Journal of Animal Research: v.6 n.1, p. 81-84. February 2016

DOI: 10.5958/2277-940X.2016.00013.9

Genetic Polymorphism of Myostatin (*MSTN*) Gene in Sheep Breeds

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Received: 09, October, 2015 **Accepted:** 28 January, 2016

ABSTRACT

Myostatin (*MSTN*) is one of the most important growth regulatory genes that resulted in higher growth of skeletal muscles in livestock species. So the study was undertaken to identify the polymorphism of myostatin (*MSTN*) gene as a genetic marker for growth traits in Madras Red, Mecheri and Nilagiri sheep breeds of Tamil Nadu. The blood samples were collected from unrelated animals of Madras Red (n = 127), Mecheri (n = 101) and Nilagiri (103) breeds of sheep from different agroclimatic regions of Tamil Nadu. Part of 5'UTR, exon 1 and part of intron 1 (797 bp) of *MSTN* gene was amplified with suitable primers. The amplified products were digested with the *Msp* I and *Hae* III restriction enzymes. But both the PCR-RFLP results of *Msp* I and *Hae* III was found to be monomorphic in all the three breeds of sheep indicating that the *MSTN* gene is highly conserved and of use in evolutionary studies.

Keywords: *MSTN* gene, PCR-RFLP, monomorphism, sheep

Marker Assisted Selection (MAS) allows for the accurate selection of specific DNA variations that have been associated with differences in growth and meat production traits and there by selection and breeding of superior individuals. The body weights (birth, weaning, 6-month and twelve-month weights) are the indicators of individual performance and they have direct effect on the health and productivity of sheep (Zaffer *et al.* 2015). Myostatin (*MSTN*) gene also called as growth differentiation factor 8 (*GDF 8*) gene is located on chromosome 2 (Archibald *et al.* 2010) of sheep. Myostatin, the protein is a member of the tumour growth factor (TGF-β) superfamily which is synthesized by a 376 amino acid precursor protein including three domains namely, a C-terminal domain, N-terminal propeptide domain and a signal sequence (McPherron *et al.* 1997). The expression of this protein 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-muscled" phenotype in different species (Grobet *et al.* 1998; Kambadur *et al.* 1997 and McPherron *et al.* 1997 in cattle; Broad *et al.* 2000 in sheep and Li *et al.* 2006 in goat). Fraudulent substitution of sheep meat with other species is easily detectable by simple molecular technique like PCR-RFLP (Saikia *et al.* 2015). However, studies on the polymorphism of *MSTN* gene and their association with growth traits are not available among Indian breeds of sheep. Hence the study was under taken to identify the polymorphism of myostatin (*MSTN*) gene and to analyse the association between the genetic variants and growth traits.

MATERIALS AND METHODS

Three breeds of sheep *viz*. Madrs Red, Mecheri and Nilagiri of three different agro-climatic zones of Tamil Nadu, were chosen for the study. The blood samples were collected from unrelated animals in the vacutainers containing EDTA as an anticoagulant for Madras Red (n = 127) from Post-Graduate Research Institute in Animal Sciences, Kattupakkam; Mecheri (n = 101) from Mecheri Sheep Research Station, Pottaneri; and Nilagiri (n = 103)

from Sheep Breeding Research Station, 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.

Table 1: *Msp* I and *Hae* III digestion mixture composition

Sl. No.	***Msp* I restriction digestion mix**		***Hae* III restriction digestion mix**	
	Component	**Volume (µl)**	**Component**	**Volume (µl)**
1	PCR product	10.0	PCR product	10.0
2	10X NE buffer 4	1.5	10X NE buffer 4	2.0
3	*Msp* I enzyme (3 units/µl)	0.1	*Hae* III enzyme (10 units/µl)	0.3
4	Nuclease free water	3.4	Nuclease free water	2.7
	Total	15.0	Total	15.0

The part of 5'UTR, exon 1 and part of intron 1 (797 bp) of *MSTN* gene was amplified by 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).

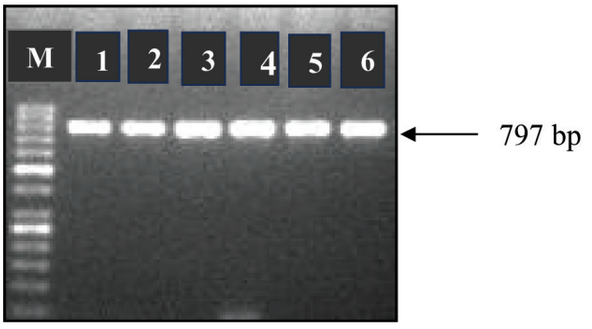

Fig. 1: PCR amplicon of part of 5'UTR, exon 1 and part of intron 1 of *MSTN* gene, Lane M: 50 bp DNA ladder, Lane 1-2: Madras Red, Lane 3-4: Mecheri, Lane 5-6: Nilagiri sheep, *MSTN* gene (797 bp)

The amplification reaction was carried out in 0.2 ml microfuge tubes using thermal cyclers (Eppendorf Mastercycler ep gradient S and Applied Biosystems 2720 models) and the PCR was performed having total reaction mixture 20 µl using 10 µl master mix (Ampliqon), 0.8 µl each of forward and reverse primers (10 pmol/µl), 1.5 µl template DNA (50 ng/µl) and 6.9 µl of nuclease free water (NFW). The amplification reaction was carried out with a program of 5 min denaturation at 95°C followed by 34 cycles of denaturation at 95°C for 35 sec, annealing at 60°C for 30 sec and extension at 72°C for 35 sec, with a final extension for 5 min at 72°C. The primer set having forward (GTCAAATGAATCAGCTCACCCT) and reverse (TCCTTACGTACAAGCCAGCAG) primers amplified for 797 bp product size.

