Association of novel polymorphisms in the bovine myocyte enhancer factor 2D (*MEF2D)* gene with carcass traits of Polish Holstein-Friesian cattle

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ABSTRACT: In the present study the polymorphism of the myocyte enhancer factor 2D (*MEF2D*) gene was analyzed as a genetic marker candidate for carcass traits in Polish Holstein-Friesian cattle. Four novel single nucleotide polymorphisms (SNPs): HQ692911:g.93C>T in exon 8, JX088659:g.69C>T in intron 8, JX0692914:g.47C>T in intron 9 as well as JX088658:g.100G>A in the 3'flanking region were identified by DNA sequencing. By applying the PCR-RFLP method, the SNPs (g.93C>T/*Hae*III and g.47C>T/*Tsp*45I) were genotyped in 401 Polish Holstein-Friesian bulls and their association with carcass traits was analyzed. Statistical analysis showed that the g.93C>T SNP was significantly associated with weight of lean (WLVC) and fat in valuable cuts (WFVC). Animals with the *TT* genotype had significantly higher WLVC ($P < 0.05$) and lower WFVC ($P < 0.05$) than those with the *CC* and *CT* genotypes. No significant relationship with carcass traits was found for the g.47C>T SNP ($P > 0.05$) in intron 9. Moreover, we found a significant effect of the combined genotypes on WFVC ($P < 0.01$), percent of lean (PLVC) ($P < 0.05$), and fat (PFVC) in valuable cuts ($P < 0.01$). This suggested that *MEF2D* is a strong candidate gene that affects carcass traits in cattle.

Keywords: *MEF2D* gene; gene polymorphism; combined genotype; meat contents; bulls

Myocyte enhancer factor 2D (MEF2D), encoded by the *MEF2D* gene, is a member of the myocyte enhancer factor 2 (MEF2) family of transcription factors which plays a pivotal role in the regulation of morphogenesis and myogenesis of skeletal, cardiac, and smooth muscle cells (Black and Olson, 1998). In vertebrates, MEF2 proteins are encoded by four genes: *MEF2A*, *MEF2B*, *MEF2C*, and *MEF2D* each of which gives rise to alternatively spliced transcripts. The MEF2 isoforms are expressed in distinct but overlapping patterns during embryogenesis and in adult tissues. MEF2 proteins bind as homo- and heterodimers to the A/T-rich DNA consensus sequences that are present in many muscle and non-muscle promoters, and the MEF-2 binding sites are present in a variety of muscle-specific genes (Molkentin and Markham, 1993). The well-established roles of MEF2 in muscle development are to control myogenesis and morphogenesis by cooperating with the myogenic basic helix-loop-helix (bHLH) transcription factors (e.g. MyoD, myogenin), homeobox proteins (e.g. tinman, Gax), and/or GATA factors (e.g. GATA4) (Black and Olson, 1998; Cripps et al., 1998; Morin et al., 2000). *MEF2* establishes an additional level of myogenic regulation by regulating the expression of microRNAs, such as miR-1 and miR-133, and posttranscriptionally represses gene expression

