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ORIGINAL ARTICLE

A genomewide association mapping study using ultrasound-scanned information identifies potential genomic regions and candidate genes affecting carcass traits in Nellore cattle

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Keywords

Bos indicus; candidate genes; fat thickness; genomewide association studies; single nucleotide polymorphisms; ultrasonography.

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Summary

The aim of this study was to identify candidate genes and genomic regions associated with ultrasound-derived measurements of the rib-eye area (REA), backfat thickness (BFT) and rumpfat thickness (RFT) in Nellore cattle. Data from 640 Nellore steers and young bulls with genotypes for 290 863 single nucleotide polymorphisms (SNPs) were used for genome-wide association mapping. Significant SNP associations were explored to find possible candidate genes related to physiological processes. Several of the significant markers detected were mapped onto functional candidate genes including *ARFGAP3, CLSTN2* and *DPYD* for REA; *OSBPL3* and *SUDS3* for BFT; and *RARRES1* and *VEPH1* for RFT. The physiological pathway related to lipid metabolism (*CLSTN2, OSBPL3, RARRES1 and VEPH1*) was identified. The significant markers within previously reported QTLs reinforce the importance of the genomic regions, and the other loci offer candidate genes that have not been related to carcass traits in previous investigations.

Introduction

The worldwide demand for high-quality animal protein is increasing, bringing meat quality to the spotlight of the beef cattle industry. The transition from a commodity market model to an industry that offers differentiated products to target markets is timely to the Brazilian beef industry, because the country is one of the main global leaders in the sector: Brazilian beef is exported to over 100 countries (FAO, 2013). The main Brazilian breed of cattle is the Nellore (*Bos indicus*), representing almost 90% of the total beef cattle in the country.

Traits to indicate the amount of meat and carcass fat deposition, such as rib-eye area (REA), backfat thickness (BFT) and rumpfat thickness (RFT), are used in many breeding programmes through the adoption of estimated breeding values for carcass traits (Reverter *et al.* 2000). However, few Nellore breeding programmes have adopted these phenotypes and estimates of genetic value need to be better exploited.

The information from genetic markers can improve these estimates. Genomewide association studies (GWAS) based on information from SNPs can be used to map genes involved in the physiological control of phenotypes (Snelling *et al.* 2010). Several GWAS have

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been carried out on the *Bos taurus* species, attempting to verify both the impact of genomic information in genetic predictions as well as a search tool for candidate genes affecting carcass traits (Bolormaa *et al.* 2011; McClure *et al.* 2012). However, more studies are needed to identify genomic regions, causal markers and candidate genes that affect carcass traits (Tizioto *et al*. 2013).

In this study, we use the GRAMMAR-Gamma association test to evaluate putative associations between SNPs distributed across all autosomal chromosomes (Chr) and REA, BFT and RFT measured in Nellore cattle. Here, we identify genomic regions and candidate genes related to physiological processes that may explain part of the variation in these phenotypes.

Material and methods

Ethical statement

No local ethical committee approval was required for the collection of non-invasive ultrasound data. Collection of DNA samples taken from each of the tests had been approved by their respective ethics committee. In all experiments, animals were handled according to the guidelines of the Brazilian College of Animal Experimentation (COBEA).

Population and phenotypes

Data of 640 Nellore (*Bos indicus*) young bulls and steers, born from 2005 to 2011, were collected in eleven different trials carried out from 2007 to 2012 in Brazil. The animals were kept in feedlot for 84 days and were evaluated at the end of this period at 23 ± 2 months of age and 500 ± 56 kg of live weight. The ultrasonography evaluation was performed using an Aloka SSD-500 ultrasound equipped with a 17.2-cm, 3.5 MHz, linear array transducer (Aloka Co. Ltd., Wallingford, CT, USA). Vegetable oil was used as coupling to optimize the contact surface between the transducer and the animal skin.

