Physiol Genomics 45: 1012–1020, 2013.
First published September 10, 2013; doi:10.1152/physiolgenomics.00066.2013.

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Genome scan for meat quality traits in Nelore beef cattle

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Submitted 1 May 2013; accepted in final form 3 September 2013

Tizioto PC, Decker JE, Taylor JF, Schnabel RD, Mudadu MA, Silva FL, Mourão GB, Coutinho LL, Tholon P, Sonstegard TS, Rosa AN, Alencar MM, Tullio RR, Medeiros SR, Nassu RT, Feijó GL, Silva LO, Torres RA, Siqueira F, Higa RH, Regitano LCA. Genome scan for meat quality traits in Nelore beef cattle. *Physiol Genomics* 45: 1012–1020, 2013. First published September 10, 2013; doi:10.1152/physiolgenomics.00066.2013.—Meat quality traits are economically important because they affect consumers' acceptance, which, in turn, influences the demand for beef. However, selection to improve meat quality is limited by the small numbers of animals on which meat tenderness can be evaluated due to the cost of performing shear force analysis and the resultant damage to the carcass. Genome wide-association studies for Warner-Bratzler shear force measured at different times of meat aging, backfat thickness, ribeye muscle area, scanning parameters [lightness, redness (a*), and yellowness] to ascertain color characteristics of meat and fat, water-holding capacity, cooking loss (CL), and muscle pH were conducted using genotype data from the Illumina BovineHD BeadChip array to identify quantitative trait loci (QTL) in all phenotyped Nelore cattle. Phenotype count for these animals ranged from 430 to 536 across traits. Meat quality traits in Nelore are controlled by numerous QTL of small effect, except for a small number of large-effect QTL identified for a*fat, CL, and pH. Genomic regions harboring these QTL and the pathways in which the genes from these regions act appear to differ from those identified in taurine cattle for meat quality traits. These results will guide future QTL mapping studies and the development of models for the prediction of genetic merit to implement genomic selection for meat quality in Nelore cattle.

GWAS; Zebu; QTL; beef; cattle

FOR DECADES, CATTLE BREEDING programs have focused on improving growth (3, 13), despite the importance of meat quality and yield traits such as meat tenderness, backfat thickness (BFT), and ribeye muscle area (REA) due to their impact on consumer satisfaction and product pricing. Less attention has been paid to the genetic improvement of these traits because they are costly and difficult to measure and are observed only after an animal has been slaughtered. Meat tenderness has been identified as a major issue of the beef industry, especially in animals with indicine ancestry. It is known that crossbreed animals with higher degrees of *Bos indicus* contribution have decreased meat tenderness (26).

Traditional breeding programs select animals based on estimated breeding values calculated from phenotypic records and pedigrees and using an estimate of the heritability of each trait; however, this method makes no attempt at identifying the genes and pathways involved in the target traits, and the process is slow if the trait can only be measured late in life or postmortem as is the case for meat tenderness (21). Research conducted primarily in *Bos taurus* cattle has identified QTL on chromosomes 1, 2, 4, 5, 7, 8, 10, 11, 15, 18, 20, 25, and 29 for meat quality traits (2, 6–9, 12, 16–18, 25, 27, 38, 39, 46, 56). However, it is not clear whether these loci contribute to variation in the same traits in *B. indicus* cattle. Furthermore, genome-wide association studies (GWAS) performed using Bayesian or genomic best linear unbiased prediction models, which may be used to estimate molecular breeding values in the deployment of genomic selection, are increasingly being used to identify quantitative trait loci (QTL) associated with complex traits (14, 15, 31, 32, 36, 52). This approach requires that thousands of molecular markers spanning the entire genome be genotyped in a population of phenotyped individuals and that the number of markers is calibrated relative to the extent of linkage disequilibrium (LD) within the population to ensure that QTL of large effect are not missed simply because they are beyond the range of LD of the nearest markers.

The success of genomic selection depends on the exploitation of LD between the markers and the QTL affecting a target trait (40). Before genetic information can be efficiently used within breeding programs, studies involving the breeds and populations targeted for improvement are essential to accurately describe the marker/QTL associations and phase relationships for important production traits in each population. Cattle breeds differ in phase relationships between marker and QTL alleles and also in allele frequencies, and consequently, the significance of QTL effects can differ between breeds. This study identifies genomic regions that putatively harbor genes related to variation in Warner-Bratzler shear force (WBSF) measured following different times of meat aging, BFT, REA,

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L*, a*, b* color parameters (L* = lightness, a* = redness, and b* = yellowness) for meat and fat, water-holding capacity (WHC), cooking loss (CL), and pH in Nelore beef cattle using genotypes produced from the Illumina BovineHD BeadChip (Illumina, San Diego, CA).

MATERIALS AND METHODS

Animal and phenotype collection. Nelore steers derived from 34 sires representing the main breeding lineages of Brazil were genotyped. Half-sib families were produced by artificial insemination of commercial and purebred Nelore dams. Animals were raised and allocated to two feedlots, as previously described (50). The animals were slaughtered at an average endpoint of 5 mm of BFT. The phenotype count for these animals ranged from 430 to 536 across traits. The research was approved by the Embrapa Pecuária Sudeste (São Carlos, São Paulo, Brazil) ethics committee.

Phenotypes for WBSF (kg), BFT (mm), REA (cm^2), WHC (%), L*, a*, b* color parameters for meat and fat, and CL (%) were measured from 2.5 cm thick steaks harvested as a cross section of the longissimus dorsi muscle between the 11th and 13th ribs collected at slaughter. The steak from the 12th rib was used to measure BFT, REA, WHC, L*, a*, b* color parameters, and CL at 24 h postmortem. Measurements of WBSF were conducted on three steaks obtained between the 11th and 13th ribs after 24 h (WBSF0), 7 days (WBSF7), and 14 days (WBSF14) of aging at 2°C in a cold chamber manufactured by Macquay Heatcraft do Brasil Ltda (São José dos Campos, São Paulo, Brazil). Briefly, the fresh steak samples were used to measure BFT, REA, WHC, and color parameters. We determined the color parameters L*, a*, and b* after exposing the steaks to atmospheric oxygen for 30 min prior to analysis, and each trait was measured at three locations across the surface of the steak using a Hunter Lab colorimeter model MiniScan XE with Universal Software v. 4.10 (Hunter Associates Laboratory, Reston, VA), illuminant D65 and 10° standard observer. Muscle pH also was measured at three locations across the steak using a Testo pH measuring instrument model 230 (Testo, Lenzkirch, Germany). WHC was determined by a compression technique in which a 0.2 kg meat sample was compressed at a force of 10 kg for 5 min, and WHC was estimated as the difference between the weight of the sample before and after compression (20). After these analyses, the steaks were weighed and cooked in a Tedesco combined oven model TC 06 (Tedesco, Caxias do Sul, RS, Brazil) at 170°C until the temperature at the center of each sample reached 70°C, controlled by thermocouples linked to FEMUX software (Flyever, São Carlos, SP, Brazil) to measure CL and WBSF. The WBSF measures were obtained with the texture analyzer TA - XT2i coupled to a Warner-Bratzler blade with 1.016 mm thickness. To measure CL we used the difference in weights before and after cooking the grilled steaks, expressed as percentage.

