The Pharma Innovation Journal 2022; SP-11(6): 2619-2621

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 2619-2621
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www.thepharmajournal.com
Received: 10-03-2022
Accepted: 14-04-2022

KRT 1.2 gene polymorphism & its association with wool traits in Rambouillet sheep

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Abstract
The present study was carried out on a total of fifty Rambouillet sheep with the objectives to determine genetic polymorphism of KRT gene and to study association of polymorphic variants of KRT gene with wool traits. The blood samples and wool records such as greasy fleece weight (GFW) (gm), staple length (SL) (cm) and fibre diameter (FD) (µ) were collected from Rambouillet sheep maintained at Sheep Breeding and Research Farm, Reasi, Jammu. DNA was isolated and amplification of KRT1.2 gene was carried out. PCR-RFLP was performed with with restriction enzyme MspI. Population genetic indexes were calculated by using POPGEN 32. Three types of genotypes AA, AB and BB were obtained with frequency of 0.36, 0.56 and 0.08, respectively. The calculated expected homozygosity (Ho), expected heterozygosity (He), Shannon's information index (I) and polymorphic information content (PIC) values were 0.535, 0.465, 0.65 and 0.35, respectively. The overall least-squares means and standard errors for different wool production traits i.e. GFW, FD and SL were 1323.08 ± 82.04 gm, 22.17 ± 0.16 µ and 5.02 ± 0.15 cm, respectively. Analysis of variations showed that the sex had significant effect only on GFW, whereas it had non-significant on FD and SL. The genotype had non-significant effect on all the traits under study. Males were superior in GFW. High GFW production was recorded for BB genotype (1487.50 ± 210.66 gm) followed by AB genotype (1255.36 ± 79.62 gm) and least by AA genotypes (1226.39 ± 99.31 gm) in Rambouillet sheep. No significant association was found in the Rambouillet population which may be due to the small number of observations for the present study.

Keywords: KRT 1.2 gene, PCR-RFLP, Shannon index, PIC, wool production traits, Rambouillet sheep

1. Introduction
The physical properties of wool fibre can be attributed to proteins from the keratin family which are the primary constituents of the fibre (Itenge-Mwezaa *et al.,* 2007) [6]. The keratin intermediate-filament proteins (KRTs) and keratin-associated protein (KAPs) are the major proteins that make up the wool fibre. The KRTs form the skeletal structure of the wool fibre (microfibrils) 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 (Powell and Rogers, 1986; Marshall *et al*., 1991 and Feughelman, 1996) [14, 10, 12]. The microfibrils consist of KRTs, also known as the "hard" a-keratins. These are low sulphur proteins, which are classified into two protein families, Type I and Type II keratins (Powell, 1996; Schweizer *et al.,* 2006) [15, 13].

The efficiency of wool processing is dependent on the consistency of wool fibre. Although selective breeding of sheep has in part reduced the variation in wool fibres, considerable variation both within and between fleeces still exists. This genetic diversity has impact on wool fibre structure and genes and proteins present in the wool fibre may be used as markers. The use of genetic markers greatly accelerates the efficiency of wool breeding programmes. Therefore, the present study was conducted to determine genetic polymorphism of KRT gene and its association with wool traits in Rambouillet sheep.

2. Materials and Methods
The blood samples and wool records such as greasy fleece weight (GFW) (gm), staple length (SL) (cm) and fibre diameter (FD) (µ) were collected from Rambouillet sheep maintained at Sheep Breeding and Research Farm, Reasi, Jammu. DNA was isolated by HiPura^{TM} SPP Blood DNA Kit (HIMEDIA). The purity of DNA was checked using spectrophotometer by taking the ratio of optical density values at 260nm and 280nm. Only good quality DNA that was having OD values ranging from 1.7 to 1.9 was used for further analysis.

A pair of primers i.e. the forward primer with sequence 5´-CACAACTGTGGCTTGGTGAACTTG-3´ and reverse primer with sequence 5´-CTTAGCCATATCTCGGATTCCCTC-3´ for amplification of KRT1.2 gene was used on the basis of published sequences as reported by Rogers *et al.* (1993) [14] that resulted in a 480 bp PCR product.

