Meat Science 123 (2017) 64–66

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effect of the g.98535683A>G SNP in the *CAST* gene on meat traits of Nellore beef cattle (*Bos indicus*) and their crosses with *Bos taurus*

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ARTICLE INFO

Article history:
Received 13 June 2016
Received in revised form 9 August 2016
Accepted 7 September 2016
Available online 10 September 2016

Keywords:
Calpastatin
Fat cover
Meat tenderness
Marbling
DNA polymorphism

ABSTRACT

The objective of this study was to estimate allele frequencies of the g.98535683A>G:BTAU7 SNP in the *CAST* gene in different genetic groups of beef cattle produced in Brazil (Nellore and their crosses with *Bos taurus*), and to evaluate associations between this polymorphism and meat traits. Five hundred animals from six different genetic groups were genotyped and phenotyped for shear force (SF), myofibrillar fragmentation index (MFI), rib eye area, backfat thickness, and total lipids. Alleles A and G of the SNP were detected in all genetic groups and the frequency of A was higher than G. Significant association ($P < 0.05$) was observed between the polymorphism and meat tenderness (SF and MFI), in which genotype AA exhibited the best values. These results demonstrate for the first time the occurrence of the studied SNP in a Zebu breed and its potential application to the genetic improvement of meat tenderness in the Nellore breed (*Bos indicus*) and its crosses with *Bos taurus*.

1. Introduction

The deposition of intramuscular and subcutaneous fat directly influences the quality of the meat produced. Marbling of the meat confers juiciness and flavor, interfering with consumption habits and the final price of the product (Killinger, Calkins, Umberger, Feuz, & Eskridge, 2004). The increase in subcutaneous fat deposition is important to guarantee carcass quality after cooling (Tait, Wilson, & Rouse, 2005). In addition to adequate carcass finishing, numerous other factors such as age, sex, breed, pre- and post-slaughter management, post-mortem enzymatic activity, muscle fiber composition, and preparation of the product by the consumer can influence meat tenderness, the most important attribute considered by consumers (Chriki et al., 2013).

The identification of quantitative trait loci (QTL) in the genome of livestock animals has focused in recent years on genome-wide association studies (GWAS), which are performed by genotyping hundreds of thousands of single nucleotide polymorphisms (SNP) using high-density chips (Hayes & Goddard, 2010). However, there is the possibility of the existence of major genes that would be responsible for most part of the variation in a trait (Zhu & Zhao, 2007).

The calcium-dependent proteolytic system is one of the main systems responsible for the variation in the extent of post-mortem tenderization (Huff Lonergan, Zhang, & Lonergan, 2010). This system comprises three components: an enzyme that requires a low calcium concentration (calpain-1), an enzyme that requires a high calcium concentration (calpain-2), and a specific calpain inhibitor, calpastatin. In this respect, the calpastatin gene (*CAST*), which is located on bovine chromosome 7 (BTAU7), is considered a strong functional candidate for meat tenderness in livestock species.

Recently, Calvo et al. (2014) identified a new polymorphism in the *CAST* gene of the taurine breeds (*Bos taurus*) Parda de Montaña and Pirenaica. This polymorphism, g.98535683A>G:BTAU7, is located in exon 7 of the gene and is responsible for a Thr182Ala amino acid substitution (change of threonine to alanine at position 182 of the amino acid sequence, NM_174003). The authors considered the possibility of this SNP being a causal polymorphism since it causes the substitution of a non-conservative non-synonymous amino acid and was found to be significantly associated with meat tenderness on post-mortem day 7 in Parda de Montaña cattle.

Since the effects of DNA polymorphisms on phenotypes are intrinsic parameters of each population, lineage or breed in a given environment and considering the possible existence of major genes controlling traits of economic interest in livestock species, the objectives of the present study were to estimate the allele and genotype frequencies of the g.98535683A>G SNP in the *CAST* gene in different genetic groups of beef cattle produced in Brazil (Nellore and their crosses with *Bos taurus*), and to evaluate possible associations between this polymorphism and meat traits.

