Journal of Applied Genetics (2018) 59:203–223
https://doi.org/10.1007/s13353-018-0436-7

ANIMAL GENETICS • ORIGINAL PAPER

Association study between copy number variation and beef fatty acid profile of Nellore cattle

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Received: 3 October 2017 / Revised: 23 January 2018 / Accepted: 26 February 2018 / Published online: 8 March 2018

Abstract

The aim of this study was to analyze the association between the copy number variation regions (CNVRs) and fatty acid profile phenotypes for saturated (SFA), monosaturated (MUFA), polyunsaturated (PUFA), ω6 and ω3 fatty acids, PUFA/SFA and ω6/ω3 ratios, as well as for their sums, in Nellore cattle (*Bos primigenius indicus*). A total of 963 males were finished in feedlot and slaughtered with approximately 2 years of age. Animals were genotyped with the BovineHD BeadChip (Illumina Inc., San Diego, CA, USA). The copy number variation (CNV) detection was performed using the PennCNV algorithm. Log *R* ratio (LRR) and allele B frequency (BAF) were used to estimate the CNVs. The association analyses were done using the CNVRuler software and applying a logistic regression model. The phenotype was adjusted using a linear model considering the fixed effects of contemporary group and the animal age at slaughter. The fatty acid profile was analyzed on samples of *longissimus thoracis* muscle using gas chromatography with a 100-m capillary column. For the association analysis, the adjusted phenotypic values were considered for the traits, while the data was adjusted for the effects of the farm and year of birth, management groups at birth, weaning, and superannuation. A total of 186 CNVRs were significant for SFA (43), MUFA (42), PUFA (66), and omega fatty acid (35) groups, totaling 278 known genes. On the basis of the results, several genes were associated with several fatty acids of different saturations. Olfactory receptor genes were associated with C12:0, C14:0, and C18:0 fatty acids. The *SAMD8* and

Communicated by: Maciej Szydlowski

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BSCL2 genes, both related to lipid metabolic process, were associated with C12:0. The *RAPGEF6* gene was found to be associated with C18:2 *cis*-9 *cis*-12 n-6, and its function is related to regulation of GTPase activity. Among the results, we highlighted the olfactory receptor activity (GO:0004984), G-protein-coupled receptor activity (GO:0004930), potassium:proton antiporter activity (GO:0015386), sodium:proton antiporter activity (GO:0015385), and odorant-binding (GO:0005549) molecular functions. A large number of genes associated with fatty acid profile within the CNVRs were identified in this study. These findings must contribute to better elucidate the genetic mechanism underlying the fatty acid profile of intramuscular fat in Nellore cattle.

Keywords Nellore · Genomic selection · Copy number variation · Fatty acids · Structural variation · *Bos indicus*

Introduction

The beef fatty acid (FA) profile of intramuscular fat participates in several biological processes, which are relevant to human health. It is also responsible for the beef flavor and juiciness since its composition has a prominent importance in the oxidative stability during the cooking process (Wood et al. 2008). Fatty acid type has a greater impact upon health issues when compared to its total amount (Wood et al. 2008; Hu et al. 2013). For humans, the ingestion of beef saturated fatty acids (SFA) can be unhealthy due to the increase in serum low-density lipoprotein (LDL) and cholesterol rates (Mensink and Katan 1992), being associated with a higher risk of cardiovascular diseases. Conversely, monounsaturated (MUFA) and polyunsaturated (PUFA) FA are highly desirable in the human diet due to their ability to reduce serum cholesterol levels (Nicklas et al. 2002) and protect the organism against some degenerative diseases (Tapiero et al. 2002).

Due to the growing demand by consumers for protein sources with a health lipid profile, livestock dietary manipulation (Faucitano et al. 2008) and genetic strategies (Liu et al. 2010; Aboujaoude et al. 2016; Berton et al. 2016) have been applied. In genetics, several variants, i.e., SNP (single nucleotide polymorphism), have been employed to identify genetic markers related to expression of FA profile in beef cattle meat (Ishii et al. 2013; Cesar et al. 2014; Sevane et al. 2014; Lemos et al. 2016). Additionally, copy number variations (CNVs) can also affect gene expression and, consequently, phenotypes by changes in gene structure and dosage (Zhang e t al. 2009). However, fewer studies have been conducted using CNVs for genetic selection in livestock. Traditional CNV discovery studies try to detect as many variable regions as possible instead of focusing on those shared by individuals (Xu et al. 2014a).

Association studies using CNVs aim to identify genetic variations in a number of copies related to phenotype expression. In humans, studies have identified associations between the CNVs and diseases, including Crohn's disease, psoriasis, schizophrenia, and autism (Fellermann et al. 2006; Sebat et al. 2007; Walsh et al. 2008; Moreno-De-Luca et al. 2010). Up to date, genome-wide association studies (GWAS) using CNVs in livestock are recent. Xu et al. (2014a, b) characterized and reported 34 CNVs significantly associated with milk production traits in Holsteins and found one deletion polymorphism associated with resistance to gastrointestinal nematodes in Angus cattle. Some authors (Zhou et al. 2016) detected 17 CNVs significantly associated with seven growth traits in Nellore cattle, and one of them (CNV100) might be involved in growth traits through the *KCNJ12* gene.

Therefore, the aim of this study was to associate CNVRs (copy number variation regions) with the FA profile of *longissimus thoracis* muscle in Nellore cattle. To our knowledge, it is the first study to detect CNVR markers from SNP microarray data in association with beef FA profile in cattle.

Material and methods

Animals and management

The database contains records from eight farms located in the southeast, northeast, and midwest of Brazil, which are part of beef cattle breeding programs. In these breeding programs, animals are selected based on growth, finishing, and sexual precocity traits. Genotypes ($n = 3794$) and phenotypes ($n = 963$) of Nellore steers with an average age of 24 months were used.

Breeding seasons are adopted at different periods on these farms. Therefore, calving seasons ranged from August to October in some farms and from November to January in others. Weaning occurred at 7 months of age. The animals were raised on grazing conditions using *Brachiaria* sp. and *Panicum* sp. forages and had free access to mineral salt, with a density varying from 1.2 to 1.6 animal unit/ha. After yearling, the breeding animals were selected and the others were kept in feedlot conditions. During the feedlot, the forage/concentrate ratio ranged from 50:50 to 70:30, according to each farm. In general, whole-plant corn or sorghum silage was used as high-quality forage. Grains of corn and/or sorghum, and soybeans, soybean meal, or sunflower seeds were used as protein concentrate.

Animals were slaughtered in commercial slaughterhouses in accordance with the Brazilian Federal Inspection Service procedures when attained 500 to 550 kg of body weight and an average age of 24 months. After stored for 48 h postmortem at 0–2 °C, meat samples were removed from the *longissimus thoracis* muscle from between the 12th and 13th ribs from each animal. Samples were stored at − 80 °C until analysis to determine the FA profile. The percentage of lipids in the *longissimus thoracis* muscle (IMF) was obtained using the method proposed by Folch et al. (1957).

CNV and CNV region (CNVR) detection

The animals were genotyped with the BovineHD BeadChip (Illumina Inc., San Diego, CA, USA) that contains 777,962 SNP markers. The CNV detection was performed using the PennCNV algorithm (Wang et al. 2007). It incorporates multiple sources of information, and it is based on a hidden Markov model for CNV detection from the high-density genotyping data. The PennCNV is the most commonly used algorithm for CNV studies since it presents a low rate of false positives (Winchester et al. 2009; Eckel-Passow et al. 2011). Initially, the PennCNV algorithm was used without any quality control parameter so as to obtain the number of CNVs in overall data. After that, to maintain the quality of the samples, the default PennCNV's quality control was applied, eliminating samples with standard deviation for LRR (log *R* ratio) higher than 0.30, BAF (frequency of allele B) higher than 0.05, and value of the waves factor higher than 0.01.

The CNVRs were generated by overlapping the identified CNVs using the CNVRuler software (Kim et al. 2012). Genomic regions with density lower than 10% were excluded ("recurrence 0.1"). The recurrence trims the CNVRs based on its frequency to avoid false positive predictions, and it defines more robust limits of the start and end regions. The option "gain/loss separated regions" was applied to evaluate the type of the event (gain, loss) in each region. Overlapping "gain" and "loss" CNVRs were merged into single regions to account for genomic regions in which both events can occur ("mixed" CNVRs).

To evaluate the location of the CNVRs, the Ensembl Biomart tool (Smedley et al. 2015) was used with the UMD3.1 reference genome assembly.

Determination of fatty acid profile

The total lipid concentration was quantified at the Animal Product Technology Laboratory in the Technology Department of FCAV/UNESP using the Bligh and Dyer method (Bligh and Dyer 1959).

Fatty acid profile for each sample was determined at the Meat Science Laboratory (LCC) in the Animal Nutrition and Production Department at FMVZ/USP using the method described by Folch et al. (1957). *Longissimus thoracis* muscle samples (~ 100 g) were collected to determine the FA profile. The lipids were extracted by homogenizing the sample with a chloroform and methanol solution (2:1). Sodium chloride (NaCl) at 1.5% was added to isolate the lipids.

The isolated lipids were then methylated and the methyl esters were formed according to Kramer et al. (1997). The FA profiles were quantified by gas chromatography (GC-2010 Plus - Shimadzu AOC 20i autoinjector) with a 100-m SP-2560 capillary column (0.25 mm in diameter with 0.02 mm thickness, Supelco, Bellefonte, PA). The initiating temperature of 70 °C was increased gradually up to 175 °C (13 °C/min), holding for 27 min, increased further up to 215 °C (4 °C/min), and then held for 31 min. Hydrogen (H2) was the carrier gas, with 40 cm^3/s. The FAs were identified by comparing the retention time of methyl esters of the samples with the standards C4–C24 (F.A.M.E mix, Sigma®), vaccenic acid C18:1 *trans*-11 (V038-1G, Sigma®), C18:2 *trans*-10 *cis*-12 (UC-61M 100 mg, Sigma®), CLA (conjugated linolenic acid) e C18:2 *cis*-9, *trans*-11 (UC-60M 100 mg, Sigma®), and tricosanoic acid (Sigma®). The FAs were quantified by normalizing the area under the curve of methyl esters using the GS solution 2.42 software (Copyright (C) 2000–2012 Shimadzu Corporation). The FAs were expressed as a percentage of the total FA methyl ester.

