Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(4): 1875-1877

Received: 27-05-2018
Accepted: 28-06-2018

Govind Singh Dhakad
M.V.Sc Research Scholar, Molecular Genetics Laboratory, Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Gyanchand Gahlot
Professor & Head, Molecular Genetics Laboratory, Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Vijay Kumar Agrawal
Ph.D. Research Scholar, Molecular Genetics Laboratory, Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

HK Narula
Principal Scientist, Central Sheep and Wool Research Institute, Arid Region Campus, Bikaner, Rajasthan, India

Urmila Pannu
Professor, Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Correspondence
Vijay Kumar Agrawal
Ph.D. Research Scholar, Molecular Genetics Laboratory, Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Genetic variation in growth regulating myostatin gene in Magra sheep of Rajasthan

Govind Singh Dhakad, Gyanchand Gahlot, Vijay Kumar Agrawal, HK Narula and Urmila Pannu

Abstract
The variation in body weight of Magra sheep depends on the gene affecting the muscular growth. Therefore the present study was carried out to investigate the genetic variation in growth regulating *MSTN* gene through PCR based SSCP marker. Genomic DNA was isolated from whole blood of randomly selected Magra sheep (N=74) maintained at Livestock Research Farm, CSWRI, Bikaner campus through spin column method. A 311bp fragment comprising of intron 2 region of *MSTN* gene was amplified through designed homologous primers (GenBank accession number JN856480) at an annealing temperature of 54 °C. SSCP marker analysis was carried on 8% non denaturing polyacrylamide gels at 120 V for 7 h at 4 ºC to detect SNP's responsible for genetic variation. The results detected three conformational patterns in the genotypic frequency of 0.73 (*AA*), 0.17 (*AB*), 0.10 (*BB*) with allele frequency of 0.818 (*A*) and 0.182 (*B*). The study concluded that nucleotide variation exist in intron 2 of myostatin gene.

Keywords: Magra sheep, MSTN intron 2, Body weight

1. Introduction
Variation in biometric traits such as body weight plays a critical role in the selection of individuals for production of future generations and an overall productivity of the livestock production system. Candidate gene such as myostatin (*MSTN*) or Growth and Differentiation Factor 8 (*GDF* 8) has been claimed as a negative determinant of body weight in many livestock species through its effect on skeletal muscle growth [1]. Quantitative trait loci studies showed that myostatin gene had a major gene effect on muscular development in different sheep breeds throughout the world [2]. Differential regulation of myogenesis and adipogenesis due to polymorphism in Ovine *MSTN* gene sequence was found to be associated with variation in muscular mass and muscular hypertrophy in sheep population [3].
Ovine *MSTN* gene is located at the end of the long arm (2q32.2 locus) of chromosome 2 [4]. The gene is identified to be composed of three exons and two introns which encode a glycoprotein that is expressed widely in skeletal muscle [5]. The different exons of the *MSTN* gene were found to be highly conserved among all species [6, 4, 7, 8]. Significant impact of polymorphism in intron 2 of *MSTN* gene on the body weight of sheep was reported [6]. The National Center for Biotechnology Information (NCBI) database also includes information for more than 40 single-nucleotide substitutions in myostatin gene of sheep which were mostly located in introns, 5'UTR and 3'UTR regions apart from only two single nucleotide substitutions in exons [9, 10] suggested that genetic variation in intronic region of myostatin gene could affect the variation in muscle weight due to change in composition of muscle fibres through altered mRNA splicing during gene expression. Highest additive estimated breeding value for weaning weight was observed in Baluchi sheep for genetically variable intronic region [11].
Polymerase chain reaction based single strand conformational polymorphism (PCR-SSCP) analysis is considered as an effective tool for the detection of genetic variation in *MSTN* gene [12]. Therefore, in order to detect effective alleles influencing body weight of Magra lambs, the present study was designed to analyze the genetic variation in intron 2 of *MSTN* gene in Magra sheep through PCR-SSCP.

