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Sequence Characterization of Coding Regions of the Myostatin Gene (GDF8) from Bakerwal Goats (*Capra hircus*) and Comparison with the Sheep (*Ovis aries*) Sequence

Wani A. Ahad[1*], Sajad A. Beigh[1], Mir Mehraj[1], Masroor Hussain[2], Hina F. Bhat[1], Riaz A. Shah[1], Ahmad S. Mudasir[1]

[1]Division of Animal Biotechnology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Shuhama Alusteng, India
[2]Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, R S Pura, India
Email: *ahad498@gmail.com

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Abstract

The Bakerwal breed of goat in Kashmir valley is a good meat purpose breed of goat. That attains an appreciable body weight under a low input production system. As these goats are mainly reared by Gujjar and Bakarwal tribes of the J & K state, so they usually are fed with the weeds, herbs, shrubs and grasses of pastures that will otherwise go waste. These goats constitute the main livestock wealth. With the good productive and reproductive potential, it makes these animals an important animal protein source for developing countries like India. The myostatin gene (GDF8) is important in the physiology of stock animals because its product produces a direct effect on muscle development and consequently also on meat production. The myostatin sequence is known in several mammalian species and shows a high degree of amino acid sequence conservation, although several alterations in the intron and exon regions have been identified. The objective of our work is to characterize the myostatin coding regions using gene sequencing and polymerase chain reaction methods of *Capra hircus* (Bakerwal breed) and to compare them with the *Ovis aries* and other livestock species of animal, looking for variations in nucleotide and protein sequences. As mutations in the myostatin gene can inactivate its expression and result in a non-functional protein, which leads to increase in muscle growth in many species. In this way, we

*Corresponding author.

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are able to identify 3 alterations on the presumed myostatin protein sequence as compared to non double-muscled ovine sequences.

Keywords

Goat, GDF8, Myostatin, Bakerwal, Intron, Exon

1. Introduction

The Bakerwal goat (*Capra hircus*) is not only an important animal but is also a major meat and/or milk producer but has been underutilized as compared to other stock animals used in husbandry, because these animals are mainly reared by Gujjar and Bakarwal tribes of the J & K state. Bakerwal goats are good in size and have massive structure as compared to other goat breeds in Kashmir valley. This growth is due to the fact that these animals are highly adaptable to temperate climates and are resistant to ectoparasites and endoparasites and show excellent nutritional efficiency combined with good productive and reproductive potential: characteristics that render these animals an interesting alternative protein source especially for poor or developing countries.

The role of protein factors responsible in the development of muscle mass in goats has been best elucidated by the study of the transforming growth factor-beta (TGF-β) superfamily of genes, which encode important factors for the regulation of embryo development and tissue homeostasis in adult animals. Within this family, growth differentiation factor 8 (GDF8) encodes the myostatin protein that is expressed during muscle development and in adult skeletal muscle [1]. Myostatin, like other members of the transforming growth factor-β (TGF-β) family, is synthesized by a 376 amino acid precursor protein including three domains namely, a C-terminal domain or active molecule, an N-terminal propeptide domain which will be cleaved at the RSRR site during maturation, and a signal sequence [1]. 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. Moreover, the only active form of the protein is the processed myostatin dimer [2].

Over a decade has passed, since myostatin was first identified by McPherron in mice and its biological function as a negative regulator of muscle growth [1]. Since then, variations in myostatin have been found to be associated with the "double-muscled" phenotype in various species of animals including mice [1], cattle [3], humans [4], dogs [5], pigs [6], and sheep [7]. The presence of variation in myostatin in other species and its association with variation in muscling suggests that further naturally occurring variation in myostatinin. Bakerwal goat can also affect muscle traits including skeletal muscle growth and meat yields. The GDF8 gene has been extensively investigated in bovines and a large number of alleles have been identified [8]. Several mutations in both the second and third exons strongly affect the phenotype and are described by [9] as responsible for musclehypertrophy (double-muscled) and have been responsible worldwide for increased meat production in several breeds.