The phenotypes obtained by ultrasonography were rib-eye area (REA), backfat thickness (BFT) and rumpfat thickness (RFT). The REA and BFT were measured between the 12th and 13th ribs in *longissimus lumborum* and RFT on the rump at the intersection of the *biceps femoris* and *gluteus medius* between ileum and ischium. Phenotypes were tested for normality using the Shapiro–Wilk test ($p = 0.05$). For the three phenotypes analysed, there was no evidence of deviation from normality ($p \geq 0.05$) according to the Shapiro–Wilk test. Values that exceeded three standard deviations around the mean were considered outliers and were omitted from the data analysis. The descriptive statistics are presented in Table 1.

DNA extraction and genotyping

All animals had blood samples taken during the ultrasound measurement. The samples were stored at 4°C and the DNA was extracted. DNA quality and quantity were tested in a biophotometer, and the acceptable values were between 1.8 and 2.0 for A260/280 ratio and a concentration of 50 ng/μl.

Animals were genotyped with the Illumina® BovineHD Genotyping BeadChip assay (HD, 328 young bulls) for 777 962 SNPs and the Illumina® Bovine SNP50 v2 BeadChip assay (50k, 312 young bulls and steers) containing 54 609 SNPs according to the manufacturer's protocol. Details of the genotyping procedure can be found in Santana *et al.* (2014). Only samples with genotype call scores greater than 0.70 and call rate greater than 90% were used.

Imputation and data filtering

Imputation from 50k to HD was performed using FIMPUTE v2.2 (Sargolzaei *et al.* 2012). SNPs from the HD panel not contained in the original 50K panel from 90 animals (defined as validation population) were omitted before imputation for the assessment of imputation accuracy. The imputation accuracy was next evaluated by comparing the imputed genotypes with the true genotypes from the original high-density panel. The overall average imputation accuracy was 98.5%. After imputation, the genotypic data were filtered, and only autosomal SNPs with a minimum allele frequency of 2%, call rate greater than 95% and Fisher's exact test for Hardy–Weinberg equilibrium p-value greater than 1×10^{-5} were kept for the association analyses, totalling 290,863 SNPs.

Association analysis

Association analyses were performed using the GRAMMAR-Gamma (GRG) association test. GRG is a variance components-based two-step method that

Table 1 Sample size, means, standard deviations (SD), minimum and maximum values of ultrasound-related traits in Nellore cattle

Trait	N	Means	SD	Minimum	Maximum
Rib-eye area (cm^2)	640	74.7	8.8	46.2	106.3
Backfat thickness (mm)	640	4.4	2.1	0.0	15.6
Rumpfat thickness (mm)	640	6.2	2.4	0.0	16.2

takes into account population structure and relatedness between individuals (Svishcheva *et al.* 2012). This methodology is an adaptation of the GRAMMAR method that uses mixed models and regression to estimate the effects of the markers (Aulchenko *et al.* 2007a). In our implementation, the first step involved fitting a mixed model to the ultrasound carcass data. The model accounted for population substructure by means of the genomic relationship matrix and included fixed effects for contemporary group, body weight and age. As a result, transformed data were produced and a Gamma correction factor was estimated. Next, in the second step, these transformed phenotypes were regressed to the genotype data and the SNP effects were estimated. Finally, the effects were corrected by dividing them by the correction factor.

The threshold adopted to declare the significant SNPs was a modified version of Bonferroni (Gao *et al.* 2008) with 5% significance level. All procedures for data filtering and GRG association were performed in GENABEL Package v1.7-6 (Aulchenko *et al.* 2007b) for the statistical environment R.

Exploratory view of significant SNPs regions

The significant SNPs for each trait were explored and possible candidate genes related to physiological processes were investigated. For this purpose, the Biomart tool was used to search for genes associated with significant SNPs using the Ensembl genes 73 database on UMD v3.1 assembly. The significant SNPs overlapped by QTLs already reported for each phenotype were also examined using the cattle QTLdb database (Hu *et al.* 2013).

