

Genetic polymorphism in 5'UTR of myostatin (MSTN) gene in Nilagiri sheep

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ABSTRACT

The study was undertaken to detect the genetic variation in the part of 5'UTR, exon 1 and part of intron 1 (797 bp) of *MSTN* gene in Nilagiri breed of sheep by Tetra-primer ARMS-PCR. Randomly, eight samples of the PCR product were sent for sequencing. The analysis revealed the presence of SNP at 711 C>A in 5'UTR of *MSTN* gene. The identified SNP was genotyped by Tetra-primer ARMS-PCR. The C711A locus of *MSTN* gene is characterized by the higher frequency of allele "C" in this breed. The effect of mutation in this gene with growth traits were analysed by association study using least-squares and had no significant effect on growth traits.

Key words: Sheep, growth traits, myostatin gene, polymorphism, tetra-primer ARMS-PCR

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INTRODUCTION

According to 19th Livestock Census, India possesses 65.06 million sheep (Anon., 2012) which constitute 12.7 per cent of the total livestock population of India and ranks third in the world sheep population. The contribution of sheep to total meat production in the country is around 7.12 per cent. India is endowed with large and biologically diverse sheep genetic resources as reflected by the availability of 40 breeds of sheep. Among these Nilagiri breed is best in terms of mutton and wool quality which is directly indicating the body growth.

Myostatin (*MSTN*) gene also called as growth differentiation factor 8 (*GDF 8*) gene is located on chromosome 2 of sheep. Myostatin, the protein whose expression is under the control of *MSTN* gene

acts as a negative regulator of muscle cell growth, where the loss of functional myostatin is known to cause the "double-muscling" phenotype in different species (Grobet et al. 1998; Kambadur et al. 1997; and McPherron and Lee, 1997 in cattle; Broad et al. 2000 in sheep and Li et al. 2006 in goat). Studies on the polymorphism of *MSTN* gene and its association with growth traits are not available among Indian breeds of sheep. Hence the objective of this study was to detect the polymorphism of myostatin (*MSTN*) gene, and to analyse the association between genetic variants and growth traits.

MATERIALS AND METHODS

The blood samples were collected in vacutainers containing EDTA as an anticoagulant from Nilagiri breed (n = 103) at Sheep Breeding Research Station,

Table 1. Primer sequences used to amplify *MSTN* gene

Sl. No.	Primer sequence (5'-3' end)	Annealing temp. (°C)	Product size
Part of 5' UTR, exon 1 and intron 1 of <i>MSTN</i>	Forward: gtc aaa tga atc agc tca ccc t Reverse: tcc tta cgt aca agc cag cag	60.0	797 bp
MSTN-711	Forward inner aaa agc aaa aga aaa gta aaa gga gga Reverse inner caa tac aat ctt ttt cct tgc tct tat tg Forward outer gga ata taa aaa gcc act tgg aat aca g Reverse outer agt ctt gag gat tta ttg ttt tgt ctc c	51.5	A allele = 225 C allele = 156 Outer = 325

Sandynallah. The genomic DNA was extracted by using standard Phenol-Chloroform extraction procedure (Sambrook et al. 1989) by using DNazol reagent, instead of SDS and proteinase K. The region 797 bp (part of 5' UTR, exon 1 and intron 1) of the *MSTN* gene was amplified by using the primer sequences designed by Fast PCR Primer designing software (Table 1).

The total 8 samples were sequenced for 797 bp fragment of *MSTN* gene. The amplicons were sequenced in both forward and reverse directions at M/s. Eurofins Genomics India Pvt. Ltd., Bangalore. The instrument used for sequencing was ABI PRISM 3730XL Genetic analyzer (Applied Biosystems, USA). Sequence data were analysed using the SeqMan program of LASERGENE software (DNASTAR Inc., USA). The sequences were assembled and screened for SNPs. Two sets of primers were designed for genotyping the individual animals with respect to the SNP from an online "Tetra-primer ARMS-PCR" designing software (http://cedar.genetics.soton.ac.uk/public_html/primer1.html; Ye et al. 2001) (Table 1). The Tetra-primer ARMS PCR conditions are given in Table 2

The genotypes were assigned on the basis of band pattern of the PCR products. The allele and genotype frequencies were calculated by standard formula (Falconer and Mackay, 1996). The χ^2 test of goodness of fit was carried out from observed and expected numbers to check whether the population was in Hardy-Weinberg equilibrium (Falconer and Mackay, 1996) at the C711A locus of *MSTN* gene. The polymorphisms observed at the C711A locus of *MSTN* gene in Nilagiri sheep breed was analysed for the association with body weights at various ages

viz., birth, weaning (3-months), 6, 9 and 12 months using least-squares procedures (Harvey, 1990).

