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Polymorphism of *MyoD1* and *Myf6* genes and associations with carcass and meat quality traits in beef cattle

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ABSTRACT. Myogenic determination factor 1 (*MyoD1*) and myogenic factor 6 (*Myf6*) genes belong to the myogenic differentiation (*MyoD*) gene family, which play key roles in growth and muscle development. The study aimed to investigate the effects of variants in cattle *MyoD1* and *Myf6* on carcass and meat traits. We screened single nucleotide polymorphisms (SNPs) of both genes in 8 cattle populations, including Simmental, Angus, Hereford, Charolais, Limousin, Qinchuan, Luxi, and Jinnan by sequencing. The G782A locus was identified in exon 1 of *MyoD1* (*MyoD1-Bgl*I) as well as the T186C locus in exon 1 of *Myf6* (*Myf6-Apa*LI). For the two SNPs, the A allele was significantly more frequent than the B allele in the populations tested. The χ^2 test showed that the *MyoD1-Bgl*I locus conformed to Hardy-Weinberg equilibrium in the 8 populations, as did the *Myf6-Apa*LI locus, with the exception of

the Simmental population ($P > 0.05$). Association analysis revealed that the *MyoD1-Bgl*I locus was significantly associated with loin muscle area (LMA) ($P < 0.05$), and the *Myf6-Apa*LI locus was significantly associated with carcass length (CL) ($P < 0.05$). Animals with BB and AB genotypes for the *MyoD1-Bgl*I locus had larger LMAs compared to animals with AA genotype. Individuals with BB genotype had longer CLs compared to those with AA and AB genotypes. We conclude that the two SNPs might provide useful genetic markers, opening up new possibilities for cattle breeding and improvements in gene-assisted selection.

Key words: Beef cattle; *MyoD1*; *Myf6*; Polymorphism; PCR-RFLP; Carcass and meat quality traits

INTRODUCTION

Carcass and meat quality traits, which are under the control of multiple genes, are economically important traits in meat producing animals. The candidate gene approach may provide a more direct understanding of the genetic basis for the expression of quantitative differences between individuals (Noguera et al., 2003), revealing genomic regions and specific markers that are associated with target traits. Recent research suggests that animals with a higher number of muscle fibers of moderate size produce meat of better quality (Rehfeldt et al., 2000; Te Pas et al., 2000). The number of muscle fibers at birth is regulated by the myogenic differentiation factor (*MyoD*) gene family, and appears to determine lean meat growth capacity in pigs (Handel and Stickland, 1988). Members of the *MyoD* gene family encode skeletal muscle-specific transcription factors, play key roles in growth and muscle development, and are considered candidate genes for meat production traits in farm animals (Te Pas et al., 1999). The myogenic determination factor 1 (*MyoD1* or *Myf3*) gene and the myogenic factor 6 (*Myf6* or *MRF4*) gene are two important members of the *MyoD* gene family, which are composed of 3 exons and share homology within the region coding for a basic helix-loop-helix domain (Olson, 1990; Fujisawa-Sehara et al., 1990). *MyoD1* has been mapped to the 37- to 40-cM interval on BTA15, where meat tenderness and carcass quantitative trait loci were located (Rexroad et al., 2001; Casas et al., 2003). *Myf6* is located on BTA5 and acts upstream of *MyoD1* to direct multipotent embryonic cells into the myogenic lineage and affects skeletal muscle contractile activity with a biphasic expression profile (Sabourin and Rudnicki, 2000). Therefore, *MyoD1* and *Myf6* are possible candidate genes for improving carcass and meat quality traits in animal breeding.

