

Original Research Article

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Polymorphism in Exon-1 of *MSTN* Gene in Boer and Bakerwal Goats and its Association with Growth Traits

Wani A. Ahad*, Sajad A. Beigh, Mehraj uddin Reshi, Rumase A. Bhat, Aadil Yousuf, John Mohammad Wani, Mudasir Andrabi and Riaz A. Shah

Division of Animal Biotechnology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama Alusteng, Ganderbal, 190006, J&K, India

*Corresponding author

ABSTRACT

Myostatin or growth and differentiation factor 8 (GDF8), is a skeletal muscle regulator factor that determines muscle mass in animals including human beings. Mutation in the myostatin gene sequence controls its regulating function and results in growth via hypertrophy of muscles. Mutation in myostatin gene has been found in various species of animals and has three exons and two introns in all species. Considering the *MSTN* gene as a candidate gene for gene assisted selection for growth traits, the present study was aimed to study its polymorphism and its association in Boer and Bakerwal goats in Kashmir valley. We screened polymorphism of the myostatin gene in 600 goats from two goat populations (Boer and Bakerwal) and applied PCR and DNA sequencing analysis to reveal single nucleotide polymorphisms (g.368A>C (p.Lys to Thr). At g.368A>C locus, the frequencies of g.368A allele were 0.70–0.80, and the frequencies of g.368C allele were 0.20–0.30 in Boer goats. And at g.368A>C locus, the frequencies of g.368A allele were 0.60–0.80, and the frequencies of g.368C allele were 0.20–0.40 in Bakerwal goats. Compared to AC genotype, those with AA genotype had superior body weight in Boer goats (12 & 14 months) (41.68 ± 0.41 vs 43.21 ± 0.31 , $P < 0.05$) (43.57 ± 0.41 vs 46.00 ± 0.05 , $P < 0.05$) respectively. In addition, the Boer goats (13 months) with AA genotype (72.50 ± 0.50 cm) had greater body height than those with AC genotype (70.37 ± 0.26 cm). Similarly, AA genotype in Bakerwal goats have high body weight than AC genotype (40.16 ± 0.54 vs 42.75 ± 0.25), $P < 0.01$ (12 months), body length (81.12 ± 0.63 vs 86.00 ± 1.00), $P < 0.05$ (14 months) and chest circumference (86.42 ± 0.61 vs 89.66 ± 0.33), $P < 0.05$ (13 months) respectively. These SNPs would provide molecular genetic material for studying the meat performance of goats.

Keywords

MSTN,
Polymorphism,
Boer, Bakerwal,
Growth traits,
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Introduction

Majority of the goat population is nondescript that is capable of producing very less meat and milk. With shrinking resources and increasing demand of goat meat and milk, there is urgent need to genetically improve and manage these animals through modern

scientific tools to enhance their productivity. Although, there is a need to utilize between-breed genetic differences for higher yields, greater emphasis is required on improvement of adapted indigenous breeds/types because of valuable adaptive traits they have developed

over long periods of time through natural selection. Breeding strategies for enhancing goat meat and milk production in the country is needed (Dekkers and Hospital, 2002). Advances in genomics and proteomics offer an opportunity to look at collective behavior of a large number of genes under a given situation. There is an urgent need to map the goat genome. Therefore there is a need to annotate the genes at the protein level by proteomic approaches.

Boer goat (*Capra hircus*) is considered to be one of the most important goat breeds for meat production. It has gained worldwide recognition for excellent body conformation, fast growing rate and good carcass quality. It has a strong impact on the meat goat industry globally (Schina 2009). Boer Goat is indigenous to South Africa and is dual purpose and claimed to bear all the attributes which a high yielding improved breed of goat should possess. It is also adapted to a wide range of climatic conditions and in addition, it produces on an average 2 kgs of milk per day containing 7-9% fat. The Boer goat has a birth weight of 2.4 kgs weaning weight 35 kgs and 115 kg adult weight in males. With the introduction of this goat in Kashmir, there would be a boost to meat production in the valley. The Bakerwal breed of goat is a good meat purpose breed that attains a significant body mass under a low input production system. The sheep and goats constitute the main livestock wealth.