2. Materials and Methods
All work was carried out in the Molecular Genetics Laboratory of Animal Genetics and Breeding Department (College of Veterinary and Animal Sciences, Bikaner, Rajasthan, India)

in collaboration with Central Sheep and Wool Research Institute, Bikaner (CSWRI, Western Campus). Seventy four (n=74) contemporary and apparently healthy male Magra lambs with no history of any illness in the past six month were randomly selected from flocks with most distant genetic background on the basis of available pedigree information from Magra Sheep Farm maintained at Livestock Research Farm of CSWRI, Bikaner. About 2ml blood sample was collected from jugular vein in sterile vacutainer tubes containing EDTA as an anticoagulant. Uniform feeding practices with full grower ration were adapted for all the studied animals. All animals were kept in optimal ventilated conditions with proper and timely vaccination and deworming.

The genomic DNA was extracted by spin column method as per manufacturer protocol through commercial kit supplied by HiMedia. The quality and the concentration of extracted DNA were checked on 0.8% agarose and Nano Drop Spectrophotometer, respectively. A pair of amplification primer (F-5'CAC ATT TTT CCC CCA GAA GAG3'; R-5'AAG ACA GTT CAG AAA ATA GCT GG3') was designed from homologous ovine *MSTN* gene sequences available at NCBI BLAST (GenBank accession No. JN856480) using primer 3 software to amplify the 311bp fragment comprising of intron 2 region of *MSTN* gene. Amplification reactions for each fragment was done by using the following constituents: in a final volume of 25µl containing 5X PCR buffer (5µL), 1 unit of *Taq* DNA polymerase (0.2µL), 10mM each of dNTPs (0.5µL), 25mM $MgCl_2$ (2.5µL), 100pMol of each primer (0.5µL) and 100ng of template DNA (2µL). Amplification was performed in a thermal cycler with the following program; after an initial denaturation step at 95 °C for 5 min, 35 cycles were programmed as follows: 94 °C for 45s, 54 °C for 60s, 72 °C for 60s and final extension at 72 °C for 10 min. The optimum number of cycles and annealing temperature were identified through gradient PCR approach. Negative controls were used during every batch of amplification to observe any contamination during process. The size and integrity of amplicons were analyzed on 1.5% agarose gel with standard molecular marker in horizontal gel electrophoresis. The gels were stained with ethidium bromide dye and visualized under ultraviolet light in gel documentation system.

SSCP marker analysis was carried out for genetic analysis and nucleotide variation in intron 2 of *MSTN* Gene in Magra sheep. 5µL PCR amplified amplicons were mixed and denatured with 10µL denaturing solution (including 800µL formamide (99%), 100µL loading dye, 100µL glycerol (98%), 3µL 0.5M EDTA, and 2µL 10M NaOH), heated for 10 min at 95 °C in Thermal cycler, and chilled on ice immediately for 20 min. Polymorphism at studied locus between different samples was detected on 8% non denaturing polyacrylamide gels at 120V for 7h at 4 ºC. The different polymorphic patterns were visualized using ethidium bromide staining method.

3. Results

The SSCP marker analysis revealed polymorphism in intron 2 region of *MSTN* gene in Magra sheep in the form of significant shift in band mobility of amplified fragments on 8% polyacrylamide gel. Three different genotypic patterns referred as '*AA*', '*AB*' and '*BB*' with varying number of bands, were detected for the locus under study (Figure 1).