Associations between single nucleotide polymorphisms (SNPs) of these two genes with carcass and meat quality traits have been described in livestock, with particular focus on the pig. Knoll et al. (1997) found a *Del*I restriction fragment length polymorphism (RFLP) locus in intron 1 of the pig *MyoD1* gene. Since then, several studies have shown that the SNPs of *MyoD1* were significantly associated with growth and meat traits in different pig populations, including back fat thickness (BF), loin muscle area (LMA), fat content, leg to hip ratio, and carcass length (CL), and the effect of the genotypes on these traits varied among different strains (Kuryl et al., 2002; Kosowska et al., 2004; Urbanski and Kuryl, 2004; Li et al., 2005; Verner et al., 2007; Liu et al., 2008; Lee et al., 2012). Ever since Ernst et al. (1994) found an *Msp*I-RFLP locus in the pig *Myf6* gene, many studies have suggested that associations between *Myf6* SNPs and meat quality

traits were breed-dependent (Wyszynska-Koko and Kuryl, 2004; Kapelanski et al., 2005; Maak et al., 2006, 2007; Wyszynska-Koko et al., 2004, 2006; Verner et al., 2007). In cattle, several studies have reported associations between the two genes with growth traits in different breeds (Bhuiyan et al., 2009; Wang et al., 2011; Chu et al., 2012). Yet, few studies have described the relationship between the SNPs of these two genes and carcass and meat quality traits in cattle.

The objectives of this study were to investigate SNPs in the two genes and to evaluate whether these polymorphisms affected carcass and meat traits in several cattle breeds. The results of this study could contribute new evidence that *MyoD1* and *Myf6* are important candidate genes for selecting carcass and meat traits in the beef cattle industry.

MATERIAL AND METHODS

Animals and carcass data

A total of 326 animals from 8 cattle breeds, including Simmental (N = 110), Angus (N = 48), Hereford (N = 30), Charolais (N = 30), Limousin (N = 25), Qinchuan (N = 29), Luxi (N = 30), and Jinnan (N = 24), were randomly selected from commercial populations for use in the association analysis. The animals (405 ± 50.5 kg; 30 ± 2 months of age at slaughter) were reared at the Gaolintun Breeding Farm (Tongliao, Inner Mongolia Autonomous Region, China). Carcass and meat quality traits were measured according to the criterion GB/T 17238-1998 of the Cutting Standard of Fresh and Chilled Beef in China (China Standard Publishing House). The following traits were measured or calculated: live weight (LW), carcass weight (CW), dressing percentage (DP), marbling score (MS), meat tenderness (MT), LMA, BF, and CL. BF and LMA were measured between the 12th and 13th rib. MS was evaluated for quality grade on a cross section of the loin muscle between the 12th and 13th rib, which is scored on a scale from 1 to 5. All experimental procedures were performed according to authorization granted by the Chinese Ministry of Agriculture.

Polymerase chain reaction (PCR) amplification and sequencing

DNA samples were extracted from blood samples according to Mullenbach (1989), which was diluted to 50 ng/μL for PCR. Using the DNA sequences of the bovine *MyoD1* gene (GenBank: NW_001493305) and *Myf6* gene (GenBank: NW_001494990), the primers were designed by the Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA). Primer sequences and their corresponding amplified fragment sizes and regions are presented in Table 1. PCR amplifications were performed in a volume of 20-μL containing 50 ng DNA template, 10 pM each primer, 0.20 mM dNTPs, 2.5 mM $MgCl_2$, and 0.5 U *Taq* DNA polymerase (TaKaRa, Dalian, China). The PCR protocol was as follows: 94°C for 5 min followed by 35 cycles of 94°C for 30 s, annealing for 30 s at 72°C, and a final extension at 72°C for 10 min. The products were purified using the Wizard Prep PCR purification kit (Shanghai Bioasia Biotechnology Co., Ltd., China).

PCR-RFLP and genotype determination

We used 5 samples (SNP discovery samples) to identify SNPs by DNA sequencing

(Beijing Aolaibo Biotechnology Co., Ltd., China) on a 3730xl DNA sequencer (Applied Biosystems, Foster City, CA, USA). SNP discovery samples consisted of one individual selected from each of the 8 breeds. After SNP detection at the sequence level, we validated the SNPs using PCR-RFLP. We used the *Bgl*I restriction enzyme (TaKaRa) to digest the *MyoD1* product and the *Apa*LI restriction enzyme (TaKaRa) digested the *Myf6* product at 37°C for 8-10 h, according to manufacturer instructions. The digested products were detected and genotyped by electrophoresis on 2% agarose gel stained with ethidium bromide.

Table 1. Sequences of primer designed for polymorphisms.