The huge Bakerwal goats fetch attractive prices on the festive occasion of Eid when people purchase them for sacrificial purposes. Apart from obtaining meat from sheep and goats, the Bakerwals also obtain milk, hides and wool from them. The Bakerwal goats augment the meat production of the otherwise meat deficient state of J&K. Indeed, they are the real custodians of our livestock (Ahad *et al.*, 2016).

Mutations within the GDF8 gene inactivate its expression or turn out a non-functional protein that results in dramatic muscularity and a “double-muscling” development in several species. Two traits, carcass quality and quantity are the most important factors in the meat industry. These traits have an effect on vital factors like genetics, climate, nutrition and so on. And presently much research is done to predict carcass traits in livestock animals. Among the factors that confirm the meat quality, genetic factors have a prominent role on meat quality (Kioumarsis *et al.*, 2008). Trend is growing towards demand for lean meat with low levels of fat, that conformable with human health standards scale. Results development of breeding programs and genetic improvement of economic animals with higher production in last decades. At recent years, there has been a beautiful progress on quantities and genetics through mapping of the economic animals’ genome and new opportunities are provided for increase meat production and market place for demand customers (Dekkers and Hospital, 2002). Numerous QTLs have been identified that influence muscle growth and carcass traits. At the current time few mutations in these QTLs are known and under survey. The mutation in myostatin gene reported that has been associated with increase in weight and carcass traits in many species (Masri *et al.*, 2011). Mutated *MSTN* gene and inactive myostatin have a major association with growth rate and carcass traits. *MSTN* gene causes increase in muscle mass. Mutation in myostatin has a very important impact on carcass quality and reduces quantity of fat in the dead body. In animals with hypertrophy phenotype, fat quantity cuts down into 50 percent and muscle mass will increase 20 percent additionally connective tissues decrease in these animals thus delicate and delicious meat is created. The most determinants of quick growth in animals is the increase of vegetative cell growth and

proliferation. Myostatin may be a negative protein that inhibits terminal differentiation of myoblasts and therefore the proliferation of myogenic cells (Thomas *et al.*, 2000). The growth traits of animals are regulated by several genes and are of primary concern throughout breeding for determining the animal's economical price. Generally, distinguishing genetic markers for growth traits is the initial and significant condition to establish a marker-assisted selection system. The gene sequence and its variations are of prime importance in studying gene expression and function. Polymorphisms in *MSTN* gene were shown to possess a major difference in several goat breeds. It was reported that the TTTTA deletion in the *MSTN* gene had emerged in several species and may be distinctive for goats, compared with sheep, cattle, Buffalo, domestic yak, pigs, and humans (Hadjipavlou *et al.*, 2008).

Many candidate genes have been detected in goats. However, few investigations have been carried out on the polymorphism of some genes involved in the control of productive traits. Candidate genes have an effect on the physiological pathway, metabolism and the expression of phenotypes. For growth traits, growth hormone (*GH*), leptin (*LEP*), insulin like growth factor I (*IGF-I*), growth hormone receptor (*GHR*), caprine pituitary specific transcription factor-1 (*POUIF1*), caprine myostatin (*MSTN*) and bone morphogenetic protein (*BMP*) genes are necessary for bone formation, birth weight, weaning weight, body condition and muscle growth (Schina 2009).

Myostatin/Growth differentiation factor (*MSTN/GDF8*) is a member of the transforming growth factor- β (TGF- β) superfamily. It is produced by skeletal muscle and acts as a negative regulator of muscle growth, which plays an essential role in the regulation of muscle growth and meat quality (Zhang *et al.*, 2013). Myostatin and activin

interact and activate a heterodimeric receptor complex with serine-threonine kinase activity, comprising a type II receptor, activin receptor 2 (ACVR2 and ACVR2B), and a type I receptor, active in receptor-like kinase 4 and 5 (ALK4 and ALK5). Myostatin signaling in myofibers is mediated by phosphorylation and nuclear translocation of transcription factors, and formation of heterodimers. Heterodimer complex binds to the promoters in the target genes & forms a transcription repressor complex. Myostatin represses the levels of myogenic regulatory factors (MyoD, Myf4, Myf5 and myogenin) leading to the inhibition of myogenic differentiation. Myostatin inhibits myogenic differentiation factor (MyoD) activity and expression via Smad3 resulting the failure of the myoblasts to differentiate into myotubes (Figure 2).