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by binding the 3'UTRs of the mRNA target and disrupting mRNA translation and stability (Zhao et al., 2005). Moreover, recent evidence suggests that MEF2 proteins, along with calcineurin, are responsible for the formation of slow-twitch fibres, since overexpression of MEF2 isoforms and calcineurin results in an increased number of type I fibres (Wu et al., 2000; Wu and Olson, 2002; Potthoff et al., 2007). After birth, *MEF2A*, *MEF2B*, and *MEF2D* are expressed ubiquitously, while *MEF2C* transcripts are restricted to the skeletal muscle, brain, and spleen (Black and Olson, 1998), and are regulated at the transcriptional, posttranscriptional, and translational levels (McKinsey et al., 2002). In addition to their crucial role in muscle development, MEF2 proteins are involved in adult cardiac hypertrophy (Black, 2007). Despite intensive studies concerning associating coronary artery disease and hypertrophic cardiomyopathy with variants in *MEF2A* (Liu et al., 2012) and *MEF2C* in humans (Alonso-Montes et al., 2012), little is known about the significant associations of *MEF2* genes polymorphisms, in particular for *MEF2D* with economic traits in livestock. Several SNPs in the *MEF2A* gene, which were associated with body and muscle weight, have been reported in cattle (Chen et al., 2010) and chickens (Zhou et al., 2010). Recently, five new SNPs in the promoter region and intron 1 of the bovine *MEF2C* gene (Juszczuk-Kubiak et al., 2011), as well as three SNPs in the promoter region of the *MEF2A* gene (Juszczuk-Kubiak et al., 2012a), have been reported. In neither case was association of the SNPs with carcass traits studied, but a significant effect of the promoter variants on the *MEF2A* mRNA level in the LD muscle of young Polish Holstein-Friesian bulls was shown.

The bovine *MEF2D* gene comprises twelve exons coding for 507 amino acids (Wu et al., 2011) and is located on BTA3, where many QTLs for intramuscular fat, thickness, and carcass traits (Casas et al., 2003; Barendse et al., 2009; Ferraz et al., 2009) have been described. In view of the key role of this protein in mammalian myogenesis, the *MEF2D* gene is a candidate gene for economically important traits in domestic animals (Onteru et al., 2012).

The aim of this study was to investigate novel polymorphisms in the bovine *MEF2D* gene and to evaluate their associations with carcass traits in Polish Holstein-Friesian cattle. The effect of the two single nucleotide polymorphisms (SNPs) and combined genotypes was estimated.

MATERIAL AND METHODS

Animals

A group of 401 Polish Holstein-Friesian (HF) bulls, the progeny of 24 artificially inseminated Holstein sires, was included in the study. The number of half-sibs varied from 3 to 9. The animals were maintained at the Polish Academy of Sciences Experimental Farm in Jastrzębiec and kept under the same housing and feeding conditions. All bulls were housed in a tie-stall and fed with silage, hay, and concentrate *ad libitum* with constant access to water. Body weight was recorded monthly throughout the whole animals' life. After 24 h of fasting, all of the bulls were slaughtered at the age of 12 months with a body weight of about 380 kg. The carcasses were chilled at 4°C for 24 h and from the right carcass side the valuable cuts (round, shoulder, tenderloin, best ribs, fore ribs) were obtained and dissected into lean, fat, and bone as described previously (Oprządek et al., 2001). The post-slaughter records included the carcass dressing percentage (CDP), weight of valuable cuts (WVC), weight of lean in valuable cuts (WLVC), weight of fat in valuable cuts (WFVC), percentage of lean in valuable cuts (PLVC), and percentage of fat in valuable cuts (PFVC). All procedures carried out on the animals were approved by the Local Ethics Commission, permission No. 29/2007.

Genomic variants detection and polymorphism analyses

In order to identify polymorphisms in the *MEF2D* gene, seven pairs of PCR primers were designed to amplify the six exons (2, 3, 4, 8, 9, and 12) with their flanking regions as well as the 3'flanking region based on the genomic sequence of the bovine chromosome 3 genomic scaffold, Bos_taurus_UMD_3.1 (GenBank Accession No. NW_003103861), using Primer 3.0 software (http://www.frodo.wi.mit.edu/) (Table 1; primers are given only for regions containing SNPs). The PCR reactions were performed in a total volume of 10 µl containing 0.5 µl of 10µM forward and reverse primers, 5 µl of HotStar Taq Master Mix Kit (Qiagen, Hilden, Germany), and approximately 100 ng of genomic DNA. The PCR amplification reactions were optimized and carried out in a C1000 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, USA) using the following cycling

Table 1. List of primers used for scanning of the polymorphism in the bovine *MEF2D* gene

