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A novel polymorphism of the myogenin gene is associated with body measurement traits in native Chinese breeds

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ABSTRACT. Using PCR-SSCP and DNA sequencing technology, we examined the association of single nucleotide polymorphisms (SNPs) in the bovine *MyoG* gene with body measurement traits in 779 individuals of six native Chinese cattle breeds, namely Luxi, Luxi × Simmental crossbred, Nanyang, Xia'nan, Jiaxian red, and Qinchuan. A novel SNP, T314C, was detected. Allelic frequencies of *MyoG*-T/C in the six breeds were 0.8308/0.1692, 0.8774/0.1226, 0.8021/0.1979, 0.8209/0.1791, 0.8630/0.1370, 0.8044/0.1956, respectively. Least squares analysis revealed a significant ($P < 0.05$) association of the *MyoG* SNP with rump length in four breeds (Luxi, Xia'nan, Jiaxian red, and Qinchuan), with hucklebone width in three breeds (Luxi × Simmental crossbred, Nanyang and Xia'nan), with waist height in two breeds (Luxi × Simmental crossbred and Nanyang) and with body length in the Luxi breed. We conclude that the *MyoG* SNP has potential as a genetic marker for economically relevant body measurement traits in native Chinese cattle breeds.

Key words: Cattle; *MyoG*; Polymorphisms; Body measurement traits

INTRODUCTION

Recent research suggests that animals with a higher number of muscle fibers of moderate size produce more meat of better quality (Rehfeldt et al., 2000; te Pas et al., 2000). The number of myofibers in muscles is strictly an embryonic process and is regulated by the *MyoD* gene family (Olson, 1990). The *MyoD* family of myogenic regulatory factors (*MRFs*) are specific to muscle and are capable of transforming a variety of cell types into myoblasts (Weintraub et al., 1989).

Members of *MRFs* include myogenic determination factor ($MyoD_1$; Davis et al., 1987), myogenin (*MyoG*; Wright et al., 1989), myogenic factor 5 (MYF_5; Braun et al., 1989), and myogenic factor 6 (MYF_6 - also called herculin or MRF_4; Buckingham et al., 2003).

MyoG has a central position within the *MyoD* gene family, not only because *MyoG* expression abrogates myoblast proliferation potential and regulates the differentiation of mononucleated myoblasts into multinucleated myofibers (Wright et al., 1989; Weintraub et al., 1991; Pas and Visscher, 1994), but also due to *MyoG* being the only member of the *MyoD* gene family that is expressed in all myogenic cell lines (Anton et al., 2002). The *MyoG* gene regulates the expression of muscle-specific genes, which encode several proteins that control the formation and apoptosis (or necrosis) of muscle fibers (Olson, 1990; te Pas et al., 1999). On the other hand, it is possible that the *MyoG* gene is also responsible for determining the number of primary fibers, which may modify the number and type of muscle fibers in the mature animal by controlling embryonic muscle development (Stickland and Handel, 1986). Therefore, the change in the expression profile of the *MyoG* gene or in its structure, caused by mutations, could influence the process of differentiation and, in the end, muscle characteristics (Wyszynska-Koko et al., 2006). Some authors suggested that the different *MyoG* genotypes are related to the variation in the number of muscle fibers and growth rate, which lead to a variation in muscle mass (Soumillion et al., 1997; te Pas et al., 1999) and, thus, body measurement.

The bovine *MyoG* gene is on chromosome 16, along with several QTLs (quantitative trait loci) for carcass weight (Casas et al., 2004). Until now, the association of *MyoG* genetic variations with body measurement has not been reported in cattle. Therefore, the objective of this study was to detect SNPs in the bovine *MyoG* gene and to explore their possible association with body measurement traits in native Chinese breeds.

MATERIAL AND METHODS

Sample collection and DNA isolation

A total of 779 adult animals at 24-48 months of age were randomly selected from breeding populations, including Qinchuan (QC, N = 473, Shaanxi Province), Nanyang (NY, N = 48, Henan Province), Jiaxian red (JR, N = 73, Henan Province), Xia'nan (XN, N = 67, Henan Province), Luxi (LX, N = 65, Shandong Province), and Simmental and Luxi crossbred steers (SL, N = 53, Shandong Province). The following traits were measured as previously described (Gilbert et al., 1993): body length, body height, withers height, rump length, hip height, chest depth, chest circumference, and hucklebone width. In order to minimize systematic error, a single person was assigned to measure one of the five traits in all animals. DNA samples were extracted from leukocytes and tissue samples using a standard phenol-chloroform protocol (Mullenbach et al., 1989).

