Survey of SSC12 regions affecting fatty acid composition of intramuscular fat using high-density SNP data

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Fatty acid composition is a critical aspect of pork because it affects sensorial and technological aspects of meat quality and it is relevant for human health. Previous studies identified significant QTLs in porcine chromosome 12 for fatty acid profile of back fat (BF) and intramuscular fat (IMF). In the present study, 374 SNPs mapped in SSC12 from the 60K Porcine SNP Beadchip were used. We have combined linkage and association analyses with expression data analysis in order to identify regions of SSC12 that could affect fatty acid composition of IMF in *longissimus* muscle. The QTL scan showed a region around the 60-cM position that significantly affects palmitic fatty acid and two related fatty acid indexes. The Iberian QTL allele increased the palmitic content (+2.6% of mean trait). This QTL does not match any of those reported in the previous study on fatty acid composition of BF, suggesting different genetic control acting at both tissues. The SNP association analyses showed significant associations with linolenic and palmitic acids besides several indexes. Among the polymorphisms that affect palmitic fatty acid and match the QTL region at 60 cM, there were three that mapped in the *Phosphatidylcholine transfer protein* (*PCTP*) gene and one in the *Acetyl-CoA Carboxylase* α gene (*ACACA*). Interestingly one of the *PCTP* SNPs also affected significantly unsaturated and double bound indexes and the ratio between polyunsaturated/monounsaturated fatty acids. Differential expression was assessed on *longissimus* muscle conditional on the genotype of the QTL and on the most significant SNPs, according to the results obtained in the former analyses. Results from the microarray expression analyses, validated by RT-qPCR, showed that *PCTP* expression levels significantly vary depending on the QTL as well as on the own *PCTP* genotype. The results obtained with the different approaches point out the *PCTP* gene as a powerful candidate underlying the QTL for palmitic content.

Keywords: SSC12, fatty acid composition, high-density SNP chip

INTRODUCTION

For several years, selection performed in most of the pig breeding schemes increased the efficiency of lean tissue deposition at the expense of back fat (BF) and intramuscular fat (IMF) content (De Vries and Kanis, 1994). These changes are unsuitable for eating quality of pig meat, because it is widely accepted that this latter largely depends on the amount and type of fat in meat (Webb and O'Neill, 2008). During the last decade, the selection objective of some pig breeding nuclei shifted toward dual goals combining carcass leanness and meat quality with a particular emphasis on IMF content (Lonergan et al., 2001; Schwab et al., 2009). Besides fat quantity, fatty acid composition is also a critical aspect of pork because it influences the sensorial and technological aspects of meat quality, such as juiciness, flavor, and tenderness (Wood et al., 2008).

The presence of a high content of polyunsaturated fatty acids (PUFA) is associated with low juiciness and can lead to abnormal flavor and rancidity in meat by excessive oxidation of free unsaturated fatty acids and production of undesirable volatile compounds (Lawrence and Fowler, 1997). Unsaturated fatty acids may undergo oxidation during the processing of dry-cured hams and lead to flavor, color, and texture deterioration (Ventanas et al., 2007). Dietary saturated fatty acids (SFA) are relevant nutrients for human health since some SFA with 14 or 16 carbon atoms chain length increase the level of plasmatic cholesterol and therefore the risk of cardiovascular disease (Lichtenstein, 2003). PUFA-rich diets play a cardiovascular health protective effect reducing cholesterol levels in blood, although nutritionists tend to focus more on the PUFA/SFA balance and the $n-6/n-3$ PUFA ratio rather than the content of particular fatty acids (Jimenez-Colmenero et al., 2010).

There are several studies reporting the role of genetic factors on fatty acid composition of porcine fats. Classical studies of QTL detection, using microsatellites genotyping data, have reported a few QTLs for BF and IMF fatty acid composition. QTLs affecting fatty acid composition of BF have been described on chromosomes

1, 2, 4, 5, 7, 8, 9, 10, 12, 15, and 17 (Kim et al., 2006; Nii et al., 2006). In addition, on porcine chromosomes 1, 3, 6, 7, 11, 12, 13, 14, 15, and 16, QTLs affecting IMF fatty acid composition have also been reported on different muscles from experimental and commercial pig populations (Sanchez et al., 2007; Quintanilla et al., 2011). Moreover, experimental research suggests that the genetic control of fatty acid composition might be different at diverse fat and muscle tissues (Muñoz et al., 2010; Uemoto et al., 2011). Among these results, SSC12 has been one of the porcine chromosomes showing consistent results. A previous work, focusing on SSC12 showed two QTLs affecting different BF fatty acids (Muñoz et al., 2007) and highly significant associations were found for two SNPs in the *Acetyl-CoA Carboxylase* ∝ gene (*ACACA*). Recent availability of a porcine genome wide SNP panel has prompted the genetic analysis of complex traits trough the use of high-density genotyping markers. In the current study, we have tried to identify regions in SSC12 influencing intramuscular fatty acid composition by using genotypes of SSC12 from high-density 60K porcine SNP Beadchip and combining linkage and association analyses approaches as well as expression data analyses.

RESULTS AND DISCUSSION

QTL DETECTION

The total number of SNPs contained in the *PorcineSNP60 Genotyping Bead Chip* and potentially located in SSC12 was around 1,200 SNPs, following *Sscrofa*10 annotation. After filtering by genotype clustering quality, the number of available SNPs decreased to 614. In addition, SNPs with a minor allele frequency less than 0.15 and those that showed mapping errors (Muñoz et al., 2011) were also discarded. In total, the genotyping information from 374 SNPs was used for the different analyses performed in this study. The SSC12 linkage map constructed with the information of 374 SNPs displayed 91.46 cM length with a 0.25-cM averaged distance between markers.

In order to perform the QTL scan along SSC12 for IMF fatty acid composition traits (**Table 1**), three different models (a first one counting for additive and dominance effects, a second one counting only for additive effects and a last one counting only for dominant effects) were used and barely significant additive effects were detected. A QTL region affecting myristic, palmitic, and arachidonic fatty acids as well as the indexes unsaturation (UI), double bound index (DBI), SFA, and the ratio between polyunsaturated/monounsaturated fatty acids (PUFA/MUFA) was detected with nominal P-values lower than 0.005. This region, around the 60-cM position, was limited by *ASGA*0054160 and *MARC*0040388 probes (59–61 cM; **Table 2**). Another QTL region affecting gadoleic fatty acid with a nominal P-value lower than 0.005 was also identified around 17 cM (15–20 cM). Moreover, the likelihood profile across chromosome 12 (**Figure 1**) showed other highly significant regions around the 40- to 50-cM position for palmitic fatty acid, UI, and SFA. However, additional analyses considering two QTL in the statistical model did not reveal any significant evidence for a second QTL affecting these traits.