Fatty acids were chosen due to their importance upon human health and their high content in feedlot animals' meat. Hence, the following identified individual FAs were selected: lauric (C12:0), myristic (C14:0), myristoleic (C14:1), palmitic (C16:0), stearic (C18:0), oleic (C18:1 *cis*-9), elaidic (C18:1 trans9), CLA-*cis* (C18:2c9t11), vaccenic (C18:1 trans11), linoleic (C18:2 cis9Cis12n6), eicosatrienoic (C20:3 n6 *cis*-8,11,14), and docosahexaenoic (DHA) (C22:6 n3). The sums of SFA (C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C21:0 + C24:0), MUFA (C16:1 + C17:1 c10 + C18:1 t11 + C15:1 c10 + C20:1 c11 + C24:1 + C22:1 n9 + C18:1n9c + C14:1 + C18:1 n9t), PUFA (C18:2 n6 + C18:3 n3 + C18:3 n6 + C20:3 n3 *cis*-11, 14, 17 + C20:3 n6 *cis*-8, 11, 14 + C20:4 n6 + C20:5 n3 + C22:6 n3), ω6 (C18:3 n6 + C20:3 n6 c8, c11, c14 + C18:2 n6 + C20:4 n6), and ω3 (C18:3 n3 + C20:3 n3 c11, c14, c17 + C22:6 n3 + C20:5 n3) were calculated. The PUFA/SFA and ω6/ω3 ratios were also calculated.

Association analyses

The phenotype was adjusted using a linear model considering the fixed effects of contemporary group (year, farm, and

management group at yearling) and the covariate age at slaughter in each trait:

$$Y = Xb + e$$

where Y is a vector of phenotypic values of a given quantitative trait, b is a vector of fixed effects and covariate, X is the incidence matrix of fixed effects, and e is the random residual vector with distribution $N \sim (0, \sigma 2e)$.

Initially, the association study using a linear model was performed considering all animals with phenotypes; however, few significant CNVRs were identified associated with FA. Then, a regression logistic model was applied, where two groups of animals with extreme phenotypes for each FA concentration were created. Extreme phenotype value groups considered 25% of the animals with the lowest (LOW) and highest (HIGH) FA concentration, receiving a score of 1 and 0, respectively.

The CNVRuler software supports the maximum likelihood test (ML), which can be used to evaluate the fit quality of logistic regression models (Kim et al. 2012). False positives (FDR) were controlled using a multiple comparison correction tests (FDR < 0.01), and minor allele frequency (MAF) lower than 0.05 was excluded.

Gene search

The significant CNVRs were placed in the cattle UMD3.1 genome assembly by surveying the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/snp/) database and Ensembl Genome Browser tool VEP database (*Bos taurus* genes UMD3.1) (http://www.ensembl.org/index.html), and all of the genes within the CNVR were annotated and used to run the posterior analyses. In these databases, it was possible to identify segments located within or close to genes that could explain the phenotypic variability of the studied traits. The classification of genes regarding their biological function was performed by the Database for Annotation, Visualization and Integrated Discovery (DAVID) v. 6.8, (Huang et al. 2009a, b) using all annotated genes in the cattle genome and the *Bos taurus* annotation (BTA) file as background. Gene ontology (GO) biological process, cellular component, and molecular function annotation datasets were used for functional enrichment analysis (P value < 0.1). Comparisons of the significant CNVRs with the described cattle QTLs were done based on the Animal QTLdb website (http://www.animalgenome.org/cgi-bin/QTLdb/BT/index).

Availability of supporting data The data sets supporting the results of this article are included within the article and its additional files.

Results and discussion

Fatty acid profile

Descriptive statistics of FA concentrations for each extreme phenotype group (LOW and HIGH) is shown in Table 1. Individual FAs with the highest concentrations were palmitic (C16:0), oleic (C18:1 *cis*-9), vaccenic (C18:1 *trans*-11), and stearic (C18:0), representing 67.3% of the overall lipid composition. The same pattern was reported by Pitchford et al. (2002) and Do Prado et al. (2003), whose results also showed the highest levels for palmitic, stearic, and oleic FAs. Also, Cesar et al. (2014) in a study with Nellore cattle finished in feedlot observed that the oleic FA displayed the highest concentration (37.46%) in intramuscular fat.

Among the sum of FAs, the group that showed the greatest concentration was the SFA, followed by MUFA and PUFA. These results were in agreement with those reported by some authors (Do Prado et al. 2003) for Nellore cattle (43.93% for SFA, 42.33% for MUFA, and 12.8% for PUFA).

Copy number variation and copy number variation region discovery

The CNV calling in 3794 samples was performed on the UMD3.1 bovine genome assembly using the PennCNV software without any quality control parameter, and a total of 399,361 CNVs were identified. After the default quality control filtering, 2902 samples remained for subsequent analysis and 195,873 CNVs were detected with average, maximum, and minimum length sizes of 54,744, 870,000, and 3000 bp, respectively. A total of 3904 CNV regions were identified in this study. The CNVR mean length size was 3,528,473 bp, ranging from 520 to 1,476,546 bp.

Saturated fatty acids

A total of 43 CNVRs were found to be significant (P value < 0.05) within the SFA group. Myristic acid (C14:0) showed the highest number of significant CNVRs (11), followed by lauric acid (C12:0) and the sum of SFA, both with ten regions. Myristic acid showed two loss regions and nine mixed regions, while lauric acid and the sum of SFA presented four and three loss regions, respectively, and six mixed regions. Additionally, none gain region was observed for myristic and lauric acids, whereas the sum of SFA showed one gain region (Table 2). In addition, these significant CNVRs harbored 141 genes distributed over the cattle genome (Table 3).

Significant CNVRs for the sum of SFA showed five genes distributed among three different chromosomes. On BTA9, the CNVR_3436_1 was found to contain the *WASF1* gene, a protein-coding gene widely and strongly expressed in the brain. This gene has been associated with the Arp2/3 complex,

a nucleating core for actin polymerization in vitro, potentially involved in the RAC-induced reorganization of the actin cytoskeleton required for membrane ruffling (Miki et al. 1998). The CNVR_4752_1 on BTA8 hosted the *C8H9orf72*, *IFNK*, and *MOB3B* genes. The *IFNK* gene encodes a member of the type I interferon family, a group of related glycoproteins that play an important role in host defenses against viral infections (Liu et al. 2008).

Lauric acid (C12:0) encompassed the highest number of genes ($n = 64$) associated with FA phenotype within significant CNVRs. The CNVR_3143_1 located on BTA28 harbored the *ASCC1*, *ANAPC16*, *DDIT4*, and *DNAJB12* genes. Among them, we highlight the *DDIT4* gene, which regulates cell growth, proliferation, and survival via inhibition of the activity of the mammalian target of rapamycin complex 1 (mTORC1). Besides, it plays an important role in responses to cellular energy levels and cellular stress, including responses to hypoxia and DNA damage (Sofer et al. 2005).

Among all CNVRs located on BTA28, the CNVR_3102_1 (C12:0) was the second most significant ($P < 0.05$). It was classified as a mixed region and all identified genes were involved in olfactory receptor functions, such as the *OR5AS1*, *OR5L2*, and *OR5D14* genes. The olfactory receptor genes comprise the largest multigene family in vertebrate genomes (Niimura and Nei 2007), with more than 1000 coding genes organized in clusters over 26 bovine chromosomes (Lee et al. 2013). They interact with odorant molecules in the snout and then initiate a neuronal response that triggers the perception of the smell (Malnic et al. 2004). Further, olfactory receptors share a 7-transmembrane domain structure with many neurotransmitters and hormone receptors responsible for the recognition and G-protein-mediated transduction of odorant signals. Olfactory receptor genes were described (Olivieri et al. 2016) to be associated with dry matter intake in Nellore cattle, and some authors (Bertics et al. 1992) reported olfactory receptors as a critical factor that could influence the development of fatty liver in pregnant cows. Hence, these findings are great evidence that olfactory receptors might be involved in pathways associated with FA synthesis.

The region encompassing the CNVR_2413_1 (C12:0) on BTA18 hosted several genes, such as the *PRX* gene that encodes a protein involved in peripheral nerve myelin upkeep (Shi et al. 2014); the *SPTBN4* gene that codifies a protein which acts on the determination of cell shape, arrangement of transmembrane proteins, and organization of organelles (Berghs et al. 2000); the *COQ8B* gene, whose function is involved in the biosynthesis of coenzyme Q (ubiquinone), an essential lipid-soluble electron transporter for aerobic cellular respiration, which probably acts as a small molecule kinase, possibly a lipid kinase that phosphorylates a prenyl lipid in the ubiquinone biosynthesis pathway (Ashraf et al. 2013); and the *MIA* gene that is associated with skin and uveal melanomas in humans (Blesh et al. 1994). Although several genes were found associated with lauric acid within this particular region, none of them have already been described related to lipid metabolism.

The CNVR_3212 (C12:0) mixed region on BTA29 harbored 17 genes, in which we detailed four of them. The *EEF1G* gene is described as a protein-coding gene related to translation elongation factor activity (GO:0003746) and acts in chain elongation during polypeptide synthesis at the ribosome (Sanders et al. 1992). The *GANAB* gene encodes the alpha subunit of glucosidase II and a member of the glycosyl hydrolase 31 family of proteins (Chi et al. 2006). The *B3GAT3* gene encodes a protein that belongs to the glucuronyl transferase gene family, that catalyzes the formation of the glycosaminoglycan-protein linkage by way of a glucuronyl transfer reaction in the final step of the biosynthesis of the linkage region of proteoglycans (Koike et al. 2014). Besides, this gene also acts on carbohydrate metabolic (GO:0005975) and glycosaminoglycan biosynthetic (GO0006024) processes. The *BSCL2* gene is associated with the lipid metabolic process (GO:0006629), lipid catabolic process (GO:0016042), lipid storage (GO:0019915), lipid particle organization (GO:0034389), and fat cell differentiation (GO:0045444). This gene codifies a regulator of lipid catabolism essential for adipocyte differentiation and may also be involved in the central regulation of energy homeostasis. The *BSCL2* gene may affect the expression of key genes which mediate triglyceride synthesis in humans, including *AGPAT2*, *LP1N1*, and *DGAT2*, thus, inhibiting the lipid accumulation of adipose tissue (Payne et al. 2008).

