POLYMORPHISM IN EXON 1 OF CAPRINE MYOSTATIN (MSTN) GENE IN MARWARI GOATS BY PCR-SSCP METHOD[#]

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ABSTRACT

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Myostatin (*MSTN*) gene plays an important role in the muscle growth of the animals. Genetic variations in *MSTN* gene were investigated by PCR-SSCP using genomic DNA of 120 Marwari goats. Polymerase chain reactions (PCR) were performed to amplify exon 1 of myostatin loci using specific primer sequences. Amplified PCR products were resolved by 8% non-denaturing polyacrylamide gel electrophoresis. Genotyping was performed according to the band pattern of resolved PCR products. The PCR amplified product for exon 1 showed similar conformation pattern in all the samples investigated, which could be an indicative of absence of mutation in the exon 1 of the myostatin gene of Marwari goat. The monomorphic pattern for exon 1 of myostatin gene in the present study could be used to identify Marwari breed from other breeds of goats. However, a definitive conclusion requires a larger number of goats to be studied.

Key words: Myostatin, Marwari goat, polymorphism, PCR-SSCP

Introduction

Marwari goat, a major meat breed of Rajasthan (India), is well adapted to the arid environment, grows faster, bred efficiently, can tolerate higher salt loads, and requires less water than many other species of this region. These unique characteristics of this breed require its molecular characterization, genetic differentiation and relationships with other breeds (Rohilla and Patel, 2003).

Myostatin (*MSTN*) or growth and differentiation factor 8 (GDF8) gene, a member of the transforming growth factor-β super family, plays an important role in the regulation of muscle growth and meat quality (Zhang *et al*., 2012). The *MSTN* gene has 3 exons and 2 introns that encode a glycoprotein that is expressed widely in skeletal muscle (Bellinge *et al*., 2005). Changes in the gene structure and expression may regulate the expression of target genes, thereby changing the composition of muscle fibres and causing a variation of muscle weight (Chen, 2008). Some studies have demonstrated that *MSTN* gene inhibits *MyoD* activity and expression via Smad3, resulting in the failure of myoblasts to differentiate into myotubes and demonstrated that *MSTN* gene plays a critical role in myogenic differentiation (Jin, 2011). Mutations in the *MSTN* gene can inactivate its expression or produce a non-functional protein, which leads to dramatic muscularity and a "double-muscling" phenomenon in many species (Grisolia *et al*., 2009). A number of studies in pigs, cattle (Walsh and Celeste, 2005; Fan *et al*., 2010) and sheep have detected the role of mutations in *MSTN* gene in muscular development (Boman *et al*., 2009). Similar investigations about the properties of the *MSTN* gene in goat are limited (Liu *et al*., 2006).

Genetic markers play an important role in detection of genetic polymorphisms in many fields of animal breeding. With the development of molecular genetic techniques, it has become possible to establish a new class of gene markers based upon the variability at DNA sequence level. PCR based Single-strand Conformation Polymorphism (SSCP) techniques are used in animal breeding domain as new and powerful tools with the aim of providing breeders an opportunity to carefully identify superior animals.

Single-strand conformation polymorphism (SSCP) technique is simple and efficient mean to detect any small alteration in PCR-amplified product. A single nucleotide change in a particular sequence, as seen in double-stranded DNA, cannot be distinguished by electrophoresis because the physical properties of the double strands are almost identical for both alleles. After denaturation, single-stranded DNA undergoes 3-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 an electrophoresis gel, even though the number of nucleotides is the same, which is, in fact an application of SSCP (Orita *et al*., 1989).

Materials and Methods

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

Experimental Animals: A random sample of 120 unrelated goats of Marwari breed from the field unit area of Deshnoke, Kalyansar, Raisar and Daiya villages of AICRP-Goat project functioning at the Department of Animal Breeding & Genetics, College of Veterinary and Animal Science, Bikaner were collected.

