

Molecular characterization of exon 3 of caprine myostatin gene in Marwari goat

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Abstract

Aim: To estimate genetic variability in exon 3 of caprine myostatin gene in Marwari goats.

Materials and Methods: A total of 120 blood samples from unrelated Marwari goats were randomly collected from different villages of Bikaner (Rajasthan), India. Genomic DNA was extracted from whole blood using blood DNA isolation kit (Himedia Ltd.) as per manufacturer's protocol. The quality of extracted genomic DNA was checked on 0.8% agarose gel. Specifically designed a primer set for caprine myostatin (*MSTN*) gene (Genebank accession no. DQ167575) was used to amplify the exon 3 region of *MSTN* gene in Marwari goat. The genetic variability in exon 3 of *MSTN* gene in Marwari goat was assessed on 8% polyacrylamide gel electrophoresis to detect single strand conformation polymorphism (SSCP) pattern.

Results: The exon 3 of *MSTN* gene in Marwari goat showed two types of conformation patterns on 8% polyacrylamide gel. One of the patterns showed only two bands and was considered as genotype AA, whereas another pattern having an extra band was designated as genotype AB. The frequencies of AA and AB genotype for exon 3 region of *MSTN* gene were calculated as 0.90 and 0.10, respectively.

Conclusion: Low level of polymorphism was observed at exon 3 region of *MSTN* gene in Marwari goat through SSCP analysis. This information could be utilized in future breeding plan to exploit the unique characteristics of Marwari goat of Rajasthan.

Keywords: exon 3, Marwari goat, myostatin gene, polymerase chain reaction-single strand conformation polymorphism.

Introduction

Marwari goat is a major meat breed of Rajasthan (India) that is well-adapted to the arid environment and can tolerate higher salt loads and requires less water. The breed is known for its faster growth and breeding efficiency [1]. The unique characteristics of this breed require its molecular characterization for growth traits. Myostatin (*MSTN*) or growth and differentiation factor 8 is a member of the transforming growth factor- β superfamily that plays an important role in the regulation of muscle growth and meat quality [2]. The three exons and two introns of *MSTN* gene encode a glycoprotein that is expressed widely in skeletal muscles [3]. *MSTN* gene plays a critical role in myogenic differentiation and results in failure of myoblast to differentiate into myotubes through inhibition of MyoD activity and expression via Smad3 [4]. The altered gene structure could affect the composition of muscle fiber and

thereby muscle weight [5]. Dramatic muscularity and a "double-muscling" phenomenon had been observed in many livestock species following mutation in the *MSTN* gene that inactivated its expression or produced a non-functional protein [6]. A number of studies in pigs, cattle [7,8], and sheep have detected the role of *MSTN* gene in muscular development [9]. However, a similar investigation about the properties of the *MSTN* gene in goat is limited [10].

Polymerase chain reaction (PCR) based single-strand conformation polymorphism (SSCP) technique has emerged as a simple, efficient, and powerful tool to detect small genetic variation in amplified product even at single base pair. Routine electrophoresis could not detect change in single nucleotide in a particular sequence of double-stranded DNA as the physical properties of the double strands are almost identical for both alleles. In SSCP technique, the double-stranded DNA after denaturation undergoes three-dimensional folding and may assume a unique conformational state based on its DNA sequence. The difference in shape between two single-stranded DNA strands with different sequences can cause them to migrate differently on polyacrylamide gel, even though the number of nucleotides is same, which is, in fact, an application of SSCP [11].

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Therefore, the present study was carried out to detect genetic variation in exon 3 of *MSTN* gene in Marwari goat using the PCR-SSCP technique.

Materials and Methods

Ethical approval

All essential procedures of sample collection were performed strictly as specified by Institutional Ethical Committee with minimal stress to animals.

Experimental animals

Random blood samples (n=120) were collected from unrelated Marwari goats from the field units of AICRP on Goat Improvement Project functioning at the Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Bikaner.

Location of study

The study was conducted at the Molecular Genetics Laboratory of Department of Animal Genetics and Breeding, College of Veterinary and Animal Science, Bikaner, Rajasthan, India.

Collection of blood samples

About 2-3 ml of venous blood was collected from jugular vein of each animal in EDTA-containing vacutainer tubes under sterile conditions. The vials were shaken gently after blood collection to facilitate thorough mixing and then kept in ice box containing ice packs. The samples were transported to the laboratory immediately and processed on the same day to extract genomic DNA.