When we calculated the chromosome-wise thresholds for correcting multiple tests, only the QTL region for palmitic fatty acid, UI, and SFA at 60 cM remained significant at FDR < 0.05 (**Table 2**). The Q allele of this QTL region, which corresponds to the Iberian parental breed in accordance to our experimental design, increased the palmitic fatty acid content in a 2.6% of the mean trait. This is the most abundant of the SFA and therefore the reported effect is consistent with those observed on SFA and UI, equivalent to a +2.4 and −5.6% of their respective mean traits. These results are in accordance with phenotypical differences between Iberian and Landrace described by Serra et al. (1998). The relevance of these results comes from the important role of SFA and in particular of palmitic fatty acid in human health and in the organoleptic properties of pork. Palmitic acid is considered less

Table 1 | Phenotypic data analyzed in F3, BC1, and BC2 generations from Iberian × Landrace intercross (IBMAP).

Trait	Mean	SD	Trait	Mean	SD
Carcass weight, CW (kg)	74.41	11.10	Eicosadienoic, C20:2 (*n*−6)	0.45	0.14
Intramuscular fat, IMF (%)	1.52	0.80	Eicosatrienoic, C20:3	0.45	0.25
Fatty acids content (%)			Arachidonic, C20:4 (*n*−6)	2.55	1.55
Myristic, C14:0	1.19	0.19	Fatty acid indexes		
Palmitic, C16:0	22.65	1.55	Average chain length, ACL	17.40	0.20
Palmitoleic, C16:1 (*n*−9)	0.36	0.08	Peroxidability, PI	26.27	9.42
Palmitoleic, C16:1 (*n*−7)	2.55	0.54	Double bond, DBI	0.84	0.09
Margaric, C17:0	0.24	0.07	Unsaturation, UI	2.24	0.35
Heptadecenoic, C17:1	0.45	0.50	SFA (%)	37.84	2.07
Stearic, C18:0	13.56	1.19	MUFA (%)	45.11	4.30
Oleic, C18:1 (*n*−9)	37.30	4.03	PUFA (%)	16.39	4.79
Vaccenic, C18:1 (*n*−7)	3.80	0.33	MUFA/SFA	1.19	0.11
Linoleic, C18:2 (*n*−6)	12.26	3.15	PUFA/SFA	0.44	0.15
Linolenic, C18:3	0.60	0.16	C20:4/C18:2	0.20	0.08
Arachidic, C20:0	0.22	0.08	(*n*−6)/(*n*−3)	29.11	8.80
Gadoleic, C20:1 (*n*−9)	0.70	0.19			

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 2 | Most relevant results of a QTL scan for fatty acid composition of intramuscular fat.

Trait	Position in cM (CI)	a_{QTL} (SE)	P-value
C14:0, %	60 (59–61)	0.07 (0.02)	0.00150
C16:0, %	60 (59–61)	0.73 (0.18)	0.00005[†]
C20:1, %	17 (15–20)	−0.05 (0.01)	0.00150
C20:4, %	60 (59–66)	−0.45 (0.14)	0.00140
DBI	60 (59–62)	−0.03 (0.01)	0.00120
UI	60 (59–61)	−0.13 (0.04)	0.00014[†]
SFA	60 (59–61)	0.90 (0.24)	0.00019[†]
PUFA/SFA	60 (59–61)	−0.04 (0.01)	0.00230

CI, confidence interval at 95%; a_{QTL}, additive effect of the QTL; [†]Threshold for P-value < 0.0005 for FDR < 0.05.
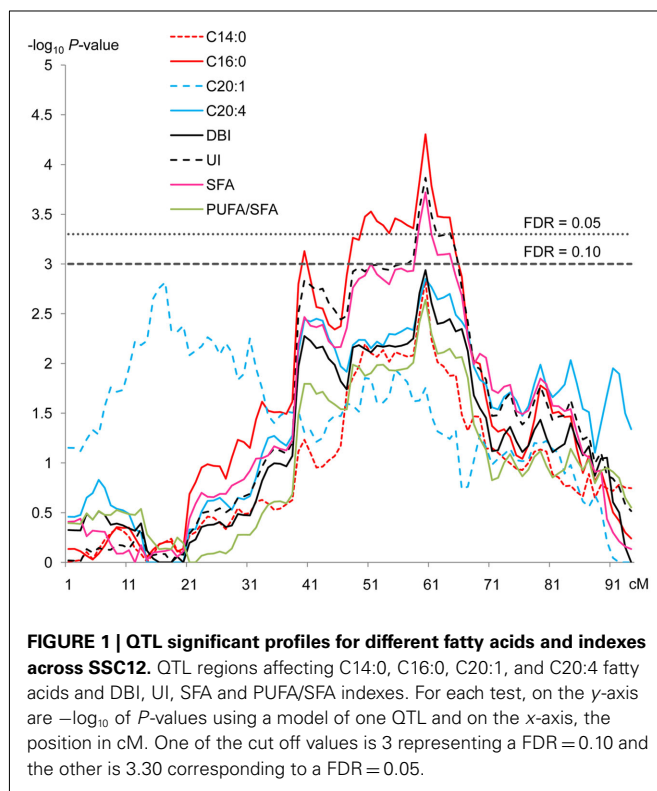
FIGURE 1 | QTL significant profiles for different fatty acids and indexes across SSC12. QTL regions affecting C14:0, C16:0, C20:1, and C20:4 fatty acids and DBI, UI, SFA and PUFA/SFA indexes. For each test, on the y-axis are −$\log_{10}$ of P-values using a model of one QTL and on the x-axis, the position in cM. One of the cut off values is 3 representing a FDR = 0.10 and the other is 3.30 corresponding to a FDR = 0.05.

hypercholesterolemic than other SFA with shorter carbon atoms chain length, such as lauric (C12:0) and myristic (C14:0; Li and Sinclair, 2002). An increase in the concentration of palmitic acid and therefore in monounsaturated fatty acids could be positively correlated with eating qualities (Cameron and Enser, 1991).

The QTL region detected in the present study does not match any of those reported in our previous study concerning BF fatty acid composition (Muñoz et al., 2007). This suggests that fatty acid composition could be under different genetic control at each tissue. Similar results were also reported by Uemoto et al. (2011) in a whole-genome QTL analysis for fatty acid composition and melting point of inner and outer subcutaneous fat, and inter and intramuscular fat in a purebred Duroc population. There are

few published studies that focus on porcine QTL for fatty acid composition but even fewer that focus on fatty acid composition of IMF (Sanchez et al., 2007; Quintanilla et al., 2011; Uemoto et al., 2011). Nevertheless, the study performed by Quintanilla et al. (2011) in Duroc pigs revealed a significant QTL in SSC12 for SFA and another suggestive QTL for palmitic fatty acid in *longissimus thoracis et lumborum* muscles at position 72–73 cM. This QTL seems to be close to our QTL region, according to the lengths of the chromosome estimated in both studies. However, the study of Quintanilla et al. (2011) was conducted with a reduced number of markers, only four microsatellites, along the chromosome which probably did not allow them to reach higher statistical power and narrow confidence intervals. The QTL region identified in our study displayed a 95% CI of 3.00 cM that corresponds to only 0.99 Mb, according to the *Sscrofa*10 annotation. This narrow CI should facilitate the identification of the candidate gene to carry the causal mutation or mutations underlying this QTL.

