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Copy number variations and genome-wide associations reveal putative genes and metabolic pathways involved with the feed conversion ratio in beef cattle

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Abstract The use of genome-wide association results combined with other genomic approaches may uncover genes and metabolic pathways related to complex traits. In this study, the phenotypic and genotypic data of 1475 Nellore (*Bos indicus*) cattle and 941,033 single nucleotide polymorphisms (SNPs) were used for genome-wide association study (GWAS) and copy number variations (CNVs) analysis in order to identify candidate genes and putative pathways involved with the feed conversion ratio (FCR). The GWAS was based on the Bayes B approach analyzing genomic windows with multiple regression models to estimate the proportion of genetic variance explained by each window. The CNVs were detected with PennCNV software using the log R ratio and B allele frequency data. CNV regions (CNVRs) were identified with CNVRuler and a linear regression was used to associate CNVRs and the FCR. Functional annotation of associated genomic regions was performed with the Database for Annotation, Visualization and Integrated Discovery (DAVID) and the metabolic pathways were obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG). We showed five genomic windows distributed over chromosomes 4, 6, 7, 8, and 24 that explain 12 % of the total genetic variance for FCR, and detected 12 CNVRs (chromosomes 1, 5, 7, 10, and 12) significantly associated [false discovery rate (FDR) < 0.05] with the FCR. Significant genomic regions (GWAS and CNV) harbor candidate genes involved in pathways related to energetic, lipid, and protein metabolism. The metabolic pathways found in this study are related to processes directly connected to feed efficiency in beef cattle. It was observed that, even though different genomic regions and genes were found between the two approaches (GWAS and CNV), the metabolic processes covered were related to each other. Therefore, a combination of the approaches complement each other and lead to a better understanding of the FCR.

Keywords CNV · Feed efficiency · Genomics · GWAS · Nellore cattle · SNPs

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Introduction

Feeding cattle is a major cost in beef production and it directly affects the overall profitability of the meat industry. Several

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strategies have been proposed to reduce this cost, focusing mainly on the improvement of feed efficiency. The feed conversion ratio (FCR) is a feed efficiency trait that measures the animal's capacity to convert feed consumed into the desired output (e.g., meat deposition or gained mass). The FCR is not a direct measurement, and it is computed as a function of the feed consumed, body weight gain, and duration of the trial (Arthur et al. 2001).

The discovery of DNA variants, such as single nucleotide polymorphisms (SNPs) and copy number variations (CNVs), which may be associated with the genetic variation of desired economic traits, has played an important role in livestock genetics (Kijas et al. 2011). In recent years, high-throughput technologies have led to the discovery of markers associated with economic traits by genome-wide association studies (GWAS), which allowed the identification of subsets of markers that explain an important portion of the variation of these traits (Barendse et al. 2007; Moore et al. 2009; Rolf et al. 2012). The use of genomic information can be a strategy for the improvement of interesting phenotypes such as the FCR by increasing the prediction accuracy of young animal candidates for genetic selection (Hayes et al. 2007), and, thus, accelerating genetic gain by reducing the generation interval. The association between markers and important phenotypes can be improved by using other genomic approaches, such as genomic prediction, to select animals (Bishop and Woolliams 2014; Kadarmideen 2014).

Additionally, several studies have reported the viability of using the information from SNPs to identify quantitative trait loci (QTL) and candidate genes associated with phenotypes of interest. Moreover, pathway analyses from GWAS results have been applied to aggregate information about genes and the physiology involved with important diseases and economical traits (Carbonetto and Stephens 2013), and to understand the molecular and physiological mechanisms involved in feed efficiency in beef cattle (Bolormaa et al. 2011; Snelling et al. 2011; Lu et al. 2013). Thus, the combination of GWAS with other approaches can be interesting to better explain these genes and pathways related to complex traits. There are alternative frameworks, such as CNVs, that can be useful to explain the variability and unveil the molecular architecture of complex traits (Hou et al. 2012b; Tamari et al. 2013; Bickhart and Liu 2014). The objective of this study was to identify candidate genes and putative pathways involved with the FCR in Nellore cattle (Bos indicus) from GWAS and CNV results.