Results

Genomewide p-values for REA, BFT and RFT are presented in Figure 1, and the significant SNPs for each

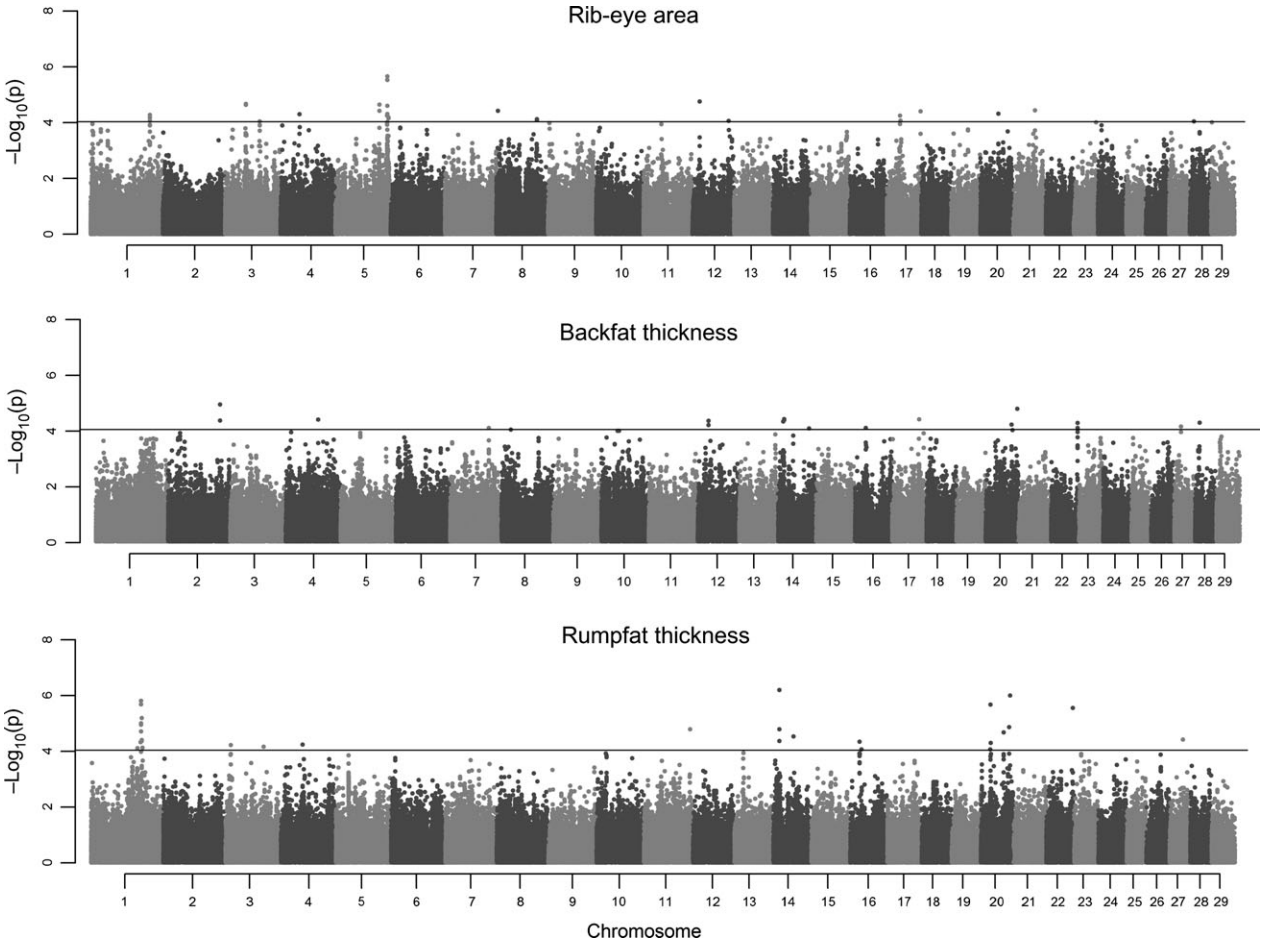

Figure 1 Manhattan plots for carcass traits using GRAMMAR-Gamma (GRG). The horizontal line represents the Modified Bonferroni threshold ($\alpha = 9.27 \times 10^{-5}$).