Collection of Blood samples: About 2-3 ml of venous blood was collected from jugular vein from each animal in the EDTA containing vacutainer tubes under sterile conditions. After collection of blood, the vials were shaken gently to facilitate

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thorough mixing of blood. The vials were then kept immediately in ice box containing ice and gel cool pack and were transported to the laboratory immediately. After reaching to laboratory, the samples were processed on the same day to extract genomic DNA.

Extraction of Genomic DNA and PCR Amplification: Genomic DNA was extracted from blood by Blood Genomic DNA Purification Kit (HIMedia Pvt. Ltd). From the purified genomic DNA, the exon 1 fragment in the *MSTN* gene was amplified using one set of primers (Forward - 5'- TAT TGA AAA CCA TGC AAA AAC TGC -3' and Reverse - 5' - TAC ACT AGA AAA GCA GTC AGC AGA -3') designed using the goat MSTN gene sequence (Gene bank accession no. DQ167575). The PCRs were performed in a final volume of 25 µL, containing 4 µL DNA template, 0.5 µL each primer, 2.0 µL dNTPs, 0.5 µL *Taq* DNA polymerase (Promega, Madison, USA), 5 µL 5X reaction buffer, 1.5 µl $MgCl_2$ and 11 µL ddH_2O. Cycling conditions for PCR were 40 cycles of 45 seconds at 94^0C, 45 seconds at annealing temperature in (Table 1) and 60 seconds at 72^0C, followed by a final extension for 10 minute at 72^0C. The PCR amplified products were checked on 1.2% agrose gel (Fig. 1).

Detection of genetic variation: SSCP method was used in the present study to detect polymorphism in the *MSTN* Exon 1 gene. Aliquots of 5 µL PCR products were mixed with 5µL denaturing solution (2X gel loading dye), heated for 8 min at 95°C, and chilled in ice immediately for 7 min. The denatured DNA was subjected to 8% polyacrylamide gel electrophoresis analysis. The gels were stained with ethidium bromide, analyzed under UV light and documented by UVP gel-doc system.

Fig. 1: Amplification of Myostatin (Exon 1) gene using the specific primer of PCR in Marwari goats visualize under UV illuminator stained with EtBr; MW: Molecular weight marker; Wells: 1-6 Myostatin (Exon 1) gene 458 bp (Marwari goat); NC: Negative control

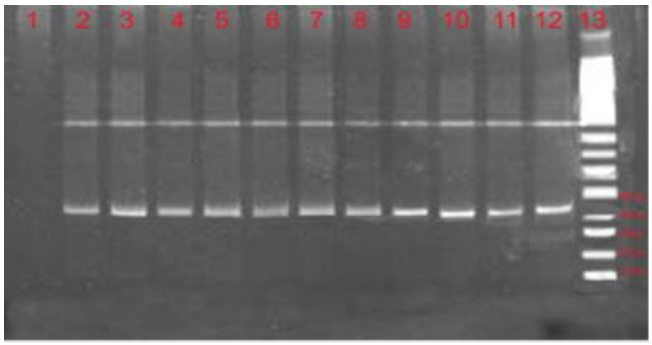

Fig. 2: 8% PAGE electrophoresis and SSCP analysis of PCR amplified of Exon 1; Lane 1: Control blank; Lane 2-12- SSCP analysis of different samples. All samples showing similar band pattern; Lane 13-DNA Marker

Results and Discussion

SSCP analysis of Exon 1: The PCR amplified product for exon 1 of *MSTN* gene showed similar conformation pattern in all the samples investigated (Fig. 2). The pattern showed only two bands and was considered as genotype AA.

Variations at DNA level play important role in the genetic characterization of livestock populations. This may also help in identification of possible hybridization events as well as past evolutionary trends. Variation in the exonic region of a gene may lead to changes in amino acid sequences which can alter the structure of expressed protein. In livestock, such variations in DNA may also be associated with, or linked to, economic traits, which are governed by polygenes, each having a small effect (Gelderman, 1997). However, the major gene model suggests that only a few genes may account for relatively large proportion of the genetic variation (Lande, 1981). Such major genes are usually involved in the biology of a trait and are candidate genes for marker identification.