Materials and methods

Ethical statement

No statement by the local ethics committee was required because the data used were from other experiments. DNA samples taken from each of the tests had been approved by the respective ethics committees of each study (Gomes et al. 2013; Santana et al. 2013; Alexandre et al. 2015).

Animals and phenotype

The phenotypic data of 1475 Nellore (B. indicus) young bulls and steers were used from 16 different studies focusing on feed efficiency conducted in Brazil from 2007 to 2013. The number of animals evaluated per test ranged from 45 to 120, and the animals had an average age of 574 ± 95 days and live weight of 381 ± 45 kg at the beginning of the experiments. These animals were from different breeding programs.

The tests were conducted in feedlots equipped with three different types of installation: two automated systems (GrowSafe and Calan Gates) and an individual pen system. Before testing, a period of adaptation to diets and facilities was conducted for no less than 21 days. Individual feed intake was measured daily for 70 to 90 days, with an average of 84 days. In addition, the feed was periodically analyzed for its chemical composition in order to adjust the dry matter intake (DMI). The diet was offered twice daily as total mixed ration. More details about the tests, diets, and managements appear in Gomes et al. (2013), Santana et al. (2013), and Alexandre et al. (2015).

During the experimental period, the animals were weighed regularly every 21 days to obtain the individual body weight (BW). These data were used to calculate the average daily gain (ADG), which was estimated as the slope of the linear regression of BW by individual experimental days. Feed efficiency was evaluated by the FCR, which was estimated by dividing the DMI by the ADG. Phenotypes (ADG, DMI, and FCR) were tested for normality (Shapiro–Wilk, $P < 0.05$) and the data that exceeded three standard deviations above or below the mean were considered outliers and excluded from further analyses.

Genotypes, imputation, and informativeness

The genotypic data from 3776 Nellore cattle were used in this study and these animals were genotyped with four different commercial products according to manufacturer: Illumina BovineSNP50® version 2 BeadChip (54,609 SNPs), Illumina BovineHD® Genotyping BeadChip (777,962 SNPs), GGP Indicus Neogen HD® (84,379 SNPs), and Affymetrix Axiom® Genome-Wide BOS 1 Array (648,874 SNPs). The genotypes were tested to ensure that they were determined correctly and clustering provided by the manufacturer was correct. First, only samples with genotype calls greater than 0.70 and call rate over 90 % were retained. Additionally, errors of duplicate samples were tested by calculating the proportion of alleles identical by state (IBS) of 10,000 SNPs randomly sampled, and all possible pairs of samples with IBS over 95 % were deleted.

In order to combine the genotypes of animals from these four different genotyping platforms and, thus, increase the sample size and marker density for association tests, a super-dense panel (SDP) was created and imputation was implemented. A total of 2604 animals were genotyped with Illumina BovineHD. Initially, the SDP (1,261,128 SNPs) was made by combining the genotypes of 279 (Affymetrix BOS 1) animals also genotyped using Illumina BovineHD (subset of 2604). Afterwards, imputation was performed for all animals with both genotypic and phenotypic data (1475 young bulls and steers). The imputation accuracy was tested by cross-validation for each SNP panel to the SDP in a parallel investigation and, for all scenarios, the concordance rate was higher than 97.5 % between the imputed and true genotypes. Imputation procedures were performed using FImpute 2.2 software (Sargolzaei et al. 2014).

After imputation, the genotypic data were evaluated for their informativeness and only SNPs in autosomal chromosomes (Chr) with minor allele frequency above 2 % and deviation from the Hardy–Weinberg equilibrium (χ^2-test, 1 df, $P > 1 \times 10^{-5}$) were kept. All quality control procedures were performed in R (R Development Core Team 2008). The final dataset had 1475 samples and 941,033 SNPs for the association test.