PCR amplification and DNA sequencing analysis

Two primer pairs (Table 1) were designed based on the bovine *MyoG* gene sequence (GenBank accession No. EF636458) to amplify exon 1 and its flanking regions using the Primer 5.0 software.

Table 1. Primer sequences and information on the bovine *MyoG* gene exon 1.

Primer	Sequences	Amplified position	Product size/bp	Tm (°C)
P_0	F: 5'-TCAGGTTTCTGTGGCGTTGG-3' R: 5'-CCGGCTGCCTTTGTCTTATCTG-3'	Part promoter +1-705	821	67.5
P_1	F: 5'-GAGGAAGTCGGTGTCTGTGGA-3' R: 5'-CCCGCTCTATGTACTGGATGG-3'	242-412	173	61.5

Tm = annealing temperature.

Twenty PCR products amplified by the primer P_0 were randomly selected for screening for DNA polymorphisms. A 15-µL PCR reaction mixture contained 1 µL (50 ng/µL) genomic DNA template, 7.5 µL 2X *Taq* PCR Master Mix, 5.9 µL ddH_2O, and 0.3 µL of each primer (10 M). The cycling protocol was 5 min at 95°C (preliminary denaturation), 35 cycles of denaturation at 94°C for 30 s, annealing (°C) as indicated in Table 1 for 40 s, and extension at 72°C for 40 s, with a final extension at 72°C for 10 min. The PCR products were purified with Axygen kits (MBI Fermentas, Canada) and sequenced in both directions using an ABI PRIZM 3730 DNA sequencer (Perkin-Elmer Shanghai Sangon Biological Engineering Technology, Ltd.), and the sequences were analyzed with the BioXM software (version 2.6). Primer P_1 was designed, focusing on the mutation site.

PCR-SSCP (single-stranded conformation polymorphism)

Aliquots of 6 µL PCR products were mixed with 10 µL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice. Denatured DNA was loaded on a 12% acrylamide/bisacrylamide (29:1) gel and electrophoresed at 110 V for 16 h in 1X TBE buffer. The gel was then stained with 0.1% silver nitrate and visualized with 2% NaOH solution (supplied with 0.1% formaldehyde) according to Zhang et al. (2007). The individual genotypes were defined according to the PCR-SSCP band patterns that were visualized on the gels with white light (Qu et al., 2005).

Statistical analysis

The following items were statistically analyzed according to previous approaches (Liu et al., 2010), including genotypic frequencies, allelic frequencies, Hardy-Weinberg equilibrium, and population genetic, indices: H_E (gene heterozygosity), H_O (gene homozygosity), N_E (effective allele numbers) and PIC (polymorphism information content). The association between SNP marker genotypes of the *MyoG* gene and records of body measurement traits (body length, body height, withers height, rump length, hip height, chest depth, chest circumference, and hucklebone width) were analyzed using the general linear model (GLM) procedure in Statistical Program for Social Sciences (SPSS 17.0, SPSS Inc.), according to the following statistical linear model:

$$Y_{ijk} = \mu + A_i + G_j + S_k + E_{ijk},$$

where Y_{ijk} is the observation for the body measurement trait, μ is the overall population mean, A_i is the fixed effect of the ith age, G_j is the fixed effect of jth genotype, S_k is fixed effect of sex, and E_{ijk} is the random error.

RESULTS

PCR-SSCP analysis of the *MyoG* gene

After a 173-bp product of the *MyoG* gene was amplified (Figure 1), three unique banding patterns were detected by PCR-SSCP analysis. Sequence analysis revealed a T>C mutation, a synonymous mutation of Asn, at position 314 of the *MyoG* gene (Figure 2). The homozygote, consistent with the sequence of GenBank accession No. EF636458, was called the TT genotype, the other homozygote was called the CC genotype, and the heterozygote was called the TC genotype.

