

# Candidate SNPs for carcass and meat traits in Nelore animals and in their crosses with *Bos taurus*

Rogério Abdallah Curi<sup>(1)</sup>, Monique Marcondes Krauskopf<sup>(1)</sup>, Janaína Conte Hadlich<sup>(1)</sup>, Marina Rufino Salinas Fortes<sup>(2)</sup>, Dianne Margaret Vankan<sup>(2)</sup>, Josineudson Augusto Il Vasconcelos Silva<sup>(1)</sup>, Henrique Nunes de Oliveira<sup>(3)</sup> and Marcílio Dias Silveira da Mota<sup>(1)</sup>

<sup>(1)</sup>Universidade Estadual Paulista (Unesp), Faculdade de Medicina Veterinária e Zootecnia, CEP 18618-000 Botucatu, SP, Brazil. E-mail: rogcuri@fmvz.unesp.br, moniquekrauskopf@gmail.com, jana\_hadlich@hotmail.com, jaugusto@fmvz.unesp.br, mdsmota@fmvz.unesp.br  
<sup>(2)</sup>The University of Queensland, School of Veterinary Science, St. Lucia, Queensland, 4072, Australia. E-mail: m.fortes@uq.edu.au, d.vankan@uq.edu.au  
<sup>(3)</sup>Unesp, Faculdade de Ciências Agrárias e Veterinárias, CEP 14884-900 Jaboticabal, SP, Brazil. E-mail: holiveira@fcav.unesp.br

**Abstract**—The objective of this work was to evaluate the effects of single-nucleotide polymorphisms (SNPs) in the genes *IGF1* (AF\_017143.1:g.198C>T), *MSTN* (AF\_320998.1:g.433C>A), *MYOD1* (NC\_007313:g.1274A>G) and *MYF5* (NC\_007303:g.1911A>G) on carcass and meat traits in Nelore (*Bos indicus*) and Nelore x *B. taurus*. A total of 300 animals were genotyped and phenotyped for rib eye area (REA), backfat thickness (BT), intramuscular fat (IF), shear force (SF) and myofibrillar fragmentation index (MFI). The effects of allele substitution for each SNP were estimated by regression of the evaluated phenotypes on the number of copies of a particular allele using the general linear model. The polymorphism at *IGF1* was non-informative in Nelore animals. In crossbred animals, the *IGF1* C allele was associated with greater REA. However, this relation was not significant after Bonferroni correction for multiple testing. The A allele of the *MSTN* polymorphism was absent in Nelore cattle and was only found in two crossbred animals. The polymorphisms of *MYOD1* and *MYF5* were little informative in Nelore animals with G allele frequency of 0.097 and A allele frequency of 0.031, respectively. These markers show no association with the analyzed traits in the total sample of evaluated animals.

**Index terms:** *Bos indicus*, candidate gene, fat deposition, meat tenderness, polymorphism.

## SNPs candidatos para características da carcaça e da carne em Nelore e nos seus cruzamentos com *Bos taurus*

**Resumo** – O objetivo deste trabalho foi avaliar os efeitos de polimorfismo de nucleotídeo único (SNP) dos genes *IGF1* (AF\_017143.1:g.198C>T), *MSTN* (AF\_320998.1:g.433C>A), *MYOD1* (NC\_007313:g.1274A>G) e *MYF5* (NC\_007303:g.1911A>G) nas características da carcaça e da carne em Nelore (*Bos indicus*) e Nelore x *B. taurus*. Trezentos animais foram genotipados e fenotipados para área de olho de lombo (REA), espessura de gordura na carcaça (BT), gordura intramuscular (IF), força de cisalhamento (SF) e índice de fragmentação miofibrilar (MFI). Os efeitos da substituição de alelos para cada SNP foram estimados por regressão dos fenótipos analisados sobre o número de cópias de um alelo em particular com uso de modelo linear geral. O polimorfismo do *IGF1* foi não informativo nos animais Nelore. Nos animais cruzados, o alelo C do *IGF1* foi associado à maior REA. No entanto, essa relação não se manteve significativa após a correção de Bonferroni para testes múltiplos. O alelo A do polimorfismo do *MSTN* mostrou-se ausente no gado Nelore e foi encontrado apenas em dois animais cruzados. Os polimorfismos do *MYOD1* e do *MYF5* foram pouco informativos nos animais Nelore com frequência do alelo G de 0,097 e do alelo A de 0,031, respectivamente. Esses marcadores não apresentam associações com as características analisadas na amostra total de animais avaliados.