The Caprine *GDF8* gene has 3 exons and 2 introns (Bellinge *et al.*, 2005) (Figure 1). 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). Mutations in sheep myostatin gene also has a significant role in muscular growth (Boman *et al.*, 2009). As a consequence, the *MSTN* gene is primarily responsible for muscle development and could be a potential candidate gene for animal muscle growth. The single nucleotide polymorphisms (SNPs) with *MSTN* would provide molecular genetic material for studying the meat performance of goat and its further exploitation for "double muscling" phenomenon.

Materials and Methods

Samples and DNA extraction

Genomic DNA samples were obtained from 600 goats: Boer goat (N = 300) and Bakerwal goat (N = 300), which were both reared at

Mountain Research Centre for Sheep and Goat (MRCSG) and Sheep Breeding Farm Banihal respectively. Blood samples were collected from goats in vacutainer tubes, homogenized and kept frozen until needed for DNA extraction, which was performed by using Phenol-Chloroform-Proteinase-K Method (Sambrook and Russell, 2001). The selection of animals was based on age (12-14 months), based on their growth characteristics (Zhang *et al.*, 2013). The growth traits of Boer and Bakerwal goats from the same farm were recorded for statistical analysis. The following traits were evaluated: body weight, body height, body length and chest circumference (12-14 months of age). Body weight (BW) was taken by using the Salter hanging spring type scale. Body height was taken between the most dorsal point of the withers and the ground level (Pesmen and Yardimci, 2008). A tailor's tape was used to determine; Body length (BL), measured from the anterior point of the shoulder joint to the posterior extremity of the pin bone (Katongole *et al.*, 1996). Chest circumference was measured by taking the circumference of the chest using a tailor's tape calibrated in cm, taken as the circumference of the body immediately behind the shoulder blades in a vertical plane, perpendicular to the long axis of the body (Pesmen and Yardimci, 2008).

Primer synthesis and PCR

After quantification and dilution of the DNA samples, the 492-bp fragment of exon-1 of caprine *MSTN* gene was amplified (Figure 3) by PCR using primer reported by Li *et al.*, (2008) as shown in table 1. Each 25 μ L reaction contained 50 ng of sample DNA, 0.4 μ M of each primer, 1X PCR buffer (10 mM Tris-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 5min 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 and statistical analysis

The PCR amplified products were sequenced at Macrogen Inc. Korea, with an automated sequencer (Applied Biosystems) (Figure 6). 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 sequence using the BLAST algorithm, to find out the polymorphism with respect to 368A>C of *MSTN* gene and the genotypes of two breeds were recorded accordingly. An association study was carried out for the SNP located in exon-1 of *MSTN* gene with goat growth traits. The data generated was subjected to Mann Whitney U test by using SPSS20.0 statistical software and significance was determined at $p < 0.05$ to estimate the association between different genotypes and growth traits.

Results and Discussion

Analysis of sequencing results

The PCR products of different genotypes were sequenced. We found single mutation located at 368A>C (Figures 4 and 5), by carrying out a BLAST search of goat *MSTN* gene sequences in GenBank sequence. The nucleotide sequences of exon-1 of the *MSTN* gene was deposited in GenBank under Accession No KU980201. The substitution 368A>C, caused a variation of amino acid lysine to threonine. The following conclusions were demonstrated by Mann Whitney U test by using SPSS20.0 statistical software, and genotypic and allelic frequencies in the 2 different goat breeds was calculated. These genotypic results indicated

that allele A was absolutely dominant in Boer goats and Bakerwal goats.