SNP	Primer sequence (5'→3')	Size (bp)	T_m (°C)	Position
*Bgl*I-*MyoD1*	GTCACCCAGGAGCACAAAT CCTGAGCAAAGTCAACGAG	633	58	Exon 1
*Apa*LI-*Myf6*	CTCCCTGCTTCGCCTAATC CTTTTTCATCCGAGCGTGC	553	62	Exon 1

Statistical analysis

The genetic characteristics of the bovine *MyoD1* and *Myf6* genes in 8 populations were analyzed using the Popgene32 software (Yeh et al., 1997). Deviations from Hardy-Weinberg equilibrium in these mutations were determined by the χ^2 test. Analysis of associations between the genotypes of SNPs and carcass and meat quality traits was carried out with the GLM procedure, using the SAS software (Statistical Analysis System 9.1, SAS Institute Inc., Cary, NC, USA) under the following statistical linear model:

$$Y_{ijk} = \mu + BF_i + M_j + G_k + e_{ijk} \quad \text{(Equation 1)}$$

where Y_{ijk} stands for the observed value; μ is the overall mean for each trait; BF_i is the effect of the ith breed and farm; M_j is the effect of the jth month of slaughtering; G_k is the kth single SNP marker genotype; e_{ijk} is the random error.

RESULTS

Identification of SNPs and genotyping

Using the DNA mixed-pool sequencing analysis method, the G782A locus was identified in exon 1 of *MyoD1* as well as at the T186C locus in exon 1 of *Myf6*. The two loci were genotyped by PCR-RFLP in the 8 populations. As shown in Figure 1, we found two alleles (A and B) by digestion of the *MyoD1* gene fragment from each individual with the *Bgl*I enzyme, so that each individual was classified as either AA (663 bp), BB (447 bp and 186 bp), or AB (663, 447, and 186 bp) with respect to the *MyoD1* genotype. As shown in Figure 2, we also found two alleles (A and B) by digestion of the *Myf6* gene fragment from each individual with *Apa*LI, so that each individual was classified as AA (553 bp), BB (410 and 143 bp), or AB (553, 410, and 143 bp) with respect to the *Myf6* genotype.

The allele and genotype frequencies of the two SNPs were estimated by genotyping 326 animals. The results showed that A was the preponderant allele in the 8 populations for the *MyoD1-Bgl*I locus; AA homozygotes (138) and AB heterozygotes (149) were significantly more

frequent than were BB homozygotes (31) (Table 2). For the *Myf6-Apa*LI locus, A was also the preponderant allele in the 8 populations; AA homozygotes (226) were significantly more frequent than were BB homozygotes (21) or AB heterozygotes (71) in the populations tested (Table 3).

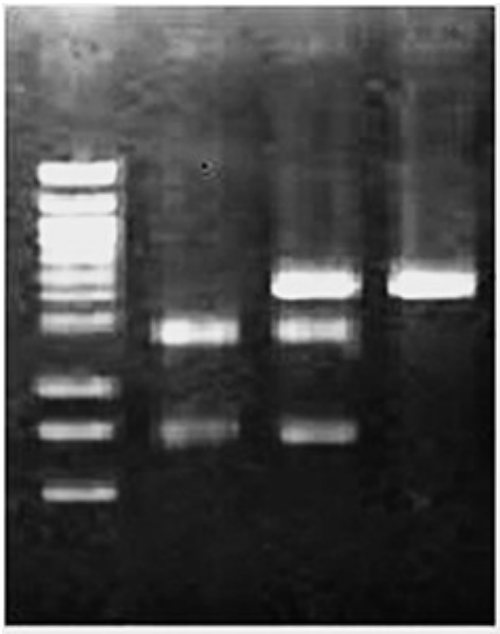

Figure 1. Agrose gel (2%) showing different genotypes of *MyoD1-Bgl*I locus. *Lane M* = 100-1500 bp ladder.