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http://dx.doi.org/10.1016/j.meatsci.2016.09.003
0309-1740/

2. Material and methods

2.1. Animals

Data of meat and carcass quality, as well as DNA samples, from 500 Nellore animals and their crosses with different taurine breeds were used. Two hundred of these animals were feedlot-finished Nellore (*Bos indicus*) males younger than 2 years (15, 17 and 19 months of age). Samples of these animals were obtained from farms that participate in the genetic breeding program of Conexão Delta G. On these farms, the animals born within a given period (<3 months) are grouped and submitted to the same management until weaning. After weaning, new groups are formed that remain under the same management conditions until yearling, when the animals are destined for breeding or feedlot finishing until slaughter.

Additionally, data from the following 256 animals were used: 114 Nellore, 67 Angus × Nellore (1/2 *Bos taurus* + 1/2 *Bos indicus*), 41 Canchim (5/8 Charolaise + 3/8 *Bos indicus*), 19 Brangus three-way cross (9/16 *Bos taurus* + 7/16 *Bos indicus*), and 15 Brown Swiss three-way cross (3/4 *Bos taurus* + 1/4 *Bos indicus*), obtained from commercial herds of seven farms in the State of São Paulo, Brazil, and feedlot finished in 2003, 2005, 2006 and 2007. In this production system, animals belonging to homogeneous batches of each farm enter the feedlot immediately after weaning, where they remain until the group has reached adequate weight and finishing for slaughter. Forty-four animals obtained by the crossing of Rubia Galega bulls (*Bos taurus*) and Nellore dams (Rubia Galega × Nellore: 1/2 *Bos taurus* + 1/2 *Bos indicus*), produced in a semi-intensive rearing system in 2006, were also used. Of these 300 animals, 32 were females and 268 were intact males, which were slaughtered at 15, 17 and 19 months of age.

2.2. Sample collection, phenotyping and genotyping

After slaughter of the animals performed at commercial slaughterhouses according to guidelines for humane slaughter of cattle, the carcasses were identified and cooled for at least 24 h. After cooling, two longissimus thoracis muscle samples with bone, approximately 2.54 cm thick, were collected between the 11th and 13th rib in the cranial direction from the left half-carcass of each animal. The samples were vacuum packed and aged for 7 days at 2 °C.

A sample collected between the 12th and 13th rib was used for the measurement of rib eye area (REA), backfat thickness (BT) and shear force (SF) using a Warner-Bratzler shear apparatus. The second sample collected between the 11th and 12th rib was used for chemical analysis, including total lipids (TL) and myofibrillar fragmentation index (MFI), and for extraction of genomic DNA. REA was measured with a dot grid (USDA - Quality and Yield Grade, 2000) and BT was measured with a caliper and was expressed in mm. The remaining phenotypic parameters (TL, MFI, and SF) were determined in the laboratory according to the methods described by Bligh & Dyer (1959), Culler, Parrish Jr., Smith, & Cross (1978) and Wheeler, Koohmaraie, & Shackelford (1995), respectively.

Genomic DNA was extracted from a 250-mg meat sample by the non-phenolic method using digestion with proteinase K and precipitation with NaCl and alcohol. The A and G alleles of the g.98535683A>G polymorphism were identified by PCR-RFLP, in which a 238-bp fragment of exon 7 of the bovine *CAST* gene was amplified and digested with the *Hha*I restriction enzyme (Invitrogen, USA) as proposed by Calvo et al. (2014).

2.3. Allele frequencies and association analysis

Differences in allele frequencies within and between the genetic groups were calculated by the complete study of contingency tables (Curi & Moraes, 1981).