Association of *MSTN* polymorphism with growth traits in Boer goats

The mutation from A to C at position 368 in the exon 1 of *MSTN* gene results in substitution of lysine by threonine of the peptide chain encoded by *MSTN* gene, indicated as 368A>C polymorphism with two alleles, two genotypes were produced, AA and AC. The two different genotypes were then associated with growth traits. There was a significant difference in body weight Boer goats (12 & 14 months) between AA and AC group ($P < 0.05$), and also there was a significant difference in body height between AA and AC group ($P < 0.05$) in case of 13 months goats. As from the above findings it was evident that AA genotype is associated with high body height of Boer goats (Table 2).

Association of *MSTN* polymorphism with growth traits Bakerwal goats

Like that of Boer breed, Bakerwal goat were also grouped into three and two groups, according to their age and genotypes respectively. The association was studied between genotypes and growth traits. There was a significant difference in body weight Bakerwal goats (12 months) between AA and AC group ($P < 0.01$) and also there was a significant difference in body length in Bakerwal goats (13 months) between AA and AC group ($P < 0.01$) and significant difference in chest circumference between AA and AC group ($P < 0.05$) (Table 3).

Boer and Bakerwal populations at 368A>C loci had moderate genetic diversity. At g.368A>C locus, the frequencies of g.368A allele were in the range of 0.70–0.80, and the frequencies of g.368C allele were in the range

of 0.20–0.30 in case of Boer goats. Frequencies of alleles found in this study for Boer goats were same to the previously reported frequencies (An *et al.*, 2011). And at g.368A>C locus, the frequencies of g.368A allele were 0.60–0.80, and the frequencies of g.368C allele were 0.20–0.40 in Bakerwal goats. However no such type of studies has been done in case of Bakerwal goats.

The genotype frequencies in Boer goats for AA and AC were found to be in the range of 0.70-0.80 and 0.20-0.30 (An *et al.*, 2011), which were similar to the genotype frequencies found in Bakerwal goats. No homozygotes CC were found in the population.

There is a factor that could contribute to this, is sampling effect because the sample sizes were relatively small (Moreau *et al.*, 1998; An *et al.*, 2010). The findings were also reported by Hadjipavlou *et al.*, (2008) and (Boman and Vage, 2009) in Charollais sheep and in two Norwegian sheep breeds. Also, findings were also reported by Esmailizadeh *et al.*, (2008) and Gill *et al.*, (2009) in *Bos Taurus*.

Compared to AC genotype, those with AA genotype had superior body weight in Boer goats (12 and 14 months) (41.68 ± 0.41 vs 43.21 ± 0.31 , $P < 0.05$) (43.57 ± 0.41 vs 46.00 ± 0.05 , $P < 0.05$) respectively.

In addition, the Boer goats (13 months) with AA genotype (72.50 ± 0.50 cm) had greater body height than those with AC genotype (70.37 ± 0.26 cm).

Similarly, AA genotype in Bakerwal goats have high body weight (40.16 ± 0.54 vs 42.75 ± 0.25), $P < 0.01$ (12 months), body length (81.12 ± 0.63 vs 86.00 ± 1.00), $P < 0.05$ (14 months) and chest circumference (86.42 ± 0.61 vs 89.66 ± 0.33), $P < 0.05$ (13 months) respectively.

