Polymorphism identification in the goat *MSTN* gene and association analysis with growth traits

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ABSTRACT: The myostatin (*MSTN*) gene was studied as a candidate genetic marker for growth traits. We investigated polymorphisms of the *MSTN* gene in 664 individuals from four goat populations and applied PCR-SSCP and DNA sequencing analysis to reveal two single nucleotide polymorphisms (DQ167575: g.368A>C (p.Lys49Thr) and g.4911C>T. At g.368A>C locus, the frequencies of g.368A allele were 0.75–0.81, and the frequencies of g.368C allele were 0.19–0.25. At g.4911C>T locus, the frequencies of g.4911C allele were 0.76–0.82, and frequencies of g.4911T allele were 0.18–0.24. Compared to the female goats with *AC* genotype, those with *AA* genotype had superior body weight in Boer goats (15.69 ± 0.28 vs. 14.51 ± 0.31, $P < 0.05$) and F_1 generation of Boer × Guanzhong dairy goats (19.39 ± 0.34 vs. 18.27 ± 0.33, $P < 0.05$). In addition, the female goats with *AA* genotype (45.80 ± 0.33 cm) had greater withers height than those with *AC* genotype (44.78 ± 0.36 cm) in F_2 generation of Boer × Guanzhong dairy goats ($P < 0.05$). Hence, the biochemical and physiological functions along with the results obtained in our investigation suggest that the *MSTN* gene might play an important role in affecting the growth traits in goats.

Keywords: *MSTN* gene; polymorphisms; growth traits; linkage disequilibrium

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The physiological regulation of muscle growth in animals is under the control of multiple genes. Polymorphisms in these genes, which show associations with specific economically important traits, are useful markers for marker-assisted selection. Single nucleotide polymorphisms (SNP) are the most frequently occurring forms of variation in the genome and they can be used to study associations between them and the production traits of individuals (Wang et al., 2010). This is an increasingly common approach to genetic association studies.

Myostatin, encoded by the *MSTN* gene, is a member of the transforming growth factor β superfamily that normally acts to limit skeletal muscle mass by regulating both the number and the growth of muscle fibres (McPherron et al., 1997). This superfamily encompasses a large number of growth and differentiation factors that play pivotal roles in regulating embryonic development and maintaining tissue homeostasis in adult animals (Hickford et al., 2010). Myostatin-deficient mice had reduced adipogenesis (Lin et al., 2002) as a result of reduced production and secretion of leptin (McPherron and Lee, 2002) and tendons that were small, brittle and hypocellular (Mendias et al., 2008). In addition, natural mutations that decrease the amounts of myostatin and/or inhibit its function have been identified in a human subject (Schuelke et al., 2004) and in several cattle (Marchitelli et al., 2003; Joulia-

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Ekaza and Cabello, 2006) and sheep (Boman et al., 2009) breeds. Since the identification of the key role of *MSTN* in skeletal muscle growth and development, increasing numbers of polymorphisms in *MSTN* gene have been intensively investigated. In sheep, the *MSTN* gene is located on chromosome 2 and a single nucleotide polymorphism (SNP) – DQ530260: g.6223G>A in intron 2 of the gene, has been shown to affect muscularity (Clop et al., 2006; Kijas et al., 2007).

Based on the above considerations, the objectives of the present study were to investigate the allelic variation of the goat *MSTN* gene. Further, we reported associations of some of these alleles with growth traits in Boer goats (BG), F_1 generation of Boer × Guanzhong dairy goats (F_1) and F_2 generation of Boer × Guanzhong dairy goats (F_2).

