PCR-SSCP Analysis of Keratin - Associated Protein (KAP) 6.1 Gene in Nilagiri and Dorset x Nilagiri Cross Breeds of Sheep

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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 managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present study was investigated to detect polymorphic patterns of the keratin-associated protein (KAP) 6.1 gene in 54 numbers of Nilagiri and 20 numbers of Dorset x Nilagiri breeds of sheep. DNA was isolated and amplified with ovine specific primers of KAP 6.1 gene. Allele frequencies of A, B, C, D and E was 0.79, 0.08, 0.15, 0.07 and 0.08 respectively with departure from Hardy-Weinberg equilibrium. KAP 6.1 gene was found to have a high degree of homozygosity (0.6668), and an effective number of alleles ($N_e$) for KAP 6.1 gene was 1.7007 in Nilagiri breeds of sheep, while the PIC value was 0.3909 with positive (0.1908) $F_{IS}$ value. From the study, PCR - SSCP analysis of KAP 6.1 gene revealed polymorphism in Nilagiri breeds whereas in Dorset x Nilagiri crossbred sheep two genotypes for the A/B with no polymorphism detected.

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1. INTRODUCTION

Keratin Associated Protein (KAP) was one of the major genes that mediate the economically important quality associated traits in wool breeds of sheep hence gene mapping studies of keratin proteins have found to be associated with wool parameters. The keratin intermediate-filament proteins (KRTs) and keratin-associated protein (KAPs) are the major keratin proteins contribute about 90 per cent of the wool fibre [1,2]. The KAP genes being small (0.6 and 1.5 kb size) have less intron sites [3]. The keratin proteins are subdivided into 3 major groups based on their amino acid compositions: KAP1.n, KAP2.n, KAP3.n with high-sulphur proteins, KAP4.n, KAP5.n, KAP10.n with ultra-high sulphur proteins, and KAP6.n, KAP7.n, KAP8.n are with high glycine-tyrosine proteins [4,5,6,7]. Among all the classes of Keratin Associated Protein gene, KAP 6 gene, which was classified under glycine-tyrosine group, was found to be polymorphic having an impact on wool characteristics and was reported by various researchers.

The Nilagiri sheep is a dual utility (fine wool and meat), native to the Nilagiri hills of Tamil Nadu whose population was reported as 8000 by Ganesakale and Rathnasabapathy [8]. Dorset X Nilagiri (DN) is a meat type breed and superior to the Nilagiri sheep in terms of body conformation, temperament, bodyweight traits and meat characteristics. The adaptability and survivability were generally good under field conditions and the acceptability of the strain among the farmers is very high. At present, Nilagiri breed is endangered with 587 numbers among which 50 percent is being maintained at Sheep Breeding Research Station, Sandynallah. The breed has been used along with Merino, in the development of another synthetic wool breed named Sandyno, which has better wool quality with fine wool properties. Considering the above facts, the study was undertaken to investigate polymorphism of KAP 6.1 gene in Nilagiri breeds of sheep because Nilagiri breed of sheep should be improved for fine wool production in their native breeding tract through Marker Assisted Selection in Bharathesree et al. [9].

2. MATERIALS AND METHODS

A total of 54 blood samples and 41 numbers of wool samples of Nilagiri and 20 blood samples of Dorset x Nilagiri crossbred sheep were collected from the Sheep Breeding Research Station (SBRS), Sandynallah. Genomic DNA was isolated from whole blood using a modified method of Montgomery and Sise [10] with slight modifications by using saturated Phenol: Chloroform: Isoamyl alcohol mixture. Purity and concentration of genomic DNA were determined by using spectrophotometry; meanwhile, horizontal gel electrophoresis was performed in a one per cent agarose gel to assess their quality. DNA samples with clear bands and good quality were selected for further analysis.

