



Short Communication

Polymorphisms of the myostatin gene (*MSTN*) and its relationship with growth traits in goat breeds

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ABSTRACT. Mutations in the myostatin (*MSTN*) gene can inactivate its expression and result in a non-functional protein, which leads to dramatic muscularity and a “double-muscling” phenomenon in many species. Using gene sequencing and polymerase chain reaction-single-strand conformation polymorphism methods, polymorphisms of the *MSTN* gene were investigated as a candidate marker for growth in 288 goats. The results showed 2 novel single nucleotide polymorphisms: DQ167575 g.197G>A and 345A>T. Three potential genotypes (AA, AB, and BB) of substitution 197G>A in the 5'-untranslated region were detected in the 2 breeds. The polymorphism (CC and CD) of substitution 345A>T in exon I was segregated. The genetic diversity analysis revealed that Boer goat and Anhui white goat possessed intermediate genetic diversity in the P1 and P3 loci. Significant

associations between the genotypes of the P3 locus and body weight, body length, and body height were observed in Boer goat and Anhui white goat ($P < 0.05$). 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.

Key words: Goat; Myostatin gene (*MSTN*); Polymorphism; PCR-SSCP; Growth traits

INTRODUCTION

Myostatin (*MSTN*) is a member of the transforming growth factor- β superfamily, which plays an essential role in the regulation of muscle growth and meat quality (Zhang et al., 2012). The *MSTN* gene has 3 exons and 2 introns (Bellinge et al., 2005), and encodes a glycoprotein that is expressed widely in skeletal muscle. Changes in the gene structure and expression may regulate the expression of target genes, thereby changing the composition of muscle fibers and causing a variation of muscle weight (Chen, 2008). Meanwhile, some studies demonstrated that the *MSTN* gene inhibited *MyoD* activity and expression via Smad3, resulting in the failure of myoblasts to differentiate into myotubes. These studies demonstrated that the *MSTN* gene plays a critical role in myogenic differentiation (Jin, 2011).

Mutations in the *MSTN* gene can inactive 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). At present, many scholars had done numerous researches in pigs and cattle (Walsh and Celeste, 2005; Fan et al., 2010). In sheep, mutations in the *MSTN* gene also had an important role in muscular development (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. However, similar investigations about the properties of the *MSTN* gene in goat breeds have been limited (Liu et al., 2006). Therefore, in this study, PCR-based single-strand conformation polymorphism (SSCP) and DNA sequencing were applied to detect single-nucleotide polymorphisms (SNPs) of the *MSTN* gene from 2 reference goat populations (Anhui white goat and Boer goat, which play an important role in the goat industry as the main meat goat breed in central China). These SNPs would provide molecular genetic material for studying the meat performance of goat.

MATERIAL AND METHODS

Samples and DNA extraction

Genomic DNA samples were obtained from 288 goats: Anhui white goat (AH, N = 184) and Boer goat (BR, N = 104), which were both reared at the Hefei Bo Da Livestock Technology Development Co., China, in 2011. Genomic DNA was extracted from all blood samples using a standard phenol-chloroform extraction method.

Primer synthesis and PCR

Using the goat *MSTN* gene sequence (DQ167575), 3 pairs of primers were designed to amplify the 5'-untranslated region (UTR) and exon I fragments in the *MSTN* gene: P1 sense - 5'-CACAGATCCCGACGACAC-3' and P1 antisense - 5'-GGAAGAAGTAAGAACAAG-3'; P2 sense - 5'-CTTGTTCTTACTTCTTCC-3' and P2 antisense - 5'-ATCCTCAAGACTAGAAGCC-3'; P3 sense - 5'-GGCTTCTAGTCTTGAGTAC-3' and P1 antisense - 5'-CGACTCTGCTGACTGCTGT-3'.

The PCRs were performed in a final volume of 20 μ L, containing 2 μ L DNA template, 0.5 μ L each primer, 2.4 μ L dNTPs, 0.5 μ L *Taq* DNA polymerase, 2 μ L 10X reaction buffer, and 12.1 μ L ddH₂O. The PCR profile was 5 min at 95°C, followed by 32 cycles of 30 s at 94°C, 30 s at 56°C, and 40 s at 72°C, and a final extension of 10 min at 72°C. The PCR products were stored at 4°C and were detected by 1% agarose gel electrophoresis.

Detecting genetic variation

The SSCP method was used to scan the mutations in the *MSTN* gene. Aliquots of 2 μ L PCR products were mixed with 8 μ L denaturing solution, heated for 10 min at 98°C, and chilled in ice immediately for 7 min. The denatured DNA was subjected to 10% PAGE analysis, which was run in 1X TBE buffer at 300 V for 30 min, then at a constant 240 V for 30 min, and finally at a constant 110 V for 12 h. The gels were stained with 0.2% silver nitrate and visualized with 3% NaOH solution. We took photographs of the results and recorded the genotypes (Jin et al., 2012).