MATERIAL AND METHODS

Sample collection and DNA extraction

The animals used in this experiment were from Boer Goat Breeding Centre, Xinong Saanen Breeding Centre and Green Century Biology Development Company in Shaanxi Province, China. A total of 664 female goats were examined in this study, including Xinong Saanen goats (SN, n = 180), Boer goats (BG, n = 176), F_1 generation of Boer × Guanzhong dairy goats (F_1, n = 123) and F_2 generation of Boer × Guanzhong dairy goats (F_2, n = 185). All the studied animals came from 38 sires, including 11 Xinong Saanen male goats and 27 Boer male goats. Twenty-seven Boer male goats were divided into three groups. Ten of them were mated with BG; further seven were mated with F_1; the rest was mated with F_2. Xinong Saanen and Guanzhong are dairy breeds, while Boer is a very important breed for goat meat production in China. The growth traits of BG, F_1 and F_2 from the same farm were recorded for statistical analysis. The following traits were evaluated: body weight, withers height, body length and chest girth at 3 months of age. Approximately 5 ml of blood per goat was collected aseptically from the jugular vein and kept in a tube containing anticoagulant ACD (10:27:38 citric acid:sodium citrate:$C_6H_{12}O_6$). All samples were delivered back to the laboratory in an ice box. The genomic DNA was extracted from white blood cells using the standard phenol-chloroform extraction protocol (Sambrook et al., 1989).

PCR conditions

According to *Capra hircus MSTN* gene (GenBank accession No. DQ167575), six pairs of primers were designed to amplify exons 1, 2 and 3 and introns 1 and 2 of *MSTN* gene and screened for polymorphisms. Primer pairs 1 and 2 are shown in Table 1; other primers with no polymorphism detected in their amplicons are not shown. The 25 µl volume contained 50 ng of genomic DNA, 12.5 µl 2× reaction mix (including 500µM dNTP each; 20mM Tris-HCl, pH 9; 100mM KCl; 3mM $MgCl_2$), 0.5µM of each primer, and 0.5 units of *Taq* DNA polymerase. The cycling protocol was 5 min at 95°C, 35 cycles of denaturing at 94°C for 30 s, annealing at X°C (Table 1) for 40 s, extending at 72°C for 40 s, with final extension at 72°C for 10 min.

Single strand conformation polymorphism (SSCP) and DNA sequencing

PCR products (4 µl) were mixed with 6 µl of denaturing solution (95% formamide, 25mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice. Denatured DNA samples were subjected to PAGE (80 × 73 × 0.75 mm) in 1× TBE buffer at constant voltage (180 V) and temperature (4°C) for 4.5 h. The gel (29:1 acrylamide:bis) was stained with 0.1% silver nitrate (Ji et al., 2007; An et al., 2010a). After the polymorphisms had been detected the PCR products of different electrophoretic patterns were sent to sequence in both directions (repeated three times) in an ABI PRISM® 377 DNA sequencer (Applied Biosystems, Foster City, USA) and the sequences were analysed by DNA STAR software (version 7.1) and Blast in NCBI (National Centre for Biotechnology Information).

Statistical analysis

The allelic frequencies, heterozygosity (He) and polymorphism information content (PIC) were calculated using Cluster Analysis Software (version 1.2). The linkage disequilibrium was performed by SHEsis software (Shi and He, 2005). The software SPSS (version 16.0) was used to analyse the relationship between genotypes and growth traits in goats. The adjusted linear model with fixed effects was established and effects of sire, dam within

Table 1. Primer sequences, annealing temperatures (T_a) and sizes of amplicons

Primer	Sequence	(°C)	Fragment length (bp)	Region
Pair 1	F: 5'-AGGCATTAACGTTTGGCTTG-3' R: 5'-ACACTAGAACAGCAGTCAGCAGA-3'	59	516	Exon 1
Pair 2	F: 5'-TCTTTAATAATGACTCCCTGCG-3' R: 5'-GAACACCCACAGCGATCTACT-3'	60	450	Exon 3

sire and genotype were included. Age was fitted as a covariate. Adjusted linear model:

$$Y_{ijlm} = \mu + S_i + D_{ij} + G_l + E_{ijlm}$$

where:

Y_{ijlm} = trait measured on each of the $ijlm^{th}$ animal

μ = overall population mean

S_i = fixed effect associated with the i^{th} sire

D_{ij} = fixed effect associated with the j^{th} dam with i sire

G_l = fixed effect associated with the l^{th} genotype

E_{ijlm} = random error

Effects associated with farm and season of birth (spring vs. autumn) are not matched in the linear model, as the preliminary statistical analyses indicated that these effects did not have a significant influence on variability of traits in the analysed populations.

