Genetic Polymorphism in a Selective Intron of Ovine Myostatin Gene and Its Putative Relation with Carcass Traits in Sheep

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ABSTRACT
The main aim of the present study is to identify a suitable polymorphic locus in the myostatin gene of sheep that could be associated with production in local sheep genetic groups of Andhra Pradesh province in India. Representative samples from three local genetic groups were used in the present study namely Nellore Jodipi, Nellore Brown and Macherla Brown. Two PCR-RFLP based SNP markers located in GDF-8 (myostatin-MYST) locus were used in the present study. A PCR-RFLP assay was developed for a SNP located in the intron1 (rs119102825) of the myostatin gene. The second marker is located in exon3 which was obtained from previous studies. The SNP located in Intron1 is polymorphic and the SNP located in exon3 is monomorphic. The polymorphism information content (PIC) of the SNP in intron1 is 0.37. Association studies with limited data showed lack of association of genotypes with body weight at different ages. However, based on bioinformatic prediction, it is likely that the SNP in the intron1 locus may be involved in meat quality determination as it is close to the donor site of intron and the variation has potentiality for enhancement or repression of the gene expression. Further association studies with meat quality traits would help in understanding functional implications of the polymorphism.

Key words: Myostatin, PCR-RFLP, Polymorphism, Sheep, SNP.

INTRODUCTION
Sheep and goat production systems are predominant ones to meet the red meat requirements of Indian consumers (Anonymous 2017). Maximizing carcass-cutting yield along with desirable meat traits from sheep is one of the primary aims in sheep breeding programs. A quest to explore genes related to “increase in muscle mass” (IMM) phenotype in sheep lead to identify certain hypomorphs at the myostatin (MYST) locus (Clop et al. 2006; Boman et al. 2010). Myostatin regulates skeletal muscle hyperplasia and hypertrophy. Hence, screening for polymorphic variants in MYST locus could provide useful insights into the increase in muscle mass and meat quality. PCR–RFLP is a simple, inexpensive and technique of choice to analyze polymorphism in fewer single nucleotide polymorphism (SNP) markers (Ota et al. 2007). A perusal of literature on PCR-RFLP studies of MYST locus showed that majority of studies verified polymorphism in the exon3 using Haelll restriction enzyme. But, in most of these studies the locus is either monomorphic or less polymorphic (Elkorshy et al. 2013; Dimitrova et al. 2016).

“Nellore” is an Indian meat type sheep breed that is native to the Andhra Pradesh. The breed occurs in three variants namely Jodipi, Brown and Palla. The predominant ones are Jodipi and Brown variants. Apart from this breed, there is a local genetic group referred as Macherla Brown or Macherla sheep named after their home tract that lies in the regions of Macherla in Andhra Pradesh. This is a meat type animal and needs to be characterized. The mature body weight of this sheep made it popular among the sheep rearing farmers of the region. The present investigation was performed to identify polymorphism in the exon3 and intron1 of MYST gene using PCR-RFLP in the three genetic groups.
Livestock Research Station (LRS), Siddarampumuram, Nellore Jodipi (n=22) sheep located at the LRS, Palamaner and the Macherla Brown sheep (n=25) that are available from different farmer flocks in their home tract in Guntur district of Andhra Pradesh, India. The Livestock Research Stations are constituent institutions of S V Veterinary University and the study area spreads across Andhra Pradesh province in India. Phenotypic data on body weights at birth, 3 months, 6 months and 9 months age was available for the Nellore Brown and Nellore Jodipi sheep.

DNA isolation and PCR

Standard protocols followed for isolating the genomic DNA (Sambrook and Russell, 2001). Primers for amplification of the SNP region in the exon3 were obtained from published literature (Elkorshy et al, 2013) and the primers for intron1 RFLP variant were designed using primer3 program (Untergasser et al, 2012). The primers used to amplify the exon3 and intron1 region are given in Table 1. Primer specificity search of primers used in literature targeting SNP in the exon3 using primer-blast indicated that two mutations in the primer binding region of the primers and hence they are modified suitably to amplify the sheep MYST region unambiguously. This discrepancy in primers may be attributed to the fact the primers designed for the first time utilization of MYST exon3 from mouse (McPherron et al, 1997).

PCR-RFLP

The exon3 primers generated a PCR product with 337bp length. This fragment upon digestion with Haell restriction enzyme produces 125bp, 118bp and 94bp fragments, which is generally designated as mm genotype. The alternative genotype 337bp is denoted as MM. While designing primers for the SNP in the intron1, an internal control site for HpyCHV restriction enzyme was included in the amplifying region, that would serve as a positive control for the restriction digestion. Presence of T at the SNP locus would result in two fragments of 269bp, 115bp and 74bp, which is designated as hh in this study. Presence of G at the position results in two fragments of 384bp and 74bp and is designated as HH.

| Primer          | Sequence 5’ >3’          | Tm  | GC %  | Length | Amiploc length | Accession No. |
|-----------------|--------------------------|-----|-------|--------|----------------|---------------|
| MYST_Exon3_F    | TAGGAGAGATTTTGGGCTTGA    | 50  | 42.9  | 21     | 337            | NC_019459     |
| MYST_Exon3_R    | TCAAGGCAACCCACAGCAGTC   | 56  | 57.1  | 21     | 458            | NC_019459     |
| MYST_Intron1_F  | GCCCTTTTATGCCCTCTAAGGAAAACAT | 52  | 37.5  | 24     | 458            | NC_019459     |
| MYST_Intron1_R  | TGCTTGGAGACAAAAACAT     | 49  | 38.1  | 21     |                |               |

