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Research Paper

Polymorphism in exon 3 of myostatin (*MSTN*) gene and its association with growth traits in Indian sheep breeds

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ABSTRACT

The polymorphism of myostatin (*MSTN*) gene was analysed as a genetic marker for growth traits in Madras Red and Mecheri sheep breeds of Tamil Nadu. PCR-RFLP revealed a SNP G5622C in exon 3 and was confirmed by sequencing. The breed-wise genotypic and allelic frequencies observed for *MM* genotype were 0.417 and 0.486; and *Mm* were 0.583 and 0.514 in Madras Red and Mecheri breeds respectively. The estimated allelic frequencies for *M* and *m* in Madras Red and Mecheri breeds were 0.7085 and 0.2915; and 0.7430 and 0.2570 respectively. Madras Red sheep with homozygous (*MM*) genotypes were significantly ($P < 0.05$) heavier than heterozygous (*Mm*) genotype by 1.162 kg and 1.227 kg at nine and 12 months weight respectively. However, no significant effect was observed for birth, weaning (three months) and six months weight in the same breed. There was no significant difference in mean birth, weaning, six, nine and 12 months weight between *MM* and *Mm* genotypes in Mecheri sheep.

1. Introduction

India is endowed with biologically diverse sheep genetic resources (40 breeds) possessing 65.06 million sheep (Livestock Census Report, 2012) which constitute 12.7% of the total livestock population of India and ranks third in the world sheep population. Sheep meat is a major source of animal protein for which sale of surplus animals with male body weight at the market age forms a major component of income in sheep husbandry. Therefore, faster growth and early maturity are important components of profitable sheep production. Growth indicated as the live body weight measured at various ages is one of the most important traits in the genetic improvement for meat sheep. Single nucleotide polymorphism of gene sequence which affects body growth starting from pre-weaning daily gain till 11 months of age acts as a basis for breeding of the economically important growth traits through marker assisted selection (Hua et al., 2009).

Myostatin, the protein whose expression is under the control of myostatin (*MSTN*) gene acts as a negative regulator of muscle cell growth, where the loss of functional myostatin is known to cause the "double-muscled" phenotype in cattle (Kambadur et al., 1997; McPherron et al., 1997 McPherron and Lee, 1997; Grobet et al., 1998), in sheep (Broad et al., 2000) and in goats (Li et al., 2006). *MSTN* also called as growth differentiation factor 8 (*GDF 8*) gene is located on chromosome 2 of sheep (Archibald et al., 2010). Mutation in *MSTN* acts in various ways like myostatin null mice causes hypertrophy and hyperplasia of skeletal muscle mass (McPherron et al., 1997) whereas deletion, missense or nonsense mutations leads double muscling in Belgian Blue and Piedmontese breeds of cattle (McPherron et al., 1997 McPherron and Lee, 1997) and hypertrophy in child due to splice site mutation in human being (Schuelke et al., 2004). However, there is no report regarding the variation in the exons of *MSTN* in Indian sheep breeds till date. Hence, the identification of the variation in coding region of ovine *MSTN* gene using PCR-RFLP followed by sequencing and the association analyses with growth traits are described in this study.

2. Material and methods

2.1. Experimental sheep flocks and PCR amplification

Madras Red and Mecheri are two large sized hairy meat breeds of Tamil Nadu, India considered for the study. The genomic DNA was isolated from Madras Red (127) and Mecheri (105) sheep using standard Phenol-Chloroform extraction procedure

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(Sambrook et al., 1989) with slight modifications by using DNAzol reagent, instead of SDS and proteinase K.

The exon 3 (380 bp) of *MSTN*, with part of intron 2 and 3′ UTR on either side was amplified as a 615 bp fragment, using the primer sequence designed by Fast PCR Primer designing (FastPCR software for PCR, *in silico* PCR, and oligonucleotide assembly and analysis) software (Kalendar et al., 2014). The primer was synthesised at M/s. Eurofins Genomics, Bangalore. The PCR was performed with volume of 20 μl using 10 μl master mix (Ampliqon), 0.6 μl each of forward primer (CGG TAG GAG AGT GTT TGG GGA T) and reverse primer (GAT GGT TAA ATG CCA ACC ATT GC), 1.3 μl template DNA (50 ng/μl) and 7.5 μl of NFW. The amplification was carried out with a program of 5 min initial denaturation at 95 °C followed by 32 cycles of 95 °C for 35 s, annealing at 61.5 °C for 30 s and extension at 72 °C for 35 s, with a final extension of 5 min at 72 °C. The product was checked by electrophoresis (2 per cent agarose) and the gel was stained with ethidium bromide, visualized under UV light.

2.2. PCR- RFLP and analysis of sequence variation

The PCR product (615 bp) was digested with *Msp*I restriction enzyme at 37 °C for 6 h in water bath with the restriction digestion mix of 10.0 μl of PCR product, 1.5 μl of 1X cut smart buffer, 0.1 μl of *Msp*I enzyme (3 units/μl) and 3.4 μl of NFW. The digested products were checked in 2% agarose gel using 50 bp DNA ladder.