Eleven CNVRs were significant for the myristic acid (C14:0) and five genes showed the functions described. The *SEMA3A* gene located on BTA4 belongs to the CNVR_896_1 mixed region, and it is involved in the development of the olfactory system (Hanchate et al. 2012). The *PRAME* gene was identified on CNVR_3378_1 on BTA17, and it encodes an antigen that is preferentially expressed in human melanomas and in testis (Epping et al. 2005). This gene was also associated with weaning gain, conformation at weaning, and conformation at yearling in a GWAS study for CNVs and body traits in Nellore cattle (Zhou et al. 2016). The CNVR_1553_2 observed on BTA7 encompassed six olfactory receptor genes: *OR2L13*, *OR2T12*, *OR2M5*, *OR2M4*, *OR2M3*, and *OR2T4*. All of them are protein-coding genes and belong to the olfactory receptor family 2, with similar functions as those previously described for the olfactory receptor genes. The last significant region was the CNVR_4411_1, a mixed region found on BTA28 with a similar length size as the CNVR_3102_1 (C12:0). Thus, both regions encompassed the same genes, which have already been described.

Although seven CNVRs were found to be significant for palmitic acid (C16:0), none of them showed genes neither related nor associated with lipid or energy metabolism. For stearic acid

Table 1 Descriptive statistics of fatty acid concentration for LOW and HIGH extreme phenotype groups

Trait[a]	Nomenclature	LOW group[b]				HIGH group[c]			
		Min	Max	Mean	SD	Min	Max	Mean	SD
Lauric	C12:0	0.04	0.04	0.04	0.001	0.06	0.06	0.06	0.001
Myristic	C14:0	0.95	1.68	1.38	0.22	2.49	3.73	2.88	0.35
Myristoleic	C14:1	0.09	0.10	0.10	0.001	0.53	0.53	0.52	0.001
Palmitic	C16:0	16.54	20.35	18.84	0.42	23.24	28.57	24.48	0.42
Stearic	C18:0	10.86	12.80	11.80	0.22	15.88	17.78	16.68	0.22
Elaidic	C18:1n9t	0.002	0.13	0.07	0.04	9.24	11.18	10.29	0.83
Oleic	C18:1n9c	25.57	29.51	27.60	0.38	34.51	37.51	35.85	0.38
Vaccenic (TVA)	C18:1t11	0.04	0.12	0.08	0.03	27.33	27.53	27.43	0.05
Linoleic (LA)	C18:2n6	2.47	4.92	4.13	0.28	8.96	11.82	9.98	0.27
Linolenic	C18:3n3	0.23	0.49	0.41	0.03	0.87	1.20	1.00	0.03
CLA-*cis*	C18:2c9t11	0.14	0.20	0.18	0.02	0.33	0.59	0.41	0.02
Docosahexaenoic (DHA)	C22:6 n3	0.64	0.65	0.65	0.003	1.40	1.42	1.41	0.008
Sum of SFA		39.91	42.05	41.10	0.32	45.37	49.62	46.87	0.32
Sum of MUFA		30.14	34.50	32.75	0.39	40.44	43.71	42.20	0.39
Sum of PUFA		4.33	8.24	7.16	0.46	15.79	20.46	17.21	0.46
Sum of ω-3		2.78	5.39	4.49	0.29	9.77	12.94	10.89	0.29
Sum of ω-6		1.24	2.65	2.18	0.20	5.14	7.62	6.14	0.20
ω6/ω3 ratio		1.45	1.64	1.57	0.03	2.15	2.63	2.27	0.03
PUFA/SFA ratio		0.09	0.18	0.16	0.01	0.36	0.51	0.40	0.01

The sum of SFA (C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C21:0 + C24:0), MUFA (C16:1 + C17:1 c10 + C18:1 t11 + C15:1 c10 + C20:1 c11 + C24:1 + C22:1 n9 + C18:1n9c + C14:1 + C18:1 n9t), PUFA (C18:2 n6 + C18:3 n3 + C18:3 n6 + C20:3 n3 *cis*-11, 14, 17 + C20:3 n6 *cis*-8, 11, 14 + C20:4 n6 + C20:5 n3 + C22:6 n3), ω6 (C18:3 n6 + C20:3 n6 c8, c11, c14 + C18:2 n6 + C20:4 n6), and ω3 (C18:3 n3 + C20:3 n3 c11, c14, c17 + C22:6 n3 + C20:5 n3)

[a] The fatty acid concentration is expressed as a percentage of the total fatty acid methyl esters (FAME)

[b] LOW group: the tenth lowest extreme phenotypes

[c] HIGH group: the tenth highest extreme phenotypes

(C18:0), five CNVRs were significant and a total of 29 genes were identified. The CNVR_2505_1 on BTA3 described the *GBP6* gene, which had previously been associated with conformation at weaning in Nellore cattle (Zhou et al. 2016). It is important also to highlight the CNVR_952_2 located on BTA15 given its importance upon the olfactory receptor groups, which have already been identified and described in the CNVRs reported above, demonstrating its important role in FA synthesis.

Monounsaturated fatty acids

A total of 42 CNVRs were significant (P value < 0.05) within the MUFA group. Myristoleic acid (C14:1) displayed the highest number of significant CNVRs (12), followed by vaccenic acid (C18:1 trans11) and the sum of MUFA, both with ten regions each. Myristoleic acid showed two loss regions and ten mixed regions, while vaccenic acid presented two loss regions and eight mixed regions, and the sum of MUFA showed only mixed regions (Table 4). Associated genes within significant CNVRs for MUFA profile are described in Table 5, totaling 52 genes with the functions described in the literature.

For the sum of MUFA, a total of ten CNVRs were significant ($P < 0.05$); however, only two regions showed associated genes. Among these regions, the CNVR_3391_2 on BTA17 was found at the same position as the CNVR_3378_1 (C14:0). Although this region did not contain any relevant gene for the present association, it should not be discarded in further studies seeking for FA profile and synthesis since it was detected twice. On BTA2, the CNVR_315_1 harbored the *ZNF804A* gene, which was found to be associated with diseases in humans, such as bipolar disorder and schizophrenia (Riley et al. 2010).

Myristoleic acid (C14:1) showed the highest number of significant CNVRs ($P < 0.05$) for the MUFA group, and a total of 31 genes were identified distributed over 11 chromosomes. The CNVR_5095_1 was found to contain genes for myristoleic acid and also for lauric acid. The mixed CNVR_7642_1 located on BTA9 possesses the *SAMD8* gene, whose function is related to lipid metabolic

Table 2 Significant copy number variation regions (CNVRs) associated with saturated fatty acid profile in intramuscular fat of *longissimus thoracis* muscle in Nellore cattle

Trait	Nomenclature	CNVRs ID	BTA	Description	Start (bp)	End (bp)	Size (bp)	Control[a]	Case[b]	*P* value	FDR
Sum of SFA		CNVR_206_2	7	Mixed	103,154,830	103,168,693	13,864	14	28	0.00401	0.24
		CNVR_3436_1	9	Mixed	40,529,468	40,667,345	137,878	18	29	0.00693	0.24
		CNVR_4253_1	21	Mixed	18,531,324	18,566,035	34,712	39	46	0.01737	0.40
		CNVR_4565_1	7	Mixed	38,359,707	38,537,300	177,594	30	28	0.02772	0.44
		CNVR_4752_1	8	Mixed	16,420,855	16,924,662	503,808	26	26	0.00398	0.44
		CNVR_766_1	8	Loss	93,854,223	93,863,828	9606	15	6	0.01398	0.50
		CNVR_732_1	11	Mixed	81,414,106	81,425,393	11,288	26	30	0.03678	0.50
		CNVR_573_1	13	Gain	5,750,278	5,800,039	49,762	71	54	0.03175	0.50
		CNVR_1052_1	11	Loss	53,933,240	53,982,995	49,756	23	35	0.03294	0.50
		CNVR_2408_1	7	Loss	36,862,487	36,865,662	3176	17	18	0.01255	0.50
Lauric	C12:0	CNVR_3143_1	28	Loss	28,447,018	28,532,009	84,992	18	3	0.00251	0.54
		CNVR_760_1	5	Mixed	27,945,443	28,025,514	80,072	17	6	0.02042	0.54
		CNVR_2425_1	18	Loss	61,894,649	61,924,592	29,944	12	3	0.02580	0.54
		CNVR_1785_1	12	Mixed	621,640	631,466	9827	30	17	0.03618	0.52
		CNVR_3102_1	28	Mixed	2,468,624	2,860,512	391,889	59	43	0.03923	0.54
		CNVR_1571_6	10	Mixed	24,766,755	24,822,212	55,458	27	15	0.04343	0.54
		CNVR_2413_1	18	Mixed	49,995,899	50,337,677	341,779	8	1	0.04489	0.52
		CNVR_3212	29	Mixed	41,533,612	41,725,223	191,612	8	1	0.04489	0.54
		CNVR_677_1	4	Loss	95,780,598	95,974,974	194,377	12	4	0.04760	0.54
		CNVR_391_1	3	Loss	14,360,623	14,419,603	58,981	9	2	0.04774	0.54
Myristic	C14:0	CNVR_1669_1	7	Loss	99,322,117	99,328,261	6145	19	38	0.00829	0.42
		CNVR_3718_1	20	Mixed	44,900,560	45,106,413	205,854	15	3	0.00929	0.42
		CNVR_1408_1	6	Mixed	79,932,750	79,987,353	54,604	23	9	0.01320	0.43
		CNVR_896_1	4	Mixed	36,527,545	36,656,495	128,951	23	9	0.01320	0.36
		CNVR_1567_1	7	Mixed	53,453,768	53,664,924	211,157	13	3	0.01957	0.42
		CNVR_3550_2	19	Mixed	3,025,966	3,029,934	3969	19	7	0.01988	0.36
		CNVR_3378_1	17	Loss	25,056,695	25,119,996	63,302	111	135	0.02585	0.42
		CNVR_1001_2	4	Mixed	90,699,813	90,705,987	6175	25	41	0.03505	0.42
		CNVR_3430_2	17	Mixed	51,115,979	51,370,688	254,710	127	14	0.03783	0.44
		CNVR_1553_2	7	Mixed	42,945,525	43,353,211	407,687	66	87	0.03856	0.42
		CNVR_4411_1	28	Mixed	2,468,624	2,860,512	391,889	91	11	0.04972	0.36
Palmitic	C16:0	CNVR_2226_3	10	Mixed	23,847,193	23,854,403	7211	55	30	0.00266	0.77
		CNVR_2226_4	10	Mixed	24,061,376	24,062,806	1431	49	27	0.00569	0.77
		CNVR_335_3	2	Mixed	11,875,973	11,974,681	98,709	13	1	0.01169	0.77
		CNVR_2257_1	10	Loss	40,734,273	40,794,738	60,466	13	4	0.03411	0.77
		CNVR_2819_1	13	Loss	14,525,354	14,533,369	8016	17	31	0.03798	0.77
		CNVR_2695_1	12	Mixed	43,164,746	43,196,111	31,366	21	36	0.03891	0.77
		CNVR_2045_1	9	Mixed	53,893,533	53,899,150	5618	14	5	0.04157	0.77
Stearic	C18:0	CNVR_2505_1	3	Mixed	54,414,408	55,017,187	602,780	43	59	0.01924	0.92
		CNVR_270_1	10	Mixed	27,086,857	27,117,598	30,742	24	10	0.02896	0.92
		CNVR_546_2	11	Mixed	93,900,408	93,922,796	22,389	64	89	0.02582	0.92
		CNVR_952_2	15	Mixed	46,666,188	46,975,690	309,503	41	51	0.02962	0.92
		CNVR_1304_2	19	Mixed	2,270,929	2,321,350	50,422	27	46	0.02750	0.92