DNA extraction and genotyping. Straws of frozen semen obtained from Brazilian artificial insemination centers were used to extract DNA from bulls by a standard phenol-chloroform method (43). For the steer progeny, 5 ml blood samples were collected, and DNA extractions were performed by a salting-out method. DNA concentration was measured by spectrophotometry, and quality was verified by the 260/280 optical density ratio, followed by inspection of integrity through agarose gel electrophoresis. All animals were genotyped using the Illumina BovineHD BeadChip (Illumina, San Diego, CA) either at the United States Department of Agriculture's Agricultural Research Service 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. Animals were filtered according to call rate (<90%) and heterozygosity (>40%). Loci were deleted if they could not be uniquely localized to an autosome or the X chromosome in the UMD3.1 sequence assembly, call rate (<85%), minor allele frequency (<0.1%), and Hardy-Weinberg equilibrium. Only effects of single nucleotide polymorphisms (SNPs) located on the autosomal chromosomes were considered for association analysis.

Genome-wide association analysis. Missing genotypes were imputed with BEAGLE (5) without the use of pedigree information. Meat quality traits were analyzed under a Bayesian model with GenSel software (15). The BayesC approach, which is less sensitive to starting values for additive genetic and residual variances was first used to estimate these variances, assuming the π parameter was zero (i.e., assuming that all SNPs contributed to explaining genetic variance in each trait). The estimated additive genetic and residual variances from the BayesC0 analyses were then used as starting values in BayesCπ analyses to estimate the π parameter for each trait. The estimated values for the additive genetic and residual variances and π were finally used to run BayesB analyses to estimate the SNPs effects. The BayesB analysis fits separate variances for every SNP in the model allowing large effect SNP to be estimated without overly regressing their effects toward zero. The statistical model included fixed effects of birth and feedlot locations, breeding season, slaughter group, and animal age at slaughter as a covariate.

The Bayesian estimation of SNP effects was performed based on the model below:

$$y = 1\mu + \sum_{j=1}^{k} xj\beta j\delta j + e$$

where y is the vector of phenotypic values, μ is an overall mean, k is the number of marker loci in the panel, x_j is the column vector representing the genotype covariate at locus j, β_j is the random allele substitution effect for locus j, which is conditional on σ^2_β and is assumed normally distributed $N(0, \sigma^2_\beta)$; when $\delta_j = 1$ but $\beta_j = 0$ when $\delta_j = 0$, δ_j is a random 0/1 variable indicating the absence (with probability π) or presence (with probability $1 - \pi$) of locus j in the model, and e is the vector of random residual effects assumed normally distributed $N(0, \sigma^2_e)$.

On the basis of the magnitude of the π parameter estimated in the BayesCπ analysis, we identified all genes within $\pm$ 10 kb of the largest effect $651{,}259 \times (1 - \hat{\pi})$ SNPs to search for candidate genes for the detected QTL. The genomic regions associated with each trait were examined for candidate genes using Map Viewer (NCBI). The enriched annotation and pathways in which genes within these regions are involved were evaluated using the Database for Annotation, Visualization and Integrated Discovery (DAVID) software (23).

RESULTS

Summary statistics. Raw means, standard deviations, variance components, heritability, and π estimates for each trait are in Table 1. The estimates of heritability are based on small sample sizes and consequently possess considerable sampling variance. Heritability estimates varied between 0.05 for L*fat and 0.28 for b*muscle.

GWAS. After selection of SNPs based on call rate, allele frequency, and Hardy-Weinberg equilibrium, as described in the methodology, genotypes were available for 651,259 SNP loci scored in both the steers and their sires and 0.80% of missing genotypes were imputed. The sire genotypes were included in the analysis to enable the estimation of molecular breeding values for these important animals. We found that the evaluated meat quality traits were primarily influenced by QTLs of small effect and that no genes of large effect such as attributed to *CAPN1* and *CAST* in taurine cattle (4, 10, 35, 44) were detected.

The software DAVID v6.7 was used to search for enriched functional clusters and pathways based upon our supplied gene lists. For WBSF0, we identified 858 candidate loci (including uncharacterized loci, pseudogenes and predicted proteins, Sup-

Table 1. *Raw means, SD, heritability, and estimated π of each trait*

Trait	*n*	Mean ± SD	σ^2a	σ^2e	h^2	π
WBSF0, kg	442	8.70 ± 2.20	0.37228	1.84566	0.1678	0.992995
WBSF7, kg	425	5.93 ± 2.16	0.523924	2.21659	0.1911	0.957823
WBSF14, kg	437	4.56 ± 1.89	0.290425	1.56359	0.1566	0.996825
BFT, mm	536	6.42 ± 2.33	0.779323	2.87337	0.2133	0.990398
REA, cm^2	534	59.98 ± 7.55	10.848	29.2516	0.2705	0.891685
L*muscle	453	38.55 ± 2.55	0.694113	2.91523	0.1923	0.994683
a*muscle	453	16.88 ± 3.96	0.533459	2.06924	0.2049	0.999995
b*muscle	453	13.51 ± 2.00	0.823522	0.322681	0.2815	0.999946
L*fat	451	75.69 ± 4.71	1.10219	20.1762	0.0517	0.952439
a*fat	452	8.22 ± 4.04	0.830185	2.30143	0.1686	0.999899
b*fat	452	17.24 ± 2.82	0.984939	2.7525	0.2635	0.954324
WHC, %	452	80.44 ± 3.19	1.0267	7.77859	0.1166	0.954324
CL, %	453	27.56 ± 5.51	1.1079	15.6947	0.0419	0.97999
pH	452	5.59 ± 0.20	0.011649	0.032997	0.2480	0.99979

Warner-Bratzler shear force (WBSF) was measured following different times of meat aging: 24 h after slaughter (WBSF0), 7 days after slaughter (WBSF7), and 14 days after slaughter (WBSF14). BFT, backfat thickness; REA, ribeye muscle area; WHC, water-holding capacity; CL, cooking loss. Color parameters for meat and fat: L*, lightness; a*, redness; b*, yellowness.

plemental Table S1) in the vicinity of the 4,563 associated SNPs selected based on the π parameter estimated for this trait (Table 1).[1] Genes that have already been reported as candidates for meat tenderness QTL were found in these analyses. One SNP associated with WBSF0 was located in the vicinity of calpain 2 (m/II) large subunit (*CAPN2*), and four were in calpain 5 (*CAPN5*); SNPs associated with WBSF0 were also found in collagen family (*COL15A1* and *COL23A1*) genes. However, no associated SNP was found within 10 kb of calpastatin (*CAST*), which has been shown to be associated with WBSF in taurine breeds. BTA7 was found to harbor SNPs, which explained the greatest amount of additive genetic variance in WBSF0 (Fig. 1); however, these SNPs were not located near any annotated genes, suggesting that the causal mutations may be regulatory in nature. The largest QTL identified for WBSF0 was located on BTA23 at 24 Mb (Table 2); the QTL in this genomic region accounted for only 0.11% of the additive genetic variance in WBSF0. There are several genes located within the vicinity of this QTL, including the glutathione S-transferase alpha gene family (*GSTA2, GSTA3, GSTA5, GSTA4*). We also identified candidate genes in other QTL regions such as *SERPIN2,* which encodes a serine protease protein and is located near to associated SNPs on BTA2. Serpin genes are known to control proteolysis in molecular pathways associated with cell survival and development (45). The DAVID functional analysis revealed clusters involved in potassium and calcium channel activity, and the enriched pathways found were neuroactive ligand-receptor interaction, TGF-β signaling, vascular smooth muscle contraction, focal adhesion, calcium signaling, and ribosome (Supplemental Table S1).