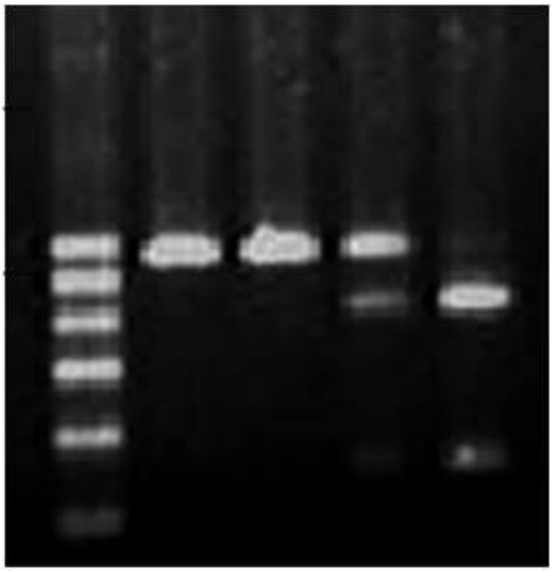

Figure 2. Agrose gel (2%) showing different genotypes *Myf6-Apa*LI locus. *Lane M* = 100-600 bp ladder.

Table 2. Genotypic and allelic frequencies of *MyoD1-Bgl*I SNP.

Breeds	N	Genotypic frequency (%) (N)			Allelic frequency (%)	
		AA	AB	BB	A	B
Angus	48	47.92 (23)	37.50 (18)	14.58 (7)	66.67	33.33
Hereford	30	33.33 (10)	50.00 (15)	16.67 (5)	58.33	41.67
Jinnan	23	17.39 (4)	69.57 (16)	13.04 (3)	52.17	47.83
Limousin	22	45.45 (10)	54.55 (12)	0.00 (0)	72.73	27.27
Luxi	29	51.72 (15)	44.83 (13)	3.45 (1)	74.14	25.86
Qinchuan	27	59.26 (16)	25.93 (7)	14.81 (4)	72.22	27.78
Simmental	109	41.28 (45)	49.54 (54)	9.17 (10)	66.06	33.94
Charolais	30	50.00 (15)	46.67 (14)	3.33 (1)	73.33	26.67
Total	318	43.40 (138)	46.80 (149)	9.75 (31)	66.82	33.18

Table 3. Genotypic and allelic frequencies of *Myf6-Apa*LI SNP.

Breeds	N	Genotypic frequency (%) (N)			Allelic frequency (%)	
		AA	AB	BB	A	B
Angus	48	81.25 (39)	14.58 (7)	4.17 (2)	88.54	11.46
Hereford	30	56.67 (17)	33.33 (10)	10.00 (3)	73.33	26.67
Jinnan	23	78.26 (18)	21.74 (5)	0 (0)	89.13	10.87
Limousin	22	77.27 (17)	18.18 (4)	4.55 (1)	86.36	13.64
Luxi	29	62.07 (18)	27.59 (8)	10.34 (3)	75.86	24.14
Qinchuan	27	59.26 (16)	33.33 (9)	7.41 (2)	75.93	24.07
Simmental	109	71.56 (78)	20.18 (22)	8.26 (9)	81.65	18.35
Charolais	30	76.67 (23)	20.00 (6)	3.33 (1)	86.67	13.33
Total	318	71.07 (226)	22.33 (71)	6.60 (21)	82.23	17.77

Genetic polymorphisms of *MyoD1* and *Myf6* and the χ^2 test

Values for polymorphism information content (PIC), heterozygosity (H_E), effective number of alleles (N_E), and the χ^2 value were determined for all 8 populations. For the *MyoD1-Bgl*I locus (Table 4), the PIC ranged from 0.3099 to 0.3745, indicating moderate polymorphism at this locus among the 8 populations. The PIC and He were higher in Jinnan cattle than in the other populations, implying that polymorphism and genetic variation were higher in Jinnan cattle than in the other populations. The χ^2 values were all less than 5.991, which indicated that this locus was in Hardy-Weinberg equilibrium ($P > 0.05$) in these populations.

Table 4. Genetic diversity of the *MyoD1-Bgl*I SNP.

Breeds	PIC	H_E	N_E	χ^2 value
Angus	0.3457	0.4444	1.800	1.1719
Hereford	0.368	0.4861	1.9459	0.0245
Jinnan	0.3745	0.4991	1.9962	3.5693
Limousin	0.3185	0.3967	1.6575	3.0938
Luxi	0.3099	0.3835	1.622	0.8282
Qinchuan	0.3207	0.4012	1.6701	3.3806
Simmental	0.3479	0.4484	1.8131	1.1955
Charolais	0.3146	0.3911	1.6423	1.1196

($P < 0.05$), $\chi_{0.05}^2 = 5.991$.