SNP	Primer sequences (GenBank Accession No. NW_003103861)	Position	Length	Annealing temperature (°C)	Detection method/ fragment length (bp)[a]
g.93C>T g.69C>T	F: GGTAAAGCAGCCCACACATC R: ACTCCCCCAAATCAACACCT	498132–498438 exon 8, intron 8	306	61	RFLP/*Hae*III C:162, 144; T: 306
g.47C>T	F: CACCTGGTCCCTGTGTCTCT R: CTTAGTGGCTCGCATGCTGT	502141–502298 intron 9	157	59	RFLP/*Tsp*45I C: 83, 74; T: 157
g.100G>A	F: CTGCTTGCAGTATTCCGACA R: GCCCTCAGAGCTCAATTCAC	504808–505032 3'flanking region	224	60	MSSCP

[a]fragment lengths for different alleles obtained after digestion with restriction enzymes

conditions: 95°C for 15 min, followed by 35–37 cycles, 94°C for 1 min, anneling temperatures (Table 1) for 40 s and 72°C for 1 min, with a final extension at 72°C for 5 min. Genomic DNA was extracted from the blood samples using a Genomic DNA Purification Kit (Promega, Madison, USA) and stored at –20°C. A total of 472 DNA samples derived from different breeds of cattle: Polish Holstein-Friesian (HF, n = 401), Charolaise (CH, n = 19), Limousine (LIM, n = 16), and Hereford (HER, n = 36) were applied to carry out polymorphism analysis and to investigate genotype distribution. Polymorphism screening was performed using the comparative resequencing approach in 20 bulls representing all of the tested breeds. The purified PCR products were directly sequenced using a 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, USA). Two SNPs, g.93C>T and g.47T>C, were genotyped using *Hae*III and *Tsp*45I restriction enzymes (New England Biolabs, Inc., Ipswich, USA) respectively, and were selected for association analysis. The g.100G>A SNP was genotyped using the multitemperature single-strand chain polymorphism (MSSCP) method. The PCR products were digested at 37°C and 65°C for 3 h with 10 U of *Hae*III or *Tsp*45I restriction nucleases (New England Biolabs Inc., Ipswich, USA), respectively. MSSCP electrophoresis was carried out in the Pointer System (Kucharczyk Co., Warsaw, Poland) at constant power (40W) for 70 min. The electrophoresis temperatures were as follows: 35, 15, and 5°C for 350 V/h. The gels were subsequently silver stained for 30 min using the Silver Stain Kit (Kucharczyk Co., Warsaw, Poland) and scanned with the Molecular Imager System FX (Bio-Rad Laboratories, Inc., Hercules, USA). Sequence alignments and identification of variations were performed using the Clustal W (http://www.ebi.ac.uk/tools/msa/clustalW2) and Chromas Lite v2.01 (http://www.technelysium.com.au/chromas) programs. The genotype and haplotype frequencies and deviation from the Hardy-Weinberg equilibrium were calculated using POPGENE v 3.1 software (http://www.ualberta.ca/~fyech).

Statistical analysis

The association between two SNPs (g.93C>T, g.47C>T) and carcass traits was analyzed by the Least Squares Means method as applied in the General Linear Models (GLM) procedure of SAS (Statistical Analysis System, Version 9.2, 2010) according to the model:

$$Y_{ijkl} = \mu + F_i + G_j + S_k + \beta(x_{ijkl} - x) - e_{ijkl}$$

where:

Y_{ijkl} = studied traits
μ = overall mean
F_i = random effect of sire (i = 1, ..., 24)
G_j = fixed effect of the *MEF2D* genotype (j = 1, ..., 3) or the combined genotype (j = 1, ..., 5)
S_k = fixed effect of season at the start of fattening (j = 1, 2)
$\beta(x_{ijkl} - x)$ = regression on cold carcass weight
e_{ijkl} = random residual effect

Frequency of the *CT-CC* combined genotype was low and was excluded from association analysis. Data in Tables 3 and 5 are presented as the Least Squares Means ± their respective standard errors. Differences between the *MEF2D* genotypes were tested using Duncan's test.