Genome-wide association study

Genome-wide associations for the FCR were made using Bayes B analyzing all SNP data simultaneously with different genetic variance for each SNP (Meuwissen et al. 2001; Habier et al. 2011). The prior genetic and residual variances were estimated using Bayes C (Garrick and Fernando 2013; Cesar et al. 2014), with π being 0.9999. The model equation was:

$$y = Xb + \sum_{j=1}^{k} a_j \beta_j \delta_j + e$$

where:

y The vector of the phenotypic values
X The incidence matrix for fixed effects
B The vector of fixed effects defined above
k The number of SNP loci (941,033)
a_j The column vector representing the SNP covariate at locus j coded as the number of B alleles

β_j was the random substitution effect for locus j, which, conditional on σ^2_β, was assumed to be normally distributed $N(0, \sigma^2_\beta)$ when $\delta_j = 1$ but $\beta_j = 0$ when $\delta_j = 0$, with δ_j being 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 the random residual effects assumed to be normally distributed $N(0, \sigma^2_e)$.

The variance σ^2_β (or σ^2_e) was *a priori* assumed to follow a scaled inverse Chi-square distribution with $v\beta = 4$ (or $ve = 10$) degrees of freedom and scale parameter S^2_β (or S^2_e), and these parameters for markers were derived as a function of the known genetic variance of the population. The genetic variance was based on the number of SNPs assumed to have non-zero effects based on parameter π being 0.9999 and the average SNP allele frequency. The posterior distributions of SNP effects was performed in GenSel software (Fernando and Garrick 2008) using Markov chain Monte Carlo (MCMC). The Bayesian multiple regression models with π = 0.9999 and about 80–105 SNP markers are fitted simultaneously in each MCMC iteration. Inference of associations in these multiple regression models is based on genomic windows rather than on single markers (Onteru et al. 2011), and these genomic windows were constructed based on the UMD3.1 bovine assembly.

In this study, 2527 windows (1 MB) were performed across the autosomal chromosomes. Samples of the proportion of genetic variance explained by each window were obtained by dividing the variance of the window by the variance of the whole genome in that iteration. The window was computed by multiplying the number of alleles that represent the SNP covariates for each consecutive SNP in a window by their sampled substitution effects in that iteration.

CNV detections and association analyses

The CNV analyses were performed in 2253 animals genotyped in Illumina BovineHD. The CNVs were identified by PennCNV (Wang et al. 2007), which uses the luminosity measure of log R ratio (LRR) and B allele frequency (BAF) in the predictions. The LRR is predicted from the ratio of the expected normalized intensity of a sample and observed normalized intensity, while the BAF is calculated from the difference between the expected position of the cluster group and the actual value (Winchester et al. 2009). A PennCNV perl script was used to eliminate calls from low-quality samples based on the standard deviation of LRR (>0.30), the default for BAF drift (>0.01), and waviness factor (>0.05).

The CNV regions (CNVRs) were determined by merging overlapping CNVs identified in two or more samples (Redon et al. 2006; Hou et al. 2012a). They were inferred by CNVRuler (Kim et al. 2012). Regions of very low density of overlapping (recurrence parameter <0.1) were not used in the analyses for a more robust definition of the beginning and end of regions. CNVRs with less than 5 % allele frequency were also not included in the association analyses. Linear regression was used to determine associations between CNVRs and the FCR. The false discovery rate (FDR) method was used for multiple comparison correction, where an FDR <0.05 was considered to have a potentially significant association.

Quantitative trait loci, candidate genes, and enrichment analysis

The top five SNP windows with the highest posterior mean proportion of genetic variance and the significant CNVR were considered the most important regions associated with FCR and were declared as promising QTL. Gene annotations were obtained from the Ensembl Genes 81 Database using BioMart software (Kinsella et al. 2011). Functional annotation was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 (Huang et al. 2009) and pathway analyses were based on data available in the Kyoto Encyclopedia of Genes and Genomes (KEGG).