For the *Myf6-Apa*LI locus (Table 5), the PIC values of Angus, Jinnan, Limousin, and Charolais were all less than 0.25, which indicated that the locus had low polymorphism in these 4 populations. In contrast, the PIC of the other populations ranged from 0.2547 to 0.3146, indicating moderate polymorphism in these 4 populations. The χ^2 value of the Simmental population was greater than 11.6128, demonstrating that polymorphisms at this locus deviated from Hardy-Weinberg equilibrium ($P < 0.01$), whereas the χ^2 values of the other populations were all less than 5.991, indicating that these populations were in Hardy-Weinberg equilibrium at this locus ($P > 0.05$).

Table 5. Genetic diversity of the *Myf6-Apa*LI SNP.

Breeds	PIC	H_E	N_E	χ^2
Angus	0.1823	0.2029	1.2546	3.7978
Hereford	0.3146	0.3911	1.6423	0.6547
Jinnan	0.1750	0.1938	1.2403	0.6547
Limousin	0.2078	0.2355	1.3081	0.6547
Luxi	0.2992	0.3662	1.5779	0.6547
Qinchuan	0.2987	0.3656	1.5762	0.2099
Simmental	0.2547	0.2996	1.4278	11.6128**
Charolais	0.2044	0.2311	1.3006	0.5436

$\chi^2_{0.05} = 5.991 = \chi^2_{0.01} = 9.21$; **Effect was significant at $P < 0.01$.

Association of the SNPs with carcass and meat quality traits

After the genotypes were identified, the effects of the two SNPs on cattle carcass and meat traits were analyzed. As shown in Table 6, the *MyoD1-Bgl*I locus was found to be associated with LMA ($P = 0.0302$), but no dominance effect was found for the other traits examined ($P > 0.05$). In addition, animals with the BB and AB genotypes had larger LMAs compared to animals with the AA genotype (Table 6).

Table 6. Associations of *MyoD1-Bgl*I locus genotypes with carcass and meat quality traits.

Traits	Genotypes (means ± SE)			P
	AA	AB	BB	
Live weight (LW, kg)	419.14 ± 55.40[a]	528.60 ± 15.65[b]	550.49 ± 8.95[b]	0.8431
Carcass weight (CW, kg)	228.11 ± 37.14	295.50 ± 10.49	307.14 ± 6.00	0.9598
Dressing percentage (DP, %)	54.26 ± 3.15	55.95 ± 0.89	55.90 ± 0.51	0.5726
Meat percent (MP, %)	47.27 ± 3.14	48.77 ± 0.89	48.79 ± 0.50	0.3353
Marbling score (MS, 1-5)	1.58 ± 0.93	2.12 ± 0.26	1.97 ± 0.15	0.5644
Loin muscle area (LMA, cm^2)	48.13 ± 10.27[a]	66.86 ± 2.90[b]	70.93 ± 1.65[b]	0.0302*
Backfat thickness (BF, cm)	0.67 ± 0.43	1.18 ± 0.12	1.11 ± 0.07	0.0592
Meat tenderness(MT, kg)	5.97 ± 1.38	4.31 ± 0.39	4.06 ± 0.22	0.4477
Carcass length (CL, cm)	123.46 ± 4.97[a]	133.89 ± 0.82[b]	137.66 ± 0.80[c]	0.2745

[a,b]Superscripts with different letters means significant difference ($P < 0.05$); *Effect was significant at $P < 0.05$.

As shown in Table 7, the *Myf6* locus was found to be associated with CL ($P = 0.0466$). Moreover, animals with the BB genotype had longer CLs compared to animals with AA or AB genotypes ($P < 0.05$). However, no significant associations were detected between the genotypes and other traits measured in this study ($P > 0.05$).

Table 7. Associations of *Myf6-Apa*LI locus genotypes with carcass and meat quality traits.