RESULTS

Twelve fragments of the bovine *MEF2D* gene encompassing the coding regions with their flanking regions and the 3'flanking region were resequenced in order to identify genetic variations in different

Table 2. Genotypic distribution and allelic frequencies of three SNPs in the *MEF2D* gene in four cattle breeds (*n* given in brackets)

SNP	Genotype/allele	Breed			
		HF	HER	CH	LIM
HQ692911:g.93C>T exon 8	*CC*	0.155 (62)[a]	0.139 (5)	0.211 (4)	0.125 (2)
	CT	0.494 (198)	0.361 (13)	0.316 (6)	0.188 (3)
	TT	0.352 (141)	0.500 (18)	0.474 (9)	0.688 (11)
	C	0.077	0.070	0.105	0.063
	T	0.247	0.180	0.158	0.093
	χ^2 (*P*-value)	0.3 (0.583)	1.03 (0.309)	1.96 (0.161)	3.26 (0.07)
JX0692914:g.47C>T intron 9	*CC*	0.329 (132)	0.556 (20)	0.368 (7)	0.375 (6)
	CT	0.461 (185)	0.333 (12)	0.526 (10)	0.563 (9)
	TT	0.209 (84)	0.111 (4)	0.105 (2)	0.063 (1)
	C	0.560	0.722	0.632	0.656
	T	0.440	0.278	0.368	0.344
	χ^2 (*P*-value)	1.64 (0.200)	1.03 (0.309)	0.33 (0.568)	1.03 (0.309)
JX088658:g.100C>T 3'flanking region	*GG*	0.963 (130)	0.944 (34)	0.947 (18)	1.000 (16)
	GA	0.030 (4)	0.056 (2)	0.053 (1)	0.000 (0)
	AA	0.007 (1)	0.000 (0)	0.000 (0)	0.000 (0)
	G	0.978	0.972	0.974	1.000
	A	0.022	0.028	0.026	0.000
	χ^2 (*P*-value)	13.67 (0.0002)*	0.03 (0.864)	0.01 (0.906)	0.000

HF = Polish Holstein Friesian, HER = Hereford, CH = Charolaise, LIM = Limousine
*$P < 0.001$

breeds of cattle. Overall, four novel SNPs, namely, HQ692911:g.93C>T in exon 8, JX088659:g.69C>T in intron 8, HQ692914:g.47C>T in intron 9 as well as one JX088658:g.100G>A transition in the 3'flanking region of the *MEF2D* gene were identified and have been deposited in the GenBank database. The SNP g.93C>T in the coding region was synonymous and caused no change of the amino acid sequence (ADX60068:G316G). Of the four SNPs identified, two SNPs (g.93C>T and g.47C>T) were confirmed by PCR-RFLP analysis with the *Hae*III and *Tsp*45I restriction enzymes, respectively, and were selected for association analysis. However, the g.69C>T SNP was not genotyped, but a close localization with the g.93C>T SNP suggested their joint occurrence as intragene haplo-

Table 3. Association of the g.93C>T and g.47C>T SNPs of the *MEF2D* gene with carcass traits in the Polish Holstein-Friesian cattle (*n* given in brackets)