Despite its important role in the control of muscle development, the Bakerwal goat myostatin gene has never been characterized, and the objective of the work described in this paper is to characterize the myostatin coding regions from Bakerwal and compare them to sheep in an effort to locate alterations in nucleotide and protein sequences.

2. Materials and Methods

Whole blood samples were obtained from ten adult Bakerwal breed goats. The blood samples were collected in Vacutainer tubes, homogenized and kept frozen once until needed for DNA extraction, which was performed by using Phenol-Chloroform-Proteinase-K Method [10].

After quantification and dilution of the DNA samples, the regions corresponding to the three exons of the Bakerwal goat *GDF*8 gene were amplified by PCR using primer pairs designed from *Capra hircus* DNA sequences (**Table 1**). Each 25 µL reaction contained 50 ng of sample DNA, 0.4 µM of each primer, 1X PCR buffer (10 mMTris-HCl, pH 8.0, 50 mM KCl), 2.0 mM MgCl2, 0.2 mM of each dNTP and 1 U of Taq DNA polymerase (Invitrogen). Amplification reactions were carried out in a thermocycler (Applied BioSystem), with 5

Table 1. Sequence of the forward (F) and reverse (R) primers used for the amplification and sequencing of Bakerwal goat myostatin exons.

Exon	Primers (5' to 3')	Reference
1	F-TGGCGTTACTCAAAAGCAAA R-AACAGCAGTCAGCAGAGTCG	[11]
2	F-TGGAGGCGTTCGTTCATT R-GATGGTAGCCCTGTACCCAA	[9]
3	F-TCTTTAATAATGACTCCCTGCG R-GAACACCCACAGCGATCTACT	[12]

min denaturation at 95˚C, 35 cycles of 94˚C for 1 min, 59˚C for 1 min and a 72˚C extension for 1 min, and a final extension at 72˚C for 4 min.

Sequencing was performed at Macrogen Inc. Korea, with an automated sequencer (Applied Biosystems) (**Figure 1**). The resulting sequences were aligned using the BioEdit program (BioEdit v5.0.9) and the obtained consensus sequences were used to compare it with GenBank caprine sequences using the BLAST algorithm [13]. The nucleotide sequences of exons 1, 2 and 3 of the GDF8 gene of Bakerwal goat were deposited in GenBank under Accession No KU980201, KU991727 and KX171679 respectively.

3. Results and Discussion

The ten goats sequenced did not show polymorphisms among themselves but 3 nucleotide variations were found when the goat myostatin gene was compared with the ovine myostatin gene. Exon 1 showed two alterations, the first was an A→G transition located at position 14 from the start codon and was responsible for the substitution of Glutamine with Arginine at position 5 of the protein chain. The second substitution was an A→G transversion at position 178 of the nucleotide sequence leading to a Isoleucine being replaced by valine at position 60 in the protein sequence. Exon 2 revealed the presence of one T→C transition at position 575 responsible for a Leu192Pro substitution in the amino acid sequence. No changes were observed in exon 3. All alterations in the presumed myostatin amino acids sequence as compared to ovine sequence are shown in **Figure 2**. And all alterations in the myostatin amino acids sequence as compared to other species of livestock animal sequences are shown in **Figure 3** and **Figure 4**.

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 [14].

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, high due to the close proximity of the species which belong to the same family Bovidae. Of the three alterations observed, none of the alteration could possibly represent a biological effect. Identifying mutations in sequences of myostatin gene using molecular techniques is an effective solution for the survey of double muscle phenotype and help the breeders to get important information in order to make the precise decision for management and selecting the best population for reproduction and it lets the breeders to have a lot of data about genetic status of *MSTN* gene in livestock animals.