Haplotype sequencing and statistical analysis

After the polymorphisms were detected, the PCR products of different electrophoresis patterns were selected for sequencing. The linear model with fixed effects was used to analyze the relationships between genotypes and growth traits of the 2 goat breeds. The linear model used was as follow: $Y_{ijk} = \mu + \text{Breed}_i + \text{Age}_j + \text{Marker}_k + e_{ijk}$, where Y_{ijk} is the observation of the trait, μ is the least-square mean, Breed_i is the effect of breed, Age_j is the effect of age, Marker_k is the effect of marker genotype, and e_{ijk} is the residual effect.

RESULTS

PCR amplification and SSCP detection

Three pairs of primers in this study were designed to amplify the goat genomic DNA, and the quality of the PCR products was checked by electrophoresis on 1% agarose gel. The PCR products had a single and clear strip, without smearing, which were appropriate for PCR-SSCP analysis. Results of PCR-SSCP showed that three genotypes (AA, AB, and BB) were present in the 5'-UTR, whereas only 2 genotypes (CC and CD) were detected in exon I.

Analysis of sequencing results

The PCR products of different genotypes were cloned and sequenced. We found 2 mutations located at 197 and 345 bp, respectively, by carrying out a BLAST search of goat *MSTN* gene sequences in GenBank (DQ167575). The substitution 197G>A was a synonymous mutation, which did not cause the mutation of amino acid. The substitution 345A>T CC genotype caused a variation of amino acid, compared with the CD genotype, as shown in Figure 1.

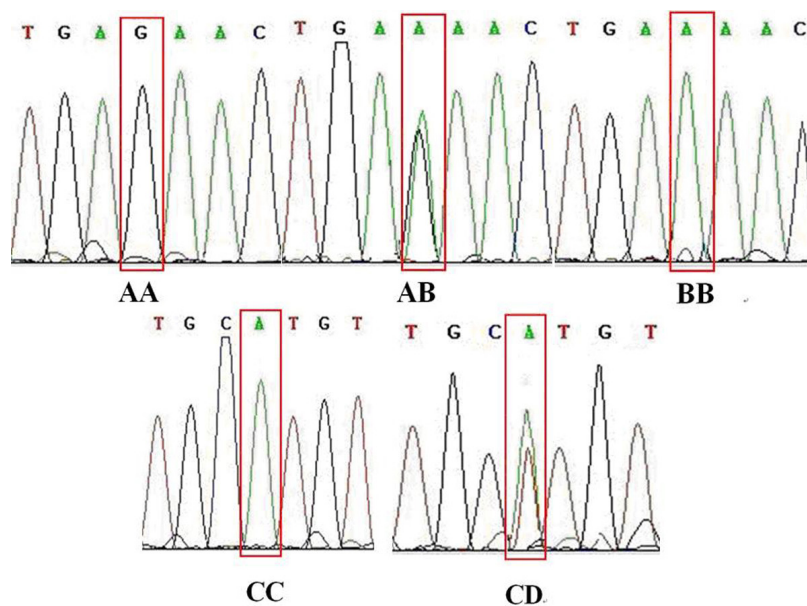


Figure 1. Sequence alignments of AA, AB, BB genotypes of primer P1, and CC and CD genotypes of primer P3 in the goat *MSTN* gene

Genetic polymorphisms of the *MSTN* gene in different goat breeds

The following conclusions were demonstrated by the chi-square test, and genotypic and allelic frequencies in the 2 different goat breeds. These genotypic results indicated that allele A was absolutely dominant in Anhui white goats, whereas allele B was the dominant allele in Boer goat populations. Genotype composition in BR goat: AA, 24.0% (25); BB, 43.3% (45); AB, 32.7% (37). Genotype composition in AH goat: AA, 56.0% (103); BB, 9.8% (18); AB, 34.2% (63). Genotype composition in BR goat: CC, 69.2% (72); CD, 30.8% (32). Genotype composition in AH goat: CC, 60.9% (112); CD, 39.1% (72). These data are shown in Table 1.

Association of genotypes in the *MSTN* gene with goat growth traits

The genetic variations within 5'-UTR and exon I in the *MSTN* gene and the association between the genotypes and the growth traits (body weight, body height, body length, and chest circumference) in goat are shown in Table 1. It shows that there are significant associations

between the genotypes of the P3 locus and body weight, body length, and body height in the BR and AH goats ($P < 0.05$). BR goats with the CC genotype outperformed those with the CD genotype by approximately 2.12 kg in body weight, 1.38 cm in body height, and 1.88 cm in body length ($P < 0.05$). Meanwhile, AH goats with the CC genotype outperformed those with the CD genotype by approximately 20.80 kg in body weight, 2.02 cm in body height, 2.58 cm in body length, and 2.05 cm in chest circumference ($P < 0.05$). Moreover, there was a tendency for individuals with the BB genotype to have better performances in body height and body length than those with AA and AB genotypes, although no significant differences were apparent ($P > 0.05$).