**Termos para indexação:** *Bos indicus*, gene candidato, deposição de gordura, maciez da carne, polimorfismo.

### Introduction

In beef cattle, variation in carcass composition and meat quality is observed within and between genetic groups, although the underlying mechanisms are not

fully understood. Recent physiological and genomic studies have made progress in this area, revealing pathways and genes associated with carcass and meat traits. The genes *insulin-like growth factor 1* (*IGF1*), *myostatin* (*MSTN*), *myogenic differentiation 1*

(*MYOD1*) and *myogenic factor 5* (*MYF5*) have been proposed as functional and positional candidate genes for carcass composition and meat quality in livestock (Ibeagha-Awemu et al., 2008; Bhuiyan et al., 2009).

Insulin-like growth factor-1 (IGF1), codified by the *IGF1* gene, has an essential role in regulating animal growth and metabolism (Hossner et al., 1997). *IGF1* was mapped to chromosome 5, position 73.5 cM, and was located near quantitative trait loci (QTL) associated with fatness and other meat traits in beef cattle (Casas et al., 2000; Li et al., 2004a). Ge et al. (1997) identified a single-nucleotide polymorphism (SNP) in the regulatory region of *IGF1*, located 512 pb upstream of its first codon (AF\_017143.1:g.198C>T). This SNP was later associated to growth and carcass composition in *Bos taurus* and in *B. taurus* x *B. indicus* crosses (Ge et al., 2001; Li et al., 2004b; Curi et al., 2005; Islam et al., 2009).

Analysis from a bovine QTL mapping study revealed a region with pleiotropic effects on a number of carcass and meat traits. The position of the detected QTL coincides with the mapped position of the *MSTN* gene on bovine chromosome 2 (Sellick et al., 2007). *MSTN* regulates skeletal muscle mass, and the SNP AF\_320998.1:g.433C>A (F94L) is a non-synonymous mutation identified in the coding region of the gene. Independent studies have indicated that F94L increases beef yield in *B. taurus* cattle carcasses without the disadvantages of double muscling effects (Alexander et al., 2007; Sellick et al., 2007; Esmailzadeh et al., 2008).

The *MYOD1* and *MYF5* genes of the MYOD family are potentially involved in muscle fiber development (Wyszyńska-Koko et al., 2006). *MYOD1* was mapped to bovine chromosome 15, between 37 and 40 cM, the same region in which a QTL for carcass and meat traits was found (Rexroad et al., 2001; Casas et al., 2003). The gene *MYF5* was mapped to bovine chromosome 5, between 0 and 30 cM (more accurately at 19 cM), in the same region as a reported QTL for growth traits (Li et al., 2002). Bhuiyan et al. (2009) studied the effect of *MYOD1* and *MYF5* polymorphisms on growth and carcass traits in the Korean breed Hanwoo (*B. taurus*). These authors observed that two intronic polymorphisms, i.e., NC\_007313:g.1274A>G, a SNP in *MYOD1*, and NC\_007303:g.1911A>G, a SNP in *MYF5*, were significantly associated to live weight and carcass weight.

However, there is still a relative lack of information in the literature regarding the potential use of molecular markers for carcass and meat quality traits in *B. indicus* animals, especially of the Nelore breed, which are relevant for beef industries in Brazil.

The objective of this work was to evaluate the effects of SNPs in the genes *IGF1* (AF\_017143.1:g.198C>T), *MSTN* (AF\_320998.1:g.433C>A), *MYOD1* (NC\_007313:g.1274A>G) and *MYF5* (NC\_007303:g.1911A>G) on carcass and meat traits in Nelore and in their crosses with *B. taurus*.

