Effects of quantitative trait loci on iron content of bovine *longissimus dorsi* muscle.

P.C. Tizioto[1], J.F. Taylor[2], J.E. Decker[2], C.F. Gromboni[3], M.A. Mudadu[4], R.D. Schnabel[2], L.L. Coutinho[5], G.B. Mourão[5], R.T. Nassu[4], F.A.B. Donatoni[4], P. Tholon[4], T.S. Sonstegard[6], M.M. Alencar[4], R.R. Tullio[4], J.M. Reecy[7], A.R.A. Nogueira[4], L.C.A. Regitano[4]

[1]Department of Genetics and Evolution, Federal University of Sao Carlos, [2]Division of Animal Sciences, University of Missouri, [3]Instituto Federal de Educação, Ciência e Tecnologia da Bahia (IFBA), [4]Embrapa Southeast Livestock, [5]Department of Animal Science, University of São Paulo/ESALQ, [6]Agricultural Research Service, United States Department of Agriculture (USDA),[7]Department of Animal Science, Iowa State University.

ABSTRACT: Beef cattle require dietary minerals to maintain their health, production and reproduction. Concentration of iron in bovine tissues is at least partially genetically determined. We genotyped 373 Nelore steers from 34 half-sib families with the Illumina BovineHD BeadChip. Genome-wide association analysis was performed for iron content of the *longissimus dorsi* muscle using a Bayesian approach. A large-effect QTL which explained 6.53% of the additive genetic variance in iron amount was detected at 72 Mb on BTA12. The candidate gene lists generated for all detected QTL were enriched for genes involved in signal transduction, signaling pathway, integral to membrane, intrinsic to membrane, regulation of transcription and metal ion binding. Our findings provide the first step towards the implementation of genomic selection for iron content in Nelore beef cattle, however validation in other populations will be required to estimate the accuracy of molecular estimates of breeding values.

Keywords: *Bos indicus*, Fe, GWAS

Introduction

Genome-wide association studies (GWAS) have been used to identify quantitative trait loci (QTL) associated with complex traits and to better understand which genes and biological mechanisms underlie phenotypic variation.

A genome scan which identified regions of the genome that are related to the iron content of bovine tissues, such as *longissimus dorsi* muscle, might provide valuable information about the mechanisms which underlie ruminant metabolic diseases (Morris et al. (2013)), assist in animal diet formulation and allow the development of beef products which improve human nutrition.

Variation in the mineral content of muscle depends on supplementation and excretion (Morris et al. (2013)), but may also be affected by environmental effects such as birthplace, age and breed (Tizioto et al. (2014)). Furthermore it has been shown that tissue content of minerals is genetically influenced and heritable (Morris and Phua (2010); Mateescu et al. (2013)). Genes may affect mineral balance through the actions of receptor, transporter and chaperone proteins (Morris et al. (2013)).

Consequently, a genome-wide association study of the iron content of beef would likely enhance knowledge of the identities of genes which underlie variation in iron balance, transportation, absorption and excretion, as well as provide a basis for implementation of genomic selection for this trait. This study identifies QTL that putatively harbor genes that are related to variation in iron levels in the *longissimus dorsi* muscle of Nelore cattle.

Materials and Methods

Nelore steers, totaling 373 animals, produced by 34 sires which represent the main breeding lineages of Brazil, were genotyped and phenotyped. Animals were raised and then allocated to two feedlots as previously described by Tizioto et al. (2012). The animals were slaughtered at an average endpoint of five mm of back fat thickness after about 90 days of confinement feeding. The research was approved by the Embrapa Southeast Livestock (São Carlos, São Paulo, Brazil) ethics committee.

Iron content was determined as previously described by Tizioto et al. (2014) using a Vista Pro-CCD ICP-OES spectrometer with radial view (Varian, Mulgrave, Australia). Excitation wavelengths were chosen to minimize spectral interference and produce the highest intensity emission for each element. A linear calibration was calculated with up to five points from preparations using standard analytical solutions.

The accuracy and precision of the utilized method was evaluated by measuring the recovery and relative standard deviation of the certified reference material (CRM): Bovine Liver 1557b and Bovine Muscle 8414 from the National Institute of Standards and Technology (NIST Gaithersburg, MD, USA).

Five mL blood samples were collected from each steer and DNA extractions were performed using a salting out method. All animals were genotyped using the Illumina BovineHD BeadChip (Illumina Inc., San Diego, CA) either at the USDA ARS Bovine Functional Genomics Laboratory in Beltsville, MD or at the ESALQ Genomics Center, Piracicaba, São Paulo, Brazil. Genotypes were called in the Illumina Genome Studio software. Animal genotypes were filtered according to call rate (< 90%) and heterozygosity (> 40%). Loci were deleted if their Illumina probe sequence could not be uniquely localized to an autosome or the X chromosome in the UMD3.1 sequence assembly, call rate (< 85%), minor allele frequency (< 0.5%), or Hardy Weinberg Equilibrium ($\chi^2_1 > 100.0$), as described by Tizioto et al. (2013). Only Single Nucleotide Polymorphisms (SNPs) located on autosomes were considered for the association analysis.

Longissiumus dorsi iron mineral content was analyzed under a Bayesian model using GenSel software (Fernando and Garrick (2009)) using a similar approach to that described by Tizioto et al. (2013) for the GWAS. The statistical model included fixed effects of contemporary group which was formed using birth and feedlot locations, breeding season and slaughter group. The animal's age at slaughter was included as a covariate.