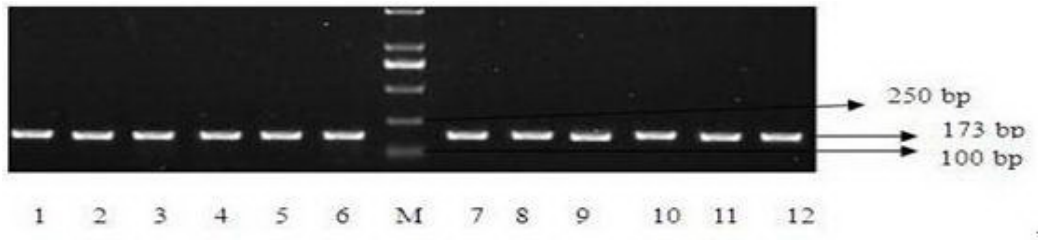

Figure 1. *MyoG* gene exon 1 PCR amplification products. *Lane M* = DL2000 Marker; *lanes 1-12* = PCR products.

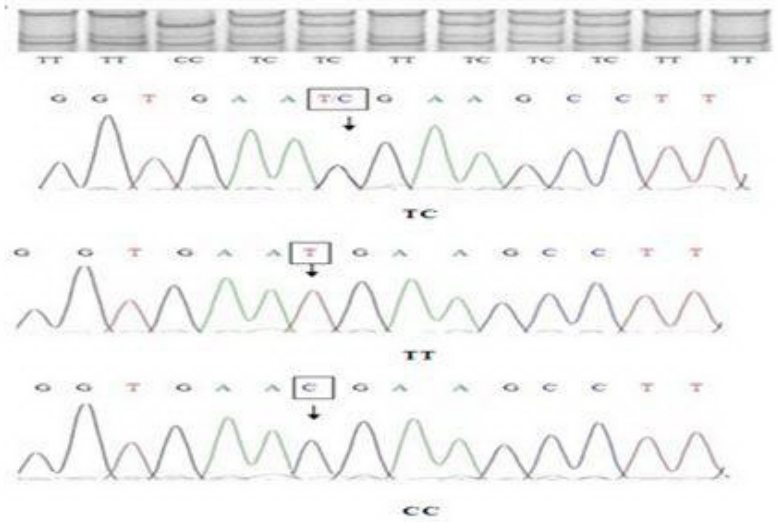

Figure 2. PCR-SSCP patterns and DNA sequencing traces of the bovine *MyoG* gene locus. Three patterns (TT, TC, CC) were observed in six Chinese indigenous cattle. Sequencing trace revealed a T>C mutation at position g.314.

Genetic polymorphism of the bovine *MyoG* gene and χ^2 test

Allele frequencies of the SNP were investigated and performed by the χ^2 test in all populations of bovine in our study (Table 2). The data shown here demonstrate that allelic frequencies of *MyoG*-T/C in LX, SL, NY, XN, JR, and QC breeds were 0.8308/0.1692, 0.8774/0.1226, 0.8021/0.1979, 0.8209/0.1791, 0.8630/0.1370, and 0.8044/0.1956, respectively. According to the χ^2 test, there was a significant difference in allelic frequency between the LX, NY and QC ($P < 0.05$) populations, suggesting that LX, NY and QC were not in Hardy-Weinberg equilibrium. H_E, N_E and PIC of the bovine *MyoG* locus in the six native Chinese cattle varied from 0.2152 (SL) to 0.3175 (NY), 1.2742 (SL) to 1.4652 (NY), and 0.1920 (SL) to 0.2671 (NY), respectively (Table 3). Generally, PIC is classified into the following three types: low polymorphism (PIC value <0.25), average polymorphism (0.25 < PIC value < 0.5), and high polymorphism (PIC value <0.5). According to this classification of PIC, the LX, SL and JR breeds belong to the low polymorphism level, while the other breeds belong to the average polymorphism level (Table 3).

Table 2. Genotype distribution and allele frequencies of the bovine *MyoG* gene exon 1.

