Genome-wide association study for omega-3 and omega-6 fatty acids in intramuscular fat of Nellore cattle using haplotypes


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Summary

The aim of this study was to identify genomic regions associated with omega 3 (n3), omega 6 (n6) and ratio of n6/n3 fatty acids in intramuscular fat of Nellore cattle using haplotype blocks. The investigated dataset contained records from 963 Nellore steers. After genomic quality control, 469,981 SNPs and 893 animals were available for the analyses. Haplotype blocks were defined based on linkage disequilibrium. Genome-wide association analyses were performed considering one haplotype at a time. A total of 83,957 haplotypes were included in the genome-wide association analyses. From those haplotypes, 115, 25 and 66 were significantly associated (genome-wide p < 0.05) with n3, n6 and ratio n6/n3, respectively. These significant regions harbor 35 genes related to fatty acids. These results may contribute to further investigations aiming to generate tools for selecting Nellore cattle with improved beef fatty acid composition for better flavor and improved human health.

Keywords: Beef quality, candidate genes, haplotypes, Nellore cattle

Introduction

There is a current concern about the impact of excessive beef consumption on human health, mainly in relation to fat composition or beef fatty acids profile. Saturated fatty acids are undesirable since they are related to cardiovascular diseases (e.g., Katan et al., 1994). Unsaturated fatty acids, such as omega 3 (n3) and omega 6 (n6), are desirable because they increase the levels of high density lipoproteins (HDL) that are responsible for lowering serum cholesterol levels (e.g., Tapiero et al., 2002).

The sequencing of the bovine genome made possible to detect QTLs for carcass and beef quality traits, such as intramuscular fatty acid profile. Haplotype are combination of alleles on the same chromosome that tend to be transmitted together and may display higher linkage
disequilibrium (LD) with causative variant than individual a locus and this may lead to better identification of causative variants. Several studies identified genomic regions and genes related to beef fatty acids profile in the Nellore (Cesar et al., 2014; Lemos et al., 2016; Berton et al., 2016). According to these authors, genomic information may help to improve the beef fatty acid profile in zebu cattle. These studies were performed based on individual SNP marker genotypes or gene expression, instead of using haplotype blocks. Thus, the aim of this study was to perform GWAS to identify genomic regions associated with n3, n6 and ratio of n6/n3 fatty acids in intramuscular fat of Nellore cattle exploring the use of haplotype blocks, and subsequently identify functional candidate genes within the identified regions.

Material and methods

Data from 963 Nellore steers about two years old, finished in feedlot (90 days) from farms from three breeding programs (DeltaGen, CRV PAINT and Nellore Qualitas) were used. Contemporary groups were formed by animals born on the same farm and year, and the same management group at yearling. Meat samples of *longissimus dorsi* muscle, between the 12th and 13th ribs of the left half-carcasses, were taken to measure the fatty acids (FAs).

To determine the fatty acid profile, the Folch et al. (1957) method was used to extract lipids and the Kramer et al. (1997) method was used for methylation. FAs were quantified by gas chromatography (GC-2010 Plus - Shimadzu AOC 20i autoinjector) using SP-2560 capillary column (100 m x 0.25 mm diameter with 0.02 mm thickness, Supelco, Bellefonte, PA).

The animals were genotyped using a high-density SNP panel (BovineHD BeadChip assay 777k, Illumina Inc., San Diego, CA). Those SNP markers with minor allele frequency lower than 0.05, call rate lower than 90%, monomorphic, located on sex chromosomes, and those with unknown position were removed from the analysis. After genomic quality control, 469,981 SNPs and 893 animals were available for the analyses. Missing genotypes were imputed and genotypes were phased to haplotypes using FImpute software (Sargolzaei et al., 2014). Haplotype blocks were defined using Gabriel et al. (2002) method based on linkage disequilibrium using HaploView software (Barrett et al., 2005).

In this preliminary genome-wide association analyses, a fixed effect model was used considering one haplotype at a time, using the MIXED procedure of SAS 9.4. The model included fixed effects of contemporary group (92 levels), haplotype effect (linear regression on number of copies of the haplotype: 0, 1 or 2), and age at slaughter as a linear covariate. Bonferroni correction was applied at 5% significance to adjust for multiple tests. Subsequently, for the significant haplotype blocks, further investigation was carried out to identify functional candidate genes within the haplotype regions. Functional analyses were performed using the Ensembl - Variant Effect Predictor tool (www.ensembl.org/Tools/VEP), the National Center for Biotechnology Information database (NCBI, www.ncbi.nlm.nih.gov/gene/), using the UMD3.1 version of the bovine genome, GeneCards Human Gene Database (www.genecards.org), and UniProtKB (www.uniprot.org).

Results and Discussion
A total of 83,957 haplotypes were included in the genome-wide association analyses. From those haplotypes, 115, 25 and 66 were significantly associated (p < 0.05) with omega 3 (n3), omega 6 (n6) and n6/n3 ratio, respectively (Figure 1, Figure 2 and Figure 3).

Most of the associations were identified on BTA4, BTA3, BTA7, BTA17, BTA1, BTA2 and BTA8. These significant haplotype regions harbour genes involved in lipid metabolism: PPT1, CALB1, PDE3B, CROT; transport and use of fatty acids and cholesterol: LURAP1, MFSD2A, GPA33, SLC6A7, TRPA1, COPA, PEX19; phospholipid and membrane hydrolysis and biosynthesis: PLSCR1, BPNT1, MYO10, SYTL3, AGAP1, GABBR2, GPC6; energy metabolism: CRYZL1, ADGRL2, POMGNT1, GALNT12, PDE10A, KIRREL1, CDC14A, ATP6V0A2; constituents of cell membranes: ENSBTAG0000034449; protein kinase synthesis: FRK, CAMK2A, EPAS1, HIF3A, AKAP13, PRKG1, DUSP23; and factors of elongation and synthesis of long chain fatty acids in different species: PLA2G10.

Cesar et al. (2014) also performed a GWAS for beef fatty acid profile in Nelore cattle, using individual SNP genotypes, and found the GPC6 gene associated with sum of monounsaturated beef fatty acids. The SLC6A7 gene identified in the present study belongs to the same family as the SLC51A and SLC4A4 genes identified in the study of Lemos et al. (2016), using GWAS for fatty acids profile with individual SNP genotypes in Nellore cattle, as well as belonging to the same family as the SLC16A7, SLC1A4 and SLC27A6 genes identified in the study by Berton et al. (2016) performing a gene expression analysis for beef fatty acids profile in Nellore cattle. Lemos et al. (2016) also identified the GALNT12 gene associated with the sum of polyunsaturated and sum of saturated fatty acids.

Conclusions

The candidate genes identified in the significant haplotype blocks associated with omega-3, omega-6 and n6/n3 ratio fatty acids are of great interest, because these fatty acids are related with human health. These results may contribute to further investigations aiming to generate tools for selecting Nelore cattle with improved beef fatty acid composition for better flavor and improved human health.

List of References


Figure 1. Manhattan plot of genome-wide association results using haplotypes for omega 3 fatty acids (n3) in intramuscular fat of Nellore cattle. The gray line shows the threshold for 5% level genome-wide significance.

Figure 2. Manhattan plot of genome-wide association results using haplotypes for omega 6 fatty acids (n6) in intramuscular fat of Nellore cattle. The gray line shows the threshold for 5% level genome-wide significance.
Figure 3. Manhattan plot of genome-wide association results using haplotypes for the ratio between omega 6 fatty acids and omega 3 fatty acids (n6/n3) in intramuscular fat of Nellore cattle. The gray line shows the threshold for 5% level genome-wide significance.