[a] Control: group of animals with the lowest averages obtained for the studied parameter

[b] Case: group of animals with the highest averages obtained for each studied parameter

Table 3 Genes within significant copy number variation regions (CNVRs) for saturated fatty acid profile in intramuscular fat of *longissimus thoracis* muscle in Nellore cattle

Trait	Nomenclature	BTA	Genes
Sum of SFA		7	–
		9	*WASF1*
		21	–
		7	–
		8	*C8H9orf72*, *IFNK*, *MOB3B*
		8	–
		11	–
		13	
		11	*LOC101903989*
		7	–
Lauric	C12:0	28	*ASCC1*, *ANAPC16*, *DDIT4*, *DNAJB12*
		5	*ATG101*, *NR4AI*, *GRASP*
		18	*LOC618662*
		12	–
		28	*LOC782694*, *LOC782769*, *OR5AS1*, *OR5L2*, *LOC513384*, *LOC787409*, *LOC100297422*, *LOC787801*, *OR5D14*, *LOC787835*, *LOC787869*, *LOC787883*, *LOC787902*, *LOC531024*, *LOC787953*
		10	*LOC767888*, *LOC101908188*
		18	*PLD3*, *HIPK4*, *TRNAG-CCC*, *PRX*, *SERTAD1*, *SERTAD3*, *BLVRB*, *SPTBN4*, *SHKBP1*, *LTBP4*, *NUMBL*, *COQ8B*, *ITPKC*, *C18H19orf54*, *SNRPA*, *MIA*, *RAB4B*, *EGLN2*
		29	*AHNAK*, *EEF1G*, *TRNAG-UCC*, *TUT1 MTA2*, *EML3*, *ROM1*, *B3GAT3*, *GANAB*, *INTS5*, *LBHD1*, *METTL12*, *UQCC3*, *UBXN1*, *LRRN4CL*, *BSCL2*, *GNG3*
		4	*MKLN1*, *TRANAE-UCC*, *LOC104972201*
		3	*MEF2D*
Myristic	C14:0	7	–
		20	–
		6	–
		4	*SEMA3A*
		7	*TMCO6*, *NDUFA2*, *IK*, *WDR55*, *DND1*, *HARS*, *HARS2*, *ZMAT2*, *PCDHA3*, *PCDHA6*, *PCDHA10*, *PCDHA13*
		19	–
		17	*PRAME*
		4	–
		17	–
		7	*LOC788041*, *LOC788055*, *LOC616716*, *LOC788079*, *OR2L13*, *OR2T12*, *OR2M5*, *OR2M4*, *OR2M3*, *OR2T4*
		28	*LOC782694*, *LOC782769*, *OR5AS1*, *OR5L2*, *LOC513384*, *LOC787409*, *LOC100297422*, *LOC787801*, *OR5D14*, *LOC787835*, *LOC787869*, *LOC787883*, *LOC787902*, *LOC531024*, *LOC787953*
Palmitic	C16:0	10	
		10	*LOC10193548*
		2	*ZNF804A*
		10	*LOC100296164*, *MDGA2*
		13	–
		12	–
		9	–
Stearic	C18:0	3	*GBP2*, *LOC785445*, *LOC781596* ,*LOC781675*, *LOC781719*, *GMP4*, *LOC786500*, *LOC104968497*, *GBP6*, *LOC510382*, *LOC100336669*, *LOC510382*, *LOC100336669*, *LOC104969803*, *LOC507055*, *LOC100336443*
		10	*LOC784260*
		11	*LOC786596*, *LOC786573*
		15	*LOC100125776*, *LOC100336980*, *LOC511622*, *OR2AG2*, *LOC783299*, *OR2AG1*, *LOC104970024*, *LOC101903126*, *LOC506989*, *LOC783920*
		19	–

(GO:0006629) and sphingolipid metabolic (GO:0006665) processes. Sphingolipid is a class of lipids containing a backbone of sphingoid bases, a set of aliphatic amino alcohols that includes sphingosine.

On BTA2, the mixed CNVR_3474_1 (C14:1) encompassed 13 genes (*FGR*, *LOC104971347*, *AHDC1*, *WASF2*, *GPR3*, *CD164L2*, *MAP3K6*, *SYTL1*, *TMEM222*, *WDTC1*, *LOC104971348*, *TRNAE-UUC*, and *SLC9A1*). Among them, we can highlight the *FGR* gene, which is related to the immune system process (GO:0002373) and protein phosphorylation (GO:0006468) and has been associated with sarcoma in humans (Chen et al. 2001).

Table 4 Significant copy number variation regions (CNVRs) associated with monounsaturated fatty acid profile in intramuscular fat of *longissimus thoracis* muscle in Nellore cattle

Trait	Nomenclature	CNVRs ID	BTA	Description	Start (bp)	End (bp)	Size (bp)	Control[a]	Case[b]	*P* value	FDR
Sum of MUFA		CNVR_2199_3	10	Mixed	23,847,193	23,854,403	7211	51	29	0.00637	0.50
		CNVR_2498_2	11	Mixed	58,249,719	58,427,956	178,238	23	44	0.00686	0.50
		CNVR_2742_1	13	Mixed	14,525,354	14,533,369	8016	14	31	0.00988	0.50
		CNVR_3391_2	17	Mixed	25,056,695	25,119,996	63,302	134	108	0.01023	0.50
		CNVR_3012_1	14	Mixed	81,841,283	81,878,266	36,984	17	5	0.01276	0.50
		CNVR_315_1	2	Mixed	11,825,478	11,958,282	132,805	12	2	0.01636	0.50
		CNVR_134_1	1	Mixed	83,218,713	83,238,102	19,390	43	25	0.01707	0.58
		CNVR_1987_2	9	Mixed	31,107,969	31,122,388	14,420	48	69	0.02815	0.58
		CNVR_3551_1	18	Mixed	45,300,008	45,330,206	30,199	60	41	0.02854	0.58
		CNVR_1386_2	6	Mixed	80,232,706	80,244,389	11,684	22	10	0.02973	0.58
Myristoleic	C14:1	CNVR_7602_2	9	Mixed	16,420,855	16,924,662	503,808	17	31	0.00623	0.35
		CNVR_5095_1	28	Mixed	28,447,018	28,532,009	84,992	25	7	0.00721	0.35
		CNVR_7642_1	9	Mixed	31,075,126	31,122,388	47,263	73	58	0.01741	0.51
		CNVR_3551_1	20	Loss	15,371,013	15,381,710	10,698	34	13	0.02017	0.51
		CNVR_6779_1	6	Mixed	114,705,514	114,716,539	11,026	24	9	0.03107	0.51
		CNVR_4209_1	23	Mixed	25,747,610	25,881,173	133,564	45	48	0.03271	0.51
		CNVR_361_2	1	Mixed	124,159,079	124,420,643	261,565	18	11	0.03286	0.51
		CNVR_3474_1	2	Mixed	126,262,898	126,731,462	468,565	14	5	0.03839	0.51
		CNVR_7530_1	8	Mixed	103,917,356	104,025,778	108,423	20	10	0.03952	0.51
		CNVR_1849_1	14	Loss	79,699,163	79,706,075	6913	15	8	0.04617	0.51
		CNVR_230_1	1	Mixed	83,218,713	83,238,102	19,390	22	56	0.04712	0.51
		CNVR_6050_1	4	Mixed	106,642,849	106,765,834	122,986	40	18	0.00577	0.52
Oleic	C18:1 *cis*-9	CNVR_2001_1	12	Mixed	61,223,484	61,229,440	5957	20	45	0.00103	0.22
		CNVR_1663_1	10	Mixed	27,106,916	27,117,598	10,683	25	11	0.01766	0.69
		CNVR_1365_1	8	Loss	54,371,946	54,435,508	63,563	14	4	0.02381	0.69
		CNVR_3033_1	X	Mixed	28,434,257	28,494,393	60,137	54	35	0.02505	0.69
		CNVR_2262_1	15	Mixed	3,275,242	3,280,326	5085	24	11	0.02527	0.69
		CNVR_2628_4	18	Mixed	49,074,618	49,109,287	34,670	30	16	0.03162	0.77
		CNVR_1349_1	8	Mixed	46,895,749	46,925,080	29,332	16	6	0.03526	0.77
Elaidic	C18:1 trans9	CNVR_3597_1	7	Mixed	6,708,218	6,718,398	10,181	9	16	0.00608	0.78
		CNVR_2280_1	23	Mixed	30,441,844	30,472,870	31,027	12	17	0.02612	0.78
		CNVR_3204_1	5	Mixed	27,945,443	28,025,514	80,072	18	6	0.04091	0.78
Vaccenic	C18:1 trans11	CNVR_4225_1	26	Loss	50,795,857	50,962,176	166,320	13	2	0.01187	0.14
		CNVR_4135_5	26	Mixed	3,968,813	3,977,095	8283	13	2	0.01187	0.14
		CNVR_4128_2	25	Mixed	41,250,992	41,371,975	120,984	15	5	0.02871	0.16
		CNVR_627_1	3	Mixed	11,962,808	11,975,091	12,284	44	64	0.03038	0.16
		CNVR_2756_2	14	Mixed	2,382,595	2,468,020	85,426	13	4	0.03472	0.17
		CNVR_1765_2	8	Mixed	33,719,567	33,725,198	5632	49	32	0.03666	0.18
		CNVR_671_6	3	Mixed	38,653,612	38,799,108	145,497	14	5	0.04237	0.20
		CNVR_1332_4	6	Mixed	33,486,467	33,496,920	10,454	14	5	0.04237	0.20
		CNVR_2614_1	12	Loss	77,799,528	77,803,453	3926	20	34	0.04652	0.21
		CNVR_317_1	2	Mixed	2,527,718	2,535,261	7544	51	70	0.04784	0.21

[a] Control: group of animals with the lowest averages obtained for the studied parameter

[b] Case: group of animals with the highest averages obtained for each studied parameter

Additionally, the *SLC9A1* gene was described as related to transport of glucose and other sugars, bile salts and organic acids, metal ions, and amine compounds and metabolism pathways (Slepkov et al. 2005).

