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Research Article

MOLECULAR CHARACTERIZATION OF MYOSTATIN GENE AFFECTING MUSCLE GROWTH IN KANKREJ CATTLE

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ABSTRACT

The regulation of muscle growth and body weight is controlled by candidate genes such as myostatin (*MSTN*). Allelic variation in *MSTN* affects development of muscle's growth in Kankrej cattle. The present study was undertaken in Kankrej cattle to characterize *MSTN* gene through polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) genotyping. Blood samples were collected aseptically from 70 unrelated Kankrej cattle maintained at Livestock Research Station, Kodamdesar, Bikaner. Genomic DNA was extracted from 3 ml of whole blood through spin column method. The quality and concentration of extracted genomic DNA was checked on 0.8% agarose gel and Nano Drop spectrophotometer, respectively. The exon 2 region of *MSTN* gene was amplified using *MSTN* primers designed from GenBank (Accession No. DQ167575). The amplified 375 bp region of *MSTN* was digested for 2 h with 10 units of *Hae*III restriction enzyme at 37ºC. The genetic variability in exon 2 region of *MSTN* was assessed on 8% polyacrylamide gel electrophoresis. All the digested samples showed 'm' allele with two fragments of 88 and 287 bp. The study concluded that all the animals were monomorphic and genotyped homozygous 'mm' for the locus studied. The result showed the suitability of PCR-RFLP technique for evaluating genetic variability in *MSTN* gene in Kankrej cattle.

Key words: Kankrej cattle, myostatin, PCR-RFLP

Indigenous cattle though well known for their adaptation and low maintenance costs, are often being blamed for slow growth, delayed puberty and low production due to low body weight than exotic cattle. Kankrej cattle is considered as the best dual purpose breed and is famous for its large body size and excellent farming ability (Ankuya *et al*., 2016). The body weight is a major indicator of health and productivity (Zaffer *et al*., 2015). The rate of genetic progress in growth traits of animals having long generation interval can be enhanced through marker assisted selection in domestic animals such as cattle (Goddard and Hayes, 2009). The allelic variation in several candidate genes largely affects the quantitative traits such as body muscle mass and ultimately the body weight in farm animals (Lacorte *et al*., 2006).

Myostatin (*MSTN*) or Growth Differentiation Factor-8 (GDF-8) is one such candidate gene that is primarily expressed in developing skeletal muscle and plays a key role in skeletal muscle growth (Tahmoorespur *et al*., 2011). The *MSTN* gene is a member of the Transforming Growth Factor-B (TGF-B) family and acts as a negative regulator of myogenesis and maintains tissue homeostasis in adults (Peng *et al*., 2013). An increase in the muscle mass due to altered expression of the *MSTN* and mutations in the coding sequences of *MSTN* has been reported in various species (Grobet *et al*., 1997; Kambadur *et al*., 1997; Smith *et al*., 2000). Homogeneous and heterogeneous individuals with mutations in *MSTN* gene have shown enlarged musculature, increased birth weight and greater growth rate (Casas *et al*., 2004; Zhang *et al*., 2012). The animals which carry a single copy of mutant allele from crossbred Belgian Blue, or crossbred Piedmontese sire, had increased longissimus muscle area and reduced external and intramuscular fat deposition compared to animals received no copies thereof (Casas *et al*., 1998).

The molecular analysis of the *MSTN* gene in different species has shown that it consists of three exons and two introns (Kurkute *et al*., 2011). The gene is found to be highly conserved in various vertebrate species, however, nine mutations in *MSTN* gene have been identified in cattle, of which five are located in coding sequences (Grobet *et al*., 1998). Zhou *et al*. (2008) identified a mutation in exon 2 region of *MSTN* gene.

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Restricted fragment length polymorphisms (RFLPs) have been demonstrated to be very useful genetic markers for candidate gene to reveal polymorphisms associated with quantitative traits (Gross *et al*., 1999). Simple molecular technique like polymerase chain reaction based RFLP (PCR-RFLP) is a useful tool to detect polymorphism at genetic level (Saikia *et al.*, 2015). Identification and characterization of *MSTN* exon-2 gene of Kankrej cattle through PCR-RFLP would be useful to detect effective alleles that may influence the body weight and to assist the future selection programs Thus, the present study was undertaken in Kankrej cattle to identify the different genotypes of the myostatin exon-2 gene.