## Materials and Methods

The experiment was done with 300 animals (32 females and 268 males), including 114 Nelore (*B. indicus*), 67 Angus x Nelore crosses (1/2 *B. taurus* + 1/2 *B. indicus*), 44 Rubia Gallega x Nelore crosses (1/2 *B. taurus* + 1/2 *B. indicus*), 41 Canchim (5/8 *B. taurus* + 3/8 *B. indicus*), 19 Brangus three-way crosses (9/16 *B. taurus* + 7/16 *B. indicus*) and 15 Braunvieh three-way crosses (3/4 *B. taurus* + 1/4 *B. indicus*). The Rubia Gallega x Nelore crosses were made in a semi-intensive cattle-raising system in 2006. All other animals, from seven commercial herds, were bred in 2003, 2005, 2006 and 2007 using an intensive system, in the feedlot sector of the Department of Animal Nutrition and Genetic Breeding from the College of Veterinary Medicine and Zootechny of the Universidade Estadual Paulista (Unesp), Botucatu, SP, Brazil. All animals were bred according to the Brazilian legislation for animal well-being (protocol no. 89/2006) approved by the ethics committee on animal experimentation of the College of Veterinary Medicine and Zootechny of Unesp, Botucatu, SP, Brazil. The animals were slaughtered at 15, 17 and 19 months of age according to the guidelines for humane slaughter of cattle.

After slaughter, the identified carcasses were chilled for 24 hours before collecting two *longissimus lumborum* muscle samples (2.54 cm thick) between the 11<sup>th</sup> and 13<sup>th</sup> rib from the left-half carcasses.

Samples collected between the 12<sup>th</sup> and 13<sup>th</sup> rib were used to measure rib eye area (REA), backfat thickness (BT) and shear force (SF). Samples collected between the 11<sup>th</sup> and 12<sup>th</sup> rib were used to determine the myofibrillar fragmentation index (MFI) and intramuscular fat (IF or percentage of total lipids), as

well as for the extraction of genomic DNA. REA was measured by the quadrant point method and BT by a ruler, both according to the methods described by the USDA Quality Grade (United States Department of Agriculture, 1997). After these first measurements at the slaughterhouse, the *longissimus lumborum* muscle samples were de-boned, vacuum-wrapped, aged at 1 to 2°C for 14 days, and then frozen at -20°C. The other phenotypic measurements (SF, MFI and IF) were determined in the laboratory according to Wheeler et al. (1995), Culler et al. (1978) and Bligh & Dyer (1959), respectively.

Genomic DNA was extracted from 250-mg meat samples by a non-phenolic method after digestion with proteinase K and precipitation with NaCl and alcohol. The *IGF1*, *MYOD1* and *MYF5* gene polymorphisms were genotyped by the polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method. The C and T alleles of the AF\_017143.1:g.198C>T SNP of *IGF1* were identified based on the amplification of a 249-bp fragment located in the regulatory region of the gene, followed by digestion with the restriction enzyme *SnaBI* (Ge et al., 2001). For identification of alleles A and G of the NC\_007313:g.1274A>G polymorphism of *MYOD1*, a fragment of 474 bp located in intron 1 was amplified and digested with *HinfI* (Bhuiyan et al., 2009). Alleles A and G of the NC\_007303:g.1911A>G SNP, located in intron 2 of *MYF5*, were identified by the amplification of a 738-bp fragment followed by digestion with the restriction enzyme *TaqI*, as described by Bhuiyan et al. (2009). The amplified and digested DNA fragments of the *IGF1* and *MYOD1* genes were separated on 3% high-resolution agarose gels and of the *MYF5* gene on 2% agarose gels. A 100-bp molecular weight standard was included in each gel in order to calculate the size of the fragments produced. The genotypes of the individuals were determined for each polymorphism by analyzing the fragment size in base pairs. The A and C alleles of the AF\_320998.1:g.433C>A polymorphism, located in exon 1 of the *MSTN* gene, were identified using two real-time PCR assays (Vankan et al., 2010). In one of these assays, extracted DNA was amplified using real-time PCR, and post-amplification analysis was done by high-resolution melt (HRM) technology. The other assay was an allele-specific real-time PCR, which was used to confirm HRM results.

Allele and genotype frequencies were calculated for each polymorphism based on the identified genotypes. Differences in allele frequencies among and within the genetic groups were evaluated using contingency tables.

