Current Journal of Applied Science and Technology

39(20): 97-102, 2020; Article no.CJAST.59423
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

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

R. Bharathesree[1*], R. Saravanan[2], M. Jeyakumar[2] and N. Murali[2]

[1]Veterinary Dispensary, Palayajayankondam, Kulithalai Division, Karur District, 639102, India.
[2]Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal, 637 001, 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 managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i2030813
Editor(s):
(1) Dr. Ahmed Mohamed El-Waziry, Alexandria University, Egypt.
(2) Dr. Maduike Chiehiura Onwubiko Ezeibe, Michael Okpara University of Agriculture, Nigeria.
Reviewers:
(1) Ruchika Kaushik, All India Institute of Medical Sciences, India.
(2) Isam Hussain T. Al-Karkhi, University of Baghdad, Iraq.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59423

Original Research Article

Received 24 March 2020
Accepted 30 May 2020
Published 31 July 2020

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.

**Corresponding author: E-mail: rbharathi92@gmail.com;*

Keywords: Keratin Associated Protein (KAP) 6.1; PCR-SSCP; polymorphism; sheep.

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 (*KRT*s) and keratin-associated protein (*KAP*s) 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: *KAP*1.n, *KAP*2.n, *KAP*3.n with high-sulphur proteins, *KAP*4.n, *KAP*5.n, *KAP*10.n with ultra-high-sulphur proteins, and *KAP*6.n, *KAP*7.n, *KAP*8.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'-CCAATGGCATGAAGGTGT-3') and reverse primer R: (5'-AAAAAGGGAAGGGTTGGTG-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 amplicons 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 Polyacrylamide 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 Polyacrylamide 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 (F_{IS}) 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].

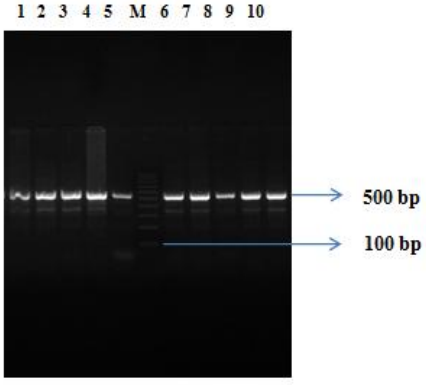

Fig. 1. Amplified product of *KAP* 6.1 gene under PCR (2% agarose gel electrophoresis) {Lane: 1 to 10 samples; Lane M: Marker (100 bp)}

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 (31)	0.14 (6)	0.15 (7)	0.06 (4)	0.04 (2)	0.06 (1)	0.59 (28.98)	0.07 (4.56)	0.00 (0.15)	0.15 (8.37)	0.01 (0.65)	0.02 (0.56)	0.06 (3.02)	0.00 (0.23)	0.00 (0.43)	0.00 (0.05)	0.06 (3.06)	0.00 (0.21)	0.00 (0.43)	0.00 (0.16)	0.00 (0.04)

Breed / Group	Total number of animals (n)	Allele frequency					χ^2 value	P value
		A	*B*	*C*	*D*	*E*		
Nilagiri	51	0.79	0.08	0.15	0.07	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	F_{IS}
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; F_{IS} = Fixation index

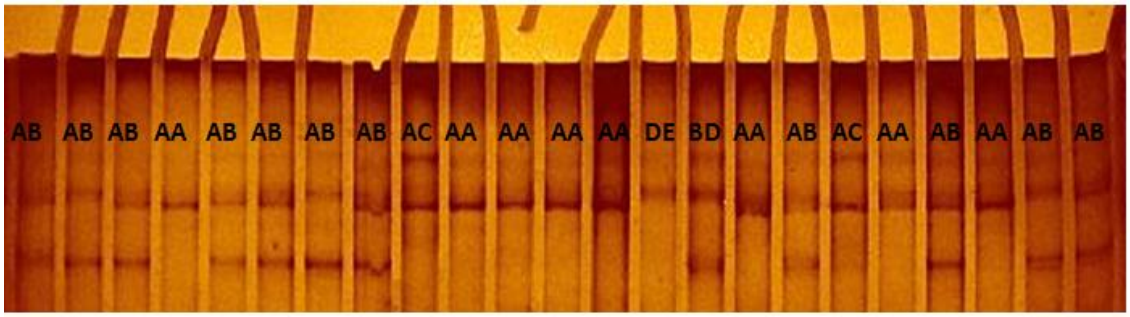

Fig. 2. PCR-SSCP patterns of *KAP* 6.1 gene in Nilagiri breed of sheep (8% PAGE electrophoresis)
Source: Bharathesree et al. [9]