MATERIALS AND METHODS

A total of 70 animals were selected randomly from the purebred herd of Kankrej cattle maintained at Livestock Research Station, Kodamdesar, Bikaner, Rajasthan (Rajasthan University of Veterinary and Animal Sciences, Bikaner) after necessary approval from the Institutional Animal Ethics Committee. About 3 ml blood samples were collected from unrelated Kankrej cattle in the vacutainers containing EDTA as an anticoagulant. The genomic DNA was extracted using spin column method as per standard protocols (Sambrook *et al.*, 1989). The quantity and quality of extracted DNA was checked on NanoDrop 2000 (Thermo Scientific) spectrophotometer and 0.8% agarose gel electrophoresis. Primer sequences used in the amplification of the relevant *MSTN* exon 2 regions of the Kankrej DNA has been represented in Table 1. The primers were designed from the gene sequences available at NCBI GenBank database (accession number DQ167575) to amplify the 375 bp fragment of exon-2 region of *MSTN* gene Amplification reactions for each fragment was done by using the following constituents: in a final volume of 25 ìl containing 5X PCR buffer, 1.25 unit of *Taq*DNA polymerase, 0.2 mM each of dNTPs, 1.5 mM $MgCl_2$, 100 pMol of each primer and 100-150 ng of template DNA. 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 30s, 54.5°C for 60s, 72 °C for 60s and final extension at 72°C for 10 min. The amplified DNA fragments were stained and visualized on 1.5% agarose gel under Gel Documentation System. The polymorphism detection and genotyping of *MSTN* exon 2 gene in all the amplified samples were carried out with *Hae*III restriction enzyme. About 10 ìl of amplified product (375 bp) of *MSTN* exon-2 gene was digested with 10 units *Hae*III restriction enzyme for 2 h at 37ºC. Then the enzyme was inactivated by increasing the incubation temperature to 80°C for 30 min. The polymorphic nature of exon-2 region of *MSTN* gene was assessed on 8% polyacrylamide gel electrophoresis with 100 bp DNA ladder in a gel documentation system. Direct counting method was used to estimate genotype and allele frequencies of genetic variants of the *MSTN* genes.

RESULTS AND DISCUSSION

The genetic variation in exon-2 region of *MSTN* gene in Kankrej cattle (*Bos indicus*) was reported in the present study. PCR-RFLP analyses were used to identify the different genotypes of *MSTN* exon 2 gene. The *in vitro* amplification of genomic DNA from all the samples revealed amplification band of 375 bp of *MSTN* exon-2 coding region using oligonucleotide primers designed from the available database. All the 70 animals included in the study did not any reveal polymorphism in *MSTN* exon 2 locus and similar genotypic pattern 'mm' were observed in all the animals studied. *Hae*III restriction enzyme digests the 'm' allele, but not 'M' allele. The digestion of the 'm' allele produced two fragments of 88 and 287 bp (Fig. 1). As a result, the polymorphic nature of 'm' allele was not observed in the present study. The genotype 'MM' and 'Mm' were not observed in any of the animal studied. The present study provides evidence that mutation is not present at *Hae*III restricted site in *MSTN* exon 2 loci in Kankrej cattle.