The allele substitution effects of the polymorphisms on the evaluated traits were estimated by regression of the analyzed phenotypes on the number of copies of a particular allele, using the general linear model (GLM) procedure of the SAS program (SAS Institute, 2004), as follows:  $Y_{ijk} = \mu + CG_i + b \times n_{jk} + \varepsilon_{ijk}$ , in which:  $Y_{ijk}$  is the measured trait;  $\mu$  is the overall mean;  $CG_i$  is the fixed effect of the  $i^{\text{th}}$  contemporary group ( $i = 1, \dots, 13$ );  $b$  is the regression coefficient;  $n$  is the number of alleles of each polymorphism; and  $\varepsilon_{ijk}$  is the random error. For the *IGF1*, *MYOD1* and *MYF5* polymorphisms, regression was performed on the T, G and A alleles, respectively. The contemporary groups considered animals of the same genetic group, sex, age at slaughter, feedlot year and farm of origin. It is important to note that these effects could not be considered separately in the model, since there is confounding among them. However, the sire effect was not included because the number of genotyped offspring from individual sires was very small. Therefore, due to the large number of parents, the possibility of confounding effects of allele and sire on the traits was minimized. To keep the error rate at 5% probability, the Bonferroni correction for multiple comparisons was applied.

## Results and Discussion

Genotype CC of the *IGF1* SNP AF\_017143.1:g.198C>T was identified by the presence of a single fragment of 249 bp. Genotype TT was characterized by two fragments of 226 and 23 bp, whereas heterozygotes (CT) showed three fragments (249, 226 and 23 bp). Allele C was fixed among Nelore animals. In addition, the frequency of this allele was significantly higher than that of T in Angus x Nelore, Canchim, and in the Brangus three-way cross ( $p < 0.05$ ). In Rubia Gallega x Nelore and in the Braunvieh three-way cross there were no differences in allele frequencies ( $p > 0.05$ ). When comparing genetic groups, the frequency of allele C was significantly higher in Nelore and Angus x Nelore than in Rubia Gallega x Nelore, Canchim, and in the Braunvieh three-way cross ( $p < 0.05$ ). Brangus three-way cross cattle showed an intermediate

frequency for allele C (Table 1). In studies with more than 20 breeds of cattle, including both dairy and beef cattle of *B. taurus* and *B. indicus*, the frequency of the C allele of *IGF1* ranged from 0.16 to 1.0 (Ge et al., 2001; Curi et al., 2005; Li et al., 2006; Islam et al., 2009). In *B. taurus* cattle, this SNP segregates – for example, in Angus, the frequency of the T allele was 0.64 (Ge et al., 2001) –, whereas in *B. indicus*, segregation was not observed. The frequency of the C allele in Nelore cattle was previously found to be 1.0 (Curi et al., 2005), which supports the results obtained in the present study. Therefore, these studies reinforce the hypothesis that the T allele could be a mutation only found in *B. taurus* breeds, since it occurred after the divergence between *B. taurus* and *B. indicus*.

Genotyping for the *MSTN* polymorphism AF\_320998.1:g.433C>A resulted in two heterozygous (AC) animals – a Rubia Galega x Nelore and a Canchim bull –, while all other were homozygous for the C allele. Normalized high-resolution melting curves were discriminated between AC and CC based on melt-curve shape and position. In allele-specific genotyping, homozygous CC genotypes showed a single-melting temperature peak, and heterozygous AC genotypes were identified by the presence of two peaks. Previous studies have shown that the mutant A allele of the *MSTN* SNP has high frequency in Limousin animals (0.83) and that it was identified in Charolais, Blonde d'Aquitane and Angus cattle (Dunner et al., 2003; Sellick et al., 2007), which are all *B. taurus*. Vankan et al. (2009) analyzed the allele frequencies of this SNP in 1,140 individuals of 15 cattle breeds in Australia. The A allele

was present in Simmental (0.008), Piedmontese (0.02), Droughtmaster (0.04) and Limousin (0.942), but was not found in Salers, Angus, Poll Hereford, Hereford, Gelbvieh, Charolais, Jersey, Brahman (*B. indicus*), Holstein, Shorthorn or Maine Anjou. These results, which are in agreement with the present work, indicate the absence of polymorphism in *B. indicus* breeds and the absence or very low frequency of the A allele in most *B. taurus* breeds, except for Limousin.