SNP ASSOCIATION ANALYSIS

The SNP association analyses based on the actual allele frequencies were carried out as an alternative approach to the classical QTL detection based on the parent line origin. The analyses were performed for the same traits that had previously been considered for the QTL detection (**Table 2**) and the same 374 selected SNPs were used. A first survey of the results showed a total of 253 associations between SNPs and IMF fatty acid percentages and indexes which displayed a nominal P-value lower than 0.05. Twenty SNPs showed association with linolenic acid (C18:3, n−3), 74 with palmitic acid (C16:0), 52 with DBI, 63 with UI, and 44 with PUFA/SFA (**Tables A1–A5** in Appendix).
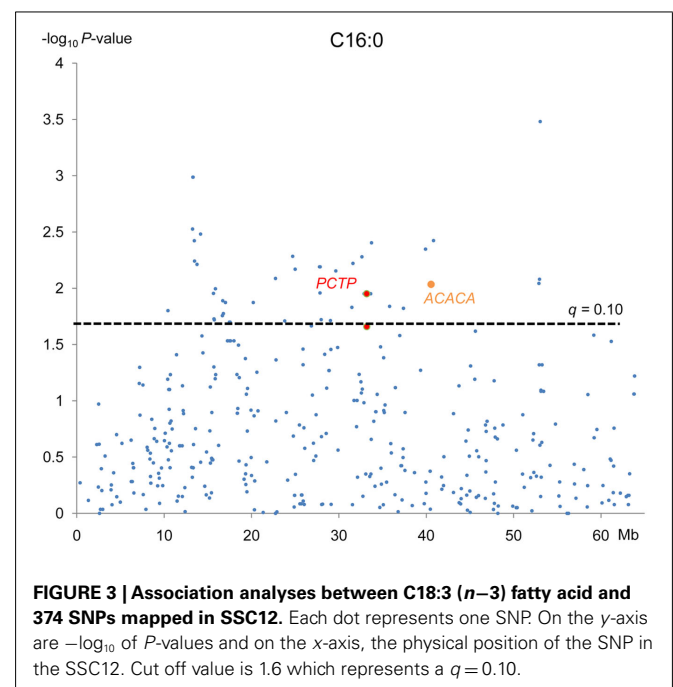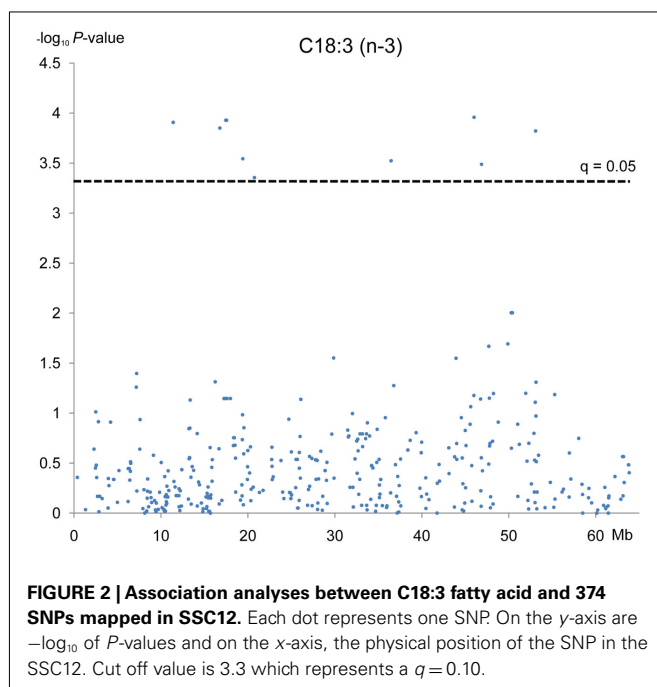
The associations detected for linolenic fatty acid content were the most statistically significants, in fact 11 out of 20 SNP associations reached a q-value lower than 0.05 (**Table A1** in Appendix). It should be noted that 7 out of these 11 SNPs map very close to each other, around physical position 11–21 Mb on the chromosome (**Figure 2**), which indicates a genome region of around 10 Mb associated to this fatty acid that it was not detected in the previous linkage analyses. Linolenic fatty acid constitutes one of the most relevant PUFA as it is considered essential from a health point of view. This fatty acid cannot be *de novo* synthesized by monogastric animals but must be obtained from the diet. Therefore, the observed genetic association could be explained by an alteration in a gene related with the absorption, transformation or transport of linolenic acid in such a way this would result in an alteration of the amount of accumulated linolenic acid in IMF. Although seven out of the associated SNPs are located in annotated genes, none of them and neither the genes included in the reported SSC12 11–21 Mb region, were related with fatty acids transport or metabolism, except *phosphatidylinositol transfer protein, cytoplasmic* 1 gene (*PITPNC*1). *PITPNC*1, also known as *M-rdgB beta*, encodes for a protein that belongs to the *Nir/rdgB* family and is implicated in a broad spectrum of cellular functions such as regulation of lipid trafficking, metabolism, and signaling among others (Lev, 2004). However, the biological association between this gene and the linolenic fatty acid content is not easy to explain because protein domain/motifs within the RdgB proteins have been identified by bioinformatic analyses and in no case

(with the possible exception of the PITP domain of DrdgBα) has their functional significance been experimentally tested (Trivedi and Padinjat, 2007).

