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MOLECULAR CHARACTERIZATION OF EXON-1 OF MYOSTATIN GENE IN BAKERWAL GOATS IN KASHMIR VALLEY

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ABSTRACT Myostatin or growth differentiation factor 8 (GDF8), is a skeletal muscle regulator factor that determines muscle mass in animals including human beings. Myostatin, a protein has a remarkable role in growth and muscular development. Mutations in myostatin gene leads to the double muscling phenomenon in my animals. The present investigation was aimed at studying the genetic variation and characterization of exon-1 of myostatin gene in Bakerwal goats in Kashmir valley by PCR-DNA Sequencing method. And a 492-bp fragment of the exon-1 of MSTN gene was amplified by PCR using oligonucleotide primers standardized for Ovis aries species. The sizes of the amplification products were similar in sheep and goat. Myostatin gene in bakerwal goats reveals monomorphism since no variation was found. The result indicates strong conservation of DNA sequence between sheep and bakerwal goats.

KEYWORDS : myostatin, PCR, bakerwal, double muscling, exon.

Introduction

Myostatin/GDF8 protein is synthesized as a 376 amino acid precursor protein molecule by the myostatin gene present in almost all animals (An *et al*., 2011). Proteasic digestion processing between the propeptide domain and the C-terminal domain results in an N-terminal propeptide and the mature form of myostatin, a 12-kDa carboxy-terminal fragment. Both mature and unprocessed myostatin form disulfide linked dimers. But the only active form of the protein is the processed myostatin dimer (Joulia-Ekaza, D. *et al*., 2006). Myostatin or growth differentiation factor 8 (GDF8), is a skeletal muscle regulator factor that determines muscular growth in animals including human beings. Mutations in the myostatin gene sequences produces a non functional protein which leads to the increased muscular growth in livestock animals and the phenomenon is reffered to as double muscling (McPherron *et al.*, 1997).

The MSTN gene has been assigned to the 2q11-q12 position of caprine chromosome. Caprine MSTN gene consists of three exons and two introns (McPherron *et al.*, 1997). Genetic polymorphism at myostatin gene mainly arises from several point mutations at the myostatin gene. Polymorphism studies of caprine *MSTN* gene have been conducted on the coding and as well as on non coding regions of the gene. The Goat contributes about 10 percent of the total meat produced in India. Although the economic importance of goats has always been known, yet very little work has been carried out to exploit the genetic potential of this animal (Schina. 2009). Though studies have been carried out on characterization in sheep, similar studies in goats are scarce (Javanmard *et al.,* 2010). The reports regarding polymorphism studies in goats are very scanty, despite the important role of myostatin gene in the control of muscle development, a limited work has been carried on goat myostatin gene. The objective of the work described in this paper is to characterize the exon-1 of myostatin gene in bakerwal goats and compare them to sheep in order to locate alterations in nucleotide and protein sequences.

Materials and Methods

The present study was conducted with 60 Bakerwal goats maintained at Mountain Research Centre for Sheep and Goat (MRCSG) Shuhama Alusteng and Sheep Breeding Farm Banihal. About 5 ml venous blood was collected from the jugular vein of each animal in a sterile 10 ml polypropylene vial containing 0.5 M EDTA as anticoagulant (Figure 1). Genomic DNA was isolated from blood samples following the phenol- chloroform extraction method described by Sambrook and Russel (2001). DNA was dissolved in TE buffer and was kept in a water bath at 60^0c for 2 hr to dissolve pellet properly in buffer. The quality of DNA was checked through spectrophotometry. DNA samples with O.D. ratio between 1.7 and 1.9 were considered as good and used for further study. The samples beyond this range were re-extracted by the phenol-chloroform extraction method. DNA quality was also checked by running the sample in 0.8 percent agarose gel electrophoresis.

DNA amplification

A 492bp fragment of exon-1 of myostatin gene was amplified with forward (5'-TGGCGTTACTCAAAAGCAAA-3') and reverse (5'-AACAGCAGTCAGCAGAGTCG-3') primer sequences. PCR was carried out in a final volume of 25 µl reaction mixture containing 80-100 ng of sample DNA, 200 mM of each dNTP, 2.5 µl 1X PCR buffer, Taq DNA polymerase, 2 mM MgCl2 and 20 pmM of each primer. Amplification reactions were carried out in a thermocycler (Applied BioSystem) in three stages. In the first stage, initial denaturation at 95˚C for 5 minutes, annealing at 59˚C for 1 minute and final extension at 72˚C for 4 minutes.