The global food demand will double in next 50 years (Steinfeld *et al*., 2010). Thus identification and characterization of genes associated with growth and body weight would help in attainment of early maturity body weight and may provide new opportunities to indirectly improve reproduction related traits like age at first calving in slow maturing indigenous cattle such as

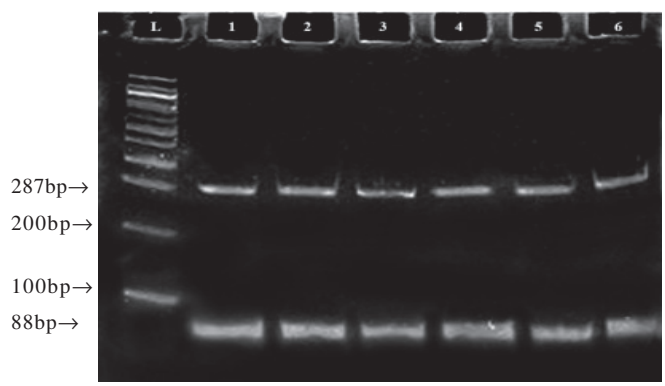

Fig 1. Restricted pattern of MSTN exon2 gene in Kankrej cattle on 8% polyacrylamide gel

Table 1
Primer sequence, PCR product size and primer annealing temperature

Gene	Primer	PCR product size	Annealing temperature
MSTN exon 2	5'-AAAAACCCAAATGTTGCTTCTTTA-3' 3'-CAGTCCTTCTTCTCCTGGTCTGG-5'	375 bp	54ºC

Means with different capital letter superscript in a row and small letter superscript in a column within a group differ significantly (P≤0.05).

Kankrej. The *MSTN* gene has been considered as an important candidate gene for growth and development of domestic animals due to its key role in muscle growth (Miranda *et al*., 2002). Candidate gene such as *MSTN* has been identified as potential regulator of body mass in different European breeds (Grobet *et al*., 1997; Kambadur *et al*., 1997; Smith *et al*., 2000). It acts as a negative regulator of skeletal muscle growth and keeps the skeletal musculature within appropriate proportions.

Breed specific haplotype in *MSTN* gene were observed in 28 European cattle breeds by Dunner *et al*. (2003). However, Crisa *et al*. (2003) found no differences among genotypes in *MSTN* gene of nine cattle breeds. Similar study in 411 animals of three Chinese cattle breeds detected very few mutant individuals (Zhang *et al*., 2007). The monomorphic nature of *MSTN* gene observed in present study is in agreement with findings of Sahin *et al*. (2013) and Aagaoglu *et al*. (2015) in native Turkish cattle breeds. The highly conserved nature of *MSTN* exon-2 gene observed in the present study in Kankrej cattle is suggestive to compare the observed information with other cattle breeds or species to reveal the presence of any intra- and inter- species polymorphism. However, studies on mutational variation in *MSTN* gene have been found to be associated with muscular mass in several species such as mice, cattle, humans, dogs, pigs and sheep (McPherron and Lee, 1997; Grobet *et al*., 1997; Han *et al*., 2013). The inconsistency in results of different workers may be ascribed to breed differences, population and sampling size, environmental factors, mating strategies, geographical position effect and frequency distribution of genetic variants.

Genotyping information on Kankrej myostatin gene would help in better understanding of muscle growth and differentiation mechanisms and provides clues for investigation of other regions. Such knowledge will be helpful in understanding the structure, function and evolution of the gene. Although a large number of alleles in *MSTN* gene have been identified in cattle, most are silent or neutral in their resultant effect. The probable effect of other mutations in *MSTN* gene including non-coding and regulatory regions on growth traits could not be neglected in Kankrej cattle. LeHir *et al*. (2003) observed the effect of introns on the transcriptional efficiency of numerous genes in a variety of organisms. The present study provides evidence that myostatin is a con-served and stable protein in Kankrej cattle. The suitability of technique PCR-RFLP employed for the generation of genotyping information in the present study is in agreement with similar study conducted by Debnath *et al*. (2016) in detection of carrier genotype in crossbred bulls. The genotyping information generated for *MSTN* gene also implies that Kankrej cattle have maintained their purity.

In conclusion, the Kankrej cattle under investigation were found monomorphic for the *MSTN* exon 2 gene by PCR-RFLP. The evidence for conservative nature *MSTN* gene suggest the investigation for other region of *MSTN* loci or other gene responsible for variation in body weight of Kankrej cattle. Hence, strategy of characterization of regions/loci responsible for growth in Kankrej should be continued to improve the growth traits.

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