economically important traits in wool sheep hence gene mapping studies of keratin proteins have identified some chromosomal regions associated with variation in wool quality and production traits [1].

The KAP genes are small, between 0.6 and 1.5 kb in size and are intron less [2]. The matrix KAPs are divided into 3 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 i.e., KAP6.n, KAP7.n, KAP8.n Barba et al. [3] Plowman, [4]; Rogers et al. [5], Schweizer et al. [6].

Among all the classes of Keratin Associated Protein gene, KAP 3.2 is found to be polymorphic having impact on wool characteristics and was reported by various researchers. The Nilagiri sheep which is a dual utility (fine wool and meat), native to the Nilagiri hills of Tamil Nadu breed has been used along with Merino, in the development of another synthetic wool breed named Sandyno, which has better wool quality and it has been improved for fine wool production through Marker Assisted Selection [7]. Considering above facts, the study was undertaken to investigate polymorphism of KAP 3.2 in Sandyno breed of sheep.

2. MATERIALS AND METHODS
A total of 51 blood samples of Sandyno breed of sheep were collected from the Sheep Breeding Research Station (SBRS), Sandynallah, the Nilgiris. Genomic DNA was isolated from whole blood using a modified method of Montgomery and Sise [8] with slight modifications by using saturated Phenol: Chloroform: Isomyl alcohol mixture. Good quality DNA samples with clear bands were selected for further study (Fig. 1).

Primers of KAP 3.2 F (5'-CCAAGACTTCTCCTAACC-3') and KAP 3.2 R (5'-GCATTAAGACTTGAGCAGCTC-3') were used for the amplification of the KAP 3.2 gene as described by Mahajan et al. [9]. PCR reactions were carried out with 20 μl of reaction mixture comprising 0.5 μl (5 picomoles) of each forward and reverse primers, 10 μl of 2 x PCR master mixes (1.5 mM MgCl2, Taq DNA polymerase, 100 μM dNTPs) and 8.5 μl of nuclease free water was aliquoted in each PCR tube containing one μl template DNA. The thermal protocol consists of an initial denaturation step at 94°C for 4 min, followed by 35 cycles of denaturation (94°C, 30 sec), annealing (56°C, 45 sec) and DNA extension (72°C, 30 sec) and a final extension step at 72°C for 10 min. To PCR products was confirmed by 2 per cent (w/v) agarose gel electrophoresis. The sizes and quantities of PCR products were verified by comparison with 100 bp DNA ladder.

To explore genetic polymorphism in KAP 3.2 gene, amplified PCR products were subjected for SSCP (Single Strand Conformation Polymorphism) through 8% Polyclaralamide gel electrophoresis (acrylamide: bisacrylamide (29:1) 13.3 ml; 5 x TBE buffer 10 ml; Ammonium persulfate (10%) 250 μl; TEMED 100 μl; Triple distilled water 26.35 ml and total volume of 50 ml). After the run was completed, silver staining was carried out according to Bassam et al. [10] with certain modifications to visualize the banding patterns (Fig. 1).

The allele and genotype frequencies were calculated and Hardy-Weinberg equilibrium was tested by comparing expected and observed genotype frequencies using a Chi-square (χ²)-test along with population genetic indexes such as gene homozygosity (Ho), gene heterozygosity (He), effective allele numbers (Ne), fixation index (Fis) and Shannon’s Information index (I) were executed in POPGENE 32 version 1.32 software [11]. The polymorphism information content (PIC) was calculated by PIC calculator.

3. RESULTS
The quantity and quality of DNA was assessed by Biophotometer and the mean yields of DNA isolated from Sandyno breed of sheep was 319.98 ± 53.33 μg/ml. The PCR amplification yielded product at 393 bp (Fig. 2) as expected for KAP 3.2 gene. PCR amplicons were subjected to SSCP analysis to detect the polymorphic patterns of KAP 3.2 gene. PCR-SSCP analysis of KAP 3.2 gene (Fig. 3) revealed AA, AB and BB genotypes with predominance of AA genotype. The genotype frequencies of AA, AB and BB were in the order of 0.84, 0.16 and 0.0 in
Sandyno breed. The A and B allele frequencies were 0.92 and 0.08 respectively in Sandyno breed of sheep (Table 1).

The present populations were consistent with Hardy-Weinberg equilibrium and had no significant difference (P > 0.05) in KAP 3.2 gene. The heterozygosity value (0.1591) in Sandyno breed was almost similar to the expected heterozygosity (0.1481) for KAP 3.2 gene (Table 2). The effective number of alleles ($N_e$) was 1.1716 and the PIC values for KAP 3.2 gene was 0.1356 in Sandyno sheep (Table 2). The $F_{IS}$ values were negative (– 0.0864) in the selected population for KAP 3.2 gene (Table 2).

4. DISCUSSION

The PCR amplification yielded product at 393 bp (Fig. 2) as expected for KAP 3.2 gene. Similarly, Mahajan et al. [9] and Wang et al. [12] also obtained products at 393 bp whereas McLaren et al. [13] observed product at 424 bp.