It could be inferred that the *MSTN* gene may be a major gene or linked to the major gene affecting the goat growth traits. The polymorphic site could be a molecular marker-assisted selection program for body weight. Mutations have effects on the *MSTN* protein structure and on the biological function of the *MSTN* protein [8]. 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. Hadjipavlou *et al.* [15] found that two SNPs in the *MSTN* gene have a significant association with the muscle depth of commercial Charollais sheep. In two Norwegian sheep breeds, two different mutations in the *MSTN* coding region are associated with carcass conformation and fatness [16]. In pigs, mutations identified in noncoding regulatory regions affect the level of *MSTN* gene expression and/or are associated with growth, muscle mass and other carcass traits [17]. Esmailizadeh *et al.* [18] found a SNP in the *MSTN* gene affecting birth, growth, carcass and beef

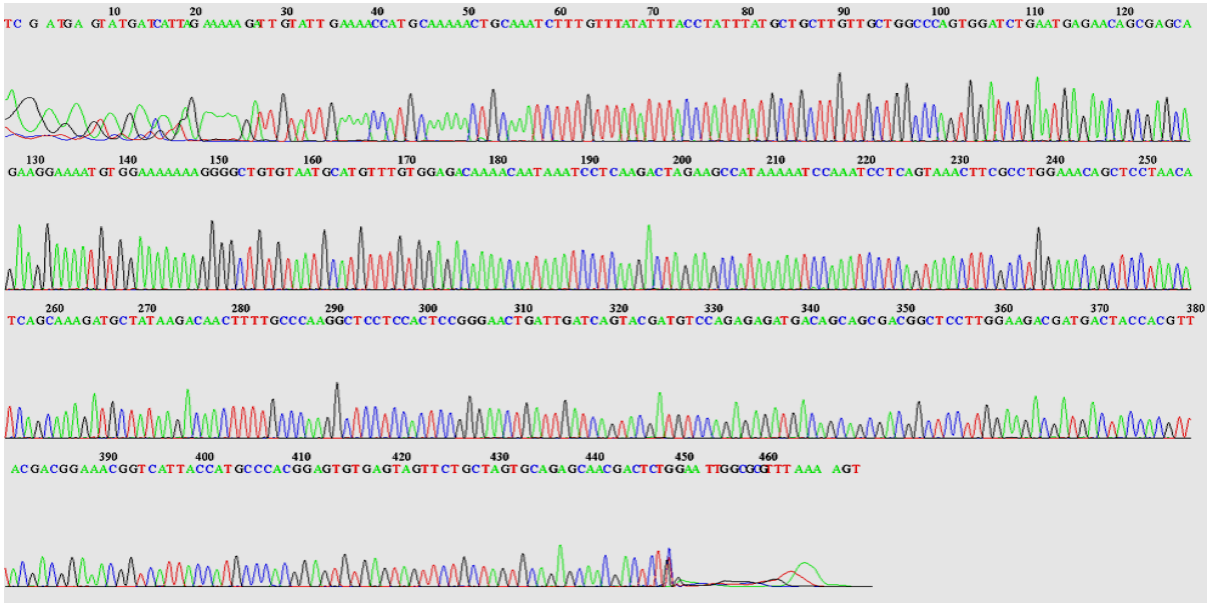

Figure 1. Sequencing results of exon-1 of *MSTN* gene.