Fig.1 Structure of *MSTN* gene

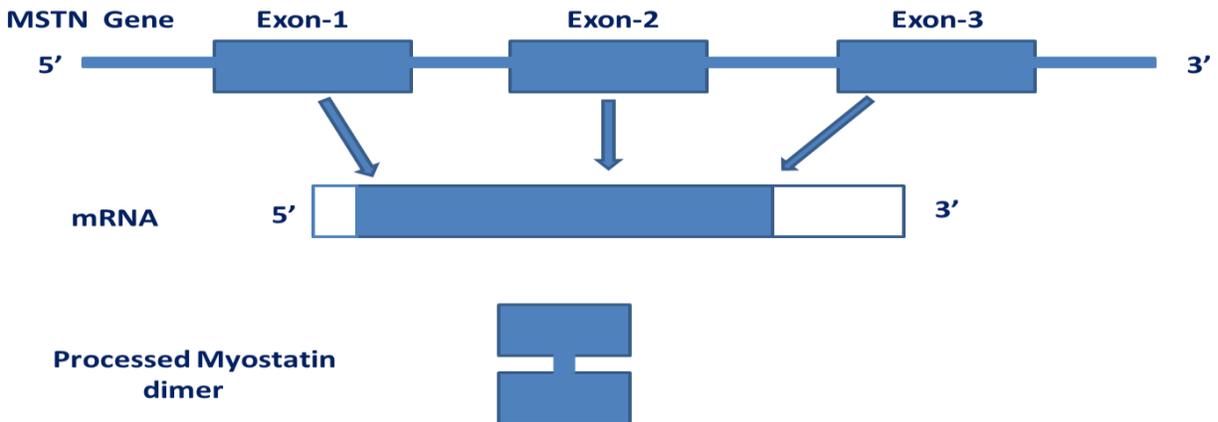


Fig.2 Physiological Action of Myostatin

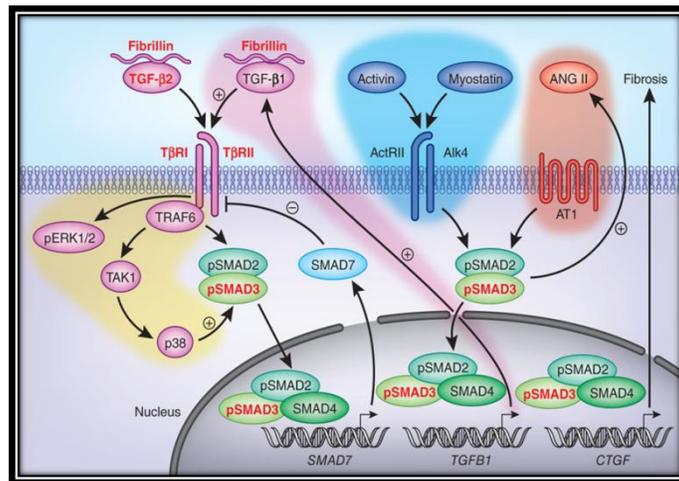


Fig.3 Gel electrophoresis picture of amplified fragment of *MSTN* gene in Boer (a) and Bakerwal (b) goat Size: 492bp

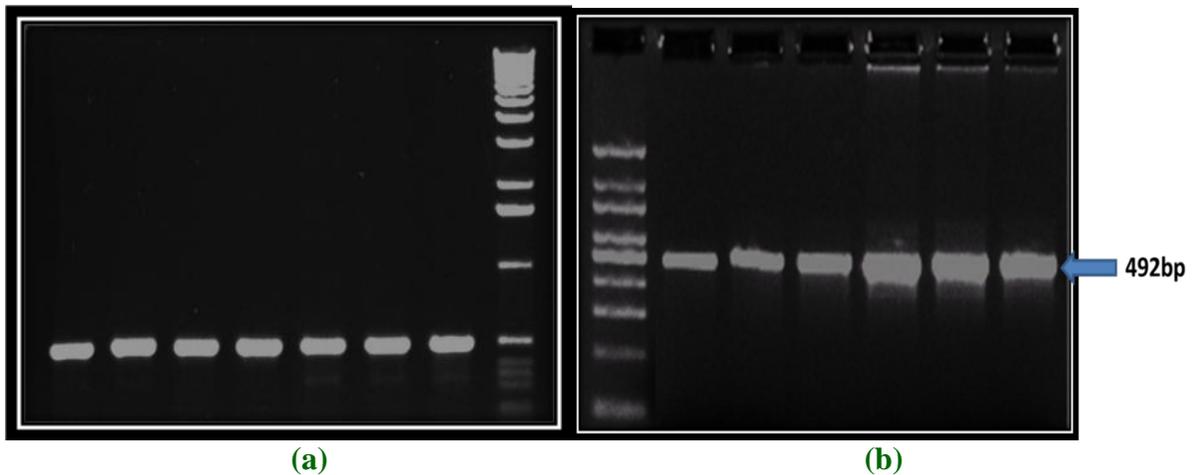


Fig.4 & 5 Chromatograms showing mutation site in Exon-1 of *MSTN* Gene in Boer and Bakerwal goats