Candidate genes have an effect on the physiological pathway, metabolism and expression of phenotypes. Growth traits are one of the important traits that are specifically considered in animal breeding programmes. Growth traits are complex traits that are controlled by many candidate genes. Growth hormone (*GH*), growth hormone receptor (*GHR*), insulin like growth factor I (*IGF-I*), leptin (*LEP*), caprine pituitary specific transcription factor-1 (*POU1F1*), caprine myostatin (*MSTN*) and bone morphogenetic protein (*BMP*) genes are few genes that play important role in bone formation, birth weight, weaning weight, body condition and muscle growth. Out of these candidate genes responsible for growth, caprine myostatin (*MSTN*) gene was investigated in the present study to characterize the genetic variation in Marwari goat breed of Rajasthan.

The growth traits of animals, which are regulated by many genes, are always of primary concern during breeding for determining the animal's economical value (Chen *et al*., 2008). Myostatin acts as a negative regulator of skeletal muscle growth and keeps the skeletal musculature within appropriate proportions (Lee and McPherron, 1999). It inhibits both the terminal differentiation of myoblasts and the proliferation of myogenic cells (Thomas *et al*., 2000; Wiener *et al*., 2009). Investigations of myostatin developmental expression in bovine skeletal muscles (Shibata *et al*., 2003) and its function in myogenesis and adipogenesis showed myostatin expression is related to animal growth (Lin *et al.,* 2002; Joulia *et al.,* 2003; Rebbapragada *et al.,* 2003; Wagner *et al.,* 2005). Casas *et al.* (1999) observed the association of an inactive MSTN allele with higher birth weights and yearling weights in Piedmontese crossbred cattle. Myostatin was considered as the candidate gene for double-muscling animals because mutations in its coding region are responsible for double-muscled Belgian Blue and Piedmontese cattle (Kambadur *et al.,* 1997).

Identification of genetic markers for growth traits is the initial and critical step to establish a marker-assisted selection system (Li *et al*., 2009). The polymorphisms of the *MSTN* gene were shown to have a significant difference in different goat breeds. It was reported that the TTTTA deletion phenomenon in *MSTN* gene had emerged in different species and might be

Gene	Forward and reverse Sequence (5' to 3')	Expected product size	Annealing temp. (^{0}C)
Myostatin (Exon 1)	Forward - 5'- TAT TGA AAA CCA TGC AAA AAC TGC -3' Reverse - 5'- TAC ACT AGA AAA GCA GTC AGC AGA -3'	458 bp	55^0

unique for goats, compared with sheep, cattle, water buffalo, domestic yak, pigs, and humans (Hadjipavlou *et al*., 2008; Grisolia *et al*., 2009; Zhang *et al*., 2013). Moreover, the diversity of the nucleic acid is a historical indicator of different individuals of goat breed so the polymorphism distribution is beneficial to study the population relationship. Li *et al*. (2008) found an important effect of a 5-bp deletion in *MSTN* gene on early body weight and sizes of goat.

However in the present study, the genetic diversity analysis of Marwari goats through PCR-SSCP revealed no genetic diversity in exon 1 of *MSTN* gene. The results observed in the present study are in agreement with similar study conducted in Boer goats by Zhang *et al*. (2013) who also reported low polymorphism in exon 1 and UTR region of Myostatin gene. Nada *et al*. (2013) also detected monomorphic pattern in myostatin gene in five Egyptian and Saudi sheep breeds using Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Dehnavi *et al*. (2012) observed polymorphic pattern in exon 3 of myostatin gene in Zel sheep using PCR-RFLP method. Soufy *et al.* (2009) also observed observed monomorphic pattern in exon 3 of myostatin gene in Sanjabi sheep. The inconsistency in the results may be ascribed to species and breed diûerences, population and sampling size, environmental factors, mating strategies, geographical position eûect and frequency distribution of genetic variants.

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