Table 2 Description of the significant SNPs for rib-eye area, identified using the GRAMMAR-Gamma method

SNP ID	Chr	Pos[1]	SNP[2]	Beta[3]	p-value[4]	Gene	Location
rs137515454	1	130.0	A/G	2.82	5.30×10^{-5}	*CLSTN2*	Intron
rs133006859	1	130.0	A/G	2.82	5.30×10^{-5}	*CLSTN2*	Intron
rs136954660	1	130.0	C/T	2.82	5.30×10^{-5}	*CLSTN2*	Intron
rs134931263	1	130.0	C/T	2.82	5.30×10^{-5}	*CLSTN2*	Intron
rs135545951	1	130.0	C/T	2.82	5.30×10^{-5}	*CLSTN2*	Intron
rs136613651	1	130.0	A/G	2.82	5.30×10^{-5}	*CLSTN2*	Intron
rs133360970	1	130.0	C/T	2.82	5.30×10^{-5}	*CLSTN2*	Intron
rs136105448	1	130.0	C/T	2.82	5.30×10^{-5}	*CLSTN2*	Intron
rs135432323	1	130.0	A/C	2.76	6.62×10^{-5}	*CLSTN2*	Intron
rs110769853	1	130.0	C/G	3.06	8.52×10^{-5}	*CLSTN2*	Intron
rs135204135	3	45.7	A/G	−2.35	2.33×10^{-5}	*DPYD*	Intron
rs111003237	3	45.7	C/T	−2.35	2.13×10^{-5}	*DPYD*	Intron
rs41573138	4	41.9	C/T	−1.76	4.99×10^{-5}	–	–
rs132942925	5	96.9	C/T	1.81	3.78×10^{-5}	–	–
rs135567748	5	96.9	C/T	−1.84	2.26×10^{-5}	–	–
rs135375969	5	96.9	A/G	1.87	2.28×10^{-5}	–	–
rs109695205	5	113.8	C/T	−3.10	5.60×10^{-5}	–	–
rs135890876	5	113.8	G/T	−3.16	4.90×10^{-5}	–	–
rs134893681	5	113.8	G/T	−3.29	2.52×10^{-5}	–	–
rs110709390	5	113.9	C/T	−3.29	2.52×10^{-5}	–	–
rs136571070	5	113.9	A/G	−3.29	2.52×10^{-5}	–	–
rs135897211	5	114.3	A/C	−3.52	2.20×10^{-6}	*ARFGAP3*	Intron
rs137777225	5	114.3	G/T	−3.46	2.98×10^{-6}	*ARFGAP3*	Exon
rs133300910	5	116.5	A/G	−2.55	6.59×10^{-5}	–	–
rs110455564	5	116.6	C/T	−2.59	6.99×10^{-5}	*FBLN1*	Intron
rs110493820	8	3.6	C/T	−3.81	3.78×10^{-5}	–	–
rs42672728	8	89.2	A/G	−4.59	7.61×10^{-5}	–	–
rs42672716	8	89.2	C/T	−4.53	8.14×10^{-5}	–	–
rs110147776	12	15.8	A/G	3.02	1.74×10^{-5}	–	–
rs135481137	12	79.7	C/T	2.16	8.63×10^{-5}	–	–
rs135228503	17	28.4	A/C	1.80	5.58×10^{-5}	–	–
rs134114725	17	29.7	C/T	4.07	8.62×10^{-5}	–	–
rs111013608	17	73.8	A/C	2.04	3.96×10^{-5}	*LOC531152*	Intron
rs135080512	20	39.2	C/T	2.30	4.81×10^{-5}	–	–
rs42598039	21	47.9	A/G	1.80	3.68×10^{-5}	–	–
rs42598037	21	47.9	C/T	1.80	3.68×10^{-5}	–	–
rs109664664	28	87.5	C/T	2.48	9.06×10^{-5}	–	–

[1]Position in Mb.
[2]Illumina TOP strand.
[3]Allele substitution effect.
[4]Probability value after correction for multiple test.

phenotype are presented in Tables 2–4. The significant SNPs mapped onto regions in chromosomes 1, 3, 5 12, 17 and 21 for REA; 2, 4, 12, 14, 17, 20, 22 and 27 for BFT; and 1, 3, 14, 16, 20, 22 and 27 for RFT. Clearly, the significant SNPs pointed to many regions, for example the 130 Mb location on Chr1 that has 10 markers associated with REA.