RESULTS AND DISCUSSION

On analysing the nucleotide sequences, it was found that there was no change in the coding region. However, in 5'UTR, a 711C>A transversion was found in this breed. Sequence analysis of C711A locus revealed the presence of all three genotypes viz., CC, CA and AA which is given in the chromatogram (Figure 1). The different sizes of amplified products

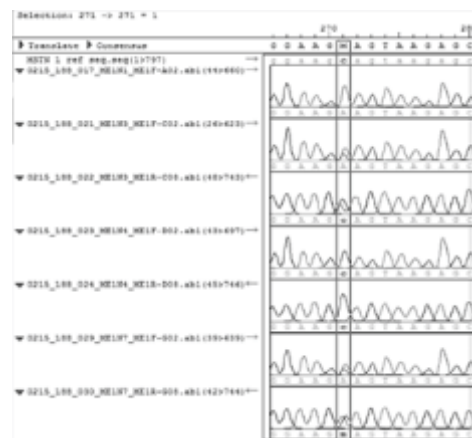


Figure 1. Chromatogram of 711C>A in 5'UTR of *MSTN* gene in Nilagiri sheep.

obtained were 156 bp, 225 bp and 325 bp (Figure 2). The CC homozygote showed two fragments viz., 156 bp and 325 bp; AA homozygote showed two fragments viz., 225 bp and 325 bp; and CA heterozygote showed all the three fragments viz., 156 bp, 225 bp and 325 bp. The genotypic frequencies of CC, CA and AA were 0.437, 0.320 and 0.243 respectively, and the allele frequencies of C and A were 0.5970 and 0.4030. The locus was significantly ($P < 0.01$) deviated from Hardy-

Table 2. PCR protocol for genotyping through Tetra-primer ARMS-PCR

Step	Process	Temperature (°C)	Duration
1	Initial denaturation	95	5 min
2	Denaturation	95	45 sec
3	Annealing	51.5	60 sec
4	Extension	72	45 sec
5	Back to steps 2 to 4	35 cycles	
6	Final extension	72	5 min
7	Hold	4	Until the samples are removed

Table 3. Least-squares means $\pm$ S.E. (kg) 711C>A in MSTN gene on growth traits

Body weight at various ages ^{NS}	Nilagiri breed			
	AA (16)	CA (21)	CC (36)	Prob
Birth weight	3.071 $\pm$ 0.126	2.900 $\pm$ 0.112	2.919 $\pm$ 0.094	0.4296
3 months weight	11.574 $\pm$ 0.622	11.201 $\pm$ 0.554	11.201 $\pm$ 0.464	0.8324
6 months weight	15.397 $\pm$ 0.668	15.746 $\pm$ 0.595	15.372 $\pm$ 0.498	0.8538
9 months weight	17.805 $\pm$ 0.709	18.960 $\pm$ 0.632	18.615 $\pm$ 0.529	0.3771
12 months weight	20.731 $\pm$ 0.816	21.759 $\pm$ 0.727	21.677 $\pm$ 0.609	0.4844

Figures in parentheses indicate number of records used for analysis, NS = not significant.

Weinberg equilibrium. This may be due to less sample size and selection forces. The 711C>A transversion in 5'UTR observed in Nilagiri breed studied is the first report in Indian sheep breeds. Similar finding (-41C→A in 5'UTR) was earlier reported by Gan et al. (2008) in different Chinese pure and synthetic breeds.

Absence of SNP in intron 1 of MSTN gene observed in this study is similar to the report of Dehnavi et al. (2012) in Zel sheep by PCR-SSCP. However, Ansary et al. (2008) identified three unique SSCP patterns for intron 1 region of GDF8 gene in Baluchi sheep. Zhou et al. (2008) also reported the transversion of G→T in intron 1 of MSTN gene and the same could not be detected in this sheep breed. The exon 1 of MSTN gene was completely identical to the reference sequence that can be used as a candidate gene for evolutionary studies.

The effects of different genotypes observed at C711A

locus in 5'UTR of MSTN gene on growth traits viz., birth, weaning (three months) six, nine and 12 months weight in Nilagiri breed was found to be non-significant (Table 3). The 711C>A in 5'UTR of MSTN gene is a novel mutation and the first report in sheep. Previous studies on association analysis with growth traits for this mutation were not reported earlier in sheep. Hence it may be concluded that the effect of this mutation on growth traits in other breeds of sheep need to be investigated.

CONCLUSION

The 5'UTR of MSTN gene is polymorphic in Nilagiri sheep breed of Tamil Nadu, India. The SNP 711 C>A is a novel and first report among Indian sheep breeds. But this mutation had no significant association with growth traits. Absence of significant association of this mutation with body weight may be due to selection, polygenic influence and deficit of grazing land.

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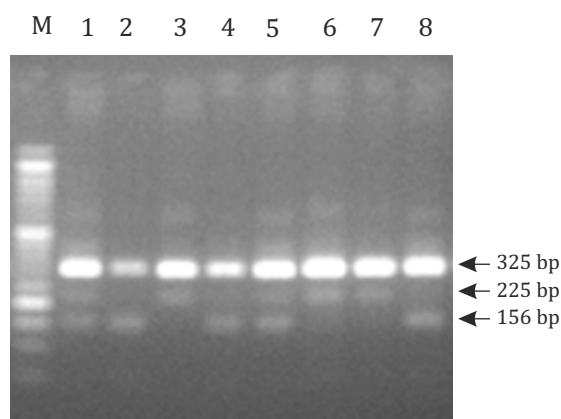


Figure 2. Tetra-primer ARMS-PCR genotyping of C711A, Lane M: 50 bp DNA ladder; Lanes 3, 6 and 7: AA (225 bp + 325 bp); 2, 4 and 8: CC (156 bp + 325 bp); and 1 and 5: CA (156 bp, 225 bp and 325 bp) of Nilagiri sheep.

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