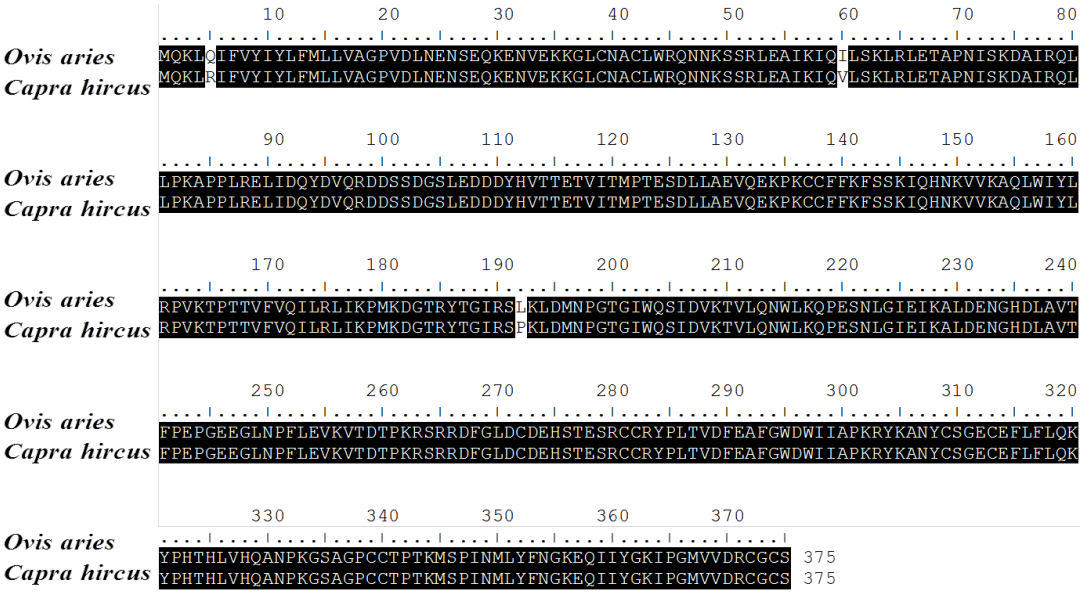

Figure 2. Comparative alignment of the presumed myostatin amino acid sequence from non double muscled *Ovis aries* (ENSOARG00000016285) and *Capra hircus*. Shaded amino acids indicate matching sequence consensus.

quality traits of *Bos taurus*. In cattle breeds, an 11-bp deletion in the coding sequence of the *MSTN* gene determines increased skeletal muscle mass, relevantly in shoulders and thighs, and the produced phenotype is known as double-muscling [19]. This supports the notion that further investigation of the *MSTN* variation in different goat breeds is needed. In goat breeds, a number of myostatin variants of different phenotypic consequence have been described across a variety of breeds [20], but there is scarcity of reports on the association analysis of SNPs with growth traits. 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.

4. Conclusion

In conclusion, we report the first characterization of the myostatin coding regions from Bakerwal goats present