The genomic regions associated with iron content were examined for candidate genes using Map Viewer (NCBI). The enriched annotations and pathways in which genes within these regions are involved were evaluated using the Database for Annotation, Visualization and Integrated Discovery (DAVID) software (Huang et al. (2009)).

Results and Discussion

After filtering the SNP based on call rate, allele frequency and Hardy-Weinberg equilibrium, and imputing 0.80% of missing genotypes, 449,364 loci were available for the GWAS.

We identified regions across the whole genome that harbor QTL related to iron content. Most of the identified QTL were small-effect QTL (i.e., accounted for less than 1% of the genetic additive variance of the trait). However, a few large-effect QTL were found. A QTL that explained 6.53% of the additive genetic variance in iron content (Table 1) was detected at 72 Mb on BTA12 (Figure 1). The genomic region that harbored this major effect QTL includes genes from the ATP-binding cassette family.

The majority of genes within the genomic regions associated with iron content of muscle function in signal transduction, signaling pathway, integral to membrane, intrinsic to membrane, regulation of transcription and metal ion binding.

Table 1. Chromosome, location, number of SNPs, and genetic variation explained by the most important QTL regions associated with iron concentration in muscle tissue of Nelore.

Chr[a]	Position (Mb)[b]	Number of SNPs[c]	Variance explained (%)[d]
12	72	94	6.53
7	32	257	4.97

[a]Chr. = Chromosome; [b]Position (Mb) = Position of the QTL on the chromosome in megabases; [c]Number of SNP within the 1-Mb window detected as harboring the QTL; [d]Percentage of the additive genetic variance explained by the 1-Mb window estimated by calculating the molecular breeding values for all animals using the SNP effects for each window

Iron is needed for several different metabolic pathways which are continuously operating at the molecular level and are essential to human life (Wood and Ronnenber, (2006)). As was found in Angus (Mateescu et al. (2013)), iron concentration is an important candidate mineral for genomic selection in *longissiumus dorsi* muscle; due to the presence of a large-effect QTL (Table 1), however, the principal genomic region identified by Mateescu et al. (2013) was not the same as detected in this study. The largest-effect QTL detected in Angus on BTA15 explained 4.76% of the additive genetic variation (Mateescu et al. (2013)). In fact, none of the large-effect QTL reported for muscle iron content identified in Angus (Mateescu et al. (2013)) colocalized with those found in this study. While a QTL mapped at 62 Mb on BTA1 explained 1.52% of the additive genetic variance in Fe content in Angus, in Nelore this genomic region explained just 0.05% of the additive genetic variance. Morris et al. (2013) conducted a study in Jersey and Limousin back-cross calves to search for QTL that influenced the amount of minerals in liver, kidney and muscle using microsatellites and the regions of the genome identified as harboring large-effect QTL for Fe concentration were also discordant with those detected in this study.

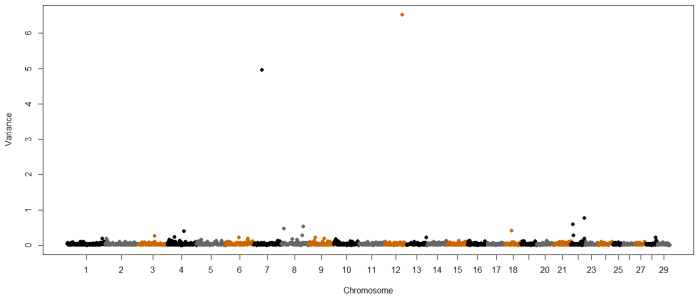

Figure 1. Genome-wide plot of additive genetic variance explained (%) by 1-Mb marker windows for iron concentration of Nelore *longissimus dorsi* muscle.

ATP-binding cassette family genes were found in the region harboring the large-effect QTL for iron in Nelore breed which mapped to BTA12. ABC proteins transport a number of substrates, including metal ions across the plasma membrane, and also across intracellular membranes (Vasiliou et al. (2009)).

A recent study has demonstrated that different genomic regions influence meat quality traits in indicine and taurine cattle (Tizioto et al. (2013)). This could be due to dissimilarities between the subspecies for allele frequencies at the causal mutations and tested SNPs and therefore the extent of LD available to detect QTL (Bolormaa et al. (2013)) or due to the divergence between indicine and taurine cattle, it may reflect the fact that polymorphisms in different genes underlie variation in these subspecies. Another reason for these inconsistences may be due to the resolution of the genotyping platform used. The Illumina BovineHD array used in this study has a considerably higher resolution than the BovineSNP50 assay and previously employed microsatellite scans which may have allowed the identification of genomic regions not identified by lower resolution scans.

Conclusions

Significant genomic regions related to the iron composition of *longissimus dorsi* muscle were identified across all chromosomes revealing the polygenic nature of this trait. The genetic improvement of tissue iron composition traits could allow the identification of animals early in life with the capacity to efficiently use the iron supply and avoid the production of nutritionally deficient beef. A large number of phenotypic records and validation in other populations will be required to accurately estimate the effects of the detected QTL before this information can efficiently be used in animal breeding programs. This study provides the first step towards genomic selection for iron content in the muscle tissue of Nelore beef cattle, which involves variability at many genes. Identification of the causal variants underlying large-effect QTL would allow cost-effective selection for improved mineral transport and metabolism in both taurine and indicine breeds of cattle.

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