Results and discussion

There was no evidence ($P = 0.38$, $P = 0.47$, and $P = 0.55$ for the FCR, DMI, and ADG, respectively) that the phenotypic data were not normally distributed according to the Shapiro–Wilk test. The top five associated genomic windows identified herein explained 6.92 %, 3.02 %, 0.83 %, 0.63 %, and 0.60 % of the genetic variance for the FCR, respectively, accounting for 12 % of the total genetic variance (Table 1).

These genomic regions are distributed over five different Chr: 4, 6, 7, 8, and 24 (Fig. 1) and harbor 135 genes based on the *Bos taurus* genome assembly UMD3.1. Of those 135 genes, 117 are annotated and used *in silico* for the functional analysis; the functional annotation tool uses gene-GO term enrichment analysis to look at the internal relationships among hundreds of other terms (Table 2).

The FCR is a measure of animal efficiency and relates the conversion of consumed feed into the desired output, such as muscle growth (protein accretion). Therefore, we identify QTL regions associated with the FCR that harbor important genes involved with pathways related to muscle development (myogenesis) and enhanced adipogenesis. The main pathways identified in GWAS were the JAK-STAT signaling pathway, cytokine–cytokine receptor interaction, ribosome and toll-like receptor signaling pathway. The cytokines is a class of endogenous feeding-regulatory substances (Plata-Salamán 2001) which activate the JAK-STAT pathway, positively or negatively regulating the differentiation of myoblasts (Jang and Baik 2013). The protein accretion and muscle growth is directly related to ribosome metabolism in that the protein synthesis is regulated by amino acids, which actively influence the protein synthesis by affecting the number of ribosomes, as reported by Wannemacher et al. (1971). On the other hand, toll-like receptors (TLRs) are associated with adipogenesis and insulin resistance (Senn 2006; Yan et al. 2010).

According to Animal QTLdb, the QTL region Chr4 overlaps with four (5271, 14675, 18437, and 18438) QTL previously reported for the FCR and two (18436 and 18435) for the

ADG (Sherman et al. 2008, 2009, 2010). In this QTL region, two potential candidate genes were identified: Carnitine O-octanoyltransferase (*CROT*) and Insulin-like growth factor 2 mRNA binding protein 3 (*IGF2BP3*). These genes are associated with the generation of precursor metabolites and energy, fatty acid metabolism, carnitine metabolic process, and regulation of cytokine biosynthesis, respectively (Table 2). The relationship between IGFs proteins and feed efficiency is well known. These proteins have been considered as promising physiological markers for feed efficiency traits since the 1990s, mainly IGF1 and IGF2 (Stick et al. 1998; Arthur et al. 2004; Moore et al. 2005; Herd and Arthur 2009). An SNP in IGF2 was previously associated with the FCR in *Bos taurus* cattle (Sherman et al. 2008).

Chr6 at 81 Mb was previously described as a suggestive region of the signature of artificial selection for production purposes (Pérez O'Brien et al. 2014). In this region, the gene *TECRL* was identified (Table 2) and is associated with lipid production in the body, i.e., lipid metabolic process (GO:0006629) and oxidation–reduction process (GO:0055114). Another gene harbored in the region Chr6 at 81 Mb is *EPHA5* (Table 2), which is related to the regulation of insulin secretion involved in the cellular response to glucose stimulus (GO:0061178), which is also important to the lipid metabolism. In EphA5 knockout mice, behavioral changes were observed due to altered concentrations of serotonin (5-HT) and the metabolite 5-HIAA in the hypothalamus, resulting in an increase of body weight (Mamiya et al. 2008).