We identified 4,161 genes within regions tagged by the SNPs that were associated with WBSF7 (Supplemental Table S1). Two associated SNPs were found in the vicinity of calpain 1 (mu/I) large subunit (*CAPN1*), four in *CAPN2*, three in *CAPN5,* and two in *CAST,* in addition to the collagen gene family members (*COL1A1, COL24A1, COL28A, COL2A1, COL4A3,* and *COL6A3*), which were also enriched in this analysis. A candidate gene (*ASAP1*: ArfGAP with SH3 domain, ankyrin repeat, and PH domain 1) previously reported in a candidate gene study employing part of this Nelore population (50) was also found in this analysis to be among those loci most strongly associated with WBSF. The QTL region that explained the greatest proportion of additive genetic variance (0.10%) was located on BTA13 at 71 Mb where the genes for protein tyrosine phosphatase, receptor type, T (*PTPRT*), and histone H2B type 1-like (*LOC614378*) are located. The single SNP on BTA11 that explained the greatest amount of variation in WBSF7 tags a region harboring two candidate genes: *RAB11FIP5* (RAB11 family interacting protein 5), which is involved in protein trafficking from apical recycling endosomes to the apical plasma membrane, and *SFXN5* (sideroflexin 5), which transports citrate. The functional clusters enriched were glycoprotein, bisulfite bound, and metal-binding. Interesting pathways including neuroactive ligand-receptor interaction, O-glycan biosynthesis, and focal adhesion were also enriched (Supplemental Table S1).

The QTL that explained the greatest amount of additive genetic variance for WBSF14 was located on BTA2 at 73 Mb and accounted for 0.19% of the additive genetic variance. Few genes are located in this QTL region but include GLI family zinc finger 2 (*GLI2*), cytoplasmic linker associated protein 1 (*CLASP1*), MKI67 (FHA domain) interacting nucleolar phosphoprotein (*MKI67IP*), and ubiquitin-conjugating enzyme E2 N-like (*LOC100294993*). From the 382 candidate genes related to WBSF14, protection of telomeres 1 homolog (*S. pombe*) (*POT1*) located on BTA4 explained the most additive genetic variance in WBSF14 and was detected by the associated markers; this gene is essential for the replication of chromosome termini. Among the significant enriched functional clusters were lipid binding, focal adhesion, and exopeptidase activity; the most enriched pathway found for this meat aging time was Fc gamma R-mediated phagocytosis.

A total of 56 genes were detected as candidates for meat tenderness from the analysis of all measures of WBSF, and the functional analysis of these concordant genes revealed three enriched functional clusters related to the regulation of transcription, membrane and metal-binding (Supplemental Table S1).

[1] The online version of this article contains supplemental material.

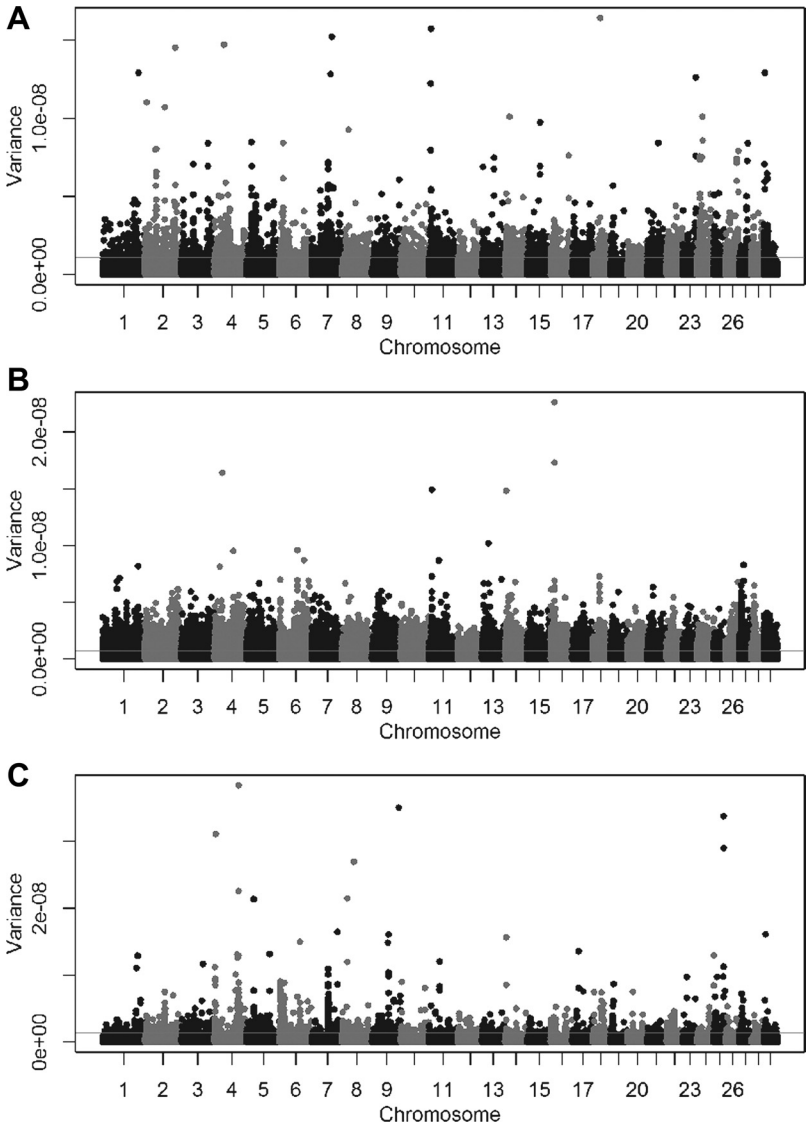

Fig. 1. Genome-wide Manhattan plots of additive genetic variance explained by each marker for Warner-Bratzler shear force after 24 h (WBSF0, *A*), after 7 days (WBSF7, *B*), and after 14 days (WBSF14, *C*).

From the GWAS for BFT (Fig. 2), a QTL located on BTA11 explained the greatest amount of variation in BFT (0.36%). Few genes and uncharacterized loci are mapped to this region (Table 2); however, none of them have a clear function in lipid anabolism or catabolism. *TTF1* (transcription termination factor, RNA polymerase I), located on BTA9, was the gene that harbors the single SNP that explains the greatest additive genetic variance in BFT. The enrichment analyses identified

Table 2. *QTL with the largest effect on variation in each trait*

Trait	Chr	Position, bp	Position, Mb	SNPs, *n*	Variance Explained, %
WBSF0	23	24,002,374. . .24,999,318	24	453	0.11
WBSF7	13	71,001,773. . .71,998,254	71	364	0.10
WBSF14	2	73,002,970. . .73,996,212	73	271	0.19
BFT	11	82,000,961. . .82,998,027	82	298	0.36
REA	23	24,002,374. . .24,999,318	24	453	0.08
L*muscle	23	24,002,374. . .24,999,318	24	453	0.14
L*fat	23	24,002,374. . .24,999,318	24	453	0.10
a*fat	12	36,010,895. . .36,994,095	23	333	1.21
b*fat	26	43,006,538. . .43,997,236	43	323	0.11
WHC	17	58,001,206. . .58,998,805	58	326	0.10
CL	23	24,002,374. . .24,999,318	24	453	0.10
pH	8	87,002,083. . .87,998,405	87	304	4.01

Chr, chromosome; position (bp), position where the quantitative trait locus (QTL) starts and finishes in the chromosome in base pairs; position (Mb), position of the QTL on the chromosome in megabases.