Extraction of genomic DNA and PCR amplification

Genomic DNA was extracted from whole blood by Blood Genomic DNA Purification Kit (HI Media Pvt. Ltd.). The exon 3 of *MSTN* gene was amplified using one set of primer designed from caprine *MSTN* gene sequence (Genebank accession no. DQ167575). The PCR reactions were carried out in a final volume of 25 μ L containing 80 ng of DNA template, 0.1 μ M of each primer, 10.0 mM dNTPs, 2.5 U of *Taq* DNA polymerase (Promega, Madison, USA), 5 μ L \times 5 reaction buffer, 1.5 mM $MgCl_2$, and 11 μ L ddH_2O . An initial denaturation at 95°C for 5 min was carried out and followed by 40 cycles of denaturation at 94°C for 45 s, annealing for 45 s (Table-1), and extension at 72°C for 60 s. A final extension of 10 min was carried out at 72°C. The PCR amplified products were checked on 1.2% agarose gel (Figure-1).

Detection of genetic variation

The polymorphism in exon 3 of *MSTN* gene was detected by SSCP analysis. Aliquot of 5 μ L PCR products were mixed with 5 μ L of denaturing \times 2 gel loading dye and heated for 8 min at 95°C and finally chilled on ice for 7 min. 8% polyacrylamide

gel electrophoresis (PAGE) was carried out with denatured amplified product to identify the different genotypic patterns. The gels were stained with ethidium bromide, analyzed under ultraviolet light, and documented by UVP gel-documentation system.

Results and Discussion

The exon 3 of *MSTN* gene in Marwari goat revealed two different conformation patterns on polyacrylamide gel (Figure-2). One of the patterns considered as genotype "AB" was observed in 12 individuals, whereas another pattern was designated as "AA" genotype and was found in most of the animals studied (n=108). The pattern AA revealed only two bands and was considered as homozygote, whereas the other pattern AB that showed three bands was designated as heterozygote. The genotypic frequencies of AA and AB genotype in the present study were found to be 0.90 and 0.10, respectively. The frequency of allele A was found to be highest (0.95) for exon 3 of *MSTN* gene in Marwari goat (Table-2). However, the present study could not detect the second homozygote.

The growth traits of animals are regulated by polygenes and are always of primary concern during breeding for determining the animal's economical value [4]. Identification of genetic markers for growth traits is the initial and critical step for the establishment of marker-assisted selection system [12]. The detection of genetic variations in DNA sequence could help in genetic characterization of livestock breeds and may help in identification of possible hybridization events or past evolutionary trends. The change in DNA sequence of exon region of genes may lead to change in amino acid sequences and ultimately the structure of expressed protein. The polymorphism in

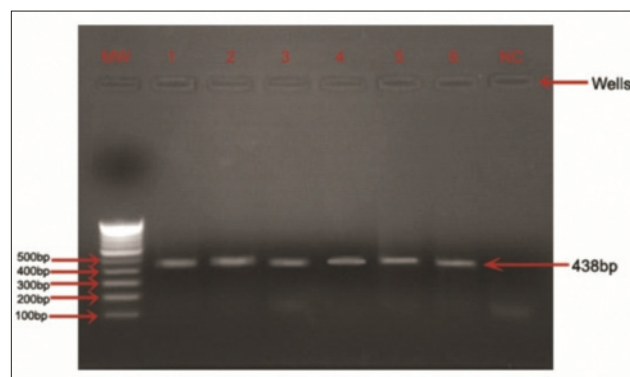


Figure-1: Amplification of myostatin (exon 3) gene using the specific primer of polymerase chain reaction in Marwari goats visualize under ultraviolet illuminator stained with ethidium bromide. MW: Molecular weight Marker, Wells: 1-6 myostatin (exon 3) gene 438 bp (Marwari goat), NC: Negative control.

Table-1: Primer sequences used to amplify myostatin gene.