Genotype AA of the *MYOD1* polymorphism NC\_007313:g.1274A>G was identified by the presence of two restriction fragments of 319 and 155 bp. The heterozygous (AG) genotype had four fragments (319, 155, 95 and 65 bp), while the homozygous (GG) one had three (319, 95 and 65 bp). Within all evaluated groups, the frequency of the A allele was significantly higher than that of the G allele ( $p < 0.05$ ). Comparisons among groups indicated that the A allele had a higher frequency in Nelore cattle ( $p < 0.05$ ), whereas no significant differences were observed among the other groups ( $p > 0.05$ ) (Table 2). The only known previous study of this *MYOD1* polymorphism also reported higher frequencies of the A allele in *B. indicus* breeds: 0.69 in Brahman and 0.90 in Red Chittagong (Bhuiyan et al., 2009). In *B. taurus* breeds (Hanwoo, Angus, Simmental, Hereford and Shorthorn), the same authors found lower frequencies of the A allele, which ranged from 0.46 to 0.63. These results are similar to those obtained in the present study, in which the A allele occurred with higher frequency in *B. indicus* (Nelore) than in the crossbreds (*B. taurus* x *B. indicus*). This indicates a difference in the allele frequency of the

**Table 1.** Allele and genotype frequencies of the AF\_017143.1:g.198C>T SNP of the bovine *IGF1* gene in the different genetic groups and in the total sample of animals<sup>(1)</sup>.

Genetic group	Allele frequency		Genotype frequency		
	C	T	CC	CT	TT
Nelore (114)	1.000Aa	0.000Bb	1.000	0.000	0.000
Angus x Nelore (67)	0.933Aa	0.067Bb	0.866	0.134	0.000
Rubia Gallega x Nelore (44)	0.591Ba	0.409Aa	0.182	0.818	0.000
Canchim (41)	0.646Ba	0.354Ab	0.439	0.415	0.146
Brangus three-way cross (19)	0.709ABa	0.291ABb	0.527	0.368	0.105
Braunvieh three-way cross (15)	0.533Ba	0.467Aa	0.200	0.667	0.133
Total (300)	0.835	0.165	0.703	0.264	0.033

<sup>(1)</sup>Frequencies followed by equal letters, uppercase in the columns and lowercase in the lines, do not differ at 5% probability. The number between parentheses indicates the number of animals in each genetic group.

**Table 2.** Allele and genotype frequencies of the NC\_007313:g.1274A>G SNP of the bovine *MYOD1* gene in the different genetic groups and in the total sample of animals<sup>(1)</sup>.

Genetic group	Allele frequency		Genotype frequency		
	A	G	AA	AG	GG
Nelore (114)	0.903Aa	0.097Bb	0.825	0.158	0.017
Angus x Nelore (67)	0.723Ba	0.277Ab	0.582	0.284	0.134
Rubia Gallega x Nelore (44)	0.625Ba	0.375Ab	0.364	0.523	0.113
Canchim (41)	0.683Ba	0.317Ab	0.536	0.293	0.171
Brangus three-way cross (19)	0.684Ba	0.316Ab	0.474	0.421	0.105
Braunvieh three-way cross (15)	0.633Ba	0.367Ab	0.467	0.333	0.200
Total (300)	0.765	0.235	0.623	0.283	0.094

<sup>(1)</sup>Frequencies followed by equal letters, uppercase in the columns and lowercase in the lines, do not differ at 5% probability. The number between parentheses indicates the number of animals in each genetic group.



*MYOD1* SNP between *B. taurus* and *B. indicus*. Allelic differences between *B. taurus* and *B. indicus* could reflect differences introduced through selection or simply genetic drift.

Genotype AA of the *MYF5* polymorphism NC\_007303:g.1911A>G was identified by the presence of one fragment with 738 bp. The genotype GG showed two fragments (580 and 158 bp), whereas heterozygous (AG) genotypes showed three. Allele G was more frequent than A ( $p < 0.05$ ) within all but two groups: Brangus three-way cross and Braunvieh three-way cross. Among groups, allele G was more frequent in Nelore and Rubia Gallega x Nelore than in the other groups ( $p < 0.05$ ) (Table 3). Despite the small number of animals in some of the genetic groups and the lack of significant associations, a lower frequency of the A allele of the *MYF5* SNP was observed in *B. indicus* (Nelore) cattle when compared to crossbreds (*B. indicus* x *B. taurus*). This result supports the notion that the A allele is more common in *B. taurus* cattle, a tendency previously observed by Bhuiyan et al. (2009), who found frequencies of the A allele ranging from 0.04 to 0.06 in *B. indicus* breeds (Brahman and Red Chittagong), and from 0.21 to 0.39 in *B. taurus* breeds (Hanwoo, Angus, Simmental, Hereford and Shorthorn).