In the Chr7 QTL region, several candidate genes were identified as *COMP*, *CRLF1*, *UBA52*, *INSL3*, *PGPEP1*, *MPV17L2*, *IFI30*, *PIK3R2*, *JAK3*, *MAP1S*, *COLGALT1*, and *SLC27A1*, as well as one QTL previously described for the FCR and one for the DMI (Nkrumah et al. 2007; Lu et al. 2013). These genes are involved in biological processes such as lipid metabolism, apoptotic process (GO:0006915), cellular protein metabolic process (GO:0044267), carbohydrate metabolic process (GO:0005975), glycogen biosynthetic process (GO:0005978), fibroblast growth factor receptor signaling pathway (GO:0008543), protein transport (GO:0015031), and cellular response to insulin stimulus (GO:0032869). These biological processes are highly associated with muscle (myogenesis) and adipose (adipogenesis) development; these are mechanisms related to animal feed efficiency. Chr8 QTL harbors two miRNAs (bta-mir-873 and bta-mir-876) and three QTL for the ADG (Santana et al. 2014a) and Chr24 QTL harbors seven genes *DOK6*, *5S_rRNA*, *CCDC102B*, *TMX3*, *HIGD1D*, *DSEL*, and *CDH19*, which were not directly associated with molecular or biological processes related to feed efficiency.

A total of 139,089 CNVs were identified by PennCNV using 2253 HD genotypes, of which about 32 % (44,558) were non-redundant, unique CNVs. CNVs were not detected in approximately 28 % (622) of the animals. Gurgul et al.

Table 1 Genomic variance explained by quantitative trait loci (QTL) regions associated with the feed conversion ratio (FCR) in Nellore cattle by Bayes B

#SNP/window	Start position	End position	Chromosome	Variance (%)	Cumulative variance(%)
389	81011906	81997627	6	6.92	6.92
373	14004499	14998872	8	3.02	9.94
249	32000405	32995535	4	0.83	10.77
335	5004457	5999368	7	0.63	11.40
557	9007160	9999594	24	0.60	12.00

(2015) reported 33 % unique CNVs in 849 Holstein animals and 48 % in animals without detection. Several CNVRs (2667) were determined in autosomal chromosomes after CNVRuler analyses, representing 9 % of those chromosomes. The pattern of the different types of CNVRs was specific for each segment, with 1111 loss, 938 gain, and 617 mixed regions. Hou et al. (2012a), working with BovineHD SNP chip in 147 Holstein animals, reported 443 CNVRs and also more loss than gain regions, with a loss to gain ratio of 1.7. Likewise, Wu et al. (2015) found more losses than gain and mixed regions in Simmental cattle.

The detected CNVRs were used in the association analyses, resulting in 16 non-fixed regions, where 12 (chromosomes 1, 5, 7, 10, and 12) had significant association (FDR < 0.05) with the FCR (Table 3). All of them were defined as mixed CNVRs, meaning that the boundary of the regions was constituted by both "gain" and "loss" of CNVs.

Compared to SNPs, CNVs can be defined as a segment of DNA that displays copy number differences by comparison with reference genomes (Redon et al. 2006; Scherer et al. 2007; Liu et al. 2010). In other words, a CNV is the variation on the number of copies of a particular genomic region or gene from one individual to another, including changing gene structure and dosage, alternating gene regulation, and exposing recessive alleles (Zhang et al. 2009; Clop et al. 2012). The PennCNV algorithm is the most common in CNVs detection from SNP arrays (Kadri et al. 2012; Hou et al. 2012a; Xu et al. 2014b). It is also considered to be a software with relatively

low false-positive rates, supporting the viability of these calls (Dellinger et al. 2010). Hou et al. (2012a) reported a high correlation between PennCNV estimations of copy number and qPCR copy number estimates.

The main goal of CNV detection is to identify their association with interested traits. Seroussi et al. (2010) were the first to work with complex traits in livestock, finding a significant association between CNVRs and protein and fat production, and herd life in Holstein cattle. Xu et al. (2014a) reported 33 candidate CNVRs using BovineSNP50 arrays in an Angus population. The authors reported one deletion in Chr7 associated with resistance to gastrointestinal nematodes. In another study, Xu et al. (2014a) conducted CNV analyses on a Holstein population and reported 34 CNVs on 22 chromosomes with significant association (P < 0.05 and FDR correction) with milk production traits.