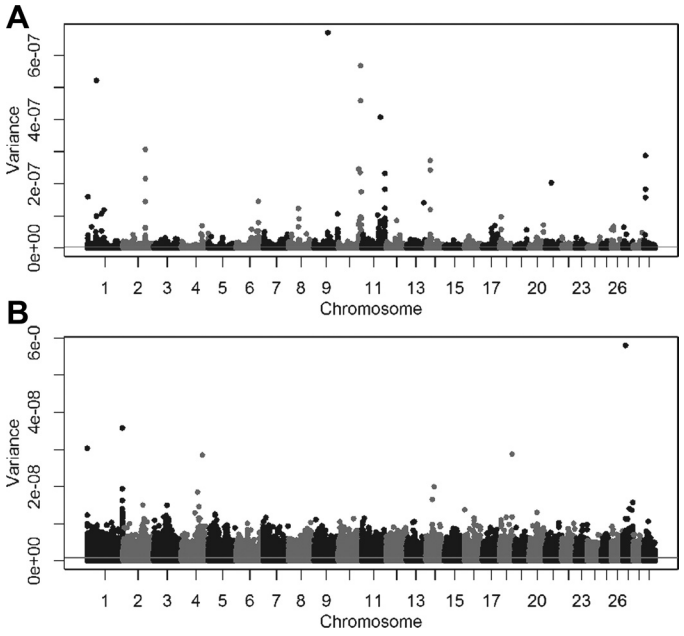

Fig. 2. Genome-wide Manhattan plot of additive genetic variance explained by each marker for backfat thickness (BFT, *A*) and ribeye muscle area (REA, *B*).

clusters related to cofactor biosynthetic process, amino-acid biosynthesis, cell death, among others (Supplemental Table S2). Previously identified candidate genes including leptin (*LEP*) and diacylglycerol O-acyltransferase 1 (*DGAT1*) (48, 49) were not identified in this analysis. Enriched pathways include drug metabolism, pentose and glucuronate interconversions, pantothenate and CoA biosynthesis, and neuroactive ligand-receptor interaction (Supplemental Table S2).

Analyses for REA identified six QTL that individually explained 0.8% of the additive genetic variance as being the most important loci (Supplemental Table S3). The same QTL described for WBSF0 at BTA23 appeared to also influence REA (Table 2). Genes related to protein kinase activity, ATP-binding, cell death, and keratin filament were found to be enriched. EH-domain containing 2 (*EHD2*) gene located on BTA18 harbors one of the single SNPs explaining the most additive genetic variance in this trait (Fig. 2). The enriched pathways were adherens junction, sphingolipid metabolism, O-glycan biosynthesis, and glycosphingolipid biosynthesis (Supplemental Table S2).

The estimated π values for a*muscle and b*muscle color parameters were higher than for the other traits (Table 1), indicating that relatively few SNPs are associated with these traits (Fig. 3). There were no annotated candidate genes identified within $\pm$ 10 kb of the associated SNPs. For L*muscle, the most strongly associated SNP was found on BTA21 (Fig. 3); this region harbors the fibronectin type III and SPRY domain containing 2 (*FSD2*) gene. (Supplemental Table S4).

The a* and b* color parameters for fat and L* for muscle seem to be influenced by similar large-effect genes (Fig. 3, Supplemental Table S4). Pathways related to lysine degradation, other glycan degradation, and cell adhesion molecules, among others appear to be important for the maintenance of color in bovine postmortem muscle (Supplemental Table S4). We identified a QTL at 58 Mb on BTA17 that has the largest effect (0.10% of additive genetic variance) on WHC (Table 2 and Supplemental Table S5). In this QTL region (Table 2) are located protein kinase, AMP-activated, beta 1 noncatalytic subunit (*PRKAB1*), and heat shock 22 kDa protein 8 (*HSPB8*). DAVID revealed clusters such as organic and catabolic processes, activation of immune response, and ubiquitin-dependent protein catabolic process for the genes in the genomic regions associated with WHC (Fig. 4). The enriched pathways were calcium signaling and neuroactive ligand-receptor interaction (Supplemental Table S5). As for WHC, the largest effect QTL identified to influence CL is located on BTA23 and explains 0.10% of the additive genetic variance; genes related to antigen processing and presentation pathway including heat shock proteins were enriched in this analysis.

A major QTL for muscle pH (24 h) was identified on chromosome 8 at 87 Mb that explained 4.01% of the additive genetic variance.

DISCUSSION

WBSF values (Table 1) are higher than those normally reported for *B. taurus* breeds (32), but this was expected and is in agreement with the observation that WBSF increases as the proportion of *B. indicus* breeding increases in crossbred animals (26). We estimated the heritability of each trait using BayesC0 analyses because BayesC is less sensitive to sample size than BayesB, which requires the joint estimation of SNP effect variances for each of the markers included in the model (15). Nevertheless, most of the heritability estimates were moderate in size, indicating that QTL exist for all of these traits in Nelore cattle.

Many important production traits in taurine cattle are polygenic and are controlled by a large number of QTL (41, 42). The identification of genes underlying variation in complex traits would enhance our understanding of the biology of phenotypic variation and would facilitate improved accuracy of selection. We performed a GWAS for 14 meat quality traits using a half-sib Nelore population that enabled us to identify many QTL underlying these traits. With the exception of CL, a*fat, and pH for which large effect QTL were identified, the detected QTLs were of very small effect. For meat tenderness, in particular, this finding is contrary to results in taurine cattle where QTL explaining 4.1–7.4% of the additive genetic variance in WBSF have been detected (32). The improvement of meat quality traits, including meat tenderness, could stimulate consumer purchases of beef because they expect desirable eating experiences and tend to divert their purchases to other sources of animal protein when they experience tough meat.