The analysis of allele substitution effects (Table 4) showed no significant effects of the *IGF1*, *MYOD1* and *MYF5* polymorphisms on the traits of interest ( $p > 0.05$ ).

Previous research on crossbreds (Simmental x Nelore, Angus x Nelore and Canchim, which are all

**Table 3.** Allele and genotype frequencies of the NC\_007303:g.1911A>G SNP of the bovine *MYF5* gene in the different genetic groups and in the total sample of animals<sup>(1)</sup>.

Genetic group	Allele frequency		Genotype frequency		
	A	G	AA	AG	GG
Nelore (114)	0.031Bb	0.969Aa	0.000	0.071	0.929
Angus x Nelore (67)	0.269Ab	0.731Ba	0.000	0.537	0.463
Rubia Gallega x Nelore (44)	0.114Bb	0.886Aa	0.000	0.227	0.773
Canchim (41)	0.318Ab	0.682Ba	0.025	0.585	0.390
Brangus three-way cross (19)	0.395Aa	0.605Ba	0.211	0.368	0.421
Braunvieh three-way cross (15)	0.467Aa	0.533Ba	0.133	0.667	0.200
Total (300)	0.182	0.818	0.023	0.317	0.660

<sup>(1)</sup>Frequencies followed by equal letters, uppercase in the columns and lowercase in the lines, do not differ at 5% probability. The number between parentheses indicates the number of animals in each genetic group.

*B. taurus* x *B. indicus* crosses) indicated that the C allele of *IGF1* is associated with heavier live weight and carcass weight, besides larger REA and BT scores (Curi et al., 2005). In the present work, the observed effect for the allele substitution of the *IGF1* SNP on REA (T for C substitution represented an increase of 3.287 cm<sup>2</sup> in REA;  $p = 0.0078$ ) was no longer significant ( $p = 0.1173$ ) after applying Bonferroni correction. Although non-significant (nominal p-value = 0.0888; p-value after adjustment by Bonferroni = 1), the effect of the *IGF1* SNP on backfat thickness (C allele increased BT by 0.342 mm) occurred in the same direction as previously observed. In a recent study, Islam et al. (2009) analyzed this same *IGF1* SNP in Angus, Charolais and hybrids (all *B. taurus*) and found significant associations for ultrasound backfat thickness, carcass average backfat and carcass lean meat yield (LMY) only in the Angus population, with genotype CC showing higher fat depth and lower LMY than genotype TT. Differences in the magnitude of the allele substitution effect can be attributed to differences in the cattle breed examined, in the linkage disequilibrium between populations (considering that the SNP might not be the casual mutation) and in epistasis. However, it is important to note that the direction of the effect was the same in all of the mentioned studies, with the C allele increasing rib eye

**Table 4.** Mean square (MS), F test (F), nominal p-values (p) and p-values after adjustment by the Bonferroni method (pB) for regression of the analyzed bovine traits in the number of alleles for each polymorphism.

Polymorphism	Trait <sup>(1)</sup>	MS	F	p	pB
<i>IGF1</i> (AF_017143.1:g.198C>T)	REA (cm <sup>2</sup> )	506.1483	7.1588	0.0078	0.1173
	BT (mm)	5.4941	2.9148	0.0888	1
	IF (%)	0.7829	1.0967	0.2958	1
	SF (kg)	0.1424	0.2603	0.6102	1
	MFI	278.3211	0.9762	0.3239	1
<i>MYOD1</i> (NC_007313:g.1274A>G)	REA (cm <sup>2</sup> )	53.3543	0.7379	0.3909	1
	BT (mm)	1.2699	0.6685	0.4142	1
	IF (%)	1.1831	1.6604	0.1985	1
	SF (kg)	0.1746	0.3193	0.5724	1
	MFI	331.8817	1.1649	0.2813	1
<i>MYF5</i> (NC_007303:g.1911A>G)	REA (cm <sup>2</sup> )	14.1488	0.1953	0.6588	1
	BT (mm)	3.7202	1.9672	0.1618	1
	IF (%)	0.1729	0.2415	0.6234	1
	SF (kg)	0.0739	0.1353	0.7132	1
	MFI	3.8591	0.0135	0.9076	1

<sup>(1)</sup>REA, rib eye area; BT, backfat thickness; IF, intramuscular fat; SF, shear force; MFI, myofibrillar fragmentation index.

area and backfat thickness. This observation supports the notion that the C allele, which creates a putative binding site for a transcription factor (Islam et al., 2009), could be a functional mutation.