Except for SNP rs137777225 (Chr5:114316915), that is located in exon 7 of *ARFGAP3*, all described significant SNPs are intronic, and the gene list included *ARFGAP3, CLSTN2, DPYD, FBLN1, LOC531152, MFSD1, OSBPL3, RARRES1, SEMA5A, SUDS3* and *VEPH1*.

Sixteen QTLs previously described to each trait in QTLdb were found, including four for REA, four for BFT and eight for RFT (Table 5). We highlighted the Chr3, Chr14 and Chr20 chromosomes with four, five and three QTLs, respectively. Therefore, the results obtained in this study concur with previously reported results.

Discussion

GRAMMAR-Gamma identified significant SNPs in autosomal chromosomes related to rib-eye area,

Table 3 Description of the significant SNPs for backfat thickness, identified using the GRAMMAR-Gamma method

SNP ID	Chr	Pos[1]	SNP[2]	Beta[3]	p-value[4]	Gene	Location
rs135775577	2	113.8	C/T	0.43	4.65×10^{-5}	–	–
rs135356741	2	113.9	A/C	0.45	1.23×10^{-5}	–	–
rs110319479	4	71.4	G/T	−0.56	4.48×10^{-5}	*OSBPL3*	Intron
rs42927849	7	84.7	C/T	0.52	8.52×10^{-5}	–	–
rs136768852	12	23.5	C/T	0.42	6.78×10^{-5}	–	–
rs109191696	12	23.5	C/T	0.43	4.74×10^{-5}	–	–
rs109191696	12	23.5	C/T	0.43	4.74×10^{-5}	–	–
rs42425395	14	12.2	A/G	0.82	4.99×10^{-5}	–	–
rs136677477	14	14.3	C/T	−0.56	4.48×10^{-5}	–	–
rs134642262	14	14.4	A/G	−0.62	4.08×10^{-5}	–	–
rs137561220	16	23.6	C/T	0.46	8.61×10^{-5}	–	–
rs135336116	17	59.1	C/T	0.41	4.19×10^{-5}	*SUDS3*	Intron
rs43155431	20	56.8	G/T	−0.80	6.49×10^{-5}	–	–
rs41960288	20	69.2	C/T	0.46	1.76×10^{-5}	–	–
rs135411488	22	58.2	A/G	−0.40	5.64×10^{-5}	–	–
rs133036606	22	58.3	A/G	0.40	8.57×10^{-5}	–	–
rs42115539	27	14.1	C/T	1.22	7.52×10^{-5}	–	–
rs109626461	28	9.6	A/G	0.56	5.48×10^{-5}	–	–

[1]Position in Mb.
[2]Illumina TOP strand.
[3]Allele substitution effect.
[4]Probability value after correction for multiple test.

backfat thickness and rumpfat thickness in *Bos indicus* cattle. Some of the markers found in this study mapped onto genes that have not been previously reported for these traits.