The least squares method and General Linear Model (GLM) procedure of the SAS v.9.1 program (SAS, 2004) were used for association analysis between the genotypes and traits of interest (REA, BT, TL, SF, and MFI). The linear model used to fit the quantitative variables of interest included the genotype effect and the effect of contemporary group as follows: $Y_{ijk} = \mu + G_i + CG_j + e_{ijk}$, where Y_{ijk} = trait of interest, μ = overall mean, G_i = fixed effect of genotype *i*, CG_j = fixed effect of contemporary group *j*, and e_{ijk} = random error. The contemporary group variable refers to animals with the same genetic group, sex, age at slaughter, animals finished in the same feedlot facilities at the same year, and were read together in the same farm of origin. These variations could not be included separately in the model because of important confounding between them, but the whole effect caused by each of them, as well as the interactions between them were considered within the CG. Genotypes whose frequencies in all animals of the sample studied were 0.10 or less were excluded from the analysis. The sire effect was not included in the linear model since the number of genotyped animals born to the same sires was very small. Thus, in view of the large number of parents, the possibility of confounding between genotype and sire effects on the production traits was diluted. Since the analyses involved multiple comparisons, FDR correction (Benjamini & Hochberg, 1995) was applied to adjust the statistical significance of the genotype and contemporary group effects on the traits of interest. The least squares means of the genotypes were compared by the Tukey test.

3. Results and discussion

As described by Calvo et al. (2014), the AA genotype of the g.98535683A>G SNP in the *CAST* gene is characterized by the presence of a 238-bp fragment. Heterozygous (AG) individuals exhibit three fragments of 238, 178 and 60 bp and homozygous (GG) individuals are identified by the presence of two fragments of 178 and 60 bp.

Alleles A and G of the g.98535683A>G polymorphism in the *CAST* gene were detected in the sample of animals studied. A higher frequency of allele A compared to allele G was observed within all genetic groups evaluated (Table 1). Despite the small number of individuals in some genetic groups, in general, no significant differences in the frequencies of alleles A or G were observed between *Bos indicus* and *Bos taurus* × *Bos indicus* animals, suggesting similar frequencies of these alleles in the subspecies *Bos indicus* and *Bos taurus*. In this respect, Calvo et al. (2014) found frequencies of allele A of 0.68 and 0.60 in Parda de Montaña and Pirenaica cattle (both *Bos taurus*), respectively.

Only genotypes AA and AG were considered in the association analysis between the genotypes of the *CAST* g.98535683A>G polymorphism and the traits of interest (Table 2). Individuals carrying genotype GG were excluded since the frequency of this genotype was only 6% in all animals of the sample. The findings showed effects of the genotypes on REA (0.8646), BT (0.8332), TL (0.9937), SF (0.0248), and MFI (0.0008). These results indicate significant associations of the genotypes with SF and MFI, with the homozygous AA genotype being more favorable than the heterozygous AG genotype. The differences found

Table 1
Allele and genotype frequencies of the g.98535683A>G polymorphism in the bovine *CAST* gene in different genetic groups.

Genetic group	Allele frequency		Genotype frequency		
	A	G	AA	AG	GG
Nellore (314)	$0.751^{AB;a}$	$0.249^{B;b}$	0.564	0.377	0.06
Angus × Nellore (67)	$0.91^{A;a}$	$0.09^{B;b}$	0.82	0.18	0.00
Rubia Galega × Nellore (44)	$0.875^{A;a}$	$0.125^{B;b}$	0.772	0.204	0.024
Canchim (41)	$0.682^{B;a}$	$0.318^{B;b}$	0.560	0.244	0.196
Brangus three-way cross (19)	$0.736^{AB;a}$	$0.264^{B;b}$	0.581	0.264	0.105
Brown Swiss three-way cross (15)	$0.90^{A;a}$	$0.10^{B;b}$	0.80	0.20	0.00

The number of animals in each genetic group is given in parentheses.
A, B: significantly different between genetic groups ($P < 0.05$); a, b: significantly different within genetic groups ($P < 0.05$).

Table 2
Least squares means and standard errors of the traits evaluated in the genotypes of the g.98535683A>G polymorphism in the *CAST* gene.