Out of the 74 SNPs associated with palmitic fatty acid, 49 of them reached a q-value lower than 0.10 (**Table A2** in Appendix). The annotation of these 49 SNPs, following *Sscrofa*10, showed that 20 are located in intergenic regions, 28 on introns of annotated genes and one is not annotated. Since our linkage analysis revealed a significant QTL for palmitic percentage, we decided to center our attention on those SNPs with significant effects on the palmitic fatty acid percentage and that could be ascribed to genes mapped within or close to the QTL region that are potentially related with fatty acid metabolism. Twenty out of the 49 SNPs are mapped around the physical position of 30–40 Mb of chromosome SSC12. This would match the QTL region identified in the previous analyses, around the 60-cM position, assuming a recombination rate of 1.43 cM/Mb in SSC12 (Muñoz et al., 2011). Among the significant SNPs included in this interval with a significant

effect on palmitic content, there were three (*ASGA*0054039 T > C, *ASGA*0054041 A > G, *ASGA*0054044 A > T) located in *Phosphatidylcholine transfer protein* (*PCTP*) gene and, another one (*ALGA*0066302 G > A) located in the *Acetyl-CoA Carboxylase* ∝ (*ACACA*) gene (**Table 3**; **Figure 3**). Both genes are related to lipid metabolism. *PCTP*, also known as *STARD*2, encodes for a protein that takes part in lipid and phospholipids transport, besides regulating genes like *PPARG*, *PPARA*, and *CREBP* that encode proteins involved in fatty acid metabolism (Wirtz, 1991; Kang et al., 2010a,b). The other gene, *ACACA*, encodes for an enzyme that catalyzes the carboxylation of acetyl-CoA to malonyl CoA and is the rate-limiting enzyme for long-chain fatty acid synthesis. These results indicate that both genes are good functional and positional candidate genes to carry the mutation or mutations underlying the QTL for IMF palmitic fatty acid percentage.

The double bond index (DBI) is a simple weighted average of the number of double bonds per fatty acid molecule, which

FIGURE 2 | Association analyses between C18:3 fatty acid and 374 SNPs mapped in SSC12. Each dot represents one SNP. On the y-axis are $-\log_{10}$ of P-values and on the x-axis, the physical position of the SNP in the SSC12. Cut off value is 3.3 which represents a $q = 0.10$.

FIGURE 3 | Association analyses between C18:3 (n−3) fatty acid and 374 SNPs mapped in SSC12. Each dot represents one SNP. On the y-axis are $-\log_{10}$ of P-values and on the x-axis, the physical position of the SNP in the SSC12. Cut off value is 1.6 which represents a $q = 0.10$.

Table 3 | Association analyses: significant effects of SNPs within candidate genes in the QTL region.

SNP	MAF	Trait	a_{SNP} (SE)	P-value	q-value
*ACACA: ALGA*0066302 G > A	0.46	C16:0, %	−0.40(0.16)	0.009	0.077
*PCTP: ASGA*0054039 T > C	0.28	C16:0, %	0.43 (0.15)	0.005	0.073
		UI	−0.08 (0.03)	0.009	0.059
*PCTP: ASGA*0054041 A > G	0.46	C16:0, %	0.32 (0.13)	0.011	0.077
		DBI	−0.02 (0.01)	0.001	0.040
		UI	−0.08 (0.02)	0.001	0.058
		PUFA/SFA	−0.03 (0.01)	0.002	0.067
*PCTP: ASGA*0054044 A >T	0.25	C16:0	0.35 (0.15)	0.022	0.091

ACACA, acetyl-CoA carboxylase α; PCTP, phosphatidylcholine transfer protein.

partially reflects, in other way, the fatty acid composition. Out of the 52 SNPs associated with DBI, seven reached a q-value lower than 0.05 and six of them matched the previously mentioned SNPs that were significantly associated with palmitic fatty acid (**Table A3** in Appendix). None of the seven SNPs map in genes related to fatty acid metabolism except one (*ASGA*0054041), located in the *PCTP* gene. This is also one of the SNPs that is associated with palmitic fatty acid in the above association analysis although with an opposite effect. The *ASGA*0054041G allele leads to a decrease of 0.02 units on the magnitude of double bonds, which in turn is consistent with the 0.32% increase observed on saturated palmitic acid (**Table 3**).

Seven out of the 63 SNPs associated with UI reached a q-value lower than 0.10 (**Table A4** in Appendix) and six out of them match the SNPs showing association with DBI (q-value < 0.05). They included again the *ASGA*0054041 SNP located in the *PCTP* gene, which showed a significant effect on this index as well consistently with the effect observed on palmitic fatty acid. The *ASGA*0054041*G* allele leads to a reduction of 0.08 units of the unsaturation index.

Finally, the analyses on the PUFA/SFA index showed that 7 out of the 44 SNP associations overcame the threshold q-value of 0.10 (**Table A5** in Appendix), the same seven SNPs that were associated with UI. The *ASGA*0054041*G* allele leads to a reduction of the ratio of 0.03 units as it was supposed to occur upon the observed effects on palmitic, DBI, and UI.

These results indicate that the effects on the fatty acid indexes are a direct consequence of the effect detected on the palmitic fatty acid, the most abundant SFA. Moreover, the overall results obtained for palmitic fatty acid, DBI, UI, and PUFA/SFA indexes support the relevance of *PCTP* as the main candidate gene to regulate the palmitic fatty acid content in IMF.
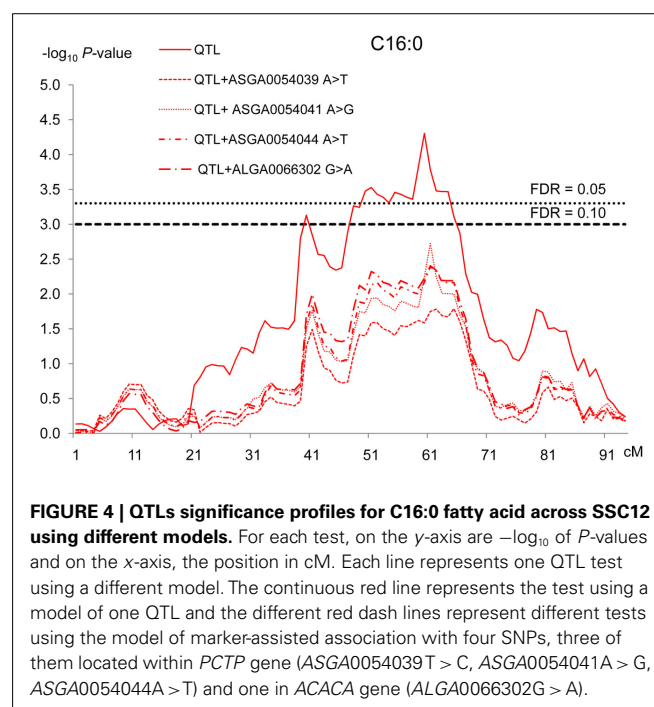
MARKER-ASSISTED ASSOCIATION TESTS

The classical QTL detection and the association analyses conducted in the present study show complementary results. A highly significant QTL for IMF palmitic fatty acid content has been identified by linkage analysis around position 30 Mb on SSC12, and the standard association analyses have detected effects of SNPs from two candidate genes mapping in this chromosomal region (*ACACA* and *PCTP*) on palmitic fatty acid content and other related indices (UI, DBI, PUFA/SFA). In addition, the association analyses have revealed another chromosomal region, around 11–21 Mb on SSC12, associated with the linolenic fatty acid content and not identified previously by the linkage analyses, although there is not a clear candidate gene to underlay this effect on IMF linolenic acid.