Significant markers in the regions of Chr 1, 3 and 5 showed the importance of these regions for REA. We identified two regions on Chr 5: (i) 96–97 Mb, and (ii) 113–116 Mb. In the first region of Chr5, another study identified a SNP at the 98 Mb position in *RPLP2* (related to protein synthesis and degradation) associated with REA in Nellore cattle (Gomes *et al.* 2013). In the second region, two SNPs are located in the *ARFGAP3* gene (ADP-ribosylation factor GTPase activating protein 3), rs135897211 in intron 7 and rs137777225 in exon 7. Additionally, close to this region, the *MGST1*, *CLEC1A* and *C12orf5* genes were upregulated in *longissimus lumborum* of Angus cattle according to the transcriptomics analysis (Baldwin *et al.* 2012). Our results provide additional evidence that candidate genes that affect muscle tissue are in this genomic region. In turn, two SNPs associated with REA in Chr 3 were in the *DPYD* gene (dihydropyrimidine dehydrogenase) that encodes an enzyme linked to the first step of the thymine and uracil catabolism (Takai *et al.* 1994). The *CLSTN2* (calsyntenin 2) is associated with obesity in mammals, particularly in the process of increasing the adipocytes in visceral tissues and subcutaneous fat (Ugi *et al.* 2014), and its expression was related to metabolic disorders such as glucose metabolism disorder, disorder of the pancreas, diabetes mellitus, non-insulin-dependent diabetes mellitus and insulin-dependent diabetes mellitus (Mas *et al.* 2013). Apparently, the *FBLN1* and *LOC531152* have no direct link with the REA.

Among the two candidate genes identified for BFT, *OSBPL3* (oxysterol223 binding protein-related protein 3) forms intracellular lipid receptors responsible for the regulation of cell adhesion and actin cytoskeleton (Lehto *et al.* 2004) and thus probably is related to the trait. The *SUDS3* (suppressor of defective silencing 3 homologue (*S. cerevisiae*)) does not appear to be directly related to the deposition of subcutaneous fat in cattle.

Several GWAS involving beef cattle highlighted regions of Chr14 for many phenotypes. We identified significant SNPs for BFT and RFT in this chromosome. These GWAS were made with carcass weight (Lee *et al.* 2013), average daily gain, REA and BFT (Peters *et al.* 2012) and feed intake (Rolf *et al.* 2012; Santana *et al.* 2014). We also identified regions on Chr14 related to RFT in the current association analysis. In an association study in Australian cattle, the authors identified an important region in the Chr14 associated with RFT, especially for markers close to 20 Mb (Bolormaa *et al.* 2011). Another study also detected a QTL on Chr14 using SNPs associated with BFT and RFT in cattle *B. indicus* and in cattle *B. taurus* (Bolormaa *et al.* 2013).

Table 4 Description of the significant SNPs for rumpfat thickness, identified using the GRAMMAR-Gamma method

SNP ID	Chr	Pos[1]	SNP[2]	Beta[3]	p-value[4]	Gene	Location
rs219607813	1	101.4	C/T	1.10	7.95×10^{-5}	–	–
rs43255441	1	108.3	C/T	1.17	4.83×10^{-5}	–	–
rs135171031	1	109.5	C/T	1.02	1.97×10^{-5}	–	–
rs136694677	1	109.5	C/T	1.17	1.15×10^{-5}	–	–
rs137267898	1	109.5	A/G	1.07	1.02×10^{-5}	*MFSD1*	Intron
rs134693968	1	109.5	C/T	1.07	1.02×10^{-5}	*MFSD1*	Intron
rs133134763	1	109.6	C/T	1.30	2.12×10^{-6}	*RARRES1*	Intron
rs109506159	1	109.6	C/T	1.22	1.59×10^{-6}	*RARRES1*	Intron
rs134242409	1	111.0	A/G	1.18	6.55×10^{-6}	*VEPH1*	Intron
rs42298690	1	111.0	A/C	1.18	6.55×10^{-6}	*VEPH1*	Intron
rs42298705	1	111.0	A/G	1.18	6.54×10^{-6}	*VEPH1*	Intron
rs135214762	1	111.1	A/G	1.00	4.05×10^{-5}	–	–
rs42298631	1	111.1	C/T	1.00	4.39×10^{-5}	–	–
rs134715778	1	112.6	A/G	0.48	7.58×10^{-5}	–	–
rs135737872	3	11.3	C/T	0.57	6.07×10^{-5}	–	–
rs137624818	3	83.7	A/G	0.50	7.00×10^{-5}	–	–
rs136808582	4	47.5	A/C	−1.54	5.91×10^{-5}	–	–
rs134117055	4	47.5	C/T	−1.54	5.91×10^{-5}	–	–
rs110128306	11	101.2	C/T	0.61	1.67×10^{-5}	–	–
rs134534175	14	14.3	A/C	−0.82	4.40×10^{-5}	–	–
rs109843532	14	14.3	C/T	−0.82	4.40×10^{-5}	–	–
rs136677477	14	14.3	C/T	−0.75	1.65×10^{-5}	–	–
rs134642262	14	14.4	A/G	−0.94	6.53×10^{-7}	–	–
rs135000490	14	45.6	G/T	0.61	2.97×10^{-5}	–	–
rs110819326	16	20.4	A/G	−0.62	4.60×10^{-5}	–	–
rs133086161	16	24.6	C/T	0.51	8.67×10^{-5}	–	–
rs135509916	20	20.4	A/G	0.49	8.78×10^{-5}	–	–
rs109008503	20	21.1	A/G	0.59	2.18×10^{-6}	–	–
rs110118806	20	21.6	C/T	−0.57	5.11×10^{-5}	–	–
rs109973513	20	49.7	A/G	−0.60	2.14×10^{-5}	–	–
rs108973107	20	61.9	C/T	−0.65	1.37×10^{-5}	–	–
rs137602025	20	64.3	A/G	−1.04	1.01×10^{-6}	*SEMA5A*	Intron
rs42284172	22	58.1	C/T	0.57	2.88×10^{-6}	–	–
rs133544468	27	29.0	C/T	0.86	3.89×10^{-5}	–	–