SNP	Combined genotype	Traits					
		CDP (%)	WVC (kg)	WLVC (kg)	WFVC (kg)	PLVC (%)	PFVC (%)
g.93C>T (exon 8)	*CC* (62)	51.8 ± 0.6	59.0 ± 0.4	40.6[a] ± 0.5	6.7[ab] ± 0.2	67.2 ± 0.9	13.3 ± 1.8
	CT (198)	52.2 ± 0.3	59.4 ± 0.2	41.6[a] ± 0.3	6.2[b] ± 0.1	68.4 ± 0.5	11.6 ± 0.7
	TT (141)	52.4 ± 0.9	59.2 ± 0.6	41.5 ± 0.7	6.0[a] ± 0.3	67.2 ± 0.7	11.1 ± 1.3
g.47C>T (intron 9)	*CC* (132)	52.6 ± 1.9	58.1 ± 1.0	40.1 ± 0.9	6.3 ± 0.1	68.0 ± 0.6	10.9 ± 1.2
	CT (185)	50.6 ± 1.9	58.7 ± 0.8	40.9 ± 0.8	6.2 ± 0.9	69.7 ± 1.3	10.5 ± 0.7
	TT (84)	51.5 ± 1.0	59.0 ± 1.4	40.6 ± 0.3	6.8 ± 1.9	69.0 ± 0.7	11.9 ± 1.0

CDP = carcass dressing percentage, WVC = weight of valuable cuts, WLVC = weight of lean in valuable cuts, WFVC = weight of fat in valuable cuts, PLVC = percentage of lean in valuable cuts, PFVC = percentage of fat in valuable cuts
[a,b]values marked with the same letter within the same column are significantly different at $P < 0.05$

Table 4. Frequency of combined genotypes (g.93C>T and g.47C>T) of the *MEF2D* gene in four breeds of cattle (*n* given in brackets)

Combined genotypes	HF (401)	HER (36)	CH (19)	LIM (16)	Total frequency (%)
CT–CT	0.461 (185)	0.278 (10)	0.316 (6)	0.500 (8)	38.9
CC–TT	0.130 (52)	0.167 (6)	0.158 (3)	0.125 (2)	14.5
TT–CC	0.328 (131)	0.389 (14)	0.474 (9)	0.375 (6)	39.1
TT–CT	0.035 (17)	0.111 (4)	0.052 (1)	–	5.2
CC–CC	0.027 (11)	0.055 (2)	–	–	2.0
CT–CC	0.012 (5)	–	–	–	0.3

HF = Polish Holstein Friesian, HER = Hereford, CH = Charolaise, LIM = Limousine

types, which was observed after the resequencing of 20 bulls with different RFLP/*Hae*III genotypes. The genotype and allele frequencies for each SNP are listed in Table 2. Genotyping performed for the g.93C>T SNP revealed a predominant frequency of the *T* allele in all of the studied breeds. At the g.47C>T locus, allele *C* was dominant in all of the tested breeds with a frequency ranging from 0.722 (HER) to 0.56 (HF). For the g.100G>A SNP, the genotyping of 206 animals showed a very low frequency of allele *A*; the *AA* genotype was found only in the HF breed (0.007). The distribution of the analyzed SNPs was consistent with the Hardy-Weinberg law, with the exception of the HF breed ($P < 0.001$). Association analysis was carried out between the g.93C>T and g.47C>T SNPs and the carcass traits in the HF breed (Table 3). The results showed that the synonymous g.93C>T SNP was significantly associated with WLVC and WFVC. Animals with the *CC* genotype had higher WFVC ($P < 0.05$) than those with the *TT* and *CT* genotype, respectively. In addition, animals with the *CC* genotype had lower WLVC than those with the *CT* ($P < 0.05$) genotype. No associations were observed for the g.47C>T SNP in intron 9 ($P > 0.05$). Six combined genotypes among these two SNPs were determined with a predominant frequency of the *TC-TC* and *CC-TT* genotype in all of the tested breeds (Table 4). As shown in Table 5, the combined genotypes had a significant effect on PLVC and WFVC. The *CT-CT* genotype was remarkably associated with higher PLVC and lower WFVC and PFVC than the *CC-TT* ($P < 0.05$) and *TT-CT* ($P < 0.05$; $P < 0.01$) genotypes, respectively. No associations of the SNPs with CDP, WVC, and WLVC were found in this study.

