Meta Gene 18 (2018) 73–78

Contents lists available at ScienceDirect

Meta Gene

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

Polymorphism analysis in genes associated with meat tenderness in Nelore cattle

Camila Urbano Braz[a,*], Jeremy Francis Taylor[b], Jared Egan Decker[b], Tiago Bresolin[a], Rafael Espigolan[a], Diogo Anastácio Garcia[a], Daniel Gustavo Mansan Gordo[a], Ana Fabrícia Braga Magalhães[a], Lucia Galvão de Albuquerque[a], Henrique Nunes de Oliveira[a]

[a] Animal Science Department, São Paulo State University (UNESP), School of Agricultural and Veterinarian Science, Jaboticabal, SP 144884-900, Brazil
[b] Division of Animal Sciences, University of Missouri, Columbia, MO 65211, USA

ARTICLE INFO

Keywords:
ASAP1
Candidate gene
CAPN1
Haplotype
Polymorphism
WBSF

ABSTRACT

The aims of this study were to identify haplotyped loci associated with meat tenderness (WBSF) in candidate genes and to search for new polymorphisms in these regions that influence such trait in Nelore cattle. Fifty-two genes that had previously been associated with WBSF or that have biological functions that may influence tenderness were chosen for the haplotype association analysis in 1657 animals. Of the 52 tested candidate genes, two haplotyped loci located in *ASAP1* and *CAPN1* were significantly associated with WBSF. The effect of the significant haplotype alleles varied from −0.44 to 0.80 and −1.03 to 1.52 kg in *CAPN1* and *ASAP1*, respectively. Exonic regions near and within these loci were chosen for sequencing to search for new polymorphisms. Four SNPs were identified in *ASAP1* and seventeen in *CAPN1*. All SNPs detected in the sequenced regions in *ASAP1* showed no association with WBSF even though two of them (*rs714391435* and *rs109256712*) were in strong linkage disequilibrium (LD) with most SNPs that composed the significant haplotyped locus. Four SNPs located in *CAPN1* were found for the first time in this study. One of them (*rs1121961662*) showed significant association with WBSF and is also in strong LD with all SNPs within the significant haplotyped locus in *CAPN1* gene. The SNP *rs1121961662* could be used as a molecular marker for this QTL and may be included in low-density arrays to improve the selection of meat tenderness in *Bos indicus* animals.

1. Introduction

Brazil has been a major global producer and exporter of beef for over a decade (Anualpec, 2016) and the Brazilian herd composition is predominantly *Bos indicus*, with Nelore being the most prominent beef breed. *Bos indicus* breeds produce beef of lower tenderness than *Bos taurus* breeds (O'Connor et al., 1997; Bianchini et al., 2007) and meat tenderness is considered to be the most important trait influencing consumer satisfaction, impacting the profitability of the entire beef industry (Huffman et al., 1996; Boleman et al., 1997; Goodson et al., 2002). However, meat tenderness is a complex trait in which many genetic and non-genetic factors affecting it (Hocquette et al., 2012) and is expressed late in the animal's life when measured *post-mortem*. As a consequence, the genetic improvement of meat tenderness in beef cattle has been slow (Gutiérrez-Gil et al., 2008; Dunner et al., 2013). Therefore, the search for trait-associated SNP markers has become an important tool to identify quantitative trait loci (QTL), candidate genes and causal mutations that influence meat tenderness (Page et al., 2004; Van den Berg et al., 2013).

Candidate gene-association mapping approach can be effectively used to analyze genes with known function related to complex traits with higher statistical power when compared to GWA studies (Kwon and Goate, 2000; Patnala et al., 2013; Amos et al., 2011). Another strategy to increase the power for the identification of significant genomic regions is through haplotype-based association methods. Haplotypes are multi-allelic, reduce the number of tests and may capture more of the available linkage disequilibrium (LD) with QTL loci (Zhao et al., 2007; Cuyabano et al., 2014). The combination of these two approaches increases the chance of detecting regions that truly influence complex traits and may be explored more deeply to search for new polymorphisms that could be useful in selection programs. Therefore, the aims of this study were to identify haplotyped loci associated with meat tenderness in candidate genes and to search for new polymorphisms in these regions that influence such trait in Nelore

* Corresponding author.
E-mail address: camila_urbano@yahoo.com.br (C.U. Braz).

https://doi.org/10.1016/j.mgene.2018.08.002
Received 24 May 2018; Received in revised form 25 July 2018; Accepted 7 August 2018
Available online 10 August 2018

cattle.

2. Material and methods

2.1. Animals

A total of 1657 Nelore bulls born between 2008 and 2011, raised on pasture and sourced from three different animal breeding programs were used in this study. The animals were finished in feedlots for approximately 90 days and then slaughtered in commercial slaughterhouses at a mean age of 729 ± 81 days. The animals belonged to 165 contemporary groups (CG) defined by the combination of farm and year of birth, management group as long-yearlings and month and year of slaughter. There were at least two animals in every CG.

2.2. Phenotype and genotype data

Briefly, to obtain the tenderness phenotypes, animals were slaughtered and the carcasses were identified and chilled for 24 to 48 hour *post-mortem*. Steaks of 2.54 cm thickness were collected from the *longissimus thoracis* muscle between the 12th and 13th ribs from the left half of the carcasses. The steaks were vacuum sealed and aged in a cold chamber for 150 h at 1 °C and then were frozen at −20 °C until they were later cooked in an oven to an internal temperature of 71 °C as proposed by Wheeler et al. (1995). After cooking, steaks were chilled overnight at 2 to 5 °C before coring. The Warner-Bratzler shear force (WBSF), a mechanical measurement of tenderness, was measured using a Salter shearing device (G-R Electric, Manhattan, KS). For precision, eight 1.27 mm meat cylinders were obtained from each sample and the average shear force was used in analysis. The mean of WBSF was 5.21 ± 1.38 kg with a minimum value of 1.56 kg and a maximum of 11.20 kg.