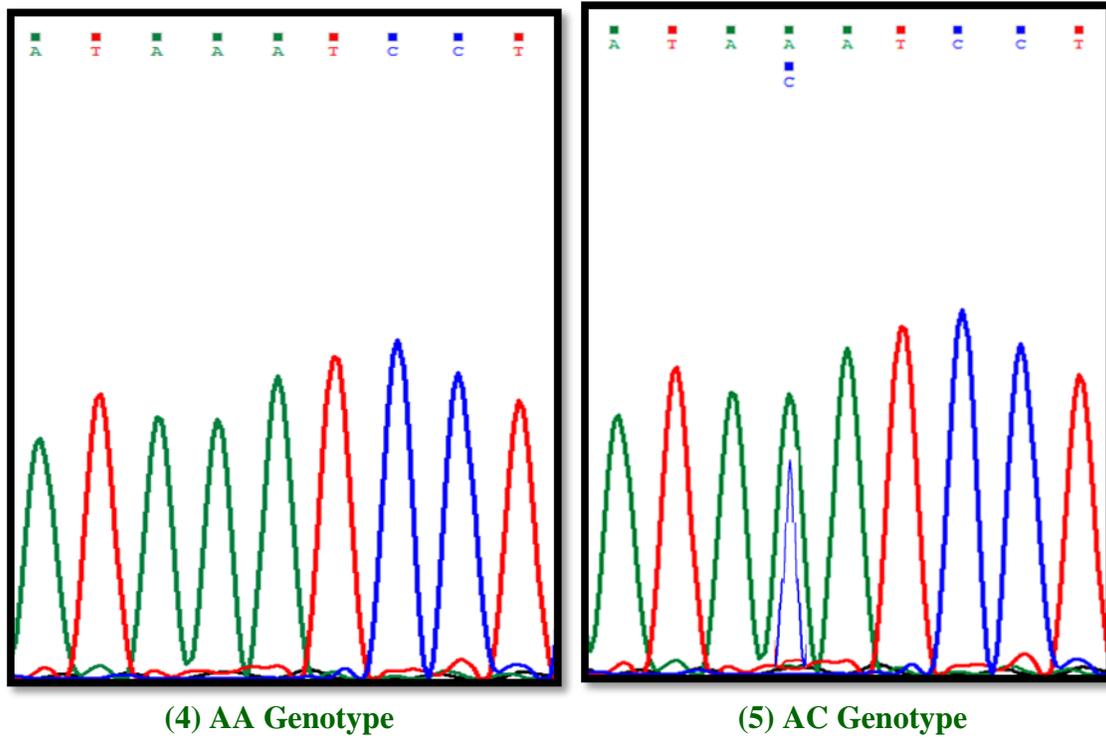


Fig.6 Sequencing result of Exon-1 of myostatin gene

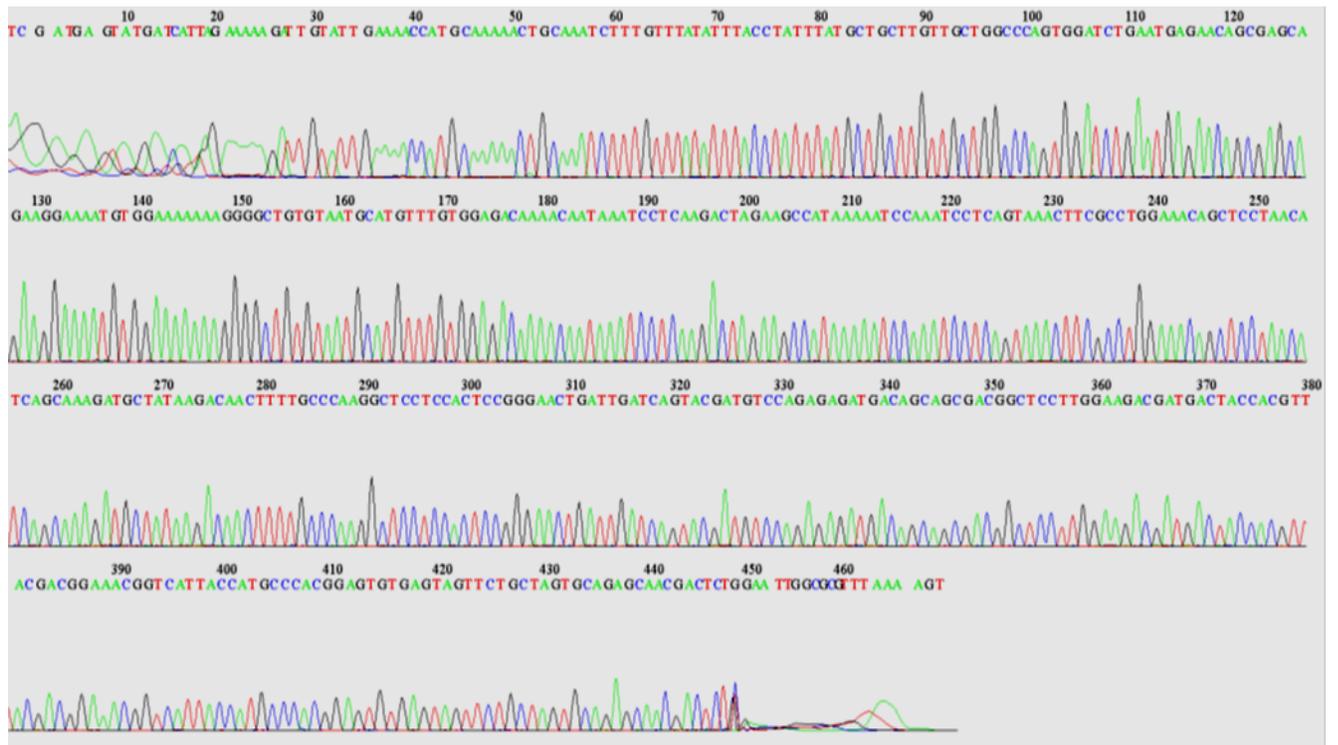


Table.1 Sequence of the forward (F) and reverse (R) primers used for the amplification of goat myostatin gene exon-1

Exon	Primers (5' to 3')	Reference
1	F-TGGCGTTACTCAAAGCAA R-AACAGCAGTCAGCAGAGTCG	Li <i>et al.</i> , 2008

Table.2 Least square means (\pm SE) analysis between genotype of polymorphism in the *MSTN* gene and growth traits of Boer goat

Breed/Age		GE	GF	Growth traits		
BOER		Body weight	Body height	Body length	Chest	Circumference
12 months	AC	30.0%	41.68 \pm 0.41 ^A	70.28 \pm 0.35	79.42 \pm 0.36	85.71 \pm 0.52
	AA	70.0%	43.21 \pm 0.31 ^B	71.33 \pm 0.33	79.66 \pm 0.33	88.00 \pm 0.57
	P		P = 0.03	P = 0.12	P = 0.71	P = 0.06
13 months	AC	20.0%	43.31 \pm 0.17	70.37 \pm 0.26 ^A	79.50 \pm 0.18	87.00 \pm 0.70
	AA	80.0%	44.83 \pm 0.76	72.50 \pm 0.50 ^B	80.50 \pm 1.50	88.50 \pm 0.50
	P		P = 0.08	P = 0.04	P = 0.66	P = 0.35
14 months	AC	30.0%	43.57 \pm 0.41 ^A	71.00 \pm 0.37	80.00 \pm 0.53	87.85 \pm 0.67
	AA	70.0%	46.00 \pm 0.05 ^B	72.33 \pm 0.33	81.33 \pm 0.66	88.66 \pm 0.88
	P		P = 0.02	P = 0.08	P = 0.18	P = 0.48

GE = Genotype; GF = Genotype Frequency; SE = standard error of the mean. Means followed by capital superscript letters differ significantly at $P < 0.05$.