Table 5 Genes within significant copy number variation regions (CNVRs) for monounsaturated fatty acid profile in intramuscular fat of *longissimus thoracis* muscle in Nellore cattle

Trait	Nomenclature	BTA	Genes
Sum of MUFA		10	–
		11	–
		13	–
		17	*PRAME*
		14	–
		2	*ZNF804A*
		1	–
		9	
		18	
		6	–
Myristoleic	C14:1	9	–
		28	*ASCC1*, *ANAPC16*, *DDIT4*, *DNAJB12*
		9	*SAMD8*
		20	–
		6	–
		23	–
		1	–
		2	*FGR*, *LOC104971347*, *AHDC1*, *WASF2*, *GPR3*, *CD164L2*, *MAP3K6*, *SYTL1*, *TMEM222*, *WDTC1*, *LOC104971348*, *TRNAE-UUC*, *SLC9A1*
		8	*LOC783399*, *LOC104969451*, *ZFP37*, *LOC104972947*
		14	*CA13*
		1	–
		4	*LOC101904045*, *LOC101903865*, *LOC101903933*, *LOC104972267*, *LOC509513*, *LOC101903672*, *LOC101903590*, *LOC101903755*
Oleic	C18:1 *cis*-9	12	–
		10	*LOC784260*
		8	*CEP78*
		X	–
		15	–
		18	*LOC614926*, *ACP7*
		8	*KLF9*
Elaidic	C18:1 trans9	7	–
		23	*LOC100296164*, *MDGA2*
		5	*LOC100337366*, *LOC100849008*
Vaccenic	C18:1 trans11	26	*LOC536342*, *ADGRA1*
		26	*PCDH15*
		25	*IQCE*, *TTYH3*, *LFNG*
		3	*LOC100139973*
		14	*ZC3H3*, *MAFA*
		8	–
		3	*LOC787637*
		6	–
		12	*HS6ST3*
		2	–

The CNVR_7530_1 (C14:1) mixed region was found to harbor the *LOC783399*, *LOC104969451*, *ZFP37*, and *LOC104972947* genes. The *ZFP37* gene encodes a transcription factor that plays a role in regulating the structures of the nucleolus and centromere in neurons in mouse (Payen et al. 1998). The CNVR_1849_1 on BTA14 hosted the *CA13* gene, which participates in the metabolism (GO:006730) and nitrogen metabolism (GO:0015701).

Seven CNVRs were found significant ($P < 0.05$) for oleic acid (C18:1 *cis*-9). Although the five genes identified within these regions, none of them showed any association neither with lipid nor energy metabolism process. The same was observed for elaidic acid (C18:1 *trans*-9). A total of 11 CNVRs were associated with vaccenic acid (C18:1 *trans*-11) and 11 genes were identified among them. The *TTYH3* gene was identified within the CNVR_4128_2 mixed region on BTA25. Its function is related to pathways involved in ion and glucose channel transport, bile salts and organic acids, metal ions, and amine compounds (Zhu et al. 2013). The *LFNG* gene was also identified in this region, and it was associated with ovarian follicle development (GO:0001541).

Polyunsaturated fatty acids

Sixty-six CNVRs were significant (P value < 0.05) for the PUFA group. Docosahexaenoic acid (C22:6 n-3) showed the highest number of significant CNVRs associated (17), of which four were classified as loss, two as gain, and 11 as

Table 6 Significant copy number variation regions (CNVRs) associated with polyunsaturated fatty acid profile in *longissimus thoracis* muscle of Nellore cattle

Trait	Nomenclature	CNVRs ID	BTA	Description	Start (bp)	End (bp)	Size (bp)	Control[a]	Case[b]	*P* value	FDR
Sum of PUFA		CNVR_3828_2	12	Mixed	57,649,064	57,684,714	35,651	30	3	0.00007	0.01
		CNVR_1465_1	4	Mixed	90,699,813	90,705,987	6175	23	48	0.00150	0.13
		CNVR_55_1	1	Mixed	15,844,150	16,000,856	156,707	30	10	0.00155	0.13
		CNVR_119_1	1	Mixed	31,670,765	31,695,808	25,044	40	66	0.00412	0.19
		CNVR_2458_1	7	Loss	88,550,049	88,553,909	3861	17	3	0.00449	0.19
		CNVR_4714_1	16	Loss	56,458,959	56,464,905	5947	13	2	0.01202	0.38
		CNVR_5419_1	20	Mixed	15,371,013	15,381,710	10,698	18	35	0.01440	0.40
		CNVR_105_3	1	Mixed	27,836,489	27,900,992	64,504	12	2	0.01691	0.42
		CNVR_3780_4	12	Mixed	39,022,698	39,098,205	75,508	13	3	0.01947	0.44
		CNVR_2506_1	7	Loss	99,776,747	99,798,476	21,730	14	4	0.02385	0.50
		CNVR_2798_1	8	Mixed	93,867,246	93,875,806	8561	35	53	0.03324	0.57
		CNVR_876_1	3	Loss	6,571,668	6,587,033	15,366	23	11	0.03603	0.57
		CNVR_2214_1	7	Mixed	6,708,218	6,718,398	10,181	16	29	0.04373	0.57
		CNVR_6481_3	26	Mixed	51,104,225	51,286,609	182,385	18	8	0.04892	0.57
Arachidonic	C20:4 n-6	CNVR_1041_1	4	Mixed	106,642,849	106,765,834	122,986	45	21	0.00680	0.63
		CNVR_4510_1	X	Gain	48,563,085	48,586,299	23,215	22	7	0.01231	0.63
		CNVR_888_2	4	Mixed	39,270,414	39,425,567	155,154	12	28	0.01688	0.63
		CNVR_1734_1	7	Loss	99,322,117	99,328,261	6145	38	20	0.01889	0.63
		CNVR_2334_5	10	Mixed	24,279,052	24,285,616	6565	47	28	0.02119	0.63
		CNVR_3820_1	18	Mixed	45,300,008	45,330,206	30,199	71	51	0.02934	0.63
		CNVR_1515_3	7	Mixed	9,409,927	9,504,448	94,522	17	7	0.03129	0.63
		CNVR_1530_1	7	Mixed	18,531,324	18,566,035	34,712	45	29	0.03422	0.63
		CNVR_3493_1	16	Mixed	39,558,124	39,589,152	31,029	62	44	0.03524	0.63
		CNVR_2026_1	9	Mixed	4,374,671	4,386,831	12,161	17	30	0.03667	0.63
		CNVR_2687_1	11	Mixed	69,975,117	70,555,942	580,826	18	8	0.04803	0.72
Linoleic	C18:2 cis9 cis12 n-6	CNVR_3012_2	15	Mixed	12,046,071	12,115,022	68,952	13	30	0.00805	0.72
		CNVR_180_3	1	Loss	105,018,867	105,264,358	245,492	42	65	0.01177	0.72
		CNVR_1346_2	6	Mixed	80,232,706	80,249,371	16,666	22	9	0.01915	0.72
		CNVR_2166_4	10	Mixed	24,061,376	24,070,828	9453	50	31	0.02115	0.72
		CNVR_1672_1	8	Mixed	1,817,817	1,860,542	42,726	18	7	0.02901	0.72
		CNVR_1446_1	7	Mixed	24,107,045	24,149,485	42,441	15	29	0.02910	0.72
		CNVR_2594_1	12	Mixed	32,054,331	32,182,960	128,630	34	19	0.03063	0.74
		CNVR_139_1	1	Mixed	83,218,713	83,238,102	19,390	41	25	0.03505	0.78
		CNVR_4549_1	27	Mixed	9,036,908	9,096,031	59,124	20	34	0.04489	078
		CNVR_4121_2	X	Mixed	26,287,961	26,369,699	81,739	30	17	0.04814	0.87
Linolenic	C18:3 n-3	CNVR_992_1	4	Mixed	83,429,553	83,463,128	33,576	58	31	0.00179	0.12
		CNVR_1042_1	4	Mixed	106,642,849	106,765,834	122,986	45	21	0.00188	0.12
		CNVR_4515_1	X	Gain	48,563,085	48,586,299	23,215	22	7	0.00647	0.23
		CNVR_1524_1	7	Mixed	11,819,446	11,876,291	56,846	52	30	0.00850	0.23
		CNVR_4457_1	X	Mixed	28,439,058	28,494,393	55,336	59	36	0.00919	0.23
		CNVR_2851_2	12	Mixed	61,223,484	61,234,845	11,362	21	40	0.00958	0.23
		CNVR_889_2	4	Mixed	39,270,414	39,425,567	155,154	12	28	0.00962	0.28
		CNVR_1737_1	7	Loss	99,322,117	99,328,261	6145	38	20	0.01337	0.28
		CNVR_2342_1	10	Mixed	27,086,857	27,117,598	30,742	27	12	0.01489	0.28
		CNVR_2712_1	11	Mixed	81,414,106	81,425,393	11,288	45	26	0.01608	0.47
		CNVR_2338_5	10	Mixed	24,279,052	24,285,616	6565	47	28	0.01847	0.47
		CNVR_446_1	2	Mixed	53,991,194	53,999,598	8405	16	6	0.03630	0.47
		CNVR_2030_1	9	Mixed	4,374,671	4,386,831	12,161	17	30	0.04631	0.47

Table 6 (continued)