PCR-SSCP analysis of KAP 3.2 gene (Fig. 3) revealed AA, AB and BB genotypes with predominance of AA genotype. The genotype frequencies of AA, AB and BB were in the order of 0.84, 0.16 and 0.0 in Sandyno breed. The A and B allele frequencies were 0.92 and 0.08 respectively in Sandyno breed of sheep (Table 1). Wang et al. [14] observed similar type of polymorphism in KAP 3.2 gene with three genotypes (AA, AB and BB) in Tibetan sheep. Similarly, Itenge-Mweza, [15] in Merino sheep and Mahajan et al. [9] in Rambouillet sheep observed three genotypes by PCR-SSCP analysis. Contrary to the present findings, Mahajan et al. [9] in Rambouillet sheep observed the genotypic frequency for KAP 3.2 gene as 0.46, 0.40 and 0.14 for AA, AB and BB genotypes respectively. Whereas, the gene frequencies of A and B alleles were 0.66 and 0.34 respectively in Rambouillet sheep.

The heterozygosity value (0.1591) in Sandyno breed was almost similar to the expected heterozygosity (0.1481) for KAP 3.2 gene (Table 2). However, Mahajan et al. [9] reported expected heterozygosity (He) value of 0.45 in Rambouillet sheep and Wang et al. [12] for Tibetan sheep (0.50) and Wang et al. [14] for Tibetan (0.50), Oula (0.47) and Qiaoke (0.29) sheep.

The effective number of alleles ($N_e$) for KAP 3.2 gene was 1.1716 in Sandyno breed of sheep (Table 2). Mahajan et al. [9] observed almost similar value of 1.81 in Rambouillet sheep. The results obtained in this study were not in agreement with those reported by Wang et al. [12] for Tibetan sheep (2.00) and Wang et al. [14] in Tibetan (2.00), Oula (1.87) and Qiaoke (1.40) sheep.

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**Fig. 1. Pictorial representation depicting the methodology of KAP 3.2 gene**
### Table 1. Genotype and allele frequencies of KAP 3.2 gene in Sandyno breed of sheep

<table>
<thead>
<tr>
<th>Breed /Group</th>
<th>Total number of animals (n)</th>
<th>Observed genotypic frequency</th>
<th>Allele frequency</th>
<th>Expected Genotype frequency</th>
<th>$\chi^2$ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>A</td>
<td>AB</td>
<td>BB</td>
<td>B</td>
</tr>
<tr>
<td>Sandyno</td>
<td>51</td>
<td>0.84 (44)</td>
<td>0.92</td>
<td>0.08</td>
<td>0.16 (7)</td>
<td>0.85 (37.24)</td>
</tr>
</tbody>
</table>

### Table 2. Heterozygosity statistics and genetic diversity at KAP 3.2 gene in Sandyno breed of sheep

<table>
<thead>
<tr>
<th>Breed</th>
<th>Gene</th>
<th>Observed homozygosity</th>
<th>Observed heterozygosity</th>
<th>Expected homozygosity</th>
<th>Expected heterozygosity</th>
<th>Ne</th>
<th>PIC</th>
<th>F&lt;sub&gt;IS&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandyno</td>
<td>KAP 3.2</td>
<td>0.8409</td>
<td>0.1591</td>
<td>0.8519</td>
<td>0.1481</td>
<td>1.1716</td>
<td>0.1356</td>
<td>-0.0864</td>
</tr>
</tbody>
</table>

*Ne = Effective number of alleles; PIC = Polymorphic information content; F<sub>IS</sub> = Fixation index*
The PIC values for KAP 3.2 gene was 0.1356 in Sandyno sheep (Table 2). However, Mahajan et al. [9] estimated polymorphic information content (PIC) values with medium polymorphism as 0.35 in Rambouillet sheep. The result is deviated from the findings of Wang et al. [12] for Tibetan sheep (0.38) and Wang et al. [14] in Tibetan (0.38), Oula (0.36) and Qiaoke (0.24) sheep.

The $F_{Is}$ values were negative (– 0.0864) in Sandyno breed for KAP 3.2 gene (Table 2). However, Mahajan et al. [9] observed Fixation index ($F_{Is}$) value of 0.11 in Rambouillet sheep. Deviation from the reported studies at KAP 3.2 gene may be due to breed differences and selective breeding practices. However, presence of few alleles at the KAP 3.2 loci in Sandyno breed of sheep indicates monomorphic situation.

5. CONCLUSION

The selected population of Sandyno breed of sheep was analysed for KAP 3.2 gene and their polymorphism. PCR-SCCP analysis revealed the monomorphic pattern in KAP 3.2 loci with three genotypes. The population genetic indices were calculated and the resulted allele frequency was
almost nearing to fixation in Sandyno sheep (0.92).

**ETHICAL APPROVAL**

Animal Ethic committee approval has been taken to carry out this study.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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