Changes in texture and sensorial properties can occur because of the postmortem degradation processes that influence the quality attributes of beef. Much attention has been paid to the calpain and calpastatin genes, which are involved in an important proteolytic system, and variation in these genes has been found to affect meat tenderness in different cattle populations (10, 11, 35, 44). Although we found SNPs in *CAPN1*, *CAPN2*, *CAPN5,* and *CAST* that were associated with WBSF measures in this population, they had smaller effects than other QTL candidates (Supplemental Table S1). This result may reflect the small sample size employed in this study. It is also possible that differences between taurine and indicine cattle for allele frequencies at the *CAPN1* and *CAST* causal mutations or the extent of LD between SNPs and these causal variants could

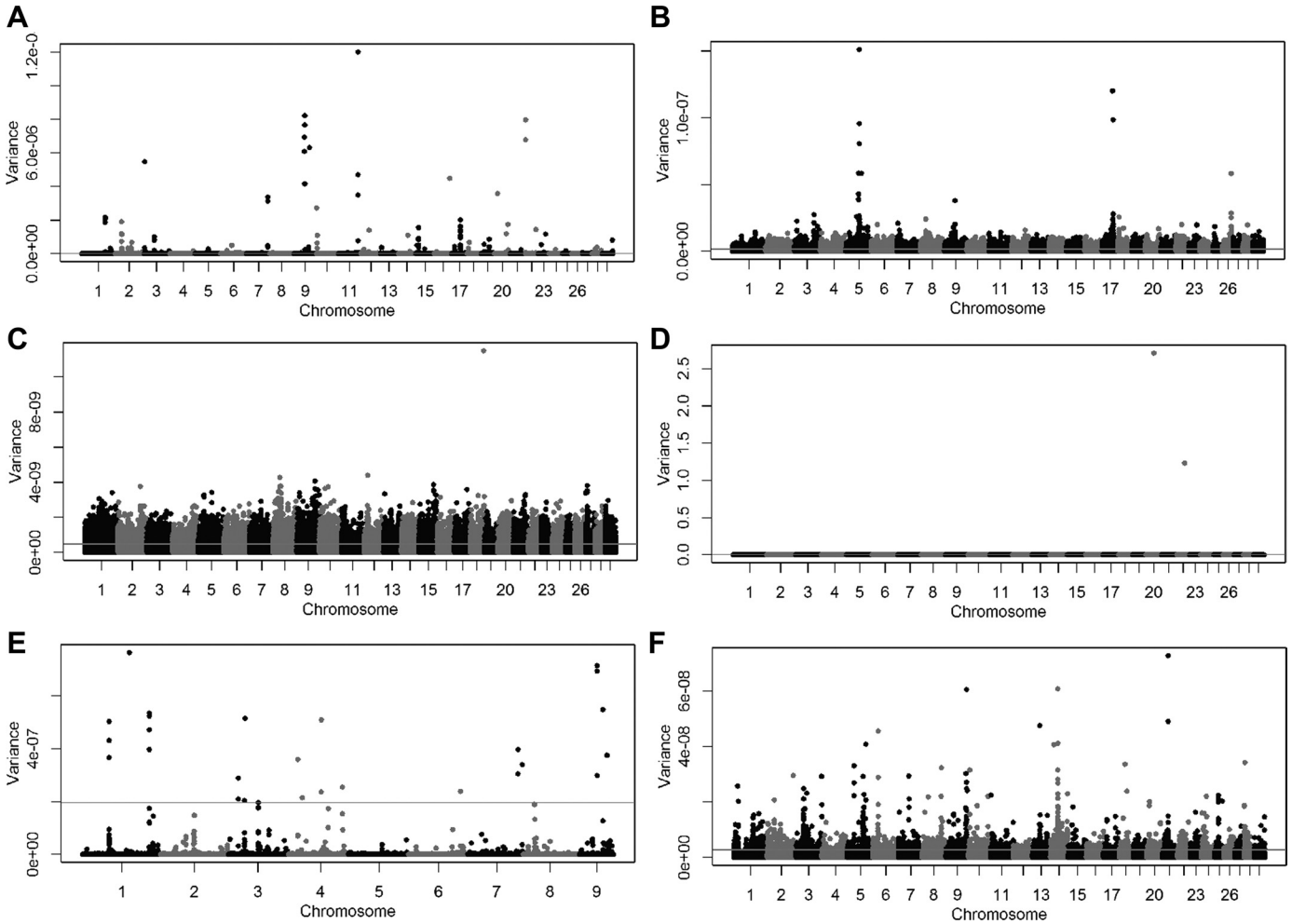

Fig. 3. Genome-wide plot of additive genetic variance explained by each marker for a*fat (*A*), b*fat (*B*), L*fat (*C*), a*muscle (*D*), b*muscle (*E*), and L*muscle (*F*). Color parameters: a*, redness; b*, yellowness; L*, lightness.

result in different marker effects being detected in different breeds. The presence of epistasis could also influence the magnitude of SNP effects across different populations, since it is known that epistatic effects can explain large amounts of the variation in quantitative traits (24).

The difference between genes and pathways identified in this Nelore study and those already reported for taurine breeds could reflect differences in metabolism or in the selection history of Zebu cattle. Functional clusters related to potassium and calcium transport as well as to metal binding were found to be enriched in our analyses of the WBSF measures. Potassium is necessary for muscle contraction and nerve impulses and also contributes to the proper balance of fluids in cells (28). Studies conducted with the same Nelore population show that potassium content in beef may affect meat tenderness (51). Furthermore, the calpain system is highly sensitive to fluctuating levels of calcium ions, pH, and temperature, and these three parameters all change rapidly immediately postmortem (47), indicating that calcium channel activity could generally influence postmortem tenderization.

Important pathways including neuroactive ligand-receptor interaction and TGF-β signaling were identified from the genes in the regions of the genome where SNP were associated with WBSF0. In the neuroactive ligand-receptor interaction pathway, several genes related to G protein-coupling were identified (Supplemental Table S1). Studies have shown that activation of G protein-coupled receptors is involved in the maintenance of skeletal muscle and also could be involved in the mediation of myofiber maturation and growth, operating through many signaling pathways to selectively stimulate protein synthesis or inhibit cytokine-dependent protein turnover (19).

The TGF-β pathway is involved in many cellular processes including apoptosis. Factor-beta (TGF-β) superfamily genes have been identified as important regulators of muscle development (33). Genes from our gene list, including *NOG* (Noggin), which is crucial for cartilage morphogenesis and joint formation and also inhibits bone morphogenetic protein (BMP) signaling, which is essential for growth and neural tube and somite patterning, and *BMP7* (bone morphogenetic protein 7), which induces cartilage and bone formation, are in this pathway (29).

The *PTPRT* gene was identified as a QTL candidate (Table 2) for WBSF7 and may be involved in both signal transduction and cellular adhesion in the central nervous system; both pathways were also found as playing an important role in variation in WBSF0. Cytokine-cytokine receptor interaction