RESULTS

MSTN gene sequences

By sequencing amplicons obtained with primer pairs 1 and 2, two polymorphisms, DQ167575: g.368A>C and DQ167575: g.4911C>T, were detected in exons 1 and 3 of the goat *MSTN* gene. The nucleotide sequence data have been submitted to the GenBank under accession numbers HM004121 and HM032894. Furthermore, g.368A>C was a missense mutation: Lys>Thr at amino acid position 49

Table 2. Genotypic distribution, allelic frequencies and linkage disequilibrium of g.368A>C and g.4911C>T loci in four goat populations

Locus			Population			
			SN	BG	F_1	F_2
g.368A>C	Genotype	*AA*	110	99	61	99
		AC	70	77	62	86
	Allele	g.368A	0.81	0.78	0.75	0.77
		g.368C	0.19	0.22	0.25	0.23
	He		0.39	0.44	0.50	0.46
	PIC		0.26	0.28	0.31	0.29
g.4911C>T	Genotype	*CC*	94	106	78	95
		CT	86	70	45	90
	Allele	g.4911C	0.76	0.80	0.82	0.76
		g.4911T	0.24	0.20	0.18	0.24
	He		0.48	0.40	0.37	0.49
	PIC		0.30	0.27	0.25	0.30
LD of L_1 and L_2		r^2	0.005	0.098	0.073	0.171

LD = linkage disequilibrium

Table 3. Association of *MSTN* genotypes with growth traits at g.368A>C locus in Boer, F_1 and F_2 goat populations

Population	Genotype	Body weight (kg)	Withers height (cm)	Body length (cm)	Chest girth (cm)
BG	*AA*	15.69 ± 0.28[a]	44.99 ± 0.31	46.68 ± 0.25[a]	53.30 ± 0.37
	AC	14.51 ± 0.31[b]	44.99 ± 0.35	45.29 ± 0.29[b]	52.82 ± 0.42
F_1	*AA*	19.39 ± 0.34[a]	50.03 ± 0.48	48.87 ± 0.54	57.90 ± 0.40[a]
	AC	18.27 ± 0.33[b]	50.68 ± 0.47	49.19 ± 0.53	56.31 ± 0.39[b]
F_2	*AA*	14.81 ± 0.29	45.80 ± 0.33[a]	46.09 ± 0.30	53.61 ± 0.31
	AC	15.49 ± 0.31	44.78 ± 0.36[b]	46.63 ± 0.32	53.77 ± 0.34

The data are expressed as least square means ± standard errors

Values with different superscripts within the same column in particular population differ significantly at $P < 0.05$

of *MSTN* in four goat populations, and g.4911C>T was a synonymous mutation.

Polymorphisms of *MSTN* gene in four goat populations

In the amplicons of six pairs of primers, only those of primers 1 and 2 exhibited polymorphism. Genotypes in g.368A>C and g.4911C>T loci are shown in Figure 1, respectively. In each of these two loci only two genotypes were detected, one homozygote and a heterozygote; the second homozygote was not observed. The frequencies of g.368A allele were 0.75–0.81, those of g.368C allele were 0.19–0.25, and the PIC was 0.26–0.31 in four goat populations (Table 2). The frequencies of g.4911C allele were 0.76–0.82, those of g.4911T allele were 0.18–0.24, and the PIC was 0.25–0.30 in four goat populations (Table 2). To reveal the linkage relationships between g.368A>C and g.4911C>T loci, linkage disequilibrium was estimated in four goat populations (Table 2). After comparing the genotype distribution within four goat populations, no significant differences were found.