DISCUSSION

Myocyte enhancer factor 2D (MEF2D), encoded by the *MEF2D* gene, is a MADS-box transcription factor which plays a pivotal role in the regulation of the myogenesis process of skeletal muscle cells in vertebrates (Black and Olson, 1998). Furthermore, the *MEF2D* gene was located nearby the QTL region affecting marbling, growth, and carcass traits in cattle (Barendse et al., 2009), which implies that the *MEF2D*

Table 5. Association of the *MEF2D* combined genotypes (g.93C>T and g.47C>T) of the *MEF2D* gene with carcass traits of the Polish Holstein-Friesian cattle (*n* given in brackets)

Breed	Combined genotypes	Traits					
		CDP (%)	WVC (kg)	WLVC (kg)	WFVC (kg)	PLVC (%)	PFVC (%)
HF (396)	*CT-CT* (185)	52.6 ± 1.9	59.9 ± 0.7	41.5 ± 0.7	5.6^{Aa} ± 0.2	69.7^{ab} ± 0.5	9.4^{Aa} ± 0.9
	CC-TT (52)	51.9 ± 0.6	58.1 ± 0.4	40.4 ± 0.4	6.4^{a} ± 0.1	66.8^{a} ± 0.8	12.6^{a} ± 1.4
	TT-CC (131)	51.2 ± 0.5	58.7 ± 0.3	40.7 ± 0.3	6.3 ± 0.4	68.7 ± 0.7	10.6 ± 1.1
	TT-CT (17)	51.0 ± 1.0	58.0 ± 1.4	40.0 ± 1.3	6.9^{A} ± 0.7	66.6^{b} ± 0.9	13.4^{A} ± 1.0
	CC-CC (11)	51.5 ± 0.4	58.9 ± 0.4	40.6 ± 0.3	6.3 ± 0.5	68.4 ± 0.6	10.9 ± 1.2

CDP = carcass dressing percentage, WVC = weight of valuable cuts, WLVC = weight of lean in valuable cuts, WFVC = weight of fat in valuable cuts, PLVC = percentage of lean in valuable cuts, PFVC = percentage of fat in valuable cuts
values marked with the same letter within the same column significantly differ at: $^{a,b}P < 0.05$ and $^{A}P < 0.01$

gene could be considered as a potential candidate gene to be a molecular marker of the carcass traits in livestock. In our previous study (Juszczuk-Kubiak et al., 2012b), three novel SNPs in the promoter and 5'UTR regions of the bovine *MEF2D* gene were identified and their impact on *MEF2D* mRNA and protein quantity was observed, but significant associations of these SNPs with carcass traits were not found.