The aim of positional candidate analyses is to verify whether a particular mutation underlies or, at least, is closely linked to the QTL. Experimental designs based on intercrosses between divergent lines have a great power to detect QTL provided by extensive linkage disequilibrium (LD) generated by the cross but they also make it difficult to distinguish between causative and neutral mutations (Varona et al., 2005). Therefore, we performed additional analyses using a model which jointly tests QTL and SNP of candidate genes, in order to reduce the rate of spurious associations. Zhao et al. (2003) outlined that this marker-assisted

association test can exclude, in some cases, the confounding effect of the extensive between-breed LD characteristic of intercrosses.

The three SNPs (*ASGA*0054039 T > C, *ASGA*0054041 A > G, *ASGA*0054044 A > T) of the *PCTP* gene and the one located in the *ACACA* gene (*ALGA*0066302 G > A) were tested using this model for the traits showing significant associations. These mutations display a similar frequency pattern in the parental breeds: fixation of one allele in the Iberian line and segregation of the other two alleles in the Landrace breed, with frequencies of the alternative allele ranging from 0.42 to 0.75. The results of these additional analyses are presented in **Table 4**. The significance of all the SNP effects was increased compared to the results of standard association analyses. In contrast, the results of the QTL scan (**Table 2**) shows that the inclusion in the model of the *ACACA* SNP reduced the significance of QTL effect on C16:0 (dropped from 10^{-5} to 10^{-3}). Similarly, *PCTP* SNPs effects reduced the significance of QTL effects for all the analyzed traits. The QTL for PUFA/SFA disappeared when the *PCTP*: *ASGA*0054041 A > G polymorphism was included in the model (**Figure 4**).
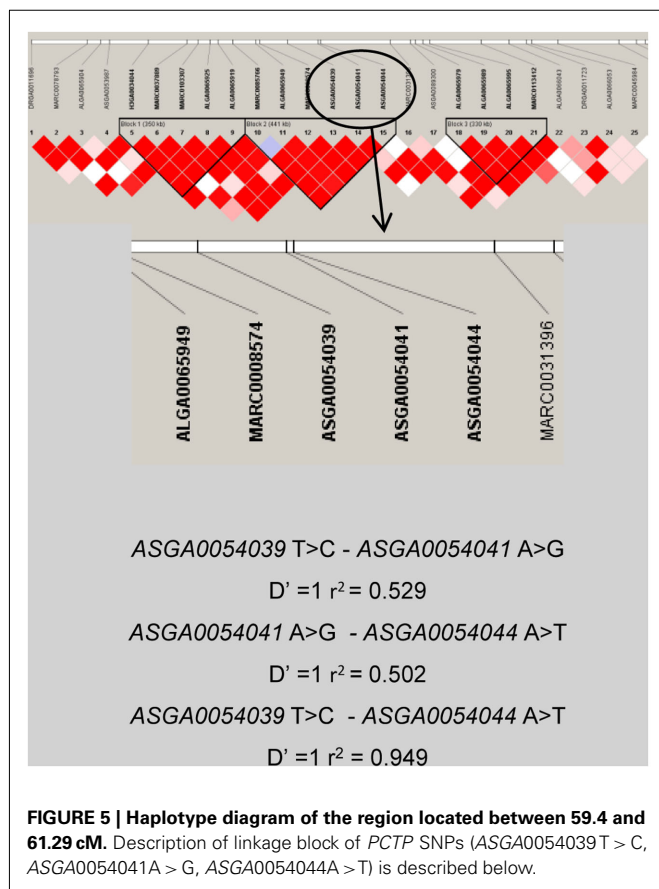
Moreover, high LD between the *PCTP* SNPs was observed (**Figure 5**) using Haploview software v4.2 (Barrett et al., 2005). Three different haplotypes could be distinguished: TAA, CGT and CAT with frequencies of 0.40, 0.44, and 0.16 respectively. Additional association analyses with both standard animal and marker-assisted models were carried out for these haplotypes and the palmitic content. The results showed that although haplotypes were significantly associated with the trait, the significance was lower than those performed with the individual SNPs. Moreover, the analysis with the joint QTL and haplotype model showed that the inclusion in the model of the haplotype effects reduced the significance of QTL effects much less than those observed with the individual SNPs. These results suggest a close linkage of these SNPs to the causative mutation that could be particularly remarkable

FIGURE 4 | QTLs significance profiles for C16:0 fatty acid across SSC12 using different models. For each test, on the y-axis are $-\log_{10}$ of P-values and on the x-axis, the position in cM. Each line represents one QTL test using a different model. The continuous red line represents the test using a model of one QTL and the different red dash lines represent different tests using the model of marker-assisted association with four SNPs, three of them located within *PCTP* gene (*ASGA*0054039 T > C, *ASGA*0054041 A > G, *ASGA*0054044 A > T) and one in *ACACA* gene (*ALGA*0066302 G > A).

Table 4 | Marker-assisted association tests: Results for *ACACA* and *PCTP* SNPs significantly associated for fatty acid composition in previous analyses.

SNP	Trait	a_{SNP} (SE)	P-value	cM	a_{QTL} (SE)	P-value
*ACACA: ALGA*0066302 G > A	C16:0	−0.43 (0.14)	0.0065	60	0.56 (0.19)	0.0040
*PCTP: ASGA*0054039 T > C	C16:0	0.47 (0.16)	0.0029	60	0.54 (0.23)	0.0162
	UI	−0.09 (0.03)	0.0044	60	−0.10 (0.05)	0.0195
*PCTP: ASGA*0054041 A > G	C16:0	−0.34 (0.13)	0.0070	60	0.68 (0.21)	0.0019
	UI	−0.09 (0.03)	0.0006	60	−0.09 (0.04)	0.0015
	DBI	−0.02 (0.01)	0.0005	60	−0.02 (0.01)	0.0740
	PUFA/SFA	−0.03 (0.01)	0.0005	60	0.03 (0.02)	0.1050
*PCTP: ASGA*0054044 A >T	C16:0	0.38 (0.16)	0.0140	60	0.71 (0.25)	0.0041

a_{SNP}, additive effect of the SNP; a_{QTL}, additive effect of QTL.

FIGURE 5 | Haplotype diagram of the region located between 59.4 and 61.29 cM. Description of linkage block of *PCTP* SNPs (*ASGA*0054039 T > C, *ASGA*0054041A > G, *ASGA*0054044A >T) is described below.

for the *ASGA*0054039 SNP of the *PCTP* gene because the magnitude of the SNP effect on C16:0 is the highest and the SNP inclusion in the marker-assisted test produces a greater decrease in the LR maximum value of the QTL than the other SNPs tested (**Table 4**).