The PCR conditions were as follows- initial denaturation at 94°C for 5 min; denaturation at 94°C for 30 sec; annealing at 59°C for 1 min; extension at 72°C for 30 sec-these followed 30 cycle and final extension at 72°C for 10 min and hold at 4°C for infinity. PCR product was digested with restriction enzyme *Msp*I in 32 µl volume at 37°C for 16 hr. After restriction digestion, the restriction product mixtures were electrophoresed on 2.5% agarose gel to get different bands and genotyping was done according to the band pattern of respective genotypes.

The frequency of KRT1.2 gene; allele A and B were estimated using the POPGENE software package (Yeh *et al.*, 1999) [18]. Population genetic indexes such as gene homozygosity (Ho), gene heterozygosity (He), observed number of alleles (N_O), effective allele numbers (Ne), fixation index (Fis) and Shannon's Information index (I) were estimated using POPGENE 32 version 1.32 software (Yeh *et al*., 1999) [18]. The polymorphism information content (PIC) was calculated according to Botstein *et al.* (1980) [2].

The data on greasy fleece weight (gm), fibre diameter (µ) and staple length (cm) were subjected to least squares analysis by using by Harvey (1990) [5]. 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 ith genotype

S_j = effect of jth sex

e_{ijk} = Random error associated with each observation and assume to be normally and independently distributed with mean zero (0) and variance (σ^2e).

3. Results and Discussions

Genotyping of individuals for the KRT1.2 gene was done by PCR-RFLP. *Msp*I restriction enzyme was used for restriction digestion. Three types of genotypic band patterns *viz.* AA (160 bp, 126 bp, 100 bp and 94 bp), AB (260 bp, 160 bp, 126 bp, 100 bp and 94 bp) and BB (260 bp, 126 bp and 94 bp) were obtained.

The genotypic frequencies for AA, AB and BB genotypes were 0.36, 0.56 and 0.08, respectively. The allele frequency of A and B alleles of KRT1.2 gene was 0.64 and 0.36, respectively (Table 1). The Chi-square test (χ^2-test) and G^2 tests showed that the values were non-significant and the population was in Hardy-Weinberg equilibrium (HWE). Singh (2017) reported higher A allele and AA genotype frequencies in Magra sheep. Arora *et al.* (2008) [1] on 15 different native sheep breeds reported three genotypes *viz.* MM, MN and NN with allele M yielding two bands of 100 and 159 bp, and allele N gave one fragment of 259 bp. Invariant fragments of 126 and 95 bp were present in both the alleles. Kumar *et al.* (2016) [7] reported three genotypes of KRT1.2 *viz.*, MM, MN and NN in all breeds/strains studied except Malpura and Avikalin in which NN genotype was not observed. Farag *et al.* (2018) [3] reported eight genotypes in KRT1.2 gene by PCR-SSCP techniques in Egyptian sheep. Meena *et al.* (2018) reported higher frequency of MM genotypes in Magra sheep. Kumar *et al.* (2016) [7] and Singh (2017) also reported non-significant χ^2- value for KRT1.2 locus in Indian sheep breeds and Magra sheep, respectively.

Table 1: Genotype distribution and allelic frequencies of KRT1.2 gene in Rambouillet sheep

Locus	Genotypes			Gene/Allele		χ^2-test
	AA	AB	BB	A	B	(HWE)
KRT1.2	0.36 (18)	0.56 (28)	0.08 (4)	0.64	0.36	2.32

Figures in parentheses are number of observations

The population genetics indexes of KRT1.2 gene have been presented in Table 2. The calculated expected homozygosity (H_o) and expected heterozygosity (H_e) values were 0.535 and 0.465, respectively. It was observed that the H_o value was more than the H_e value. It indicates that the homozygosity was more for KRT1.2 gene in the Rambouillet population. Arora *et al.* (2008) [1] reported lower H_e value of 15 different native Indian sheep of the KRT1.2 gene.