In the present study we explored new SNPs in the coding and non-coding regions of the bovine *MEF2D* gene and analyzed the association of the SNPs with carcass traits. Overall, four novel SNPs were identified, but no mutations with an effect on the protein structure were described, which implies that the protein encoded by the *MEF2D* gene is highly evolutionarily conserved (Wu et al., 2011). No polymorphism was found in exons 2 and 3 encoding the MADS-box and MEF2 domains, which indicates that these regions are functionally important and that there could exist a system ensuring efficient repair of any spontaneously occurring changes in the gene sequence, which might influence MEF2D activity (Black and Olson, 1998). The silent g.93C>T SNP detected in exon 8 of the *MEF2D* (G316G) does not alter the encoded amino acid and has no effect on the resulting protein sequence, but may affect the splicing, mRNA stability, and protein functions (Kimchi-Sarfaty et al., 2007). In addition, exon 8 encodes the transcriptional activation domain of the MEF2D protein, which includes multiple phosphorylation motifs (Potthoff et al., 2007). It is known that phosphorylation plays an important role in the regulation of MEF2 expression and it is likely that causal mutation in these sites might affect the ability of the MADS and MEF domains to bind with DNA sequences (Edmondson et al., 1994). Association results showed that the g.93C>T SNP was significantly associated with WLVC and WFVC. Favourable values for these traits, i.e. associated with higher WLVC and lower WFVC, were noted for the *TT* genotype, which suggests that allele *T* might have a positive effect on carcass composition. The results indicate that g.93C>T SNP of the *MEF2D* gene can both accelerate muscle growth and reduce fat content. This makes the results even more interesting since it might be possible to use the mutation to overcome the antagonism between meat and fat content in carcass. No significant associations between g.47C>T SNP and the examined carcass traits were observed. However, when in combination, two SNPs showed association with WFVC, PLVC, and PFVC, which indicates that associations of combined genotypes with phenotypic traits were more accurate than those of single SNP. A high frequency of combined genotypes suggests the existence of preferable intragenic haplotypes within the bovine *MEF2D* locus. It is very likely that the silent g.93C>T SNP may be linked to the nearby QTL, or that causative mutations in other regions of the *MEF2D* gene are responsible for these phenotypic alterations, thus confirming its potential of being applied as a marker for molecular marker-assisted (MAS) selection of carcass traits in cattle (Onteru et al., 2012). Many studies have shown that *MEF2* transcripts were predominantly expressed in the muscle tissues of humans, mice, and pigs (Naya and Olson 1999; Potthoff et al., 2007; Liu et al., 2011). In skeletal muscle cells in culture, *MEF2D* has been reported to be expressed in proliferating myoblasts prior to the onset of differentiation (Breitbart et al., 1993) and to play a crucial role in muscle growth and maturation during porcine myogenesis (Zhao et al., 2011). Moreover, it has been reported that MEF2 proteins regulate the promoter activity of several negative regulators of myogenesis, e.g. myostatin (Li et al., 2012) and TRIM72 (Jung and Ko, 2010).

In our results the relationship between *MEF2D* polymorphism and fat content in carcass may also be due to the *MEF2D* gene's participation in lipid metabolism in muscle tissue. Several experimental approaches have revealed that *MEF2* factors play an important role in the regulation of gluconeogenesis and adipocyte differentiation by regulating the expression of target genes, such as glucose transporter protein 4 (*GLUT4*) (Thai et al., 1998; Silva et al., 2005) and CCAAT/enhancer-binding protein α (*C/EBPα*) (Zhao et al., 2011) in skeletal muscle, heart, and adipose tissue. It has been shown that the level of *GLUT4* expression is dependent on the binding of the *MEF2A-MEF2D* heterodimer complex to the MEF2 DNA-binding site in human and mice *GLUT4* promoters; since a reduction in *MEF2* expression is correlated with reduced *GLUT4* promoter activity (Mora et al., 2001; Santalucia et al., 2001). Recently, it has been suggested that the transcriptional coactivator peroxisome proliferator-activated receptor-β (*PPAR-β*) participates in *GLUT4* gene transcription by interacting with *MEF2* transcription factors (Gan et al., 2011).

On the other hand, the *MEF2D* gene is located on BTA3, near the leptin receptor (*LEPR*) loci (Ferraz et al., 2009), and it is likely that association of the *MEF2D* gene with fatness traits might be the re-

sult of the linkage disequilibrium between g.93C>T SNP and causative mutations in the *LEPR* gene (Mackowski et al., 2005; Guo et al., 2008), which then affect the variability of carcass traits in cattle.

Thus, our findings are consistent with the possible biological functions of the *MEF2D* gene and suggest that polymorphism of the *MEF2D* gene may affect carcass traits in cattle.

CONCLUSION

In summary, new SNPs were found in the bovine *MEF2D* gene and significant associations were identified between the g.93C>T SNP in exon 8 and carcass traits such as WLVC, PLVC, WFVC, and PFVC. Our results provide evidence that the *MEF2D* gene might have potential effects on carcass traits in cattle. However, in order to confirm the observed associations, further analysis should be extended to a large and more homogeneous population of cattle with the use of a reference family. Nevertheless, these data could serve as a basis for further insight into this bovine gene.

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