Nucleotide sequencing

The PCR products were concentrated to 50 ng/µl by pooling several tubes to precipitate by the isopropanol procedure. In order to obtain clean sequencing results, quantification was done by loading one µl of sample in 1% agarose gel and comparing with standard molecular marker (100 bp DNA ladder). Only samples with good concentration

(>50 ng/µl) were selected and subjected to sequencing. Sequencing was performed at Macrogen Inc. Korea, with an automated sequencer (Applied Biosystems) (Figure 2 & 4). The resulting sequences were aligned using the BioEdit program (BioEdit v5.0.9), DNASTAR software and the database search of sequences for a possible match to the DNA sequence of myostatin gene was conducted using the BLAST algorithm available at the National Center for Biotechnology Information (NCBI) (Figure 3). (Altschul et al., 1990). Translated protein sequences of different myostatin gene were also subjected to the BLAST algorithm.

Results and Discussion

Nucleotide sequencing

The nucleotide sequence obtained as well as the derived amino acid sequence of exon-1 of myostatin gene of bakerwal goat (Gene Bank) have been depicted in Figure 2 & 4. The nucleotide sequences of exon-1 of the GDF8 gene of Bakerwal goat were deposited in GenBank under Accesion number KU980201. Sequence variations and amino acid variation were observed at the myostatin gene exon-1 between Capra hircus and Ovis aries (Figure 3).

The bakerwal goats sequences did not show any kind of polymorphism among themselves and also with the nucleotide sequences of exon-1 of myostatin gene of sheep. The observation of amplified fragments of the expected sizes and subsequent analysis of the obtained sequences confirmed the amplification of the regions of interest, demonstrating the complete transferability of the primer pairs developed for Ovis aries to Capra hircus. This is important in terms of reducing the costs of genome analysis of closely related species, as has been widely demonstrated for a large number of organisms, especially plants (Katzir *et al*., 1996). Our result shows a high degree of conservation in Bakerwal goat myostatin compared to the ovine protein. The similarity and identity between the nucleotide and amino acid sequences from Ovis aries and Capra hircus were as expected due to the close proximity of the species which belong to the same family Bovidae. Finding the mutations in the myostatin gene sequences using molecular techniques can be an effective solution for identifying the double muscling phenotype and help the breeders to get necessary information in order to make the accurate decision for management and selecting the best sires for reproduction and it also helps the breeders to have a lot of data about genetic status of MSTN gene in livestock animals (Ahad *et al*., 2016).

It could be concluded that the GDF8 gene can be a major gene affecting the growth traits in goats. The polymorphic site could be a molecular marker-assisted selection program for body weight. Polymorphism in myostatin gene has effects on the myostatin protein structure and on the biological function of the myostatin protein (Kambadur *et al.*, 1997). In addition, missense mutation (p.Lys to Thr) locus have a direct effect on the MSTN gene expression or be closely correlated with traits affected by loci in the nearby region, but further verification is needed. In Charollais sheep two SNPs in the myostatin gene has a significant association with the muscle growth and muscle depth (Hadjipavlou *et al*., 2008). Two different mutations in the MSTN coding region of two Norwegian sheep breeds are associated with carcass conformation and fatness (Boman, I.A *et al.*, 2009). Mutations found in noncoding regions of myostatin gene affect the level of myostatin gene expression and are associated with growth, muscle mass and other carcass traits in pigs (Stinckens et al., 2008). Esmailizadeh et al., 2008 found a SNP in the MSTN gene affecting birth, growth, carcass and beef quality traits of Bos taurus. The biochemical and physiological functions, indicate that the MSTN gene might play important roles in affecting the growth traits in goats and other species of productive livestock animals.

Conclusion

Ovine myostatin gene specific primers amplified the caprine myostatin gene exon-1 and PCR amplification yielded a 492 bp fragment from goat DNA homologous to that of sheep. We report the first characterization of the exon-1 of myostatin coding regions from Bakerwal goats present in the Kashmir Valley.

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Figure 1: Blood sample collection from jugular vein

Nucleotide sequence:

TGGCGTTACTCAAAGCAAAAGAAAAGTAAAAGGAAGAAGTAAGAACAAGGAAAAAGATTGTATTG
AAAACCATGCAAAAACTGCAAATCTTTGTTTATATTTACCTATTTATGCTGCTTGTTGCTGGCCCAGTG
GATCTGAATGAGAACAGCGAGCAGAAGGAAAATGTGGAAAAAAAGGGGCTGTGTAATGCATGTTTGT
GGAGACAAAACAATAAATCCTCAAGACTAGAAGCCATAAAAATCCAAATCCTCAGTAAACTTCGCCT
GGAAACAGCTCCTAACATCAGCAAAGATGCTATAAGACAACTTTGCCCAAGGCTCCTCCACTCCGGG
AACTGATTGATCAGTACGATGTCCAGAGAGATGACAGCAGCGACGGCTCCTTGGAAGACGATGACTA
CCACGTTACGACGGAAACGGTCATTACCATGCCCACGGAGTGTGAGTAGTTCTGCTAGTGCAGAGCAA
CGACTCTGCTGACTGCTGTT