Trait	Nomenclature	CNVRs ID	BTA	Description	Start (bp)	End (bp)	Size (bp)	Control[a]	Case[b]	*P* value	FDR
		CNVR_1502_1	7	Mixed	114,705,514	114,716,539	11,026	12	18	0.04074	0.46
Docosahexaenoic	C22:6 n-3	CNVR_1044_1	4	Mixed	106,642,849	106,765,834	122,986	45	20	0.00116	0.24
		CNVR_3954_1	20	Loss	15,371,013	15,381,710	10,698	39	18	0.00386	0.27
		CNVR_2335_2	10	Mixed	27,106,916	27,117,598	10,683	27	10	0.00523	0.27
		CNVR_1340_3	6	Mixed	28,989,049	28,994,000	4952	22	7	0.00649	0.27
		CNVR_4488_1	X	Gain	48,563,085	48,586,299	23,215	22	7	0.00649	0.27
		CNVR_1003_1	4	Mixed	90,699,813	90,705,987	6175	45	25	0.01096	0.32
		CNVR_1513_2	7	Mixed	9,409,927	9,504,448	94,522	20	7	0.01396	0.32
		CNVR_2024_1	9	Mixed	4,374,671	4,386,831	12,161	18	35	0.01401	0.32
		CNVR_4434_2	X	Mixed	28,434,257	28,494,393	60,137	58	37	0.01745	0.32
		CNVR_3512_1	16	Gain	60,462,670	60,502,932	40,263	13	3	0.01993	0.35
		CNVR_4460_1	X	Loss	36,544,204	36,555,271	11,068	16	5	0.02039	0.35
		CNVR_3152_1	14	Mixed	54,123,999	54,126,508	2510	40	23	0.02376	0.35
		CNVR_163_1	1	Mixed	83,218,713	83,238,102	19,390	25	42	0.02492	0.37
		CNVR_1739_1	7	Loss	99,322,117	99,328,261	6145	34	19	0.03187	0.37
		CNVR_4431_1	X	Mixed	25,335,659	25,443,589	107,931	115	92	0.03533	0.44
		CNVR_1868_1	8	Loss	44,934,828	44,944,671	9844	16	6	0.03633	0.44
		CNVR_1417_1	6	Mixed	80,232,706	80,249,371	16,666	13	25	0.04423	0.44

[a] Control: group of animals with the lowest averages obtained for the studied parameter

[b] Case: group of animals with the highest averages obtained for each studied parameter

mixed regions. Linolenic acid (C18:3 n-3) and the sum of PUFA displayed 14 CNVRs associated, and both presented a great number of mixed regions, with values of 12 and 10, respectively (Table 6).

The sum of PUFA shared 64 genes within the identified CNVRs (Table 7). On BTA16, the CNVR_4714_1 loss region harbored the *SLC9C2* gene. This gene belongs to the solute carrier family 9, and it is related to pathways such as transport of glucose and other sugars, bile salts and organic acids, metal ions, and amine compounds. This family gene plays an important role to transport hexose to the mammalian cells since these sugars are unable to diffuse across cellular membranes, and require transporter proteins to enter into and out of the cells (He et al. 2009).

The second CNVRs associated with the sum of PUFA were the mixed CNRV_6481_3 on BTA26, in which we identified the NKX6-2 and INPP5A genes. The NKX6-2 is a protein-coding gene related to multicellular organism development (GO:0007275), while the INPP5A gene codifies a protein that mobilizes intracellular calcium and acts as a second messenger mediating cell responses to several stimulations (Mills et al. 2008).

Significant CNVRs for arachidonic acid (C20:4 n-6) encompassed 11 CNVRs, and 20 LOC (genes of uncertain functions) were found within these regions. The CNVR_1041_1, located on BTA4, was found exactly at same significant region observed for the CNVR_6050_1 (C14:1), as described above. Only the *ALK* gene was identified with the function described, and it was located on CNVR_2687_1 on BTA11. The same pattern was observed for linoleic acid (C18:2 *cis*-9 *cis*-12 n-6). This FA displayed ten significant CNVRs with fewer LOC and only one gene identified, the *RAPGEF6* gene, located on BTA7 in the mixed CNVR_1446_1. The *RAPGEF6* gene is related to regulation of GTPase activity (GO:0043087).

The PUFA which presented the highest number of CNVRs associated was linolenic (C18:3 n-3); however, only LOC regions lacking functional information were identified. It is worth to mention that the CNVR_1446_1 located on BTA7, as reported above, was also significant for the linolenic and docosahexaenoic acids.

Omega 3 and 6 fatty acids

A total of 35 CNVRs were found to be significant ($P < 0.05$) for the ω3, ω6, and ω6/ω3 ratio FA groups (Table 8). The sum of ω3 acids presented the highest number of significant CNVRs (13) in comparison to ω6 (10) and ω6/ω3 ratio (12), and most of the regions were characterized as mixed regions. These significant regions harbored 48 genes over the Nellore cattle genome, including LOC and genes with the functions described (Table 9).

The sum of ω3 showed five significant (P value < 0.05) CNVRs. On BTA7, the CNVR_2257_1 was found at the same region as did for the CNVR_1515_3 (C20:4 n-6) and CNVR_1513_2 (C22:6 n3). On BTA29, the CNVR_3212 was described to contain 17 genes that have been previously associated with lauric acid; thus, they have already been

Table 7 Genes within significant copy number variation regions (CNVRs) for polyunsaturated fatty acid profile in intramuscular fat of *longissimus thoracis* muscle in Nellore cattle

Trait	Nomenclature	BTA	Genes
Sum of PUFA		12	–
		4	–
		1	–
		1	–
		7	–
		16	*SLC9C2*
		20	–
		1	–
		12	–
		7	–
		8	–
		3	–
		7	–
		26	*NKX6-2*, *INPP5A*
Arachidonic	C20:4 n-6	4	*LOC101904045, LOC101903865, LOC101903933, LOC104972267, LOC509513, LOC101903672, LOC101903590, LOC101903755*
		X	–
		4	*LOC782609*
		7	–
		10	*LOC100295747*
		18	–
		7	*LOC100299465*, *LOC508826*, *LOC509641*, *LOC787503*, *LOC104972795*, *LOC504888*
		7	*LOC1019053227*, *LOC100337044*
		16	*LOC517828*
		9	–
		11	*LOC104968430*, *ALK*
Linoleic	C18:2 cis9 cis12 n6	15	–
		1	*LOC781650*, *TRNAS-GGA*, *LOC782622*
		6	–
		10	*LOC101903548*
		8	*LOC520638*
		7	*RAPGEF6*
		12	*LOC100847339*, *LOC101902112*
		1	–
		27	–
		X	–
Linolenic	C18:3 n3	4	*TRGC3*, *TRGC4*
		4	*LOC101904045, LOC101903865, LOC101903933, LOC104972267, LOC509513, LOC101903672, LOC101903590, LOC101903755*
		X	–
		7	*LOC100848374*, *LOC101909051*, *LOC789203*, *LOC101906711*
		X	–
		12	
		4	*LOC782609*
		7	–
		10	–
		11	–

Table 7 (continued)

Trait	Nomenclature	BTA	Genes
		10	–
		2	–
		9	–
		7	*LOC100337366*, *LOC100849008*
Docosahexaenoic	C22:6 n3	4	*LOC101904045, LOC101903865, LOC101903933, LOC104972267, LOC509513, LOC101903672, LOC101903590, LOC101903755*
		20	–
		10	*LOC784260*
		6	–
		X	–
		4	–
		7	*LOC100299465, LOC508826, LOC509641, LOC787503, LOC104972795, LOC504888*
		9	–
		X	–
		16	–
		X	–
		14	–
		1	–
		7	–
		X	–
		8	–

described above. The CNVR_3298_1 on BTA10 harbored genes with known function, such as the *COL4A3BP* gene. Its function is related to cell morphogenesis (GO:0000902), protein phosphorylation (GO:0006468), and ceramide metabolic (GO:0006672) processes.

A total of ten CNVRs were associated with the ω6 acid; however, only three of them showed genes with the functions described. The CNVR_5420_1 located on BTA4 was classified as a loss region and it was also associated with lauric acid, which has been already described above. Fewer LOC were identified in the remaining CNVRs and none of them had functions described.

Twelve CNVRs were associated with the ω6/ω3 ratio and seven genes were identified within six different CNVRs. On BTA4, the mixed CNVR_3175_3 encompassed the *ZC2HC1A* and *IL7* genes. The *IL7* gene produces an important cytokine for B and T cell development. This cytokine works in cooperation with the hepatocyte growth factor (HGF) to form a heterodimer that acts as a pre-pro-B cell growth-stimulating factor. Studies in mice suggested that this cytokine plays an essential role in lymphoid cell survival (Markley and Sadelain 2010). On BTA1, the CNVR_206_1 hosted the *SI* gene, which encodes a sucrase-isomaltase enzyme that is expressed in the intestinal brush border and it is related to carbohydrate metabolic process (GO:0005975), metabolic process (GO:0008152), and polysaccharide digestion (GO:0044245). The *COL11A1* gene was identified in the CNVR_669_1 on BTA3, which encodes a minor fibrillar collagen and it is also related to cartilage condensation (GO:0001502) and ossification (GO:0001503).

Functional analysis

The analysis set comprised 213 genes, of which 197 presented DAVID ID and were used to perform the functional analysis. Gene ontology terms (cellular components, molecular functions, and biological processes) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analyses were performed by the DAVID tool (Huang et al. 2009a, b) to give an insight into the predicted gene networks (Table 10). Among the results, we highlighted the olfactory receptor activity (GO:0004984), G-protein-coupled receptor activity (GO:0004930), potassium:proton antiporter activity (GO:0015386), sodium:proton antiporter activity (GO:0015385), and odorant-binding (GO:0005549) molecular functions.

The olfactory receptor activity (GO:0004984) term is defined as a combination of the olfactory receptor with an odorant to transmit a signal from one side of the membrane to the other to initiate a change in cell activity in response to detection of smell (Ashburner et al. 2011).