Breed	Genotype frequencies (number)			Sample size	Allele frequencies		χ^2 (HW)
	TT	TC	CC		T	C	
LX	0.7385 (48)	0.1846 (12)	0.0769 (5)	65	0.8308	0.1692	7.6666*
SL	0.7925 (42)	0.1698 (9)	0.0377 (2)	53	0.8774	0.1226	0.9502
NY	0.7083 (34)	0.1875 (9)	0.1042 (5)	48	0.8021	0.1979	8.0465*
XN	0.7015 (47)	0.2388 (16)	0.0597 (4)	67	0.8209	0.1791	1.4096
JR	0.7534 (55)	0.2192 (16)	0.0274 (2)	73	0.8630	0.1370	0.0462
QC	0.6913 (327)	0.2262 (107)	0.0825 (39)	473	0.8044	0.1956	37.3531*
Total	0.7099 (553)	0.2169 (169)	0.0732 (57)	779	0.8184	0.1816	56.9060*

HW = Hardy-Weinberg equilibrium; LX = Luxi; SL = Simmental and Luxi crossbred steers; NY = Nanyang; XN = Xia'nan; JR = Jiaxian red; QC = Qinchuan. *P < 0.05.

Table 3. Genetic diversity of the bovine *MyoG* gene exon 1.

Breed	Homozygosity	Heterozygosity	Effective allele number	PIC
LX	0.7188	0.2812	1.3912	0.2417
SL	0.7848	0.2152	1.2742	0.1920
NY	0.6843	0.3175	1.4652	0.2671
XN	0.7059	0.2941	1.4165	0.2508
JR	0.7636	0.2364	1.3097	0.2085
QC	0.6854	0.3146	1.4591	0.2651
Total	0.7027	0.2973	1.4231	0.2531

PIC = polymorphism information content. For breed abbreviations, see legend to Table 2.

Effect of the *MyoG* gene genotypes on body measurement traits

Eight body measurement traits were analyzed by the comparison between the genotypes of 779 individuals and their phenotypic data. The results of association analysis of the gene-specific SNP marker are shown in Table 4. Least squares analysis revealed a significant ($P < 0.05$) association of the *MyoG* SNP with rump length in four breeds (LX, XN, JR, and QC), with hucklebone width in three breeds (SL, NY and XN), with waist height in two breeds

(SL and NY) and with body length in the LX breed. No significant correlations were observed between any of the marker genotypes at T314C and other traits (data not shown).

Table 4. Associations of bovine *MyoG* gene exon 1 genotypes with body measurement traits.

Breed	Genotype	Body measurement traits			
		Body length	Waist height	Rump length	Hucklebone width
LX	TT	139.594 ± 1.628^{a}	131.219 ± 0.948	44.375 ± 0.854^{a}	21.531 ± 0.312
	TC	132.857 ± 3.255^{ab}	128.500 ± 1.896	42.625 ± 1.707^{ab}	20.875 ± 0.624
	CC	126.000 ± 6.511^{b}	125.500 ± 3.793	37.000 ± 3.414^{b}	20.500 ± 1.247
SL	TT	129.286 ± 0.923	124.119 ± 0.683^{a}	39.405 ± 0.509	21.214 ± 0.408^{a}
	TC	126.889 ± 1.993	123.667 ± 1.476^{a}	39.667 ± 1.100	20.778 ± 0.881^{ab}
	CC	124.500 ± 4.228	117.000 ± 3.131^{b}	39.000 ± 2.333	17.500 ± 1.868^{b}
NY	TT	151.281 ± 1.054	131.031 ± 0.769^{a}	40.469 ± 0.537	27.922 ± 0.370^{a}
	TC	149.100 ± 1.885	127.900 ± 1.376^{ab}	39.400 ± 0.960	26.900 ± 0.662^{ab}
	CC	146.800 ± 2.666	126.600 ± 1.946^{b}	38.600 ± 1.358	25.800 ± 0.936^{b}
XN	TT	153.687 ± 2.953	137.500 ± 1.294	49.375 ± 1.012^{a}	25.281 ± 0.691^{a}
	TC	143.200 ± 5.283	136.000 ± 2.315	48.000 ± 1.810^{ab}	23.000 ± 1.237^{ab}
	CC	138.500 ± 8.353	135.000 ± 3.661	42.000 ± 2.862^{b}	20.000 ± 1.956^{b}
JR	TT	130.151 ± 1.308	123.792 ± 0.714	42.906 ± 0.416^{a}	21.170 ± 0.405
	TC	126.733 ± 2.458	123.733 ± 1.343	40.800 ± 0.781^{ab}	21.000 ± 0.761
	CC	121.500 ± 6.733	122.000 ± 3.677	38.500 ± 2.140^{b}	20.000 ± 2.083
QC	TT	127.908 ± 1.407	123.939 ± 1.139^{a}	42.051 ± 0.574^{a}	23.786 ± 0.682
	TC	123.824 ± 2.390	121.853 ± 1.933^{ab}	41.588 ± 0.974^{ab}	22.824 ± 1.157
	CC	119.667 ± 4.022	115.667 ± 3.254^{b}	38.500 ± 1.640^{b}	22.667 ± 1.948