Gene	Forward and reverse sequence (5' to 3')	Expected product size	Annealing temp (°C)
<i>MSTN</i> (Exon 3)	F-5'-TTTCTTTAATAATGACTCCCTGCG-3' R-5'- TCTACTACCATGCCTGGAATCTTC -3''	438 bp	55°

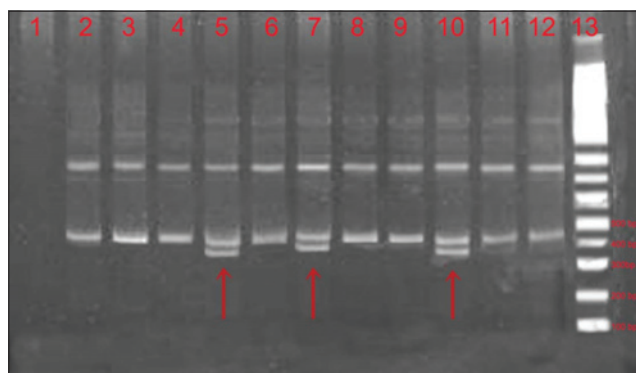


Figure-2: 8% polyacrylamide gel electrophoresis and single strand conformation polymorphism (SSCP) analysis of polymerase chain reaction (PCR) amplified product of exon 3. Lane 1: Control blank, Lane 2-12: SSCP analysis of different samples. Arrow indicating a PCR product producing an extra lower band, Lane 13: DNA marker.

Table-2: Depiction of gene and genotype frequency of Exon 3 of *MSTN* gene by SSCP.

Gene	Genotype frequency			Gene frequency	
	AA	AB	BB	A	B
Exon 3	0.90 (n=108)	0.10 (n=12)	-	0.95	0.05

SSCP=Single strand conformation polymorphism

DNA could be linked to economic traits in many livestock species [13].

A major gene model suggests that few candidate genes are responsible for relatively large proportion of genetic variation and could be utilized as marker [14]. Such major genes are usually involved in the biology of a trait and have an effect on the physiological pathway, metabolism, and expression of phenotypes. Growth hormone (GH), GH receptor, insulin-like growth factor I, leptin, caprine pituitary-specific transcription factor-1, caprine myostatin (*MSTN*), and bone morphogenetic protein genes are few candidate genes that play an important role in bone formation, birth weight, weaning weight, body condition, and muscle growth.

Myostatin protein acts as a negative regulator of skeletal muscle growth and keeps the skeletal musculature within appropriate proportions [15]. The protein inhibits terminal differentiation of myoblasts and the proliferation of myogenic cells [16,17]. Investigations of myostatin developmental expression in bovine skeletal muscles [18] and its function in myogenesis and adipogenesis had shown the relationship of myostatin expression with animal growth [19-23] observed the association of an inactive *MSTN* allele with higher birth weights and yearling weights in Piedmontese crossbred cattle. A double-muscling phenomenon was observed in Belgian Blue and Piedmontese cattle following mutation in coding region of *MSTN* gene.

The polymorphisms of *MSTN* gene were shown to have significant difference among goat breeds. It was reported that TTTTA deletion phenomenon occurred in *MSTN* gene was unique for goats when

compared with sheep, cattle, water buffalo, domestic yak, pigs, and humans [6,24-26] found an important effect of a 5-bp deletion on early body weight and size of a goat.

The present study focused on identification of possible genetic variants in exon 3 of *MSTN* gene in Marwari goat keeping in view the scanty information available for caprine *MSTN* gene. The present study in Marwari goat revealed low genetic diversity in exon 3 of *MSTN* gene which is in agreement with the similar study conducted in Saanen and Boer × Guanzhong goats by An *et al.* [27], who also observed one homozygote and a heterozygote genotypic pattern revealed by SSCP in exon 3 of caprine *MSTN* gene. However, a similar type of study in Sanjabi and Zel sheep by Soufy *et al.* [28] and Dehnavi *et al.* [29] revealed a monomorphic genotypic pattern in exon 3 of *MSTN* gene that suggests the fixation of this locus in sheep. The results of the present study indicate that low level of polymorphism still exists in this meat type of breed. The inconsistency in the results may be ascribed to species and breed differences, population and sampling size, environmental factors, mating strategies, geographical position effect, and frequency distribution of genetic variants. The present study could provide a base for further investigation of caprine *MSTN* gene in Marwari goats and other native breeds using large number of samples.

Conclusion

The PCR-SSCP analysis of exon 3 of *MSTN* gene in Marwari goat revealed low level of polymorphism. This information could be utilized in future breeding plan to exploit the genetic potential of Marwari goat.

Authors' Contributions

GCG and JPK designed the work plan. JPK, K, and ASD collected and processed the blood samples. JPK, MA, and VKA carried out PCR, electrophoresis, and PAGE. GCG, JPK, and P compiled, tabulated, transformed, and analyzed the data. JPK and GCG interpreted the results. JPK, VKA, and K prepared the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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