Table 8 Significant copy number variation regions (CNVRs) associated with the sum of ω3 and ω6 and ω6/ω3 ratio fatty acid profile in *longissimus thoracis* of Nellore cattle

Trait	CNVRs ID	BTA	Description	Start (bp)	End (bp)	Size (bp)	Control[a]	Case[b]	*P* value	FDR
Sum of ω3	CNVR_2257_1	7	Mixed	9,409,927	9,504,448	94,522	21	5	0.00291	0.55
	CNVR_3356_1	10	Loss	27,086,857	27,117,598	30,742	27	12	0.01442	0.55
	CNVR_3212	29	Mixed	41,592,079	41,725,223	133,145	13	3	0.01958	0.55
	CNVR_6294_1	X	Gain	48,563,085	48,586,299	23,215	17	6	0.02425	0.55
	CNVR_5695_2	20	Mixed	46,839,480	47,116,159	276,680	21	9	0.02765	0.55
	CNVR_5694_1	20	Mixed	45,910,295	45,990,206	79,912	18	7	0.02903	0.55
	CNVR_3036_3	9	Mixed	31,368,505	31,370,192	1688	13	4	0.03546	0.55
	CNVR_3036_2	9	Mixed	31,316,956	31,327,903	10,948	13	4	0.03546	0.55
	CNVR_2749_1	8	Loss	44,934,828	44,944,671	9844	13	4	0.03546	0.55
	CNVR_4892_1	16	Gain	60,462,670	60,502,932	40,263	13	4	0.03546	0.55
	CNVR_3298_1	10	Mixed	6,815,725	6,824,247	8523	48	31	0.03709	0.55
	CNVR_6221_1	X	Mixed	28,434,257	28,494,393	60,137	56	38	0.03870	0.55
	CNVR_4852_1	16	Mixed	39,558,124	39,589,152	31,029	67	48	0.04193	0.55
Sum of ω6	CNVR_4675_1	29	mixed	11,800,467	11,845,269	44,803	21	34	0.02617	0.82
	CNVR_1026_1	12	Mixed	936,045	944,850	8806	77	109	0.02804	0.82
	CNVR_6852_1	9	Mixed	16,420,855	16,924,662	503,808	20	37	0.03310	0.82
	CNVR_6037_3	6	Mixed	77,164,347	77,294,195	129,849	12	2	0.03727	0.82
	CNVR_956_1	11	Mixed	81,414,106	81,425,393	11,288	37	29	0.03761	0.82
	CNVR_2609_1	19	Mixed	2,252,450	2,344,351	91,902	27	39	0.03918	0.82
	CNVR_5420_1	4	Loss	95,780,598	95,974,974	194,377	15	6	0.03974	0.82
	CNVR_1186_2	12	Mixed	57,649,064	57,684,714	35,651	32	13	0.04137	0.82
	CNVR_3793_1	23	Mixed	29,385,602	29,493,694	108,093	18	29	0.04845	0.82
	CNVR_6759_1	8	Mixed	93,873,095	93,875,806	2712	34	52	0.04874	0.82
ω6/ω3 ratio	CNVR_2071_3	9	Mixed	6,225,419	6,252,169	26,751	28	6	0.00032	0.07
	CNVR_3175_3	14	Mixed	44,047,994	44,134,488	86,495	25	11	0.01676	0.33
	CNVR_206_1	1	Mixed	103,098,743	103,168,693	69,951	31	16	0.02145	0.33
	CNVR_4228_1	21	Loss	36,862,487	36,865,662	3176	24	11	0.02402	0.33
	CNVR_2372_5	10	Mixed	24,275,820	24,285,616	9797	33	52	0.02635	0.33
	CNVR_4508_2	X	Mixed	26,287,961	26,369,699	81,739	20	36	0.02655	0.33
	CNVR_1923_1	8	Mixed	46,893,088	46,929,974	36,887	15	5	0.02813	0.33
	CNVR_4250_1	21	Mixed	53,293,514	53,310,758	17,245	26	13	0.03062	0.33
	CNVR_1534_1	6	Loss	114,705,514	114,716,539	11,026	24	12	0.03849	0.40
	CNVR_2793_1	12	Mixed	1,167,394	1,185,172	17,779	55	38	0.04462	0.41
	CNVR_669_1	3	Mixed	40,529,468	40,667,345	137,878	30	17	0.04484	0.41
	CNVR_4116_2	20	Mixed	47,008,760	47,032,305	23,546	15	6	0.04975	0.42

[a] Control: group of animals with the lowest averages obtained for each studied parameter

[b] Case: group of animals with the highest averages obtained for each studied parameter

Only one KEGG pathway was identified overrepresented (*P* value = 9.05E-10) for this set of genes, identified as the bta04740:olfactory transduction (Fig. 1). Functional enrichment analysis of CNVRs in Qinchuan cattle revealed the olfactory transduction pathway as the most enriched (Zhang et al. 2015). The cellular and molecular machinery for olfactory transduction is located in the olfactory cilia. Odorant transduction begins with odorant binding to specific receptors on the external surface of cilia. Binding may occur directly, or by way of proteins in the mucus (called odorant-binding proteins) that sequester the odorant and shuttle it to the receptor (Strotmann and Breer 2011). Olfactory transduction pathways act in the perception of odor through olfactory receptors and biochemical signaling events, which influence food consumption (Ma 2007). This pathway has also been identified as overrepresented in studies assessing feed efficiency and performance in crossbred beef cattle (Abo-ismail et al. 2014) and residual feed intake in pigs (Do et al. 2014).

Table 9 Genes associated within significant copy number variation regions (CNVRs) for ω3, ω6, and ω6/ω3 ratio fatty acids in intramuscular fat of *longissimus thoracis* muscle of Nellore cattle

Trait	BTA	Genes
Sum of ω3	7	*LOC100299465*, *LOC508826*, *LOC509641*, *LOC787503*, *LOC104972795*, *LOC504888*
	10	*LOC784260*
	29	*AHNAK*, *EEF1G*, *TRNAG-UCC*, *TUT1*, *MTA2*, *EML3*, *ROM1*, *B3GAT3*, *GANAB*, *INTS5*, *LBHD1*, *METTL12*, *UQCC3*, *UBXN1*, *LRRN4CL*, *BSCL2*, *GNG3*
	X	–
	20	–
	20	–
	9	–
	9	–
	8	
	16	*LOC104974482*
	10	*COL4A3BP*
	X	–
	16	*LOC517828*
Sum of ω6	29	*LOC505383*
	12	–
	9	–
	6	–
	11	–
	19	–
	4	*MKLN1*, *TRNAE-UCC*, *LOC104972201*
	12	–
	23	*LOC515704*, *LOC782379*, *LOC785479*, *LOC785557*, *LOC509155*, *LOC84614*, *LOC784652*, *LOC784681*, *LOC528343*
	8	–
ω6/ω3ratio	9	–
	14	*ZC2HC1A IL7*
	1	*SI*
	21	*LOC101905544*
	10	*LOC100295747*
	X	–
	8	*KLF9*
	21	–
	6	–
	12	–
	3	*COL11A1*
	20	–

Plasma membranes isolated from the bovine olfactory epithelium containing large numbers of olfactory receptor cells are characterized by high lipid content, especially phospholipid. Odorant molecules are lipoid-soluble, which suggests that the interaction of odorant molecules with the lipid layer of the olfactory receptor membrane is important in olfactory reception (Koyama and Kurihara 1972).

The importance of olfactory receptor genes has been previously studied in pigs (Paudel et al. 2015). They compared and contrasted CNV patterns among different pig populations and species so as to investigate the role that CNVRs may play in this ongoing speciation process. As a result, the majority of the copy number variable genes were olfactory receptors known to play a prominent role in food foraging and mate recognition in pigs.

Several studies have mainly focused on genome wild association SNPs, and fewer studies have investigated the influence of structural variations (SVs) on phenotypic

Table 10 Gene ontology (GO) enriched terms (***P*** value < 0.1) from the set of genes previously identified

GO term	*P* value	Genes
Biological process		
GO:0007608~sensory perception of smell	2.47-E-12	*LOC784652*, *LOC528343*, *OR2AG1*, *OR2AG2*, *LOC515704*, *OR2T4*, *LOC100849008*, *OR2L13*, *OR2M4*, *OR2M5*, *LOC100336980*, *OR2T12*, *LOC788079*, *LOC784681*, *LOC616716*, *LOC788055*
GO:0007186~G-protein-coupled receptor signaling pathway	4.06E-11	*LOC784652*, *LOC528343*, *OR2AG1*, *OR2AG2*, *LOC504888*, *OR5D14*, *LOC515704*, *OR2T4*, *LOC509641*, *OR5L2*, *LOC100849008*, *OR2M4*, *OR2L13*, *OR2M5*, *LOC100336980*, *LOC100299465*, *LOC786596*, *OR2T12*, *LOC788079*, *OR5AS1*, *LOC784681*, *GNG3*, *LOC513384*, *LOC788055*, *LOC616716*
GO:0006427~histidyl-tRNA aminoacylation	0.01297	*HARS2*, *HARS*
GO:0007156~homophilic cell adhesion via plasma membrane adhesion molecules	0.02536	*PCDHA6*, *PCDHA3*, *PCDH15*, *PCDHA13*
GO:0072673~lamellipodium morphogenesis	0.03843	*WASF1*, *WASF2*
GO:0050911~detection of chemical stimulus involved in sensory perception of smell	0.04196	*LOC100299465*, *LOC504888*, *LOC509641*
GO:0098719~sodium ion import across plasma membrane	0.06324	*SLC9C2*, *SLC9A1*
GO:0016180~snRNA processing	0.08144	*TUT1*, *INTS5*
GO:0016601~Rac protein signal transduction	0.08144	*WASF1*, *WASF2*
GO:0050910~detection of mechanical stimulus involved in sensory perception of sound	0.08144	*PCDH15*, *COL11A1*
Molecular function		
GO:0004984~olfactory receptor activity	1.02-E-10	*LOC784652*, *LOC528343*, *OR2AG1*, *LOC100125776*, *OR2AG2*, *LOC504888*, *OR5D14*, *LOC515704*, *OR2T4*, *LOC509641*, *OR5L2*, *LOC100849008*, *OR2M4*, *OR2L13*, *OR2M5*, *LOC100336980*, *LOC100299465*, *LOC786596*, *OR2T12*, *LOC788079*, *OR5AS1*, *LOC784681*, *LOC513384*, *LOC788055*, *LOC616716*
GO:0004930~G-protein-coupled receptor activity	6.89-E-10	*LOC784652*, *LOC515704*, *OR5D14*, *LOC504888*, *OR2T4*, *OR5L2*, *LOC100849008*, *OR2L13*, *GPR3*, *LOC786596*, *LOC100299465*, *OR2T12*, *OR5AS1*, *LOC788079*, *LOC784681*, *LOC788055*, *LOC528343*, *OR2AG1*, *OR2AG2*, *LOC100125776*, *LOC509641*, *OR2M4*, *OR2M5*, *LOC100336980*, *ADGRA1*, *LOC616716*
GO:0004821~histidine-tRNA ligase activity	0.01309	*HARS2*, *HARS*
GO:0015386~potassium:proton antiporter activity	0.06994	*SLC9C2*, *SLC9A1*
GO:0015385~sodium:proton antiporter activity	0.06994	*SLC9C2*, *SLC9A1*
GO:0005549~odorant binding	0.09453	*OR5D14*, *OR5AS1*, *OR5L2*, *LOC513384*
Cellular component		
GO:0005886~plasma membrane	3.84-E-07	*PRX*, *PCDHA6*, *LOC784652*, *LOC504888*, *LOC515704*, *OR5D14*, *PCDHA3*, *OR2T4*, *OR5L2*, *LOC100849008*, *OR2L13*, *LOC100299465*, *TTYH3*, *LOC786596*, *OR2T12*, *OR5AS1*, *LOC788079*, *LOC784681*, *PCDHA13*, *LOC788055*, *LOC528343*, *OR2AG1*, *OR2AG2*, *LOC100125776*, *KLF9*, *LOC509641*, *PCDH15*, *OR2M4*, *OR2M5*, *LOC100336980*, *BLVRB*, *SLC9C2*, *SYTL1*, *GRASP*, *LOC616716*, *SLC9A1*
GO:0016021~integral component of membrane	0.00109	*PCDHA6*, *LOC784652*, *LOC504888*, *PCDHA3*, *OR5D14*, *LOC515704*, *OR2T4*, *OR5L2*, *BSCL2*, *DNAJB12*, *LOC100849008*, *OR2L13*, *GPR3*, *LOC100299465*, *LOC786596*, *OR2T12*, *OR5AS1*, *LOC788079*, *LOC784681*, *IFNK*, *LOC513384*, *PCDHA13*, *LOC509513*, *LOC788055*, *LOC528343*, *OR2AG1*, *TMEM222*, *LRRN4CL*, *OR2AG2*, *LOC100125776*, *MDGA2*, *LOC509641*, *SI*, *PCDH15*, *ALK*, *OR2M4*, *OR2M5*, *LOC100336980*, *SLC9C2*, *ADGRA1*, *LOC616716*, *ROM1*, *SLC9A1*
GO:0031209~SCAR complex	0.04485	*WASF1*, *WASF2*
GO:0005847~mRNA cleavage and polyadenylation specificity factor complex	0.08770	*TUT1*, *ZC3H3*