Recent studies have shown significant phenotypic effects associated with the F94L polymorphism of *MSTN*. Muscle mass was increased from 5.9 to 7.3% in heterozygous AC calves and from 12 to 15% in homozygous AA calves, with a reduction in carcass fat (Alexander et al., 2007; Sellick et al., 2007; Esmailzadeh et al., 2008). Unfortunately, the apparent non-existence of the A allele of the F94L SNP of the *MSTN* gene in pure breed Nelore and its very low frequency in only two of the other genetic groups imply that this SNP is inadequate for association studies in most Brazilian herds.

Another reason to investigate the associations between the *MYOD1* SNP and the fatness (BT and IF) and tenderness (SF and MFI) traits was the reported existence of a QTL for meat tenderness and a percentage of kidney, heart and pelvic fat identified between 17 and 40 cM of chromosome 15 (Rexroad et al., 2001; Casas et al., 2003). This QTL location coincides with the *MYOD1* location. However, the reported lack of association does not support this gene as a candidate for the QTL. REA was the most likely trait to be associated with a *MYOD1* SNP. Measurements of REA are correlated to meat yield, which is influenced by the number of muscle fibers formed during embryonic development, a process regulated by genes of the MYOD family (Olson, 1990). However, no association was found between REA and this *MYOD1* SNP, which is in accordance with the lack of association observed in Korean Hanwoo cattle (*B. taurus*) (Bhuiyan et al., 2009).

Genes of the MYOD family have not been largely evaluated in cattle, but *MYF5* is the most frequently reported in the literature. Other polymorphisms of *MYF5* were associated with growth traits in Canadian commercial beef cattle (Li et al., 2004b), in Korean Hanwoo cattle (Chung & Kim 2005; Bhuiyan et al., 2009) and in the Chinese cattle breed Qinchuan (Zhang et al., 2007), which are all *B. taurus*. The specific *MYF5* SNP observed in the present study was initially described by Drogemuller & Kempers (2000) and is located in the intron 2. The intron location indicates the possibility of direct and indirect effects of the SNP on phenotypes. This SNP of *MYF5* was associated

with average daily gain, and pre- and postweaning in *B. taurus* (Li et al., 2004b). In another study, the SNP was associated to live weight at 24 months of age, in Hanwoo cattle (*B. taurus*) (Bhuiyan et al., 2009). Hanwoo animals with AA genotype were heavier than those with GG and GA genotypes, and their carcass also tended to be heavier (Bhuiyan et al., 2009). However, no association was observed between this SNP in the intron 2 and the area of the muscle *longissimus lumborum* (Bhuiyan et al., 2009), as observed in the present work for REA in Nelore (*B. indicus*) and Nelore x *B. taurus*. The protein coded by *MYF5* plays an important physiological role in myogenic lineage determination and myocyte differentiation (Braun et al., 1989). Consequently, associations of the *MYF5* SNP with REA, SF and MFI could occur, but associations with BT and IF would not be expected. None of the QTL mapped close to *MYF5* (Cattle QTL database, 2010) were related to fat deposition traits, such as BT or IF.

## Conclusions

1. In Nelore, the fixation of C alleles in *IGF1* (AF\_017143.1:g.198C>T) and *MSTN* (AF\_320998.1:g.433C>A), as well as the low frequency of the G allele in *MYOD1* (NC\_007313:g.1274A>G) and of the A allele in *MYF5* (NC\_007303:g.1911A>G) make impossible or difficult the association studies between these markers and phenotypes.
2. There is no association between the *IGF1* SNP and rib eye area in crossbred animals.
3. The evaluated SNPs of *MSTN*, *MYOD1* and *MYF5* are inadequate for marker-assisted selection in cattle with breed compositions similar to those evaluated.

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