Table.3 Least square means (\pm SE) analysis between genotype of polymorphism in the *MSTN* gene and growth traits of Bakerwal goat

Breed/Age		GE	GF	Growth traits		
BAKERWAL		Body weight	Body height	Body length	Chest	Circumference
12 months	AC	40.0%	40.16 \pm 0.54 ^a	70.66 \pm 0.49	79.16 \pm 0.30	85.50 \pm 0.42
	AA	60.0%	42.75 \pm 0.25 ^b	71.50 \pm 0.64	81.25 \pm 0.85	87.50 \pm 0.64
	P		P = 0.01	P = 0.38	P = 0.07	P = 0.05
13 months	AC	30.0%	44.44 \pm 0.49	71.14 \pm 0.40	79.42 \pm 0.20 ^a	86.42 \pm 0.61 ^A
	AA	70.0%	46.41 \pm 0.77	72.66 \pm 1.20	83.00 \pm 1.15 ^b	89.66 \pm 0.33 ^B
	P		P = 0.11	P = 0.23	P = 0.01	P = 0.02
14 months	AC	20.0%	45.71 \pm 0.55	74.00 \pm 0.46	81.12 \pm 0.63 ^A	88.00 \pm 0.65
	AA	80.0%	48.25 \pm 0.25	76.00 \pm 1.00	86.00 \pm 1.00 ^B	91.50 \pm 1.50
	P		P = 0.05	P = 0.14	P = 0.04	P = 0.11

GE = Genotype; GF = Genotype Frequency; SE = standard error of the mean. Means followed by capital superscript letters differ significantly at $P < 0.05$. Means followed by small case superscript letters differ significantly at $P < 0.01$.

Hence, the physiological functions and the results obtained in our investigation suggest that the myostatin gene play a significant role in expression of growth traits in goats. It could be interpreted that the myostatin gene may be a major gene affecting the goat economic traits. The polymorphic site can be a molecular marker-assisted selection program for body weight. The results of relationship between the different genotypes and growth traits showed that the 368A>C locus mutation was significantly associated with the growth traits $P < 0.05$. The missense mutation (p.Lys to Thr) has effects on the myostatin protein structure and on the biological function of the myostatin protein (Grobet *et al.*, 1997, 1998). Hadjipavlou *et al.*, (2008) found that two SNPs in the myostatin gene have a significant association with the muscle depth of Charollais sheep. Two different mutations in the myostatin coding region in two Norwegian sheep breeds are associated with carcass conformation (Boman and Vage, 2009). In pigs, mutations identified in noncoding regulatory regions affect the level of myostatingene expression and are associated with muscle mass and other carcass traits (Stinckens *et al.*, 2008). Esmailzadeh *et al.*, (2008) found a SNP in the myostatingene affecting growth, carcass and beef quality traits of *Bostaurus*. In cattle breeds, an 11-bp deletion in the coding sequence of the myostatin gene determines increased skeletal muscle mass and the produced phenotype is known as double-muscling (Gill *et al.*, 2009). This supports that further investigation of the myostatin variation in different goat breeds is needed. In goats number of myostatin mutations of different phenotypic expressions has been described in variety of breeds (Javanmard *et al.*, 2010), but there is scarcity of reports on the association analysis of SNPs with growth traits. The biochemical and physiological functions and the results obtained in our study indicate that the myostatin gene may play

significant role in affecting the economic traits in goats. The polymorphisms we have identified in exon-1 of the myostatin gene (368A>C locus) could be potential genetic markers for growth traits in goats.

The obtained results indicate that the exon-1 of myostatin gene evaluated in the present study is polymorphic in boer and bakerwal goats and can be noticed for the marker assisted selection, especially to improve the body weight. Further characterization of mutations responsible for muscle hypertrophy in goats will not only allow producers to incorporate gene-assisted selection (GAS) into their breeding programs, but it will also contribute to our basic understanding of muscle formation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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