traits in livestock (Lemos et al. 2016; Zhou et al. 2016). CNVRs can be a major mechanism driving gene and genome evolution by duplicating and deleting segments of the genome, creating novel gene functions, disrupting

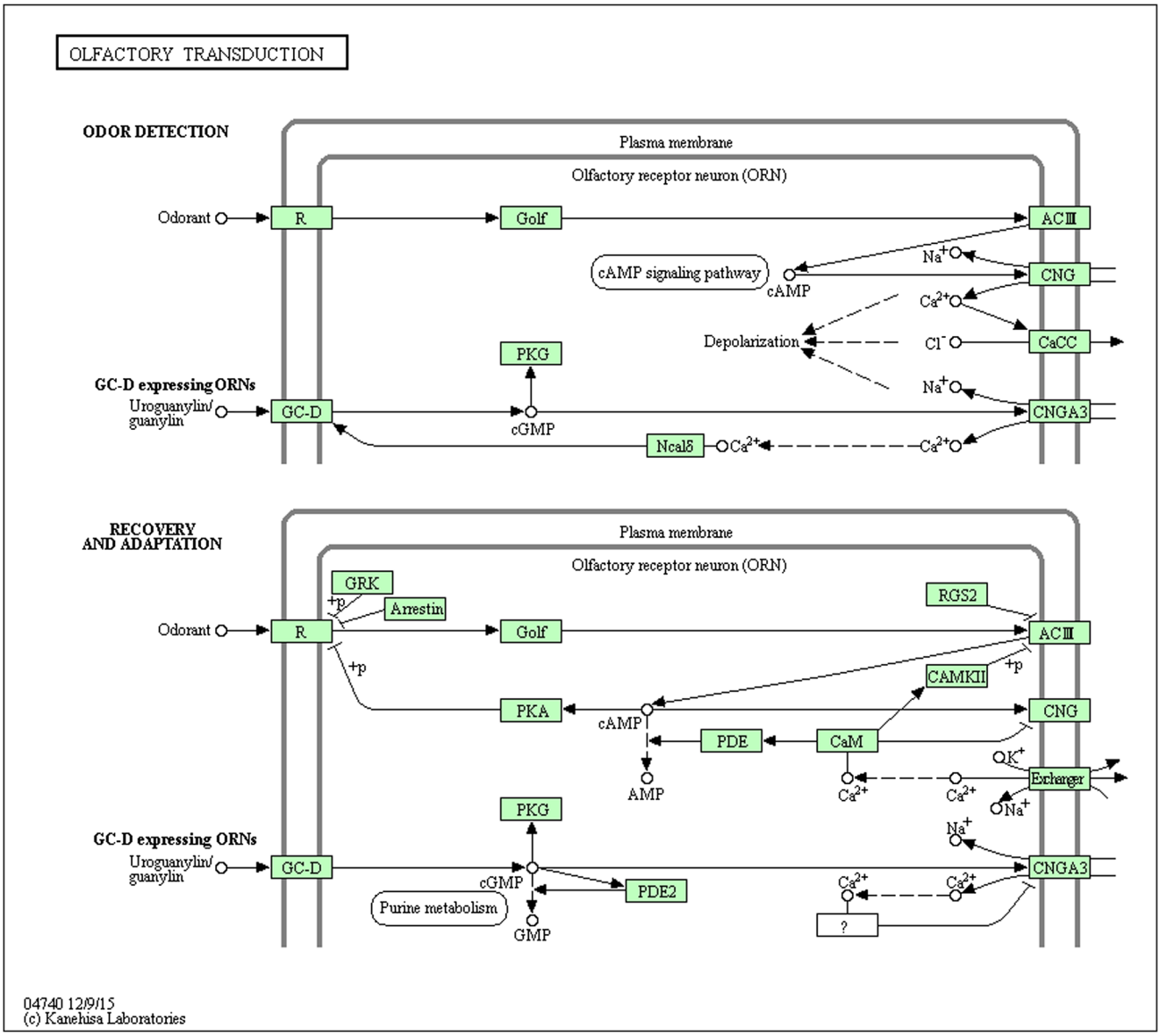

Fig. 1 KEGG olfactory transduction

gene functions, or affecting regulatory mechanisms in the genome (Paudel et al. 2015).

In this study, several CNVRs were found near QTL regions associated with SFA, MUFA, and PUFA groups in Nellore *longissimus thoracis* muscle. Some of them harbored interesting genes involved in lipid metabolic process, sphingolipid metabolic process, olfactory receptors, metabolic process, cell morphogenesis, transport of lipids, immune system, energy metabolism, nitrogen metabolism, transport of glucose and other sugars, lipid metabolic process, lipid catabolic process, lipid storage, lipid particle organization, and fat cell differentiation.

Among the several genes identified in this study, the *BSCL2* gene associated with lauric acid (C12:0) is highlighted since it is necessary for correcting lipid storage and lipid droplet maintenance and may affect the expression of the *DGAT2* gene in humans (Payne et al. 2008). The CNVR that harbors the *BSCL2* gene is a mixed region; thus, this gene may be acting in a way to increase or decrease its dosage.

The *DGAT2* gene contributes to the triacylglycerol synthesis through its acyltransferase activity. This encodes one of two enzymes which catalyze the final reaction in the synthesis of triglycerides in which diacylglycerol is covalently bound to long chain fatty acyl-CoAs (Kantartzis et al. 2009). A positive and moderate correlation between the level of marbling and the expression of *DGAT2* gene in beef meat (Buchanan et al. 2014) and a negative correlation between marbling and concentrations of stearic, linoleic acid, and PUFA (Xie et al. 1996) were reported previously in some studies. Also, this gene was

upregulated for palmitic and downregulated for linoleic acid and PUFA/SFA ratio in a study with Nellore cattle (Berton et al. 2016).

The results of this study pointed out some genes that were identified associated with several FAs of different saturations. The olfactory receptor genes were associated with the lauric, myristic, and stearic acids. The *SAMD8* and *BSCL2* genes, related to lipid metabolic process, were associated with lauric acid; the *RAPGEF6* gene is related to regulation of GTPase activity and was associated with linoleic acid. The large number of genes identified within the CNVRs, which were associated with FA profile in this study, should help to better understand the genetic mechanism underlying FA profile of intramuscular fat in Nellore cattle. Strategies such as genomic selection using or considering the variability among markers at the same time would be appropriate to improve the FA profile of bovine meat.

Conclusion

Several CNVRs were associated with fatty acid profile, and these regions pointed out some genes that might have influence on fatty acid composition and metabolism. The identification of such CNVRs and its respective candidate genes associated with lipid metabolic process and regulation of GTPase activity, i.e., *SAMD8*, *BSCL2*, and *RAPGEF6* genes, as well as their respective metabolic pathways, should contribute to improve genetic knowledge regarding the fatty acid profile of Nellore (*Bos indicus*) and help to improve the selection of such traits upon human health. The CNV information described in this study may contribute to future fine mapping studies and also can be incorporated in genetic improvement programs.

Authors' contributions MVAL, FLBF, MPB, ASCP, and FB conceived and designed the experiment; MVAL, MPB, HLJC, FLBF, EP, SK, BFO, NBS, AMF, LFM, RLT, LGA, and HN performed the experiments; MVAL, HN, ASCP, NBS, and FB did analysis and interpretation of results; MVAL, ACSP, and FB drafted the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) with grant numbers (#2013/11853-4, #2011/2141-0, and #2009/16118-5). MVA Lemos, FLB Feitosa, and E Peripolli received support from FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo. HLJ Chiaia, MP Berton, S Kluska, and BF Olivieri received scholarships from the Coordination Office for Advancement of University-level Personnel (CAPES; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) in conjunction with the Postgraduate Program on Genetics and Animal Breeding, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (FCAV, UNESP). F Baldi received support from FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo grant #2011/21241-0 and Lucia G. Albuquerque from FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo grant #2009/16118-5.

Compliance with ethical standards

Ethics approval This study was approved by the ethics committee of the Faculdade de Ciências Agrárias e Veterinárias (FCAV), Universidade Estadual Paulista (UNESP), Jaboticabal-SP, Brazil.

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no competing interests.

Abbreviations *MUFA*, Sum of monounsaturated fatty acids; *FA*, Fatty acid; *CNV*, Copy number variation; *CNVR*, Copy number variation regions; *GWAS*, Genome-wide association study; *QTL*, Quantitative trait loci; *PUFA*, Sum of polyunsaturated fatty acids; *CLA*, Conjugated linoleic acid; *GO*, Gene ontology; *MAF*, Minor allele frequency; *SFA*, Sum of saturated fatty acids; *ω3*, Sum of omega 3 acids; *ω6*, Sum of omega 6 acids; *BAF*, Allele B frequency; *IMF*, Intramuscular fat; *LDL*, Low-density lipoprotein; *BTA*, *Bos Taurus* chromosome; *LRR*, Log *R* ratio; *SNP*, Single nucleotide polymorphism

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