These results contrast with those obtained in our previous study conducted on BF where the most significant associations were found for two *ACACA* SNPs with stearic, palmitoleic, and vaccenic fatty acid concentrations (Muñoz et al., 2007). Gallardo et al. (2009) also detected significant associations between two linked *ACACA* SNPs and percentages of saturated (myristic,

palmitic, and stearic) and polyunsaturated (linoleic) fatty acids in the *longissimus thoracis* and *lumborum muscle* of Duroc pigs. These results support the hypothesis that there is a different genetic control of fatty acid composition acting at IMF and BF tissues. This is the first time that significant and consistent effects on the IMF fatty acid composition are described for the *PCTP* gene.

GENE EXPRESSION ANALYSIS
The combination of gene expression with linkage and association data offers new options to maximize the success in the selection of candidate genes. In the present study, we have evaluated the *longissimus* muscle differential gene expression following two strategies: (a) conditional on the QTL genotype at the 60-cM genetic position of SSC12, and (b) conditional on the genotype of the most relevant SNPs according to the previous analyses.

In the first analysis, individuals were classified into two groups based on their probability of being *Qq* or *qq* for the QTL detected at 60 cM in SSC12. All the animals could be classified in one of the groups. This was considered a discontinuous effect in the statistical analysis model with two levels: (1) if the probability of being *Qq* was >0.9 and (2) if the probability of being *qq* was >0.9. Note that the gene expression analyses were carried out in samples of *longissimus* muscle from the BC2 pigs, which corresponds to the backcross of F1 animals (*Qq*) with Landrace (*qq*), therefore only *Qq* and *qq* genotypes could be used. A total of 18 differentially expressed (DE) probes were detected with posterior probability (*PP*) lower than 0.001 and an expression ratio over 1.1 (**Table A6** in Appendix). All except one of the DE probes were annotated to known genes; 11 out of them were assigned to SSC12. Moreover, three of these genes map close or within the CI of the QTL, and one of them, the *PCTP* gene, is related to the fatty acid metabolism. This *PCTP* probe (Ssc.22988.1.S1_at) displayed an expression ratio between *Qq* and *qq* genotypes of 0.77.

The second strategy for the analysis of the gene expression was based on the genotype of one SNP located in the *ACACA* gene (*ALGA*0066302 G > A) and three SNPs in the *PCTP* gene (*ASGA*0054039 T > C, *ASGA*0054041A > G, *ASGA*0054044 A > T). The genotype was introduced as a covariate into the statistical model that assumes values 0, 1, or 2 for the number of allelic copies. The analyses of the differential gene expression conditional on the *ACACA* SNP (*ALGA*0066302 G > A) showed 13 DE probes with a *PP* < 0.001 and an expression ratio

over 1.1. All the probes were annotated but none of the genes have been biologically related with fatty acid metabolism (**Tables A7** in Appendix and **Table 5**).

The analyses of the differential gene expression conditional on the three *PCTP* SNPs showed consistent results. Note that, the SNPs *PCTP ASGA0054044A > T* and *ASGA54039T > C* appeared cosegregating in the BC2 animals used for the expression analyses. The analysis conditional on the *PCTP ASGA54039C* allele copy number showed seven DE probes with $PP < 0.001$ and an expression ratio over 1.1 (**Table A8** in Appendix). All the seven DE probes could be annotated and mapped. Among the identified genes, two map on SSC12 close to the CI of the QTL at 60 cM, which were *Cytochrome C oxidase assembly homolog* gene (*COX*11) and again the *PCTP* gene. Moreover, the *PCTP* gene resulted the most significantly DE. The expression ratio in the untransformed scale of 1.21 for *PCTP* transcript indicates an increase in expression of 21% produced by 1 SD ($\sigma = 0.51$ copies), therefore one copy of the *ASGA54039C* allele would lead to an increased expression of up to 41% (**Table 5**).

Similar results were obtained in the analysis performed according to the *PCTP ASGA54041G* allele copy number. A total of 21 probes showed differential expression with $PP < 0.001$ and an expression ratio over 1.1 (**Table A9** in Appendix). All the probes could be annotated and six genes were assigned to SSC12, from which five map on the region close to CI of the QTL at 60 cM. Again *PCTP* and *COX*11 probes resulted the most significantly DE (**Table 5**). The expression ratio of 1.34 for the *PCTP* transcript indicates an increase of its expression (+31%) produced by 1 SD ($\sigma = 0.66$ copies), therefore one copy of the *ASGA54041G* allele would lead to an increased expression of up to 50% and two copies would duplicate the effect (**Table 5**).

In human both genes, *PCTP* and *COX*11, map very close to each other and also to a third gene, the *hepatic leukemia factor* (*HLF*) that codes the transcription factor HLF. Flowers et al. (2008) compared the DE of healthy mice to that of mice deficient in stearoyl-CoA desaturase-1 (*Scd*1) and observed that the latter displayed reduced expression levels for genes involved in detoxification and several facets of fatty acid metabolism including biosynthesis, elongation, desaturation, oxidation, transport, and ketogenesis. Among them, the *HLF* gene was one of the repressed genes. In addition, it is known that the coexpression of closing mapping genes involved in a similar mechanism could indicate a participation of a shared common transcription factor in the regulation of such genes. Our expression results could indicate that a common expression regulation mechanism, as a transcription factor such as *HLF*, could regulate both *PCTP* and *COX*11 gene expressions.
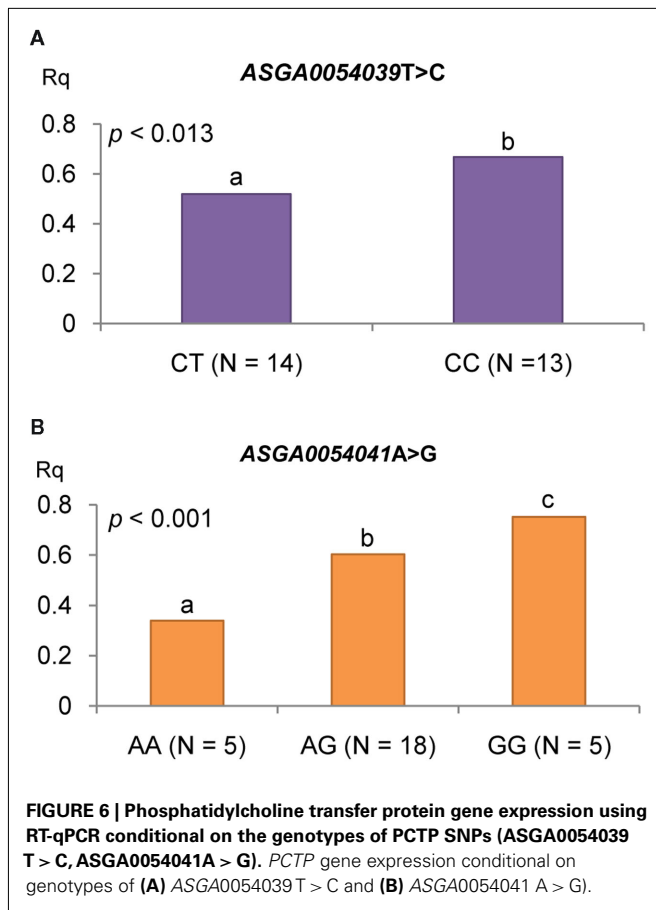
In order to validate the results of the microarray analysis, *PCTP* gene expression was quantified by using Real Time PCR in 28 BC2 pigs classified according to their genotypes for *ASGA54039T > C* and *ASGA54041A > G* SNPs. The three genotypes of *ASGA54041A > G* were represented in the sample set but for *ASGA54039T > C* only CC homozygous and CT heterozygous were available. The analysis of the expression data confirmed the results obtained with the microarray assay. The expression of the *PCTP* transcript was higher in *ASGA54039CC* animals than in CT ones ($P < 0.05$). Moreover, the analysis conditional on the *ASGA54041A > G* genotype revealed an increase of the *PCTP* transcript expression with each copy of the G allele ($P < 0.001$; **Figure 6**).