Amino acid sequence:

MQKLQIFVYIYLFMLLVAGPVDLNENSEQKENVEKKGLCNACLWRQNNKSSRLEAIKIQILSKLRLETAPNISKDAIRQLLPKAPPLRE
LIDQYDVQRDDSSDGSLEDDDYHVTTETVITMPTE

Figure 2: Sequence report of amplified 492 bp fragment of myostatin gene exon 1, *Capra hircus* breed bakerwal (Gene bank acc no. KU980201).

ENSOARG00000016285.1 Ovis aries myostatin (MSTN) gene, exon 1
Sequence ID: Query_136541 Length: 492 Number of Matches: 1
Range 1: 1 to 492 Graphics

Score	Expect	Identities	Gaps	Strand
888 bits(984)	0.0	492/492(100%)	0/492(0%)	Plus/Plus

```
Query  1    TGGCGTTACTCaaaagcaaaagaaaagtaaaaggaagaagtaagaacaaggaaaaagatt  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TGGCGTTACTCAAAAGCAAAAGAAAAGTAAAAGGAAGAAGTAAGAACAAGGAAAAAGATT  60
Query  61   gtattgaaaaccatgcaaaaaCTGCAAATCTTTGTTTATATTTACCTATTTATGCTGCTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GTATTGAAAACCATGCAAAAACTGCAAATCTTTGTTTATATTTACCTATTTATGCTGCTT  120
Query  121  GTTGCTGGCCCAGTGGATCTGAATGAGAACAGCGAGCAGAAGGAAAATGTGGaaaaaaaG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GTTGCTGGCCCAGTGGATCTGAATGAGAACAGCGAGCAGAAGGAAAATGTGGAAAAAAAG  180
Query  181  GGGCTGTGTAATGCATGTTTGTGGAGACAAAACAATAAATCCTCAAGACTAGAAGCCATA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  GGGCTGTGTAATGCATGTTTGTGGAGACAAAACAATAAATCCTCAAGACTAGAAGCCATA  240
Query  241  AAAATCCAAATCCTCAGTAAACTTCGCCTGGAAACAGCTCCTAACATCAGCAAAGATGCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  AAAATCCAAATCCTCAGTAAACTTCGCCTGGAAACAGCTCCTAACATCAGCAAAGATGCT  300
Query  301  ATAAGACAACTTTGCCCAAGGCTCCTCCACTCCGGGAACTGATTGATCAGTACGATGTC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  ATAAGACAACTTTGCCCAAGGCTCCTCCACTCCGGGAACTGATTGATCAGTACGATGTC  360
Query  361  CAGAGAGATGACAGCAGCGACGGCTCCTTGGAAGACGATGACTACCACGTTACGACGGAA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CAGAGAGATGACAGCAGCGACGGCTCCTTGGAAGACGATGACTACCACGTTACGACGGAA  420
Query  421  ACGGTCATTACCATGCCCACGGAGTGTGAGTAGTTCTGCTAGTGCAGAGCAACGACTCTG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  ACGGTCATTACCATGCCCACGGAGTGTGAGTAGTTCTGCTAGTGCAGAGCAACGACTCTG  480
Query  481  CTGACTGCTGTT  492
            ||||||||||||
Sbjct  481  CTGACTGCTGTT  492
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Figure 3: BLASTn of exon-1 of myostatin nucleotide sequence with Sheep exon-1 myostatin nucleotide sequence

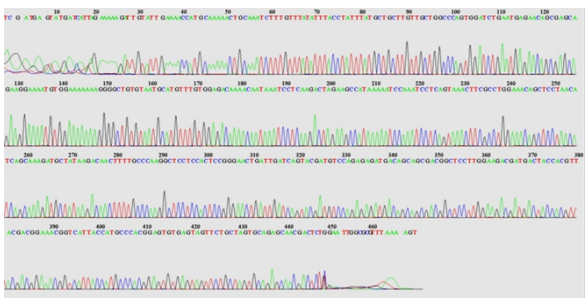

Figure 4: Sequence report of amplified 492 bp fragment of myostatin gene exon 1, *Capra hircus* breed Bakerwal

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