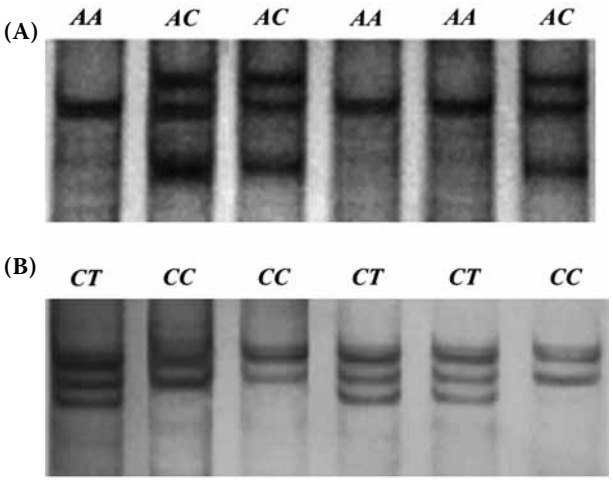

Figure 1. SSCP analysis showing polymorphisms in g.368A>C (A) and g.4911C>T (B) loci of goat *MSTN* (The genotypes are indicated above each lane)

Association of polymorphism with growth traits in three goat populations

In BG, F_1 and F_2 goat populations, the genotypes of 484 individuals were analysed for correlations with phenotypic data for four growth traits at g.368A>C locus (Table 3). At g.4911C>T locus, the records of growth traits did not show any significant correlation with genotypes (data not shown). The linkage disequilibrium between the two SNP loci was estimated, which indicated that they were not in strong linkage disequilibrium ($r^2 < 0.33$, Table 2). In BG population, the does with *AA* genotype had greater body weight and length than those with *AC* genotype ($P < 0.05$) (Table 3). In F_1 goat population, the does with *AA* genotype had greater body weight and chest girth than those with *AC* genotype ($P < 0.05$). In F_2 goat population, the does with *AA* genotype had greater withers height than those with *AC* genotype ($P < 0.05$). Other records did not reveal a significant difference (Table 3).

DISCUSSION

According to the classification of PIC (low polymorphism if PIC value < 0.25, moderate poly-mor-

phism if 0.25 < PIC value < 0.50, and high polymorphism if PIC > 0.50) (Ma et al., 2011), SN, BG and F_2 populations at g.368A>C and g.4911C>T loci had moderate genetic diversity. The results in our study indicated that g.368A>C and g.4911C>T loci were not in strong linkage disequilibrium ($r^2 < 0.33$). Li et al. (2006a) reported that the SNPs of the *MSTN* gene found at positions g.1980A>G, g.1981G>C, g.1982A>G, g.1984G>T and g.2121A>G (GenBank accession No. AY032689) may be in complete linkage disequilibrium.

In this study, in 664 goats of four populations no mutation homozygotes were observed at g.368A>C and g.4911C>T loci. There are two factors that could contribute to this. Firstly, there could be a sampling effect because the sample sizes were relatively small and very few animals homozygous for these alleles were expected. Secondly, there could be a selection effect as these alleles have negative effects on individual performance, therefore individuals with missing genotypes may have been eliminated in the breeding process (Moreau et al., 1998; Beuzen et al., 2000; An et al., 2010b). The results of relationship between the different genotypes and growth traits showed that the g.368A>C locus mutation was significantly associated with the growth traits. The reason could be that the missense mutation (p.Lys49Thr) has effects on the *MSTN* protein structure and on the biological function of the *MSTN* protein (Grobet et al., 1997, 1998). In addition, the analysis failed to reveal an association of g.4911C>T locus with growth traits, and we suppose that the synonymous mutation cannot 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. (2008) 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 (Boman and Vage, 2009). In pigs, mutations identified in non-coding regulatory regions affect the level of *MSTN* gene expression and/or are associated with growth, muscle mass and other carcass traits (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*. 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 (Grobet et al., 1998; Gill et al., 2009). 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 (Li et al., 2006b; Javanmard et al., 2010), but there is scarcity of reports on the association analysis of SNPs with growth traits. The biochemical and physiological functions, together with the results obtained in our study, indicate that the *MSTN* gene might play important roles in affecting the growth traits in goats.

In conclusion, the polymorphisms we have identified in exon 1 of the *MSTN* gene (g.368A>C locus) could be potential genetic markers for growth traits in goats. Differences in trait associations with genetic markers may exist among different populations. Therefore, more tests are needed to confirm the associated effects of the genetic markers on other populations, and the exact mechanism remains to be elucidated.

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