Searching for genes in BioMart software (Kinsella et al. 2011) on these 12 significant regions resulted in 51 genes spread over four Chr (5, 7, 10, and 12). The metabolic pathways found in KEGG related with these genes were endocytosis (*FLJ20531*, *IKZF4*, *RNF126*, *ZC3H10*, *ZNF496*, *ZNF672*), glycerophospholipid metabolism (*DGKA*, *PPAP2C*), insulin signaling pathway (*SHC2*), histidine metabolism (*AMDHD1*, *HAL*), olfactory transduction (*OR10A7*, *OR10P1*, *OR11L1*, *OR14C36*, *OR2AJ1*, *OR2AK2*, *OR2AP1*, *OR2B11*, *OR2G2*, *OR2G3*, *OR2L13*, *OR2M4*, *OR2T1*, *OR2T6*, *OR6C1*, *OR6C2*, *OR6C75*, *OR6C76*, *OR6F1*, *OR6J1*), oxidative phosphorylation (*ATP5D*, *NDUFS7*), and

Fig. 1 Manhattan plot of the genome-wide association study (GWAS) for the feed conversion ratio (FCR) in Nellore cattle by Bayes B
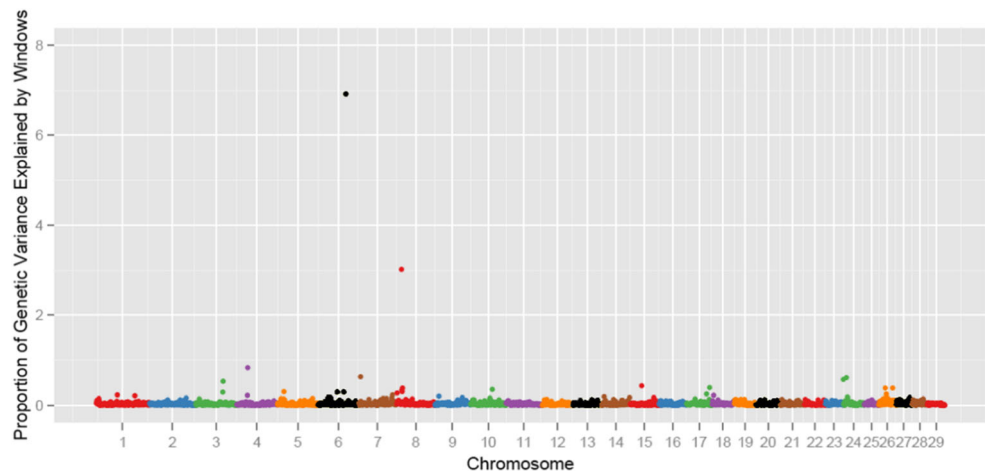
Table 2 Symbol, name, and gene ontology of candidate genes identified from the genome-wide association study (GWAS) for the feed conversion ratio (FCR) in Nellore cattle