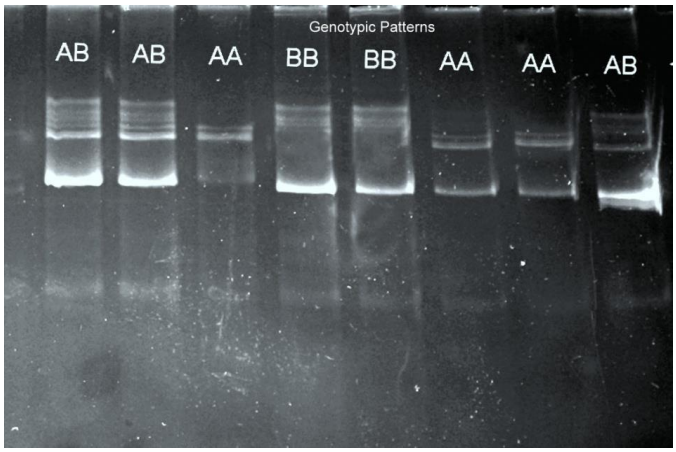

Fig 1: SSCP conformational patterns of intron 2 region of *MSTN* gene on 8% polyacrylamide gel

The genotypic pattern '*AA*' was observed to be dominant in most of the animals under study. The genotypic patterns '*AB*' and '*BB*' were present in low number. The frequency of different genotypic patterns and their allele is presented in Table 1. Likewise, the frequency of '*A*' allele was observed to be predominant in Magra sheep. Significant deviation in gene and genotypic frequency observed for the studied locus in Magra sheep indicates Hardy Weinberg disequilibrium and suggest the role of natural selection.

Table 1: Gene and genotypic frequency of *MSTN* intron 2 gene in Magra sheep

Genotypic Frequency		Gene Frequency		Chi^2 Test
		A	B	
AA	0.73 (n=54)	0.818	0.182	12.50**
AB	0.17 (n=13)			
BB	0.10 (n=7)			

4. Discussion

Growth traits are one of the important traits that are specifically considered in animal breeding programmes. In addition, fast growth rate in small ruminants ultimately determines the meat producing capability up to marketing age and economic success of the production system [13]. Magra sheep is considered as one of the best ovine genetic resources of semi arid region of Rajasthan in terms of body weight of the lambs [14] and could be considered as a potential meat genotype of the country. Knowledge of the genetic diversity in genes affecting the growth traits in Magra sheep is essential for the improvement of breed. Identification of polymorphism in candidate gene related to growth traits such as *MSTN* gene could explain partial source of genetic variation in growth traits [15]. The findings of the present investigation is in strong corroboration with the findings in Zel sheep breed who also observed frequency of 73.50% for *AA* genotypic pattern [6]. Similar results were observed [16, 17]. The present study also observed PCR-SSCP marker analysis as a robust and a reliable tool [18] to screen the sheep population for variability in *MSTN* intron 2 genes. The present study concluded that genetic variability in intron 2 region of *MSTN* gene in Magra lamb may affect the body weight in Magra sheep at different stages of life and could be useful for developing marker assisted selection programmes for improving weight gain in Magra sheep.

5. Acknowledgment
Authors are thankful to Rajasthan University of Veterinary and Animal Sciences, Bikaner (Rajasthan) for providing financial support and necessary infrastructure during M.V.Sc research work.