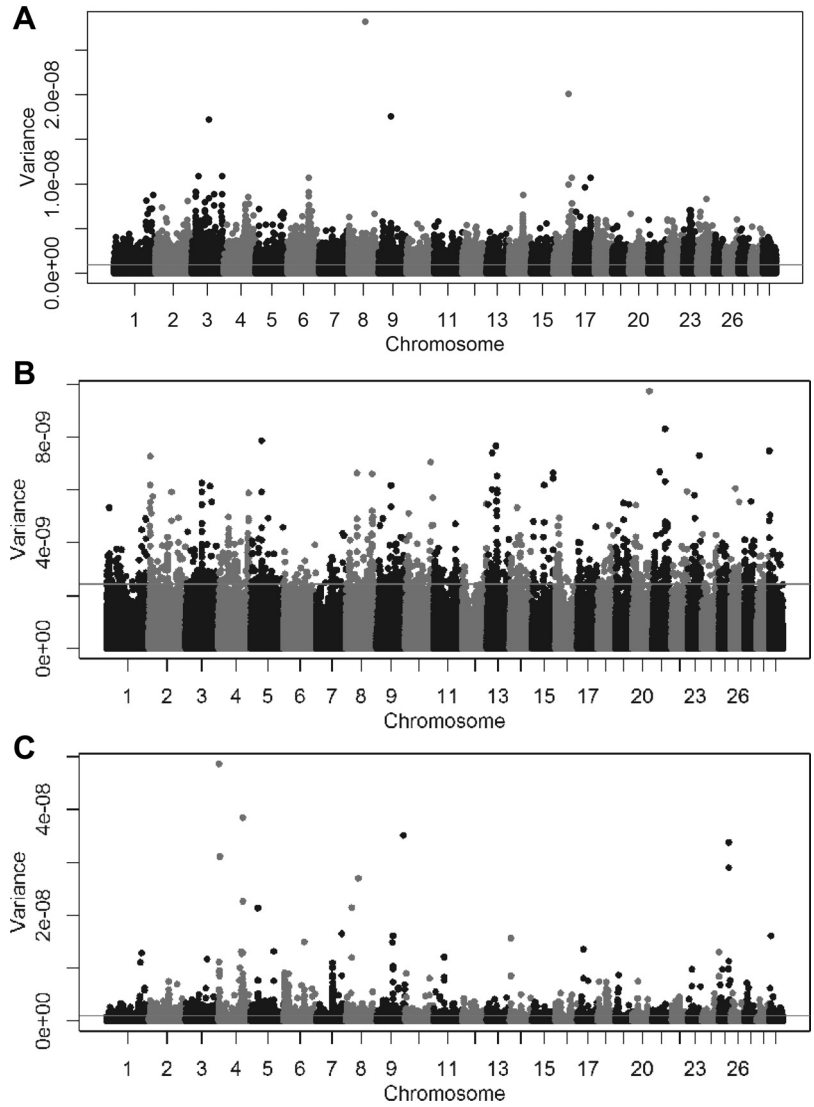

Fig. 4. Genome-wide plot of additive genetic variance explained by each marker for water-holding capacity (WHC, *A*), cooking loss (CL, *B*), and pH (*C*).

and chemokine signaling pathways were enriched for genes tagged by SNPs influencing WBSF7, suggesting that alternative and unobvious mechanisms may be acting on meat tenderness besides proteolysis. Some studies have proposed that heat shock proteins may play a role in meat tenderness (22, 37), and another study has suggested that genes involved in immune response may also be involved (55).

The O-glycan biosynthesis pathway was also enriched among the genes associated with WBSF7 and is involved in modifications of serine or threonine residues of proteins (53). The nonenzymatic glycosylation of tissue protein helps the formation of crosslinks, as O-linked oligosaccharide, that can lead to the structural and functional deterioration of collagen (34). The formation and accumulation of these crosslinks can contribute to the toughness of meat from aged animals. O-glycan biosynthesis is involved in glycosylation, which may affect collagen and other protein synthesis and could be the most common and complex form of posttranslational modification (54). From the analysis of genes within common regions associated with all WBSF measures, we infer that biological processes of regulation of transcription, glycosylation, and metal-binding are important to meat tenderness in Nelore cattle. Finally, for WBSF14 gene clusters involved in cell adhesion were found. Cell adhesion proteins appear to play an important role in the meat tenderness of this population.

The neuroactive ligand-receptor interaction pathway was enriched among the genes associated with BFT, indicating genes related to this pathway play a role in fat deposition in Nelore. The adherens junction sphingolipid metabolism, O-glycan biosynthesis, and glycosphingolipid biosynthesis pathways appear to have roles in muscle growth since they were also enriched in the REA analysis.

A possible pleiotropic QTL window on BTA23 had the largest effect on L*muscle and L*fat meat color parameters, WBSF0, REA, and CL. Further studies mining this region could help identify whether this is an effect of one or more variants that would be useful for simultaneously improving four meat quality traits in Nelore.

Postmortem chilling and pH, atmospheres used for packaging, antimicrobial interventions, and cooking can all influence meat color parameters. QTL were identified for all of these traits, suggesting that there are loci of large effect underlying these traits (30). The major QTL region found for a*fat (Table 2) harbors few genes; however, ruling out the implication of

these genes on this trait is difficult since there is little knowledge available on the biological mechanisms that regulate this fat color trait. Pathways influencing meat and fat color parameters include the cell adhesion molecules pathway that was detected for more than one color trait. Cell interactions are mediated by different families of receptors, including targeting cell adhesion to extracellular matrix proteins and to ligands on adjacent cells, and could influence many processes such as cellular growth, differentiation, junction formation, and polarity (1).

WHC of fresh meat is important because it affects both the yield and the quality of commercialized beef. It appears that proteolysis affects WHC and also plays a fundamental role in meat tenderness. The functional clusters organic acid catabolic process and proteolysis were enriched among genes in regions associated with WHC (Supplemental Table S5), and proteases including calpains *CAPN2, CAPN12, CAPN13,* and *CAPN14* were identified as candidate genes. The calcium signaling pathway was the most enriched pathway, which indicates that WHC may be affected by proteases such as the calpains, which are dependent on calcium. Changes in connective tissue during the cooking process may have a tenderizing effect. It has already been proposed that heat shock proteins may play a role in meat tenderization (22, 37). In our analysis, heat shock proteins were implicated in variation in CL, which is important for the juiciness of cooked beef.

The largest effect QTL identified for pH suggests that there is a major gene in this genomic region that influences the maintenance of a physiologically balanced internal environment.

Genetic variants have been largely explored in explaining variation in meat quality traits, but the underlying mechanisms affecting these traits remain poorly understood. Since the meat quality traits evaluated in this study in Nelore cattle appear to be controlled mainly by many QTL of small effect, identifying the relevant genes will be difficult, because each causal gene has a small contribution to overall variation. Thus, genomic selection, which explores the variability at many genes simultaneously, will be a better strategy for improving these traits than marker assisted selection.

This study provides the first step toward applying genomic selection for meat quality traits in Nelore cattle. We have identified important metabolic pathways related to meat quality traits that have not been reported in *B. taurus* cattle. These results may be biased since the magnitude of the estimated QTL effects is influenced by sample size. Studies with other populations from the Nelore breed will be required to validate the results of this study and will also be helpful for the development of models for the prediction of genetic merit to implement genomic selection for meat quality in Nelore cattle.

ACKNOWLEDGMENTS

We thank Flavia Aline Bressani, Wilson Malagó Jr. and Avelardo U. C. Ferreira for technical assistance, and Dr. Michele Lopes do Nascimento, Dr. Andrea Souza, and Amália Saturnino for monitoring the feedlots, Dr. Michel E. B. Yamaguishi and Fabio D. Vieira for technical assistance on data base maintenance, Dr. Dorian Garrick for data analysis support, the University of Missouri for accepting the first author as a visiting scholar, the National Council for Scientific and Technological Development (CNPq) for providing fellowships to M. M. de Alencar, L. L. Coutinho, and L. C. A. Regitano, and the São Paulo Research Foundation (FAPESP) for providing a scholarship to P. C. Tizioto.