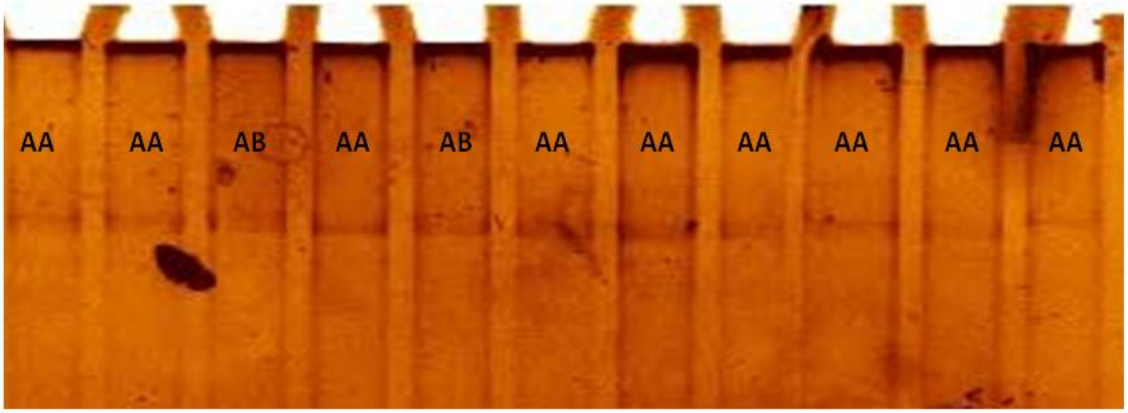

Fig. 3. PCR-SSCP patterns of *KAP* 6.1 gene in Dorset x Nilagiri crossbred sheep
Source: Bharathesree et al. [9]

KAP 6.1 gene was found to have high degree of homozygosity (0.6668) in Nilagiri sheep. The effective number of alleles (N_e) was 1.7007 (Table 2) whereas the PIC value was 0.3909 for *KAP* 6.1 gene in Nilagiri breed of sheep. F_{IS} 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.

REFERENCES

1. McLaren RJ, Geraldine R, Rogers, Kizanne P, Davies Jill, Maddox F, Grant W. Montgomery. Linkage mapping of wool keratin and keratin-associated protein genes in sheep. Mamm Genome. 1997;8:938-940.

2. Feughelman M. Mechanical properties and structure of alpha-keratin fibres: wool, human hair and related fibres. UNSW Press Sydney, Australia; 1956.
3. Powell BC. The keratin proteins and genes of wool and hair. Wool Tech Sheep Bree. 1996;44:100–118.
4. Plowman JE. Proteomic database of wool components. J. Chromatogr. B. 2003;787:63–76.
5. Rogers MA, Langbein L, Praetzel WS, Winter H, Schweizer J. Human hair keratin associated proteins (KAPs). Int. Rev. Cytol. 2006;251:209–263.
6. Barba C, Mendez S, Martí M, Parra JL, Coderch L. Water content of hair and nails. Thermochim Acta. 2009;494:136–40.
7. Schweizer J, Bowden PE, Coulombe PA, Langbein L, Lane EB, Magin TM, Maltais L, Omary MB, Parry DA, Rogers MA, Wright MW. New consensus nomenclature for mammalian keratins. J. Cell Biol. 2006;174:169–174.
8. Ganesakale D, Rathnasabapathy V. Sheep breeds of Tamil Nadu. Cheiron. 1973;2:146–155.
9. Bharathesree R., Murali N., Saravanan R, Anilkumar R. Polymorphism of Keratin-Associated Protein (KAP) 6.1 gene and its association with wool traits of Sandyno and Nilagiri breeds of sheep. Indian Journal of Animal Research. 2019;53(12).
10. Montgomery GW, Sise JA. Extraction of DNA from sheep white blood cells. New Zeal J Agr Res. 1990;33(3):437-441.
11. Bassam BJ, Caetano-Anolles G, Gresshoff PM. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem. 1991;196:80-83.
12. Yeh FC, Yang RC, Boyle BJT, Ye ZH, Mao XJ. POPGENE 32 version 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada; 1999. Available;(http://www.ualberta.ca/~fyeh/fyeh).
13. Liu YX., Shi GQ, Wang HX, Wan PC, Tang H, Yang H, Guan F. Polymorphisms of KAP6, KAP7, and KAP8 genes in four Chinese sheep breeds. Genet Mol Res. 2014;13(2):3438-3445.
14. Gong H, Zhou H, Yu, Dyer J, Plowman JE, Hickford JGH. Identification of the ovine keratin-associated protein *KAP*1-2 gene (*KRTAP*1-2). Exp. Dermatol. 2011;20:815–819.
15. Parsons YM, Cooper DW, Piper LR, Powell BC. A Bam HI polymorphism in ovine glycine/ tyrosine-rich type II (*KRTAP*6) keratin sequences. Anim Genet. 1993;24:218.
16. Feng J, Song TZ, Yang H, Yang J, Han M, Yang Y, Shi G, Xu C, Huang, X. Genetic polymorphism of *KAP* 6.1 gene in sheep. Southwest China Journal of Agricultural Sciences; 2012. Available:http://en.cnki.com.cn/Articleen/CJFDTotalXNYX201201059.htm.
17. Zhou H, Gong H, Li S, Luo Y, Hickford, JGH. A 57-bp deletion in the ovine *KAP* 6-1 gene affects wool fibre diameter. J Anim Breed Genet. 2015;132:301-307.
18. Pramod S, Kumarasamy P, Rosalyn Mary Chandra A, Sridevi P, Rahumathulla PS. Molecular characterization of Vembur sheep (*Ovis aries*) of South India based on microsatellites. Indian. J. Sci. Technol. 2009;2:55-58.
19. Itenge-Mweza TO. Identification of polymorphism in the keratin genes (*KAP3.2, KAP6.1, KAP7, KAP8*) and Microsatellite *BfMS* in Merino Sheep Using Polymerase Chain Reaction-Single Strand Conformational Polymorphism (PCR-SSCP) analysis; 2012. Available:http://dx.doi.org/10.5772/45732.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/59423