In the association analysis, the false discovery rate applied for correcting multiple test only took into account the number of tested SNPs, although a more rigorous analysis should also consider the number of analyzed traits, which are very correlated. Nevertheless, the magnitude of the observed effects of *PCTP* and *ACACA* SNPs, let us to perform further analyses that supported the reliability of our analyses. The joint results obtained from the association and expression analyses point out the *PCTP* gene as a powerful gene to carry the mutation underlying the QTL detected in the present study for IMF palmitic fatty acid content at 60 cM in SSC12. Since all the analyzed *PCTP* SNPs map in intronic regions it is unlikely that they have any functional effect. However we cannot discard the possibility that intronic polymorphisms could affect transcriptional regulation if they reside in enhancer regions or might create alternative splicing sites and thereby change protein structure or function if they were near exon–intron boundaries. Another possibility, according to the expression results, may be that the causal mutation, in LD with the analyzed SNPs, would be localized in a nearby regulatory element shared by both *PCTP* and *COX*11 genes, such as the *HLF* transcription factor. In any case, a comprehensive analysis of the *PCTP* gene including characterization of his coding, untranslated, and promoters/enhancers regions is needed in order to identify the causal mutation underlying the QTL affecting IMF fatty acid composition.

Table 5 | Relevant DE genes on *longissimus* muscle according to the SNP genotypes of *ACACA* and *PCTP* genes affecting palmitic acid content.

SNP	D.E. genes			
	Affymetrix probe ID	Gene	Posterior probability	Ratio[a]
*ACACA: ALGA*0066302 G > A	–	–	–	–
*PCTP: ASGA*0054039 T > C	Ssc.22988.1.S1_at	*PCTP*	[†]3.34×10^{-11}	1.21
	Ssc.5902.1.A1_at	*COX*11	[†]1.16×10^{-8}	0.83
*PCTP ASGA*0054041 A > G	Ssc.22988.1.S1_at	*PCTP*	[†]4.25×10^{-14}	1.34
	Ssc.5902.1.A1_at	*COX*11	[†]7.40×10^{-9}	0.78

[a]*Effect on expression of the SD of the SNP allele copy number in the untransformed scale; $\sigma = 0.51$ for ASGA0054039 T > C and $\sigma = 0.66$ for ASGA0054041 A > G* [†] *FDR < 0.10.*

FIGURE 6 | Phosphatidylcholine transfer protein gene expression using RT-qPCR conditional on the genotypes of PCTP SNPs (ASGA0054039 T > C, ASGA0054041A > G). *PCTP* gene expression conditional on genotypes of **(A)** *ASGA*0054039 T > C and **(B)** *ASGA*0054041 A > G).

CONCLUSION

Different approaches have been conducted employing the SNPs information of SSC12 contained in the high-density 60K porcine SNP array with the aim of identifying SSC12 regions affecting the IMF fatty acid composition. The different methods have yielded consistent results. A significant QTL at the 60-cM position of SSC12 has been detected, mainly affecting the IMF palmitic fatty acid (C16:0) percentage. This QTL does not match any of the ones reported in our own previous study concerning BF fatty acid composition, suggesting different genetic control mechanism acting at both tissues. The overall association analyses results suggest that the *PCTP* gene is a powerful candidate to carry the mutation underlying this QTL. Particularly remarkable is the significant association of the *PCTP ASGA*0054039 SNP with palmitic fatty acid percentage. Gene expression analyses conditional on the QTL genotypes as well as conditional on *PCTP* SNP genotypes have revealed differential expression of the own *PCTP* transcript. Since all the analyzed *PCTP* SNPs are located in intronic regions it is unlikely that they have any functional effect, therefore the potential causative mutation would probably be located in a regulatory region of *PCTP* gene.

MATERIALS AND METHODS
ANIMAL SAMPLES AND GENOTYPING
Animals from an experimental cross of Iberian × Landrace pig lines, known as IBMAP material (Ovilo et al., 2000, 2010), were used. Animal management and experimental assays were performed with standard procedures following internationally recognized guidelines and with the approval of the funding institutions ethics committee. The population was established from 3 F0 Iberian sires and 30 F0 Landrace dams and includes 70 F1, 403 F2, 56 F3, and 227 individuals from two backcrosses (79 from BC1 and 148 from BC2). Intramuscular fatty acid composition records were measured by gas chromatography in 200 g of *longissimus dorsi* samples taken from F3 and backcrosses animals (**Table 1**). Indexes (UI, AC, DBI, PI, and C20:4/C18:2 ratio) were calculated as described in Pamplona et al. (1998). A total number of 416 animals, from 62 families, belonging to the F3 and backcross generations of the IBMAP experimental cross were genotyped with the *PorcineSNP*60 *Genotyping Bead Chip* (Illumina) using the Infinium HD Assay Ultra protocol (Illumina). Raw individual data had high-genotyping quality (call rate > 0.99). The SNPs with call rates less than 0.85, those with a minor allele frequency less than 0.15, those located in sex chromosomes or those not mapped in the *Sscrofa*10 assembly were removed. Genotype quality filtering was performed using GenomeStudio software and the SNPs selection, filtering by allele frequency and position was done using PLINK software (Purcell et al., 2007).