6. References

1. Zhang C, Liu Y, Xu D, Wen Q. Polymorphisms of myostatin gene (MSTN) in four goat breeds and their effects on Boer goat growth performance. Molecular Biology and Reproduction. 2012; 39:3081-3087.
2. Elkorshy N, Karima F, Mahrous, Lamiaa MS. Genetic polymorphism detection in four genes in Egyptian and Saudi sheep breeds. World Applied Science Journal. 2013; 27(1): 33-43.
DOI: 10.5829/idosi.wasj.2013.27.01.8191.
3. Han J, Forrest RH, Hickford JGH. Genetic variations in the myostatin gene (MSTN) in New Zealand sheep breeds. Molecular Biology and Reproduction. 2013; 40:6379–6384.
4. Dimitrova I, Bozhilova-Sakova M, Stancheva N, Tzonev T. Molecular analysis of ovine myostatin gene (MSTN) in northeast Bulgarian Merino sheep breed using PCR-RFLP. Bulgarian Journal of Agricultural Sciences. 2016; 22(2):1-3.
5. Bellinge RH, Liberles DA, Iaschi SP, Brien PA. Myostatin and its implications on animal breeding: a review. Animal Genetics. 2005; 36:1-6.
6. Dehnavi E, Azari AM, Hasani S, Nassiry MR, Mohajer M, Khan AA *et al*. Polymorphism of myostatin gene in intron 1, 2 and exon 3 and their associations with yearling weight using PCR-RFLP and PCR-SSCP techniques in Zel sheep. Biotechnology Research International 2012; 1:1-5.
7. Agrawal VK, Gahlot GC, Ashraf M, Khicher JP, Thakur S. Sequence analysis and phylogenetic relationship of myostatin gene of Bikaneri camel (*Camelus dromedarius*). Journal of Camel Practice and Research. 2017a; 24(1):73-76.
8. Agrawal VK, Gahlot GC, Gupta S, Yadav SP, Ashraf M, Thakur S. Molecular characterization of myostatin gene affecting muscle growth in Kankrej cattle. Haryana Veterinarian. 2017b; 56(1):25-28.
9. Trukhachev V, Belyaev, Kvochko A, Kulichenko A, Kovalev D, Sergey P, Volynkina A *et al*. Myostatin gene (*MSTN*) polymorphism with a negative effect on meat productivity in Dzhalginsky Merino sheep breed. Journal of Biosciences and Biotechnology. 2015; 4(2):191-199.
10. Chen TT, Polymorphisms of MSTN, IGFBP-3 gene and the related research with growth performance of Tianfu Goat, Sichuan Agricultural University, Ya'an. Asian Journal of Animal and Veterinary Advances. 2008; 7:802-811.
11. Ansary M, Tahmoorespur M, Nassiry MR, Taheri A, Valeh V. Polymorphism in intron-I of *Myostatin* gene and its association with estimated breeding values of growth traits in Baluchi sheep (*Ovis aries*). Indian Journal of Animal Science. 2011; 81(8):75-100.
12. Farhadian M, Hashemi A. Molecular characterization and phylogeny based analysis of intron I sequence of Myostatin (MSTN) gene in Iranian Makuei sheep breed. Annals of Animal Science. 2016; 16(4):1007–1018. DOI: 10.1515/aoas-2016-0013.
13. Abbasi MA, Abdolllahi-Arpanahi R, Maghsoudi A, Vaez Torshizi R, Nejati-Javaremi A. Evaluation of models for estimation of genetic parameters and maternal effects for early growth traits of Iranian Baluchi sheep. Small Ruminant Research. 2012; 104:62-69.
14. Narula HK, Yadav SBS, Sharma PR, Mehrotra V. Growth and reproductive performance of Magra sheep of Rajasthan. Indian Journal of Animal Sciences. 2009; 79:639-641.
15. Tahmoorespur M, Taheri A, Gholami H, Ansary M. PCR-SSCP variation of GH and STAT 5A genes and their association with estimated breeding values of growth traits in Baluchi sheep. Animal Biotechnology. 2011; 22:37-43.
16. Masoudi A, Hemrani H, Abbasi A. Using PCR-SSCP techniques to study polymorphism of myostatin gene and its association with production traits in Baluchi sheep, in Proceedings of the 4th National Biotechnology Congress of Iran, Kerman, Iran, August, 2005.
17. Ansary M, Tahmoorespour M, Valeh V, Nassiry, MR, Shahroudi FE. Investigation of polymorphism of GDF-8 gene and its association with average daily gain in Baluchi sheep in Proceedings of the 3rd Congress on Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran, 2008.
18. Hickford JGH, Forrest RH, Zhou H, Fang Q, Han J, Frampton CM, Horrel AL. Polymorphisms in the ovine myostatin gene (MSTN) and their association with growth and carcass traits in New Zealand Romney sheep. Animal Genetics. 2009; 41:64-72.