The KAP 6.1 gene was amplified by using forward primer F: (5'-CCATGGCATGAAGGTGT-3') and reverse primer R: (5'-AAAAAGGAAGGTTGGTG-3') as described by McLaren et al. [1]. PCR was carried out in a final reaction volume of 20 μl. Nineteen μl of reaction mixture comprising 5 p. moles of each forward (KAP 6.1 F) and reverse primers (KAP 6.1 R), 2 X PCR master mixes (1.5mM MgCl2, 100 μM dNTPs) (Gene Technologies, Chennai) and nuclease free water was aliquoted in each PCR tube and one μl template DNA was added to each tube to make the final volume. KAP 6.1 gene was amplified by the following PCR thermal protocol with initial denaturation step at 94°C for 4 min, continued by 35 cycles of denaturation (94°C, 30 sec), annealing (62°C, 45 sec), extension (72°C, 30 sec) and a final extension step at 72°C for 10 min. Agarose gel electrophoresis (2%) was done to visualize the amplified PCR products with a volume of five μl of PCR ampicons with 0.5 μg/ml ethidium bromide in 1x TAE buffer at a constant voltage of 5 V/cm for 40 min. The amplified sizes of PCR products were verified and compared with 100 bp DNA ladder.

Amplified PCR products were then subjected for SSCP (Single Stranded Conformation Polymorphism) to explore genetic polymorphism or genetic variation in KAP 6.1 gene through 8 per cent Polycrylamide gel electrophoresis (PAGE). The composition includes acrylamide: bisacrylamide (29:1) solution of 13.3 ml; TBE buffer (5x) of 10 ml; Ammonium persulfate (10%) of 250 μl; TEMED (Tetramethylethylenediamine) of 100 μl; Triple distilled water of 26.35 ml to make up the total volume of 50 ml. The Polycrylamide gel electrophoresis was performed at 4°C (refrigerated) for 24 hours at 120 V for 20×20 cm plate size. The gel was removed from the glass plates after 24 hours of run, and silver staining procedure was referred
out as described by Bassam et al. [11] with certain modifications to visualize the polymorphic patterns.

The significance of the departure from Hardy-Weinberg equilibrium was tested by Chi-square test. Population genetic parameters such as gene homozygosity (Ho) and heterozygosity (He), effective allele numbers (Ne), fixation index (Fis) and Shannon’s Information index (I) were calculated in POPGENE 32 version 1.32 software [12] whereas the PIC calculator was used to assess the polymorphism information content (PIC).

3. RESULTS AND DISCUSSION

A Good quality genomic DNA samples (KAP 6.1 gene) on PCR amplification yielded product at 528 bp (Fig. 1) similar to the observation of McLaren et al. [1], Liu et al. [13] in contrast reported that amplification of KAP 6 gene produced product of 498 bp size. The A, B, C, D and E allele frequencies in Nilagiri sheep were 0.79, 0.08, 0.15, 0.07 and 0.08 respectively. Gong et al. [14] observed the same with six alleles in Merino, Romney, Coopworth breeds of sheep. However, Parsons et al. [15] observed two alleles A1 and A2 in Medium Peppin flock by PCR RFLP method. The PCR-SSCP pattern of 528 bp fragment of KAP 6.1 gene yielded (Fig. 2) six genotypes viz. AA, AB, AC, AD, CC, EE with frequencies were in the order of 0.56, 0.14, 0.15, 0.06, 0.04 and 0.06 respectively in Nilagiri breed (Table 1). The Genotype AA was more prevalent in Nilagiri (0.56) breed and the chi-square values indicate that there was a significant difference in their allele and genotype frequency.

Rather, the reports of Feng et al. [16] observed three polymorphic variants (AA, AB and BB) in Chinese Merino sheep with the highest frequency in BB genotype (0.457). Whereas, Zhou et al. [17] found three sequence variants in Merino cross lambs with the highest frequency for AB genotype (0.405). Deviation of present study at KAP 6.1 gene may be deviated from the above-quoted reference studies due to the size of the population, breed differences and selective breeding practices.