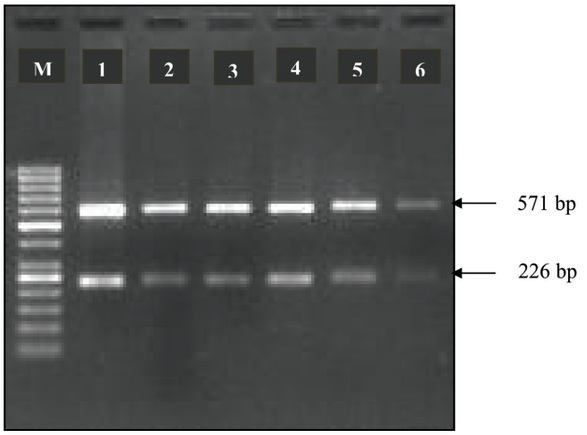

Fig. 2: *Msp* I-RFLP of part of 5'UTR, exon 1 and part of intron 1 of *MSTN* gene, Lane M: 50 bp DNA ladder, Lane 1-2: Madras Red, Lane 3-4: Mecheri, Lane 5-6: Nilagiri sheep, *MSTN* gene (226 bp and 571 bp)

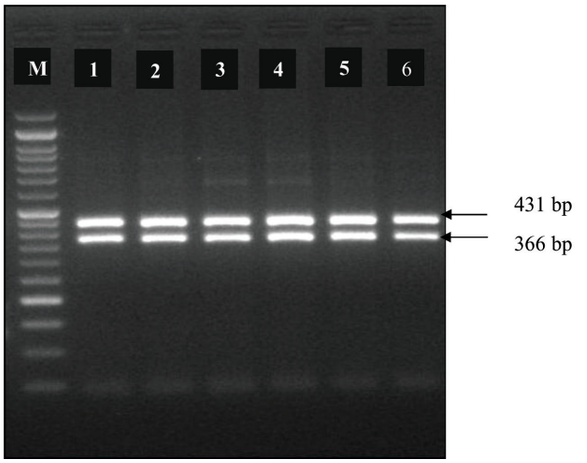

Fig. 3: *Hae* III-RFLP of part of 5'UTR, exon 1 and part of intron 1 of *MSTN* gene. Lane M: 50 bp DNA ladder, Lane 1-2: Madras Red, Lane 3-4: Mecheri, Lane 5-6: Nilagiri sheep *MSTN* gene (366 bp and 431 bp)

Msp I and *Hae* III restriction enzymes were used for digestion at 37°C for 2 hours (Table 1). Then both the enzymes were inactivated by increasing the incubation temperature to 80°C for 20 minutes. The digested products were checked in 2% agarose gel with 50 bp DNA ladder in a gel documentation system.

RESULTS AND DISCUSSION

The PCR amplification of *MSTN* gene with the designed primer resulted in amplified product of size (797 bp) which includes part of 5'UTR, exon 1 and part of intron 1 in Madras Red, Mecheri and Nilagiri breeds of sheep (Figure 1). The restriction endonuclease, *Msp* I had cleavage site (C/CGG) at nucleotide position 571 of the amplified region which yielded two fragments *viz*. 226 bp and 571 bp in all the three sheep breeds studied (Figure 2). So all the parts of the gene studied did not show any polymorphism.

The restriction endonuclease *Hae* III with recognition sequence GG/CC on digestion of amplicon yielded two fragments 366 bp and 431 bp (Figure 3) suggested monomorphism, which indicates the *MSTN* gene is highly conserved among all the three breeds studied and might be required to study the other indigenous sheep breeds for presence of any polymorphism.

Absence of polymorphism in the exon 1 of *MSTN* gene is in agreement with the reports on different European breeds (Clop *et al.* 2006; Kijas *et al.* 2007; Hadjipavlou *et al.* 2008; Johnson *et al.* 2009; Boman *et al.* 2010; Haynes *et al.* 2013; Hope *et al.* 2013); Chinese breeds (Gan *et al.* 2008) and Iranian breeds (Dehnavi *et al.* 2012) of sheep. Contrary to the present findings, Zhou *et al*. (2008) observed the polymorphism in exon 1 of *GDF8* gene and their corresponding change of codon from glutamic acid to glycine substitution in New Zealand Cross bred Romney sheep. Studies on polymorphism of *MSTN* gene are not available in Indian breeds of sheep.

CONCLUSION

Part of 5'UTR, exon 1 and intron 1 of *MSTN* gene was investigated to detect the polymorphism in Tamil Nadu breeds of sheep *viz*. Madrs Red, Mecheri and Nilagiri. The absence of polymorphism suggested that *MSTN* gene is highly conserved and could be of use in evolutionary studies.

ACKNOWLEDGEMENTS

Authors are thankful to the Post-Graduate Research Institute in Animal Sciences, Kattupakkam; Mecheri Sheep Research Station, Pottaneri and Sheep Breeding Research Station, Sandynallah for their necessary support to carry out the research work.

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