Table 1. Least square means (\pm SE) analysis between genotype of polymorphism in the *MSTN* gene and growth traits of goat.

Loci	Breeds	GE	GF	Growth traits (12 months)			
				Body weight	Body height	Body length	Chest circumference
P1	BR	AA	24.0% (25)	42.25 \pm 0.64	71.78 \pm 0.25	79.92 \pm 0.29	88.41 \pm 0.37
		AB	32.7% (34)	41.36 \pm 0.27	71.36 \pm 0.44	79.44 \pm 0.51	89.15 \pm 0.64
		BB	43.3% (45)	43.81 \pm 0.43	70.00 \pm 1.73	80.00 \pm 1.96	87.50 \pm 2.51
		P		P = 0.411	P = 0.45	P = 0.71	P = 0.610
	AH	AA	56.0% (103)	35.86 \pm 0.28	65.00 \pm 1.07	73.60 \pm 0.27	85.58 \pm 0.31
		AB	34.2% (56)	36.96 \pm 0.32	64.97 \pm 0.22	71.90 \pm 1.43	83.81 \pm 1.61
		BB	9.8% (18)	36.61 \pm 0.21	64.56 \pm 0.32	73.38 \pm 0.43	85.70 \pm 0.49
		P		P = 0.342	P = 0.562	P = 0.470	P = 0.871
P3	BR	CC	69.2% (72)	43.68 \pm 0.38 ^A	71.71 \pm 0.63 ^A	81.46 \pm 0.73 ^a	89.08 \pm 0.93
		CD	30.8% (32)	41.56 \pm 0.29 ^B	70.33 \pm 0.22 ^B	79.58 \pm 0.26 ^b	88.47 \pm 0.33
		P		P = 0.007	P = 0.000	P = 0.021	P = 0.480
		P		P = 0.003	P = 0.013	P = 0.000	P = 0.035
	AH	CC	60.9% (112)	36.86 \pm 0.32 ^A	66.73 \pm 0.51 ^a	76.80 \pm 0.67 ^A	87.52 \pm 0.78 ^a
		CD	39.1% (72)	36.06 \pm 0.21 ^B	64.71 \pm 0.18 ^b	74.22 \pm 0.24 ^B	85.47 \pm 0.27 ^b
		P		P = 0.003	P = 0.013	P = 0.000	P = 0.035
		P		P = 0.003	P = 0.013	P = 0.000	P = 0.035

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

DISCUSSION

The main determinants of fast growth in mammals are the increases of muscle cell growth and proliferation. Myostatin is a negative growth factor that inhibits both the terminal differentiation of myoblasts and the proliferation of myogenic cells (Thomas et al., 2000; Wiener et al., 2009). Our study focused on the identification of possible genetic variations of the *MSTN* gene and an evaluation of their genetic effects on goat production traits.

In the animal industry, 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., 2012). Generally, identifying genetic markers for growth traits is the initial and critical condition to establishing a marker-assisted selection system (Li et al., 2009). The gene sequence and its variations are the entry point to studying gene expression and function. In the latest study, 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 of the *MSTN* gene had emerged in different species and might be 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., 2012).

Moreover, the diversity of the nucleic acid is a historical indicator of different goat

breeds, so the polymorphism distribution is beneficial to study the population relationship. The polymorphisms of the *MSTN* gene have important implications on growth performance in 3 goat breeds. Li et al. (2008) found an important effect of a 5-bp deletion on goat early body weight and sizes, and that the heterozygote populations have better growth traits than homozygotes.

In this study, the genetic diversity analysis revealed that BR and AH goats possessed intermediate genetic diversity in the P1 and P3 loci. No mutation information was detected in the P2 locus. In the P1 locus, no significant associations were detected between any variant sites and body length, body height, and chest circumference ($P > 0.05$). However, significant associations between the genotypes of the P3 locus and body weight, body length, and body height were observed in AH and BR goats ($P < 0.05$). Furthermore, we suggest that the CC genotype in the *MSTN* gene could be regarded as a molecular marker for superior body weight. However, these results should be further investigated before they are used in goat breeding and genetics.

These results indicate that the *MSTN* gene is a candidate gene for goat growth performance, and plays a significant role in the body weight of goats ($P < 0.05$). It could be inferred that the *MSTN* gene may be a major gene or linked to the major gene affecting goat growth traits. The polymorphic site could be a molecular marker-assisted selection program for body weight.

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