[1]Position in Mb.
[2]Illumina TOP strand.
[3]Allele substitution effect.
[4]Probability value after correction for multiple test.

For RFT, we highlight the *RARRES1* and *VEPH1* as candidate genes. *VEPH1* (ventricular zone expressed PH domain containing 1) is associated with lipid metabolism and has been reported to be important for diabetes in humans (Collares *et al.* 2013) and fat deposition in pigs (Fowler *et al.* 2013). *RARRES1* (retinoic acid receptor protein respond 1) encodes the retinoic acid receptor that regulates cell differentiation and proliferation of adipocyte-like cells (Zhang *et al.* 2004). The retinoic acid is a vitamin A metabolite that is important for the formation of subcutaneous adipose tissue (Renaville *et al.* 2002) and for growth hormone on the pituitary gland. This hormone reduces the fat deposition in the bovine carcass (Oka *et al.* 1998). No direct physiological relationships were found between RFT with *MFSD1* (major facilitator superfamily domain containing 1) and *SEMA5A* (semaphorin-5A) genes.

It is interesting to note of that the appointed candidate genes, four of them are related in some way to lipid metabolism: *CLSTN2* (obesity), *OSBPL3* (intracellular lipid receptor), *RARRES1* (proliferation of adipocyte-like cells) and *VEPH1* (diabetes). These genes offer additional insights into the role of lipid metabolism in the physiological control of these phenotypes. However, this metabolic pathway only relates to a portion of all the physiology that controls carcass traits in beef cattle, and further studies are necessary to validate these results.

Table 5 Previously described quantitative trait loci (QTLs) that overlapped the genomic region of the significant SNPs

Trait	QTL[1]	Chromosome	Range (Mb)
Rib-eye area	#10690	3	40.6–56.2
	#11733	12	15.3–15.8
	#11110	20	29.8–40.1
	#11134	21	43.2–49.4
Backfat thickness	#23154	14	12.0–12.3
	#20703	14	10.9–12.8
	#11125	27	11.7–20.9
	#11269	28	8.7–14.1
Rumpfat thickness	#10067	3	8.1–11.3
	#10068	3	8.1–11.3
	#10069	3	8.1–11.3
	#20874	14	35.8–45.6
	#20875	14	35.8–45.6
	#20876	14	35.8–45.6
	#20735	20	18.8–31.7
	#20736	20	18.8–31.7

[1]Identification of QTL according to the cattle QTLdb database (Hu *et al.* 2013).

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