Candidate gene symbol	Name	Gene ontology/biological process
COLGALT1	Collagen beta(1-O)galactosyltransferase 1	Extracellular matrix organization (GO:0030198)
COMP	Cartilage oligomeric matrix protein	Skeletal system development (GO:0001501); growth plate cartilage development (GO:0003417); apoptotic process (GO:0006915); cell adhesion (GO:0007155); organ morphogenesis (GO:0009887); extracellular matrix organization (GO:0030198); negative regulation of apoptotic process (GO:0043066)
CRLF1	Cytokine receptor-like factor 1	Positive regulation of cell proliferation (GO:0008284); positive regulation of tyrosine phosphorylation of Stat3 protein (GO:0042517); negative regulation of neuron apoptotic process (GO:0043524)
CROT	Carnitine O-octanoyltransferase	Generation of precursor metabolites and energy GO:0006091; fatty acid metabolic process (GO:0006631); carnitine metabolic process (GO:0006635); fatty acid beta-oxidation (GO:0009437)
EPHA5	EPH receptor A5	Regulation of insulin secretion involved in cellular response to glucose stimulus (GO:0061178)
IFI30	Interferon, gamma-inducible protein 30	Cytokine-mediated signaling pathway (GO:0019221); negative regulation of fibroblast proliferation (GO:0048147); protein stabilization (GO:0050821); oxidation–reduction process (GO:0055114)
IGF2BP3	Insulin-like growth factor 2 mRNA binding protein 3	Regulation of cytokine biosynthetic process (GO:0042035); anatomical structure morphogenesis (GO:0009653)
INSL3	Insulin-like 3	Inositol biosynthetic process (GO:0006021); lipid metabolic process (GO:0006629); biological process (GO:0008150); phospholipid biosynthetic process (GO:0008654)
JAK3	Janus kinase 3	JAK-STAT cascade involved in growth hormone signaling pathway (GO:0060397)
MAP1S	Microtubule-associated protein 1S	Deoxyribonuclease activity (GO:0004536); protein binding (GO:0005515); microtubule binding (GO:0008017); microtubule binding (GO:0008017); tubulin binding (GO:0015631); beta-tubulin binding (GO:0048487); actin filament binding (GO:0051015); actin filament binding (GO:0051015)
MPV17L2	MPV17 mitochondrial membrane protein-like 2	Mitochondrial ribosome assembly (GO:0061668); positive regulation of mitochondrial translation (GO:0070131)
PGPEP1	Pyroglutamyl-peptidase I	Proteolysis (GO:0006508)
PIK3R2	Phosphoinositide-3-kinase, regulatory subunit 2	Cellular glucose homeostasis (GO:0001678); phospholipid metabolic process (GO:0006644); phosphatidylinositol biosynthetic process (GO:0006661); insulin receptor signaling pathway (GO:0008286); insulin receptor signaling pathway (GO:0008286); fibroblast growth factor receptor signaling pathway (GO:0008543); protein transport (GO:0015031); cellular response to insulin stimulus (GO:0032869); response to endoplasmic reticulum stress (GO:0034976); phosphatidylinositol-3-phosphate biosynthetic process (GO:0036092)
SLC27A1	Solute carrier family 27 (fatty acid transporter), member 1	Medium-chain fatty acid transport (GO:0001579); long-chain atty acid metabolic process (GO:0001676); phosphatidylethanolamine biosynthetic process (GO:0006646); phosphatidic acid biosynthetic process (GO:0006654); phosphatidylglycerol biosynthetic process (GO:0006655); phosphatidylcholine biosynthetic process (GO:0006656); phosphatidylserine biosynthetic process (GO:0006659); long-chain fatty acid transport (GO:0015909); response to insulin (GO:0032868); adiponectin-activated signaling pathway (GO:0033211); cellular lipid metabolic process

Table 2 (continued)

Candidate gene symbol	Name	Gene ontology/biological process
		(GO:0044255); positive regulation of protein serine/threonine kinase activity (GO:0071902)
TECRL	Trans-2,3-enoyl-CoA reductase-like	Lipid metabolic process (GO:0006629); oxidation–reduction process (GO:0055114)
UBA52	Ubiquitin A-52 residue ribosomal protein fusion product 1	G1/S transition of mitotic cell cycle (GO:0000082); G2/M transition of mitotic cell cycle (GO:0000086); activation of MAPK activity (GO:0000187); protein polyubiquitination (GO:0000209); mitotic cell cycle (GO:0000278); toll-like receptor signaling pathway (GO:0002224); MyD88-dependent toll-like receptor signaling pathway (GO:0002755); carbohydrate metabolic process (GO:0005975); glycogen biosynthetic process (GO:0005978); glucose metabolic process (GO:0006006); cellular protein metabolic process (GO:0044267)

retinol metabolism (*RDH5*). Retinol metabolism was related to the rump fat thickness in another GWAS in Nellore cattle. In that study, the authors indicated that this metabolic pathway participates in cell proliferation and differentiation, increased fat deposition, and release of growth hormone in the pituitary (Santana et al. 2015). Lipid and protein metabolism, which are related to body composition and protein turnover, are widely known and discussed as important bases for the physiology of feed efficiency (Richardson and Herd 2004; Moore et al. 2009; Herd and Arthur 2009). Glycerophospholipid and histidine pathways found in this study reinforce the physiological linkage of lipid and protein metabolism with feed efficiency. Likewise, the endocytosis pathway was previously associated with the DMI in Angus cattle (Rolf et al. 2012), and differences in the gene expression profile of Yorkshire pigs related to this molecular mechanism were also observed (Lkhagvadorj et al. 2010). Interestingly, all of the genes in this study for the endocytosis pathway are genes that are encoding zinc finger-type proteins. These proteins have been identified as candidate genes for the DMI in Nellore cattle (Santana et al. 2014b) and Holstein–Friesian dairy cows (Veerkamp et al. 2012).