Amplicons of eight samples from each breed (Madras Red and Mecheri) were sequenced for 615 bp fragment of *MSTN* gene. The amplicon was 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 version 7.1.0 (44) (DNASTAR Inc., USA).

2.3. Statistical analysis

The genotypes were assigned on the basis of band patterns of the PCR products. The genotype and gene frequencies were estimated and the locus was tested for Hardy-Weinberg equilibrium. The polymorphism observed at the G5622C locus of *MSTN* gene in Madras Red and Mecheri sheep breeds was analysed separately for their association with body weights at various ages *viz*., birth, weaning (three month), six, nine and 12 months using least-squares procedures (Harvey, 1990). The data on number of animals used for association analysis was lower than the number of animals genotyped due to the non-availability of complete set of data on body weights and deletion of outliers.

Data were classified into two periods of birth *viz*., period 1 (2007–2010) and period 2 (2011–2014) in Madras Red; and period 1 (2008–2010) and period 2 (2011–2013) in Mecheri breed of sheep; sex as males and females; and different genotypes at G5622C locus of *MSTN* gene. Weight of dam at lambing was also included as a covariable in the model as below:

$$Y_{ijkl} = \mu + P_i + S_j + G_k + b(WM_{ijk} - WM) + e_{ijkl}$$

Where,

Y_{ijkl} = is the body weight of the l^{th} animal of the k^{th} genotype of the jth sex born in the ith period of lambing

μ = overallmean

P_i = fixed effect of i^{th} period of lambing (i = 1and2)

S_j = fixed effect of j^{th} sex of the lamb (j = 1and2)

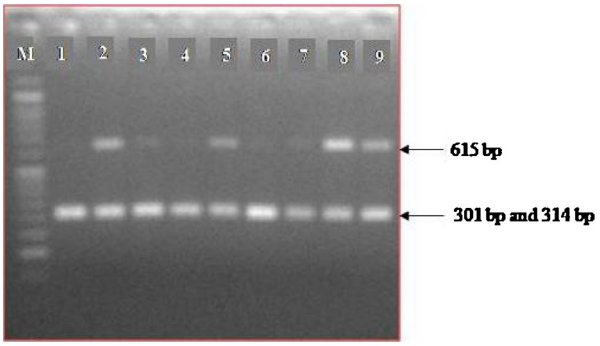

Fig. 1. *Msp*I-RFLP of PCR amplicon (615 bp) in exon 3 of *MSTN* gene. Lane M: 50 bp DNA ladder, Lanes 1–5: Madras Red, and Lanes 6–9: Mecheri sheep. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

G_k = fixed effect of k^{th} genotype(k = 1and2)

b ($WM_{ijk} - \bar{WM}$) = regression of Y on dam′s weight at lambing

e_{ijkl} = residual random error, NID ($0, \sigma^2$)

3. Results and discussion

The restriction enzyme, *Msp*I had cleavage site (C/CGG) at nucleotide position 314 of the amplified region (615 bp) and showed polymorphism in both sheep breeds studied. Restriction digestion revealed the existence of two genotypes *viz., MM* (301 bp and 314 bp) and *Mm* (301 bp, 314 bp and 615 bp) and the locus was characterized by the presence of two alleles *M* and *m* (Fig. 1). The *mm* genotype was absent in both breeds. The breed-wise genotypic and allelic frequencies observed (Table 1) for *MM* genotype were 0.417 and 0.486; and *Mm* were 0.583 and 0.514 in Madras Red and Mecheri breeds respectively. The estimated allelic frequencies for *M* and *m* in Madras Red and Mecheri breeds were 0.7085 and 0.2915; and 0.7430 and 0.2570 respectively. The frequency of *MM* genotype was higher in Mecheri than Madras Red and vice versa for *Mm* genotype.

The sequences were assembled and screened for single nucleotide polymorphisms (SNPs). On analysing the amplicon (615 bp), one SNP was found in the exon 3 of *MSTN* at locus G5622C, characterized by transversion of G > C, but no change in amino acid was observed in both the sheep breeds. Two genotypes *viz*., GG and GC were observed as shown in chromatogram (Fig. 2). The 5622G > C transversion observed in the present study is contrary to the reports in the New Zealand Romney sheep (Zhou et al., 2008) and in Chinese breeds of sheep (Gan et al., 2008), where exon 3 was monomorphic. The estimated allelic frequency for *M* was higher than *m* in both the breeds of sheep studied. The locus 5622G > C has significantly ($P < 0.01$) deviated from genetic equilibrium in both sheep breeds.

The effect of 5622 G > C mutation in exon 3 of *MSTN* gene on nine and twelve months weights was significant ($P < 0.05$) in Madras Red sheep. Animals with homozygous (*MM*) genotype were heavier than heterozygous (*Mm*) genotype by 1.162 kg and 1.227 kg at nine and 12 months weight respectively. However, a non-significant effect of this mutation was observed for birth, weaning (three months) and six months weight in the same breed. There was no