Traits	Genotypes (means ± SE)			P
	AA	AB	BB	
Live weight (LW, kg)	562.44 ± 9.08	559.09 ± 9.13	576.87 ± 10.88	0.11862
Carcass weight (CW, kg)	312.86 ± 5.88	309.22 ± 5.91	321.71 ± 7.04	0.1483
Dressing percentage (DP, %)	55.62 ± 0.48	55.32 ± 0.48	55.79 ± 0.58	0.6170
Meat percent(MP, %)	48.52 ± 0.48	48.12 ± 0.48	48.50 ± 0.57	0.5809
Marbling score (MS, 1-5)	2.23 ± 0.21	2.22 ± 0.14	2.17 ± 0.15	0.1259
Loin muscle area (LMA, cm^2)	70.81 ± 1.64	71.25 ± 1.65	68.27 ± 1.96	0.2238
Backfat thickness (BF, cm)	1.15 ± 0.06	1.15 ± 0.06	1.22 ± 0.08	0.4938
Meat tenderness(MT, kg)	4.12 ± 0.20	4.29 ± 0.20	4.19 ± 0.23	0.5883
Carcass length (CL, cm)	138.57 ± 0.81[a]	138.53 ± 0.81[a]	140.47 ± 0.97[b]	0.0466*

[a,b]Means of traits with different superscripts were significantly different (P<0.05); *effect was significant at P < 0.05; **effect was significant at P < 0.01.

DISCUSSION

Currently, breeding goals are shifting from targeting high yield traits toward selecting for greater meat quality traits (van Wijk et al., 2005). Studies investigating trait associations with a candidate gene is a step toward better comprehension of the genetic basis underlying productive traits (Ovilo et al., 2006). *MyoD1* and *Myf6* are two important candidate genes for selecting carcass and meat quality traits in cattle breeding research. However, few studies have focused on these two genes in cattle to date. In this study, *MyoD1-Bgl*I *and Myf6-Apa*LI polymorphisms loci were detected by PCR-RFLP in 8 commercial cattle breeds. For the *MyoD1* gene, the association analysis showed that the *MyoD1-Bgl*I locus was significantly associated with LMA, and individuals with BB and AB genotypes had larger LMAs compared to individuals with the AA genotype (P < 0.05). This result was similar to that of a previous study of association analysis of the *Taq*I-RFLP locus in intron 2; individuals with genotype AA had higher values of LW, CW, and LMA compared to individuals with the AB genotype (P < 0.05) (Tian et al., 2007). In contrast, Ujan et al. (2011) found that the g.624C>G polymorphism locus in exon 1 had no influence on any meat quality traits in 5 indigenous Chinese cattle breeds. Several studies have demonstrated associations between *MyoD1* and growth traits. Association analysis between growth traits (LW and CW) and the g.1274A>G locus revealed that heterozygous animals appeared advantageous for LW and CW compared to their homozygous counterparts (Bhuiyan et al., 2009). In addition, the C→T mutation locus at the 3' untranslated region showed significant associations with heart girth and body weight in yak populations (Chu et al., 2012).

Myf6 plays a particularly important role in the growth of muscle hypertrophy, thereby affecting the quality of meat. Wang et al. (2011) reported that the *Xba*I-RFLP locus was significantly associated with growth traits in Qinchuan cattle and related hybrids, and individuals with genotype AA had higher body weights, withers heights, heart girths and heights at hip cross compared to those with genotypes AB or BB (P < 0.05). Our results showed that the *Myf6-Apa*LI locus was significantly related to CL, and individuals of the BB genotype had significantly longer CLs compared to those of AA and AB genotypes (P < 0.05). This result is in contrast to the g.183T>C polymorphism in Hanwoo that did not show any significant associations with growth and carcass traits evaluated (Bhuiyan et al., 2009). Presently, few studies have focused on the relationship between the *Myf6* gene and carcass and meat quality traits in cattle.

Results from this study indicate that *MyoD1* and *Myf6* have potential effects on carcass and meat quality traits, opening up possibilities for improvements in cattle breeding and gene-assisted selection. In conclusion, this preliminary study on polymorphisms of the *MyoD1-Bgl*I and *Myf6-Apa*LI loci in cattle revealed that these two SNPs might be useful genetic markers. Therefore, further study will be necessary to evaluate the use of these SNPs in marker-assisted selection programs in larger populations and to investigate whether these two genes do indeed play key roles in carcass and meat traits.

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