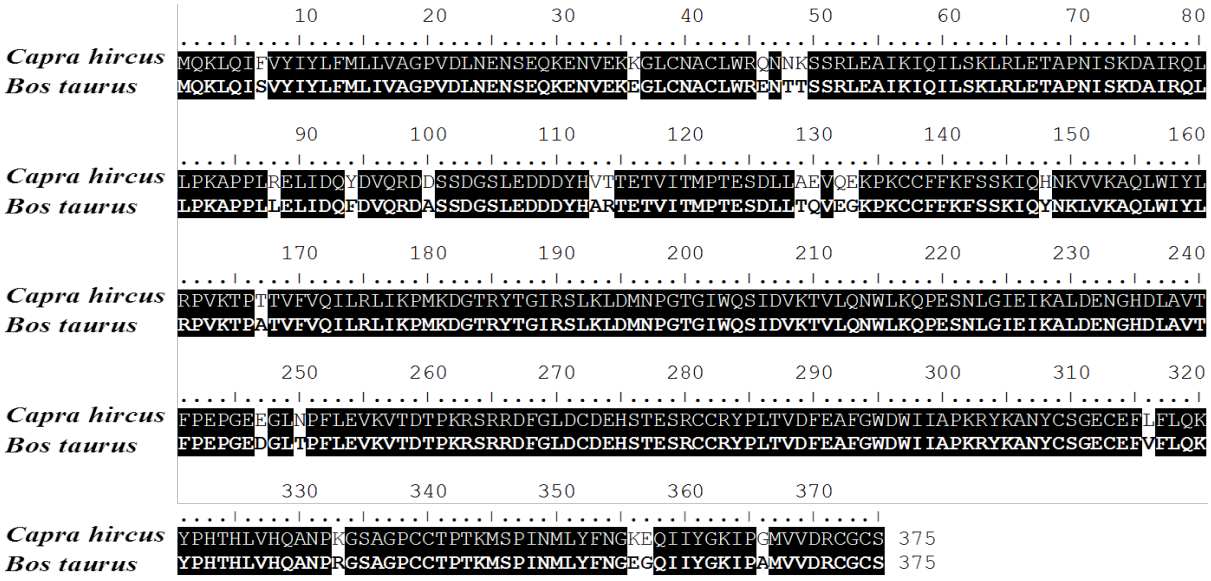

Figure 3. Comparative alignment of the myostatin amino acid sequence from *Bos taurus* and *Capra hircus*. Shaded amino acids indicate matching sequence consensus.

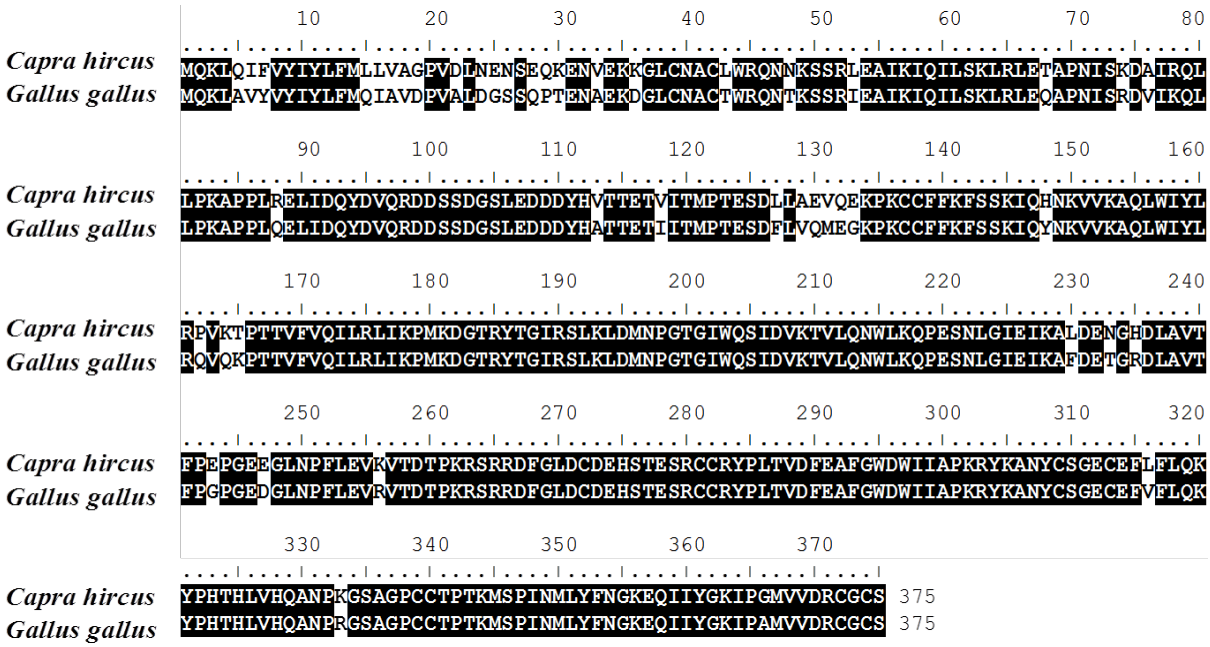

Figure 4. Comparative alignment of the myostatin amino acid sequence from *Gallus gallus* and *Capra hircus*. Shaded amino acids indicate matching sequence consensus.

in Kashmir valley and also its comparison with other livestock species of animals.

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