Table 2: Population genetic indexes of KRT1.2 gene in Rambouillet sheep

Ho	He	n_a	n_e	I	F_{IS}	SE	PIC
0.535	0.465	2.00	1.85	0.65	-0.22	0.03	0.35

Ho = Expected homozygosity,
He = Expected heterozygosity (Levene, 1949),
n_a = Observed number of alleles,
n_e = Effective number of alleles,
I = Shannon's Information index by Lewontin (1972),
Fis = Fixation index of individual with sub-population by Wright's (1978),
SE = Standard error and
PIC = Polymorphic information content

The calculated observed number of alleles (n_a) and effective number of alleles (n_e) values were 2.00 and 1.85, respectively. The Shannon's information index (I) was 0.65. It was observed that Shannon's information index (I) was higher in the population statistics for KRT1.2 gene. Shannon's diversity index indicates that there are different types of alleles present in population. Fixation index of individual with sub-population (F_{IS}) was negative value (-0.22) which implies that an excess of heterozygotes may occur for KRT1.2 gene. Standard error (SE) of the mean allelic frequencies was 0.03. The calculated polymorphic information content (PIC) value was 0.35. The PIC value of the present study indicates the marker is moderately informative. The chi-square value for genotypes was non-significant. Kumar *et al.* (2016) [7] reported lower mean effective number of alleles in Indian sheep breeds. However, lower estimate of SE value was obtained by Arora *et al.* (2008) [1] for KRT1.2 alleles.

Analysis of variations showed that the sex had significant effect only on GFW, whereas it had non-significant on FD and SL. The genotype had non-significant effect on all the traits under study (Table 3). Males were superior in GFW (1502.58 ± 101.40 gm); whereas, female were found to be superior in FD (22.18 ± 0.20 µ) and SL (5.13 ± 0.19 cm). Non-significant differences between the least squares means of various genotypes were observed for GFW, FD and SL. High GFW production was recorded for BB genotype

(1487.50 ± 210.66 gm) followed by AB genotype (1255.36 ± 79.62 gm) and least by AA genotypes (1226.39 ± 99.31 gm) in Rambouillet sheep. Itenge-Mweza *et al*. (2007) [6] reported non-significant effect of A and B alleles of KRT1.2 gene on GFW and significant effect of alleles of KRT1.2 gene for FD in New Zealand. Singh (2017) reported non-significant effect of KRT1.2 in SL whereas, significant effect in FD in Magra sheep. On Contrary to the present findings, Farag *et al*. (2018) [3] reported significant effect on genotypes on SL, FD and clean FW in Egyptian sheep.

Table 3: Least-squares means along with standard errors for greasy fleece weight (GFW), fibre diameter (FD) and staple length (SL) in Rambouillet sheep

	GFW (gm)	FD (µ)	SL (cm)
Overall (50)	1323.08 ± 82.04	22.17 ± 0.16	5.02 ± 0.15
Sex	**	NS	NS
M (25)	1502.58 ±101.40	22.17 ± 0.20	4.91 ± 0.19
F (25)	1143.58 ± 101.40	22.18 ± 0.20	5.13 ± 0.19
Genotype	NS	NS	NS
AA (18)	1226.39 ± 99.31	22.07 ± 0.19	5.20 ± 0.19
AB (28)	1255.36 ± 79.62	22.07 ± 0.15	5.36 ± 0.15
BB (04)	1487.50 ± 210.66	22.37 ± 0.41	4.50 ± 0.39

Figures in parentheses indicates number of observations
**P<0.01NS- Non-significant

4. Conclusions

The present study on KRT1.2 gene was carried out to study the association of KRT1.2 polymorphic variants with wool traits in Rambouillet sheep. Genotyping of KRT1.2 gene by PCR-RFLP showed that KRT1.2 gene is polymorphic in the studied Rambouillet sheep population. The non-significant chi-square value for genotypes indicates that the population was in Hardy-Weinberg equilibrium for KRT1.2 gene. The higher Shannon's information index (I) and negative F_{IS} value for KRT1.2 gene in the population indicates there is an excess of heterozygotes which indicates proper breeding strategies of the farm. The non-significant effect of genotypes for all the traits was not sufficient to establish the KRT1.2 gene as a suitable marker for wool traits in this Rambouillet population which may be due to the small number of observations for the present study. Further study should be conducted on large population to explore KRT1.2 gene as a marker for wool traits.