SSCP analysis of KAP 6.1 gene in Dorset x Nilagiri crossbred sheep resulted in two genotypes for the A/B with no polymorphism (Fig. 3). Absence of polymorphism in these two loci in Dorset x Nilagiri crossbred sheep intended for meat production further strengthens the evidence that the proposed KAP genes are possible candidate genes for wool traits rather than meat quality traits and can be utilized as markers for selection of fine wool quality. No studies on polymorphism of KAP gene in Dorset breed of sheep could be found in the literature for comparison with the present study.

Regarding population genetic indices, KAP 6.1 gene had significant (P < 0.01) difference in genotype and allelic frequencies for Nilagiri breed. The departure from Hardy-Weinberg equilibrium could be due to the presence of null alleles, the practice of non-random mating, selection of population over the years and shrinkage in population size [18].

![Fig. 1. Amplified product of KAP 6.1 gene under PCR (2% agarose gel electrophoresis) (Lane: 1 to 10 samples; Lane M: Marker (100 bp))](source: Bharathesree et al. [9])
Table 1 a,b. Genotype and allele frequencies of KAP 6.1 gene in Nilagiri breed of sheep

| Breed     | Total number of animals (n) | Observed Genotypic frequency | Expected Genotype frequency |
|-----------|----------------------------|-----------------------------|-----------------------------|
|           | AA | AB  | AC  | AD  | CC  | EE  | AA | AB  | BB  | AC  | BC  | CC  | AD  | BD  | CD  | DD  | AE  | BE  | CE  | DE  | EE  |
| Nilagiri  | 51 | 0.56| 0.14| 0.15| 0.06| 0.04| 0.06| 0.59| 0.07| 0.00| 0.15| 0.01| 0.02| 0.06| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00|
|           |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|           |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|           |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

Breed / Group | Total number of animals (n) | Allele frequency | χ² value | P value
Nilagiri      | 51                          | A: 0.79, B: 0.08, C: 0.15, D: 0.07, E: 0.08 | 75.70     | 0.00

Figures in parentheses indicate the number of animals
*Highly significant (P < 0.01), NS: Not significant

Table 2. Heterozygosity statistics of KAP 6.1 gene in Nilagiri breed of sheep

| Breed     | Gene  | Observed homozygosity | Observed heterozygosity | Expected homozygosity | Expected heterozygosity | Ne   | PIC  | Fis  |
|-----------|-------|-----------------------|-------------------------|-----------------------|-------------------------|------|------|------|
| Nilagiri  | KAP 6.1| 0.6668                | 0.3332                  | 0.5840                | 0.4160                  | 1.7007 | 0.3909 | 0.1908 |

Ne = Effective number of alleles; PIC = Polymorphic information content; Fis = Fixation index
**Fig. 2.** PCR-SSCP patterns of *KAP* 6.1 gene in Nilagiri breed of sheep (8% PAGE electrophoresis)
*Source: Bharathesree et al. [9]*

**Fig. 3.** PCR-SSCP patterns of *KAP* 6.1 gene in Dorset x Nilagiri crossbred sheep
*Source: Bharathesree et al. [9]*

*KAP* 6.1 gene was found to have high degree of homozygosity (0.6668) in Nilagiri sheep. The effective number of alleles (*N*<sub>e</sub>) was 1.7007 (Table 2) whereas the PIC value was 0.3909 for *KAP* 6.1 gene in Nilagiri breed of sheep. *F*<sub>IS</sub> values for *KAP* 6.1 gene was positive (0.1908) (Table 2) in studied population indicates heterozygote deficiency in the Nilagiri population. In contrast, Itenge-Mweza [19] reported *KAP* 6.1 gene as non-polymorphic and un-informative in Merino cross sheep.

### 4. CONCLUSIONS

The PCR-SSCP analysis of *KAP* 6.1 gene revealed six genotypes in Nilagiri sheep with no polymorphism in Dorset x Nilagiri crossbred sheep. Considering above facts and population parameters, *KAP* 6.1 gene was selected as a candidate gene in Nilagiri breeds to investigate their polymorphic patterns. Polymorphism in *KAP* 6.1 gene observed might be a potential molecular marker for Nilagiri breed of sheep in the selection of breeds against fine wool quality traits [9].

### ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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