Tissues from the *longissimus thoracis* muscle were used to extract DNA using a DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Genotyping was performed by high-density bead array technology using the Illumina (San Diego, CA) BovineHD Infinium Assay® with an Illumina HiScan System®. The BovineHD BeadChip contains 777,962 SNP markers evenly distributed throughout the genome with a 3.43 kilobase (kb) average separation between the markers. The Illumina Genome Studio software was used to analyze the HiScan images to call genotypes. Forty-one samples for which the genotype call rate was < 90% were removed. The SNP markers with a call rate of < 95% or with a minor allele frequency < 5%, Hardy Weinberg equilibrium test statistic probability $< 10^{-5}$ or that were unmapped to autosomes or sex-linked were also excluded. After quality control a total of 1616 samples remained for analysis.

2.3. Candidate gene analysis

Fifty-two genes that had previously been associated with meat quality or that have biological functions that may influence tenderness were chosen for analysis and are described in Supplementary Table 1. The genomic coordinates for each gene based on the *Bos taurus* UMD3.1 reference assembly were expanded by 1500 bp upstream and downstream due to their potential for harboring regulatory elements and missing exons within the assembly (Whitacre et al., 2015). A total of 985 SNPs on the BovineHD assay were identified within these regions.

The software fastPHASE (Scheet and Stephens, 2006) was used for haplotype reconstruction and missing genotype estimation. The linkage disequilibrium (LD) calculation between SNPs and the haplotype block definition were performed using HaploView software (Barrett et al., 2005) based on estimates of D' (Gabriel et al., 2002).

Statistical analyses incorporating haplotype information were performed using the MIXED procedure of the SAS 9.3 (SAS Institute, 2011), for each haplotyped locus, as follows:

$$y_{ijk} = c_i + s_i + \sum_{l=1}^{n-1} b_l x_{li} + e_i$$

where, y is the WBSF phenotypes for animal i; c is the fixed effect of CG for animal i; s is the random effect of sire for animal i; b is the regression coefficient of the number of copies of the allele l; x is the number of copies of the allele l for animal i; e is the random error for animal i; and n is the haplotype allele number. The Bonferroni test was applied at an experiment-wise 10% ($P \leq 0.00028$) error rate to adjust for multiple testing.

2.4. Sequencing

Exons of each candidate gene located within, or adjacent to, the haplotyped loci that were significantly associated with WBSF were chosen for sequencing in 25 animals with the lowest degree of relatedness among the 1616 animals to identify polymorphic regions, since they are more likely to be genetically different from each other. After, the polymorphic regions were sequenced in 298 animals that belonging to the CGs from the initial 25 sequenced animals.

Primers were designed to amplify these regions (based on Gene ID: 281661 and 327705) using Primer3 software and their specificity was verified using OligoAnalyzer 3.1. The polymerase chain reaction amplification mixture contained 1.0 μL DNA (60 ng/μl), 1.5 μL of each primer (15 pM), 7.5 μL GoTaq Colorless Master Mix and 3.5 μL nuclease-free water in a final volume of 15 μL. A Master Cycler Gradient 5331 thermal cycler was used to perform the amplification using the conditions: denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing temperature dependent on each primer (Supplementary Table 2) for 1 min and extension at 72 °C for 1 min, with a final extension step at 72 °C for 5 min. Twenty-five unrelated animals were individually sequenced to identify variants in the amplicons produced by each primer pair. Sequencing was performed by the dideoxynucleotide chain termination reaction on an ABI 3730 XL sequencer using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA). Polymorphisms within each amplicon were identified using the CodonCode Aligner program.

2.5. Polymorphism association analyses

In order to increase the number of individuals analyzed, genotypes called from the sequence data produced for the SNPs discovered in the sequenced genes were merged with the SNPs from the BovineHD assay and genotypes were imputed for all 1616 animals using BEAGLE v3.3.2 (Browning and Browning, 2007). HaploView software was used to perform the tests of Hardy-Weinberg equilibrium (HWE) for each SNP and to calculate the LD between SNPs. The same statistical analysis described for candidate genes analysis (above) was performed to the SNPs discovered in the sequenced genes. The SNP *rs714391435* were not included in the association analysis due to its very low MAF (0.02). The Bonferroni correction was applied at the 5% level to adjust for multiple testing ($P \leq 0.0025$).

3. Results and discussion

Two haplotyped loci were significantly associated with meat tenderness using the Bonferroni correction ($P \leq 0.00028$) and were located in the *ASAP1* and *CAPN1* genes (Table 1). The haplotyped locus in *ASAP1* was composed of 21 SNPs spanned 36,884 bp in intron 3 and the haplotyped locus in *CAPN1* spanned 3,128 bp with 3 SNPs between intron 13 and 19. The haplotype alleles, their frequencies and average effect are shown in Table 2. In *ASAP1*, the most favorable haplotype allele (GGGTACCAGCGGGCCAAAGTG) decreased 1.03 kg in WBSF and was observed at 7.6% frequency in this population. In *CAPN1*, the haplotype allele ATG showed the most favorable effect (−0.44 kg) on