Table 2: Genotypic frequencies, Heterozygosity and PIC of the SNP rs119102825 in the intron 1 of MYST gene.

| Genetic group   | N  | Observed genotypic frequency | Expected genotypic frequency | Heterozygosity | PIC  |
|-----------------|----|------------------------------|-----------------------------|----------------|------|
|                 |    | HH  | Hh  | hh  | HH  | Hh  | hh  |                 |      |
| Nellore Jodipi  | 22 | 5   | 16  | 1   | 7.56| 10.88| 3.56| 0.48            | 0.37 |
| Nellore Brown   | 18 | 3   | 14  | 1   | 5.43| 9.14 | 3.43| 0.49            | 0.37 |
| Macherla Brown  | 25 | 6   | 19  | 0   | 9.49| 12.02| 3.49| 0.47            | 0.36 |

RESULTS AND DISCUSSION

PCR-RFLP and genetic polymorphism

Sixty-five samples were used to amplify both exon3 and intron1 targeted SNPs. All individuals in the present study are monomorphic at the SNP located in the exon3 locus with mm genotype, while the SNP located in intron1 locus is polymorphic. The exon3 SNP monomorphism is in accordance with the previous studies (Georgieva et al, 2015; Dimitrova et al, 2016). The PCR-RFLP designed for the SNP located in the intron1 is polymorphic in the three genetic groups studied. The genetic diversity estimates of the SNP in the intron1 locus are shown in Table 2. The PIC of the marker is 0.37 indicating that the marker is informative and useful in population studies. The frequency of hh genotype in the present study is 0.05, frequency of heterozygotes is 0.8 in the case of Nellore Jodipi and Nellore Brown sheep, whereas hh genotype is not detected in the Macherla Brown sheep. The three populations tested were departing from Hardy-Weinberg equilibrium assumptions (p<0.05).

Association of the RFLP variants with body weight

The body weights at birth, 3-months, 6-months, 9-months and 12-months for the Nellore Jodipi and Nellore brown sheep for the genotyped animals was tested for plausible association with the PCR-RFLP variants of the SNP g.118144833G>T genotypes. The results indicated that there is no association of the SNP variants with the body weights at different ages (p>0.05) (Table 3). However, the results should be cautiously interpreted as small samples sizes...
are likely to give false negative results (Sullivan et al. 2016).

**Functional role of SNP variants of g.118144833G>T in intron1**

The SNP g.118144833G>T in the intron1 is located near the donor site of the intron and the site is involved in SP1 transcription factor binding site and act as splice enhancer when the allele is G at the position (Sjakste et al. 2011). Whereas, when the allele at the locus is T, the allele becomes potential transcriptional repressor through demethylation. In addition, the mutation was predicted to involve in pre-mRNA secondary structure perturbations, thus presence of T nucleotide at the locus could be potential for enhancing meat production. The mutation is previously shown to be associated with increased loin yield and decreased leg, loin and total yield of total lean meat and no association with body weights at birth and weaning weights in New Zealand Romney sheep (Hickford et al. 2010) and with somebody measurements in the case of Dzhalginsky Merino sheep of Russia (Trukhachev et al. 2015). The genotypic frequency pattern in the present study is indicating probably balancing selection is acting on the locus to maintain the allelic diversity in the population and heterozygote may be advantageous over the homozygotes for the T allele (hh genotype) or G allele (HH genotype). It should be noted that myostatin polymorphism associated with increased meat production in cattle has been implicated in increased dystocia in cattle (Arthur et al., 1988). Though there are no reports indicating similar problems in sheep, the allele frequency pattern and high heterozygosity indicates that natural selection could be shaping the locus to balance between increased muscle mass and reproductive problems. Further studies in detail with carcass quality traits and body weights would provide better understanding of locus and its associated role in meat quality traits and body weight.

**CONCLUSION**

In the present study, a PCR-RFLP method to identify the variants of SNP g.118144833G>T located in the intron1 of the ovine MYST gene. The locus is polymorphic in the three genetic groups i.e. Nellore Jodipi, Nellore brown and Macherla brown sheep. Though the available information on body weights of the Nellore Jodipi and Nellore Brown sheep failed to detect significant association with the SNP variants, bioinformatic predictions and perusal of literature suggest that the SNP is likely to play a role in meat quality in sheep. Further studies including meat quality traits in the three genetic groups and PCR-RFLP would be useful in understanding putative role of the SNP variants in meat quality.

**Data availability**

The data used for analysis in the present study is available upon request from the corresponding author.

**Author contributions**

All authors contributed substantially to the work and manuscript preparation. RV, KS, SJR and MM conceived the study. DVP collected the data and performed DNA isolation and SNP genotyping. RV, KS, SJR and MM analyzed the data and prepared the manuscript.

**Competing interests**

The authors declare that they have no conflict of interest.

**ACKNOWLEDGEMENT**

The authors wish to thank the heads of the Livestock Research Stations for facilitating samples collection and the Director of Research, SVVU for permitting to use the samples. The authors wish to thank Dr. I. Hyder for proofreading and helpful comments on the manuscript.

**Financial support**

DVP received financial assistance from the SVVU as fellowship to pursue master’s program in the Department of Animal Genetics and Breeding, NTR College of Veterinary Science, SVVU, Gannavaram India. The financial support for the research is met from the contingencies allocated to the Department of Animal Genetics and Breeding, NTR College of Veterinary Science, SVVU, Gannavaram India.

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