Data are reported as measn ± SE in cm. ^{a,b}Different superscript letters in the same column indicate significant difference ($P < 0.05$). For breed abbreviations, see legend to Table 2.

DISCUSSION

As we have pointed out, the *MyoG* gene has a crucial role during myogenesis. Studies on polymorphisms of the *MyoG* gene have been conducted in pigs and goats. PCR-RFLP analysis using *Nla*IV revealed that the *MyoG* gene existed as at least in two different restricted nuclease polymorphic fragments (Mendez et al., 1997). Southern blot analysis of 105 unrelated pigs revealed that there were three polymorphic *Msp*I sites (Soumillion et al., 1997; te Pas et al., 1999). The significant effect of the *MyoG* gene on birth weight, growth rate and lean weight has been detected by te Pas et al. (1999). These results have been further confirmed by SNP studies of the *MyoG* gene (Kapelanski et al., 2005; Wyszynska-Koko et al., 2006; Verner et al., 2007). Bhuiyan et al. (2009) detected a G>C mutation in exon 1 among six breeds, including Hanwoo, Angus, Simmental, Hereford, Shorthorn, Brahman, and Red Chittagong.

Therefore, considering the evolutionary conservation among cattle, pigs and goats, we applied the results of the research cited above to determine the polymorphism and genetic effect of the cattle *MyoG* gene exon 1. In this study, the possible relationship between the *MyoG* polymorphism and body measurement traits was evaluated by using blood samples from 779 cattle belonging to six different cattle populations. The frequencies of the CC genotype were low in the six native Chinese cattle. The possibility of this observation is the occurrence of gene drift due to the low frequency of allele C. Among the six populations included in the study, comparison of body measurement traits between individuals with the TT genotype revealed significant effects on rump length, hucklebone width, waist height, and body length ($P < 0.05$). Individuals with genotype TT had superior body measurement traits, which indicates

that allele T may be the beneficial allele for body measurement traits in the native Chinese breed types. Allele C could be a recent mutation, where the presence of the C allele in the homozygote individuals significantly decreases their body measurement traits ($P < 0.05$).

MyoG is expressed in all myoblasts from the start of differentiation, and its expression continues during cell fusion. *MyoG* expression also marks the end of the proliferation of myoblasts. Thus, when *MyoG* is expressed, muscle fibers develop from the myoblasts that were previously formed. Therefore, different *MyoG* function or timing of expression could have a major influence on the number of muscle fibers that develop during myogenesis. *MyoG* knock-out mice show no muscle fiber development, and heterozygous *MyoG* knock-out mice show half the number of muscle fibers of wild-type mice (Hasty et al., 1993).

The novel SNP (T314C) could result in a synonymous mutation in the *MyoG* protein. The degeneracy of the genetic code enables the same amino acid sequences to be encoded and translated in many different ways (Komar, 2007). We know that the genome is highly redundant in terms of tRNA species for each amino acid but enigmatically underrepresents a number of specific codons (Shah et al., 2008). Thus, in the synthesis of the *MyoG* protein, if the base change at the third position of the codon is not represented by a corresponding anti-codon within the nuclear tRNA, the rate of expression of the *MyoG* protein could change. Therefore, we hypothesize that naturally occurring genetic variation in *MyoG* could affect muscle fiber numbers and, thus, body measurement.

In conclusion, we identified an SNP in the *MyoG* gene and investigated its relevance in six native Chinese cattle. Our results provide evidence that the *MyoG* gene may have potential effects on body measurement traits in native Chinese cattle. Therefore, further studies are necessary before using this SNP for marker-assisted selection in larger populations. It is also important to determine whether the *MyoG* gene plays a role in the development of those traits and whether it is involved in linkage disequilibrium with other causative mutations.

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