GRANTS

We thank Embrapa, CNPq, and FAPESP for financial support.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: P.C.T. and L.C.A.R. conception and design of research; P.C.T., T.S.S., A.N.R., M.M.A., R.R.T., S.R.M., R.T.N., G.L.D.F., L.O.S., R.A.T., F.S., and L.C.A.R. performed experiments; P.C.T., J.E.D., J.F.T., R.D.S., M.A.M., F.L.S., G.B.M., P.T., R.H.H., and L.C.A.R. analyzed data; P.C.T., J.E.D., J.F.T., L.L.C., and L.C.A.R. interpreted results of experiments; P.C.T. and J.E.D. prepared figures; P.C.T., J.E.D., J.F.T., and L.C.A.R. drafted manuscript.

REFERENCES

1. **Albelda SM, Buck CA.** Integrins and other cell adhesion molecules. *FASEB J* 4: 2868–2880, 1990.
2. **Alexander LJ, MacNeil MD, Geary TW, Snelling WM, Rule DC, Scanga JA.** Quantitative trait loci with additive effects on palatability and fatty acid composition of meat in a Wagyu-Limousin F2 population. *Anim Genet* 38: 506–513, 2007.
3. **Arthur PF, Renand G, Krauss D.** Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. *Livestock Prod Sci* 68: 131–139, 2001.
4. **Barendse W.** *DNA markers for meat tenderness.* International patent application PCT/AU02/00122. International patent publication WO 02/064820 A1, 2002.
5. **Browning SR, Browning BL.** Rapid and accurate haplotype phasing and missing data inference for whole genome association studies using localized haplotype clustering. *Am J Hum Genet* 81: 1084–1097, 2007.
6. **Casas E, Keele JW, Shackelford SD, Koohmaraie M, Sonstegard TS, Smith TP, Kappes SM, Stone RT.** Association of the muscle hypertrophy locus with carcass traits in beef cattle. *J Anim Sci* 76: 468–473, 1998.
7. **Casas E, Shackelford SD, Keele JW, Stone RT, Kappes SM, Koohmaraie M.** Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. *J Anim Sci* 78: 560–569, 2000.
8. **Casas E, Stone RT, Keele JW, Shackelford SD, Kappes SM, Koohmaraie MA.** Comprehensive search for quantitative trait loci affecting growth, and carcass composition of cattle segregating alternative forms of the myostatin gene. *J Anim Sci* 79: 854–860, 2001.
9. **Casas E, Shackelford SD, Keele JW, Koohmaraie M, Smith TP, Stone RT.** Detection of quantitative trait loci for growth and carcass composition in cattle. *J Anim Sci* 81: 2976–2983, 2003.
10. **Casas E, White SN, Wheeler TL, Shackelford SD, Koohmaraie M, Riley DG, Chase CC, Johnson DD, Smith TPL.** Effects of calpastatin and μ-calpain markers in beef cattle on tenderness traits. *J Anim Sci* 84: 520–525, 2006.
11. **Corva P, Soria L, Schor A, Villareal E, Cenci MP, Motter M, Mezzadra C, Melluci L, Miquel C, Paván E, Depetris G, Santini F, Naón JG.** Association of CAPN1 and CAST gene polymorphisms with meat tenderness in Bos taurus beef cattle from Argentina. *Genet Mol Biol* 30: 1064–1069, 2007.
12. **Davis GP, Moore SS, Drinkwater RD, Shorthose WR, Loxton ID, Barendse W, Hetzel DJ.** QTL for meat tenderness in the M. longissimus lumborum of cattle. *Anim Genet* 39: 40–45, 2008.
13. **Decker JE, Vasco DA, McKay1 SD, McClure MC, Rolf MM, Kim J, Northcutt SL, Bauck S, Woodward BW, Schnabel BD, Taylor JF.** A novel analytical method, Birth Date Selection Mapping, detects response of the Angus (Bos taurus) genome to selection on complex traits. *BMC Genom* 13: 606, 2012.
14. **Fan B, Onteru SK, Du ZQ, Garrick DJ, Stalder KJ, Rothschild MF.** Genome-wide association study identifies loci for body composition and structural soundness traits in pigs. *PLoS One* 6: e14726, 2011.
15. **Fernando RL, Garrick DJ.** *GenSel - User manual for a portfolio of genomic selection related analyses* (3rd ed.). Ames, IA: Animal Breeding and Genetics, Iowa State Univ., 2009. Accessed January 23, 2013, http://www.biomedcentral.com/content/supplementary/1471-2105-12-186-s1.pdf.
16. **Gill JL, Bishop SC, McCorquodale C, Williams JL, Wiener P.** Association of selected SNP with carcass and taste panel assessed meat quality