Genotype	Trait				
	REA (cm^2)	BT (mm)	TL (%)	SF (Newton)	MFI
AA (312)	68.58 ±0.81^A	5.79 ±0.22^A	1.23 ±0.16^A	37.18 ±0.78^A	59.80 ±1.44^A
AG (158)	68.72 ±0.95^A	5.82 ±0.26^A	1.22 ±0.09^A	39.04 ±0.88^B	54.50 ±1.70^B

The number of animals with each genotype is given in parentheses.
REA: rib eye area; BT: backfat thickness; TL: total lipids; SF: shear force; MFI: myofibrillar fragmentation index.
A, B: significantly different ($P < 0.05$).

between genotypes AA and AG (AA − AG) were –1.86 N for SF ($P = 0.0248$) and 5.31 for MFI ($P = 0.0008$).

After correction of statistical significance, the associations of SF and MFI with the genotypes were close to significance ($P = 0.124$) and significant ($P = 0.004$), respectively. Taken together, these results demonstrate the existence of an effect of the g.98535683A>G polymorphism in the *CAST* gene on the tenderness of aged meat produced by the sample of cattle studied, with allele A being more favorable than allele G. The lack of association between the allelic forms of the *CAST* gene and traits related to growth (REA) and fat deposition (BT and TL) was expected since its protein product does not appear to participate in the physiology of these traits. When the association analyses were performed in genetic groups with a larger number of individuals (Nellore and Angus × Nellore), significant results ($P < 0.05$) were obtained for SF in Angus × Nellore and for MFI in Angus × Nellore and in Nellore.

These associations are similar to those reported by Calvo et al. (2014), who studied 144 animals of the taurine breed Parda de Montaña and found that individuals carrying allele A were superior to those carrying allele G in terms of the tenderness of meat aged for 7 days (measured by SF testing). Individuals carrying genotype GG exhibited less tender meat than those carrying genotypes AA and AG. In the present study, GG individuals were excluded from the association analyses since the small number of individuals with this genotype ($n = 3$) could lead to inconsistent results. In the study of Calvo et al. (2014), no differences in meat tenderness were observed between AA and AG individuals, probably because of the small number of animals used. Therefore, based on the results of these two studies, it was not possible to determine the type of interaction between alleles of this locus.

According to Calvo et al. (2014), the proportion of phenotypic variation in SF explained by the *CAST* g.98535683A>G polymorphism in Parda de Montaña cattle was 18.41%, characterizing the gene as a major gene. However, this proportion is considerably higher than those found in the present study for the set of animals studied: SF – 1.20% and MFI - 2.71%. This difference in magnitude may be explained by differences in genetic background and/or epistatic interactions between the candidate gene and other genes in the genome of the animals investigated in the two studies. Differences in sample size may be another explanation.

The corroboration of the results of the association between the g.98535683A>G SNP in the *CAST* gene and meat tenderness in beef cattle of different genetic background indicates that this trait is in linkage or in strong linkage disequilibrium with the functional polymorphism. There is also the possibility of the polymorphism itself exerting a function since it is located in a coding region (exon 7) where it is responsible for the substitution of a non-conservative non-synonymous amino acid (Thr182Ala – threonine to alanine at position 182 of the amino acid sequence, NM_174003). A study using a subsample of the present sample (Curi et al., 2009) found a significant association between an SNP in the 3′ UTR region of the *CAST* gene (g.98579663A>G) and meat tenderness measured by SF testing ($P = 0.004$) and MFI ($P = 0.006$). Analysis of linkage disequilibrium between the *CAST* gene polymorphism studied here and that in the 3′ UTR region provided r^2 values of 0.1725 and 0.2229 considering only Nellore animals and all animals of the sample, respectively. These results indicate weak linkage disequilibrium between the two loci (<0.3), demonstrating independence of the two SNPs and, consequently, of their effects on meat tenderness.

4. Conclusion

The results of the present study show for the first time the presence of the g.98535683A>G:BTAU7 SNP in Zebu cattle and, in view of the relationship of its genotypes with the tenderness of aged meat and of the distribution of favorable alleles, suggest its potential application to the selection and genetic improvement of the trait in the Nellore breed (*Bos indicus*). At the same time, the findings validate association results previously reported in the literature for Nellore cattle and their crosses with *Bos taurus*.

Acknowledgments

The authors thank the financial support of Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2009/16118-5 and FAPESP 2015/13021-1) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

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