Energy metabolism was also observed in our CNVR analysis by the enriched oxidative phosphorylation and insulin pathways. The importance of energy metabolism for feed efficiency is well known. Processes such as oxidative phosphorylation are essential for cellular energetic efficiency and, therefore, to the energy required by the animal for basal metabolic maintenance. The relation between energy maintenance and feed efficiency has been widely discussed (Castro Bulle et al. 2007; Hoque et al. 2009). In *Bos taurus* cattle, differential gene expression of the oxidative phosphorylation pathway with feed efficiency was demonstrated and the authors suggested that there is a relationship between cellular energetic efficiency and residual feed intake (Kelly et al. 2011).

Another interesting molecular process that was found in this study was an insulin pathway, one of the main hormones involved in energy and lipid metabolism, which plays an important role in controlling feed intake and efficiency in beef cattle (Richardson and Herd 2004; Rolf et al. 2012; Karisa et al. 2014). Several studies have shown the importance of insulin in feed intake. Do et al. (2014) found an association between insulin, olfactory response, and feed efficiency in pigs. The authors suggested that insulin secretion could be an intermediate stimulus to the olfactory pathway influencing feed efficiency. In fact, it has been shown that insulin levels may modulate the response of odor perception by smell (Palouzier-Paulignan et al. 2012), and this perception may indicate the importance of genes that affect smell and taste in cattle intake (Veerkamp et al. 2012). Moreover, the genes linked to the olfactory pathway may participate in nutrient uptake by acting as chemical sensors in the gut (Veerkamp et al. 2012). In this study, most of the genes found in the CNVR

Table 3 Copy number variation regions (CNVRs) associated with the FCR

CNVR	Chromosome	Start	End	Size	FDR
1	5	58386640	58441130	54491	<0.01*
2	12	74844575	74942860	98286	<0.01*
3	5	117356476	117639815	283340	<0.01*
4	5	59421039	59627471	206433	<0.01*
5	12	75016673	75141024	124352	<0.01*
6	7	44437375	44444959	7585	<0.01*
7	12	72748544	72882991	134448	<0.01*
8	10	22709100	22952910	243811	0.01*
9	1	93730576	93819471	88896	0.01*
10	12	73657824	73666963	9140	0.01*
11	12	72518489	72739627	221139	0.02*
12	7	42736530	43353211	616682	0.04*

FDR False discovery rate

*FDR < 0.05

analysis were linked to the olfactory pathway. Two other studies have also found the potential relationship of the olfactory pathway with feed intake in cattle (Veerkamp et al. 2012; Lindholm-Perry et al. 2015).

Combining different methods of analysis (e.g., GWAS with CNV) can identify more effectively the genomic regions and pathways associated with complex traits (Ritchie et al. 2015), such as feed efficiency. This genome-wide study identified QTL and CNV regions that were associated with the FCR. Our results showed that the two approaches found distinct genomic regions, but the identified genes are part of the same biological processes, mainly lipid, protein, and energetic metabolism. These findings contribute to the knowledge of the genetic basis of feed efficiency. The benefits of using more than one type of approach has been demonstrated, especially when it is possible to integrate multi-omics data in a systems biology approach (Ritchie et al. 2015). This integration can be critical for a full understanding of complex traits from the physiological point of view. More biologically interesting and promising results could be obtained using a multi-stage multi-omic approach (Kadarmideen 2014), which includes genome, epigenome, transcriptome, proteome, and metabolome in the same integrated analysis.

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