traits in a commercial population of Aberdeen Angus-sired beef cattle. *Genet Select Evol* 41: 36, 2009.
17. **Gill JL, Bishop SC, McCorquodale C, Williams JL, Wiener P.** Associations between single nucleotide polymorphisms in multiple candidate genes and carcass and meat quality traits in a commercial Angus-cross population. *Meat Sci* 86: 985–993, 2010.
18. **Gutierrez-Gil B, Wiener P, Nute GR, Burton D, Gill JL, Wood JD, Williams JL.** Detection of quantitative trait loci for meat quality traits in cattle. *Anim Genet* 39: 51–61, 2008.
19. **Guttridge DC.** Making muscles grow by G protein-coupled receptor signaling. *Sci Sign* 4: pe45, 2001.
20. **Hamm R.** Functional properties of the myofibrillar system and their measurement. In: *Muscle as Food*, edited by Bechtel PJ. Orlando: Academic Press, 1986, p. 135–199.
21. **Hayes BJ, Goddard ME.** Mapping genes for complex traits in domestic animals and their use in breeding programmes. *Nat Rev* 10: 381–391, 2009.
22. **Hocquette JF, Capel CB, Vidal V, Jesson B, Levéziel H, Renand G, Malek IC.** The Genotend chip: a new tool to analyse gene expression in muscle of beef cattle for beef quality prediction. *BMC Vet Res* 8: 135, 2012.
23. **Huang DW, Sherman BT, Lempicki RA.** Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Prot* 4: 44–57, 2009.
24. **Huang W, Richards S, Carbone MA, Zhu D, Anholt RR, Ayroles JF, Duncan L, Jordan KW, Lawrence F, Magwire MM, Warner CB, Blankenburg K, Han Y, Javaid M, Jayaseelan J, Jhangiani SN, Muzny D, Ongeri F, Perales L, Wu YQ, Zhang Y, Zou X, Stone EA, Gibbs RA, Mackay TF.** Epistasis dominates the genetic architecture of Drosophila quantitative traits. *Proc Natl Acad Sci USA* 109: 15553–15559, 2012.
25. **Imumorin IG, Kim EH, Lee YM, De Koning DJ, van Arandonk JA, De Donato M, Taylor JF, Kim JJ.** Genome scan for parent-of-origin QTL effects on bovine growth and carcass traits. *Front Genet* 2: 44, 2011.
26. **Johnson DD, Huffman RD, Williams SE.** Effects of percentage Brahman and Angus breeding, age-season of feeding and slaughter end point on meat palatability and muscle characteristics. *J Anim Sci* 68: 1980–1986, 1990.
27. **Keele JW, Shackelford SD, Kappes SM, Koohmaraie M, Stone RT.** A region on bovine chromosome 15 influences beef longissimus tenderness in steers. *J Anim Sci* 77: 1364–1371, 1999.
28. **Knochel JP, Schlein EM.** On the mechanism of rhabdomyolysisin potassium depletion. *J Clin Invest* 51: 1750–1758, 1972.
29. **La Rosa I, Camargo L, Pereira MM, Fernandez-Martin R, Paz DA, Salamone DF.** Effects of bone morphogenic protein 4 (BMP4) and its inhibitor, Noggin, on in vitro maturation and culture of bovine preimplantation embryos. *Reprod Biol Endocrinol* 9: 18, 2011.
30. **Mancini RA, Hunt MC.** Current research in meat color. *Meat Sci* 71: 100–121, 2005.
31. **Meuwissen TH, Hayes BJ, Goddard ME.** Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157: 1819–1829, 2001.
32. **McClure MC, Ramey HR, Rolf MM, McKay SD, Decker JE, Chapple RH, Kim JW, Taxis TM, Weaber RL, Schnabel RD, Taylor JF.** Genome-wide association analysis for quantitative trait loci influencing Warner-Bratzler shear force in five taurine cattle breeds. *Anim Genet* 43: 662–673, 2012.
33. **McPherron AC, Lawler AM, Lee SJ.** Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature* 387: 83–90, 1997.
34. **Monnier VM, Kohn RR, Cerami A.** Accelerated age-related browning of human collagen in diabetes mellitus. *Proc Natl Acad Sci USA* 81: 583–587, 1984.
35. **Page BT, Casas E, Heaton MP, Cullen NG, Hyndman DL, Morris CA, Crawford AM, Wheeler TL, Koohmaraie M, Keele JW, Smith TPL.** Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *J Anim Sci* 80: 3077–3085, 2002.
36. **Peters SO, Kizilkaya K, Garrick DJ, Fernando RL, Reecy JM, Weaber RL, Silver GA, Thomas MG.** Bayesian genome-wide association analysis of growth and yearling ultrasound measures of carcass traits in Brangus heifers. *J Anim Sci* 90: 3398–3409, 2012.
37. **Pulford DJ, Vazquez SF, Frost DF, Fraser-Smith E, Dobbie P, Rosenvold K.** The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH. *Meat Sci* 79: 623–630, 2008.
38. **Reardon W, Mullen AM, Sweeney T, Hamill RM.** Association of polymorphisms in candidate genes with colour, water-holding capacity, and composition traits in bovine M. longissimus and M semimembranosus. *Meat Sci* 86: 270–275, 2010.
39. **Rexroad CE 3rd, Bennett GL, Stone RT, Keele JW, Fahrenkrug SC, Freking BA, Kappes SM, Smith TP.** Comparative mapping of BTA15 and HSA11 including a region containing a QTL for meat tenderness. *Mammal Genome* 12: 561–565, 2001.
40. **Rincon G, Weber KL, Van Eenennam AL, Golden BL, Medrano JF.** Hot topic: performance of bovine high-density genotyping platforms in Holsteins and Jerseys. *J Dairy Sci* 94: 6116–6121, 2011.
41. **Rolf MM, Taylor JF, Schnabel RD, McKay SD, McClure MC, Northcutt SL, Kerley MS, Weaber RL.** Genome-wide association analysis for feed efficiency in Angus cattle. *Anim Genet* 43: 367–374, 2012.
42. **Saatchi M, Schnabel RD, Rolf MM, Taylor JF, Garrick DJ.** Accuracy of direct genomic breeding values for nationally evaluated traits in US Limousin and Simmental beef cattle. *Genet Sel Evol* 44: 38, 2012.
43. **Sambrook J, Fritsch EF, Maniatis T.** *Molecular Cloning: A Laboratory Manual* (2nd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1990.
44. **Schenkel FS, Miller SP, Jiang Z, Mandell IB, Ye X, Li H, Wilton JW.** Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *J Anim Sci* 84: 291–299, 2006.
45. **Silverman GA, Whisstock JC, Bottomley SP, Huntington JA, Kaiserman D, Luke CJ, Pak SC, Reichhart JM, Bird PI.** Serpins flex their muscle: putting the clamps on proteolysis in diverse biological systems. *J Biol Chem* 285: 24299–24305, 2010.
46. **Stone RT, Casas E, Smith TP, Keele JW, Harhay G, Bennett GL, Koohmaraie M, Wheeler TL, Shackelford SD, Snelling WM.** Identification of genetic markers for fat deposition and meat tenderness on bovine chromosome 5: development of a low-density single nucleotide polymorphism map. *J Anim Sci* 83: 2280–2288, 2005.
47. **Suzuki K, Sorimachi H, Yoshizawa T, Kinbara K, Ishiura S.** Calpain: novel family members, activation, and physiologic function. *Biol Chem Hoppe Seyler* 376: 523–529, 1995.
48. **Taniguchi Y, Itoh T, Yamada T, Sasaki Y.** Genomic structure and promoter analysis of the bovine leptin gene. *IUBMB Life* 53: 131–135, 2002.
49. **Thaller G, Kuhn C, Winter A, Ewald G, Bellmann O, Wegner J, Xuhlke H, Fries R.** DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. *Anim Genet* 34: 354–357, 2003.
50. **Tizioto PC, Meirelles SL, Veneroni GB, Tullio RR, Rosa AN, Alencar MM, Medeiros SR, Siqueira F, Feijó GL, Silva LO, Torres-Júnior RA, Regitano LCA.** A SNP in ASAP1 gene is associated with meat quality and production traits in Nelore breed. *Meat Sci* 92: 855–857, 2012.
51. **Tizioto PC, Gromboni CF, de Araujo Nogueira AR, de Souza MM, de Alvarenga Mudadu M, Tholon P, Rosa AD, Tullio RR, Medeiros SR, Nassu RT, de Almeida Regitano LC.** Calcium and potassium content in beef: influences on tenderness and associations with molecular markers in Nellore cattle. *Meat Sci* 96: 436–440, 2013.
52. **VanRaden PM.** Efficient methods to compute genomic predictions. *J Dairy Sci* 91: 4414–4423, 2008.
53. **Varki A, Cummings R, Esko J, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME.** *Essentials of Glycobiology*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1999.
54. **Wopereis S, Lefeber DJ, Morava E, Wevers RA.** Mechanisms in protein O-glycan biosynthesis and clinical and molecular aspects of protein O-glycan biosynthesis defects: a review. *Clin Chem* 52: 574–600, 2006.
55. **Zhao C, Tian F, Yu Y, Luo J, Mitra A, Zhan F, Hou Y, Liu G, Zan L, Updike MS, Song J.** Functional genomic analysis of variation on beef tenderness induced by acute stress in Angus cattle. *Comp Funct Genom* 2012: 756284, 2012.
56. **Zhou G, Dudgeon C, Li M, Cao Y, Zhang L, Jin H.** Molecular cloning of the HGD gene and association of SNPs with meat quality traits in Chinese red cattle. *Mol Biol Rep* 37: 603–611, 2009.