5. Acknowledgements

Authors are thankful to Director, Sheep Husbandry, Jammu, and the In-charge and staff of Government Sheep Breeding and Research Farm, Reasi, Jammu for providing facilities and help for the present study.

6. References

1. Arora R, Bhatia S, Sehrawat A, Pandey AK, Sharma R, Mishra BP. Genetic polymorphism of type 1 intermediate filament wool keratin gene in native Indian sheep breeds. Biochemical Genetics. 2008;46:549-556.
2. Botstein D, White RL, Skolnick M and Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. The American Journal of Human Genetics. 1980;32:314-331.
3. Farag IM, Darwish HR, Darwish AM, Eshak, MG and Ahmed RW. Genetic Polymorphism of KRT1.2 Gene and its Association with Improving of Some Wool Characteristics in Egyptian Sheep. Asian Journal of Scientific Research. 2018;11(2):295-300. DOI: 10.3923/ajsr.2018.295.300.
4. Feughelman M. Mechanical properties and structure of alpha- keratin fibres: wool, human hair and related fibres. UNSW Press Sydney, Australia, 1996.
5. Harvey WR. Mixed Model Least Squares and Maximum Likelihood Computer Program (LSMLMW), PC-1 version, 1990.
6. Itenge-Mwezaa TA, Forresta RHJ, McKenzie GW, Hogana A, Abbott J, Amoafoa O. Polymorphism of KAP1.1, KAP1.3 and K33 genes in Merino sheep. Molecular and Cellular Probes. 2007;21:338-342.
7. Kumar R, Meena AS, Kumari R, Jyotsana B, Prince LLL, Kumar S. Polymorphism of KRT1.2 and KAP1.3 genes in Indian sheep breeds. Indian Journal of Small Ruminants. 2016;22(1):28-31. DOI: 10.5958/0973-9718.2016.00018.0.
8. Levene H. On a matching problem arising in genetics. The Annals of Mathematical Statistics. 1949;20:91-94.
9. Lewontin RC. Testing the theory of natural selection. Nature. 1972;236:181-182.
10. Marshall RC, Orwin DFC, Gillespie JM. Structure and biochemistry of mammailian hard keratin. Electron Microscopy Reviews, 1991; 4: 47-83.
11. Meena AS, Kumar R, Jyotsana B, Narula HK and Kumar S. Genetic Polymorphism of KRT 1.2, KAP 1.3 and THH gene in Magra sheep. Indian Journal of Small Ruminants. 2018;24(1):27-30.
DOI: 10.5958/0973-9718.2018.00015.6.
12. Powell BC, Rogers GE. Hair keratin: composition, structure and biogenesis. In: Bereiter-Hahn J, Matoltsy AG and Richards KS. (eds) Biology of the Integument., Springer-Verlag, Berlin. 1986; 2:695-721.
13. Powell BC. The keratin proteins and genes of wool and hair. Wool Technology Sheep Breeding. 1996;44:100-118.
14. Rogers GR, Hickford JGH and Bickerstaffe R. *Msp1* RFLP in the gene for a Type I intermediate filament wool keratin. Animal Genetics. 1993;24:218.
15. Schweizer J, Bowden PE, Coulombe PA, Langbein L, Lane EB, Magin TM. New consensus nomenclature for mammalian keratins. Journal of Cell Biology. 2006;174:169-174.
16. Singh H. Genetic analysis of wool traits and its association with keratin genes in Magra sheep. Ph D thesis submitted to Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, 2017.
17. Wright S. Evolution and the genetics of populations, Variability within and among natural populations. University of Chicago Press, Chicago, IL, 1978, 4.
18. 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. URL:<http://www.ualberta.ca/~fyeh/ fyeh >.