LINKAGE MAPPING AND QTL DETECTION
A sex-averaged SSC12 linkage map was constructed using the option Fixed of the updated CRI-MAP v2.502[1] (Green et al., 1990) as described in Muñoz et al. (2011). The order given to the SNPs within the chromosome followed the physical order of the *Sscrofa*10 assembly. QTL scanning was performed with the following models:

$$y_i = \text{sex}_i + \text{batch}_i + \beta_c c_i + P_{ai} a_{QTL} + P_{di} d_{QTL} + u_i + e_i$$
$$y_i = \text{sex}_i + \text{batch}_i + \beta_c c_i + P_{ai} a_{QTL} + u_i + e_i$$
$$y_i = \text{sex}_i + \text{batch}_i + \beta_c c_i + P_{di} d_{QTL} + u_i + e_i$$

where y_i is the i-th individual record; *batch* is the slaughter batch (eight in total); β_c is a covariate coefficient with c_i being carcass weight; a_{QTL} is the QTL additive effect; P_{ai} is the additive coefficient calculated as $P_{ai} = Pr(QQ) - Pr(qq)$, the probability of the i-th individual being homozygous for alleles of Iberian origin minus the probability of being homozygous of alleles of Landrace origin; d_{QTL} is the QTL dominant effect; P_{di} is the additive dominant calculated as $P_{di} = Pr(Qq)$; u_i is the infinitesimal genetic effect; and e_i is the random residual. A similar model fitting two different QTL effects was used for performing complementary analyses to check the hypothesis of a second QTL mapping in the same chromosome:

$$y_i = \text{sex}_i + \text{batch}_i + \beta_c c_i + P_{a1i} a_{QTL1} + P_{a2i} a_{QTL2} + u_i + e_i$$

All the statistical analyses were performed using the Qxpak v.5.1 software (Perez-Enciso and Misztal, 2011). A total number of 374 markers were employed for calculating P_{ai} and P_{di} coefficients. Likelihood ratio tests (LRT) were calculated comparing

[1]http://www.animalgenome.org/bioinfo/tools/share/crimap/

the full model and a reduced model without the corresponding QTL effect. The nominal *P*-values were calculated assuming a χ^2 distribution of the LRT with the degrees of freedom given by the difference between the number of estimated parameters in the reduced and full models. The procedure of Benjamini and Yekutieli (2005) based on the *P*-values of the multiple tests was used for controlling the false discovery rate (FDR) at a desired level. As it is recommended for QTL analysis, the chromosome positions with FDR < 0.05 were considered harboring significant QTL and those with FDR < 0.10 would fit suggestive QTL.

SNP ASSOCIATION ANALYSIS

For the association analysis of the 374 available markers, the following standard animal model was used:

$$y_i = \text{sex}_i + \text{batch}_i + \beta_c c_i + \lambda_{ik} g_k + u_i + e_{ik}$$

where λ_{ik} is a vector 374×1 that includes an indicative variable related with the number of copies of one of the alleles of the *k*th SNP, which takes values of 1 or −1 when the *i*-th animal was homozygous for each allele or 0 if the animal was heterozygous; g_k represented the additive effect of *k*th SNP. Multiple testing was performed and the number of tests (374) was the same as the number of analyzed SNPs. False discovery rate (FDR) was applied for correcting the multiple testing. The *Q*-value 1.0 software (Storey and Tibshirani, 2003) was used to calculate a FDR-based *q*-value in order to measure the statistical significance at the chromosome-wide level. The cut off of significant association at chromosome level was set at *q*-value ≤ 0.10. Additional analyses were performed for specific SNPs with significant association results in the previous analysis, using the following model of marker-assisted association test proposed by Zhao et al. (2003) in which both the QTL and SNP effects are considered:

$$y_i = \text{sex}_i + \text{batch}_i + \beta_c c_i + P_{ai} a_{\text{QTL}} + \lambda_{ik}\, g_k + u_i + e_{ik}$$

Likelihood ratio tests for QTL and SNP effects were separately calculated comparing the appropriate full and reduced models. The SNPs significantly associated were mapped in the *Sscrofa*10 assembly. Gene annotations were retrieved from the Ensembl Genes 62 Database and confirmed after BLAST search against the human database[2].

MICROARRAY DATA ANALYSIS

Expression data were obtained of *longissimus dorsi* muscle samples from 100 RC2 individuals, using GeneChip Porcine Genome arrays (Affymetrix, Boston, MA, USA). Total RNA extraction, microarray hybridization, and scanning were performed according to Affymetrix protocols by Casellas et al. (2011). Expression data were generated with Affymetrix GCOS 1.1.1 software. Microarray data quality evaluation was carried out with AffyPLM software (Bioconductor)[3]. Data normalization was conducted to reduce technical variations between chips through Gene Chip Robust Multi-Array Average algorithm using BRB-Array Tools software

(version 3.6.0)[4]. Normalized microarray expression data (background corrected and base-2 logarithmic-transformed) were analyzed through Bayesian inference using the GEAMM v.1.6 program (Casellas et al., 2008). The following model was used for searching the effects on expression data of different QTL or SNP genotypes:

$$y = Xa + \sum_{i=1}^{n} Z_i D_i + \sum_{j=1}^{m} c_j \beta_j + e$$

where *y* ($pq \times 1$ elements) is the vector of gene expression data sorted by array ($q = 100$) and probe within array ($P = 24{,}123$), and influenced by the overall effect of each array (*a*), as well as discrete (*D_i*), and continuous (*β_j*) within-probe effects both with dimensions $1 \times P$. Sex and batch discrete effects and carcass weight continuous effect are fitted in the model for all the performed analyses. Different classifications were adopted conditional on the QTL genotypes (as discrete effect) and on SNP genotypes (as continuous effect with values 0, 1, or 2 according to the number of copies of the most frequent allele). *A priori* distribution for *D_i* and β_j are assumed multivariate normal and *a priori* distributions for the remaining parameters in the model are assumed flat. All the unknowns in the model were sampled from their joint posterior distribution by Gibbs sampling (Gelfand and Smith, 1990). Additional details of the performed Bayesian procedure are reported by Casellas et al. (2008).

Inferences were made on the probe-specific difference between *D_i* levels or on the regression coefficients in β_j from the appropriate posterior distributions summarized by its mean, SD, and *PP* above (negative mean) or below (positive mean) zero. Posterior probabilities were treated as *P*-values for calculating their maximum value under multiple testing within the FDR approach of Benjamini and Hochberg (1995). The probes that displayed FDR < 0.10, *PP* < 0.001, and whose expression ratio in the untransformed scale was over 1.10 were assumed to present differential expression.

Expression probe annotation was conducted using the updated data file of Tsai et al. (2006). A functional assignation of the DE genes was performed using gene ontology (GO) information. The resulting list was assessed through database for annotation, visualization, and integrated discovery (DAVID)[5] in order to investigate their functional implications.

RNA ISOLATION AND QUANTITATIVE REAL-TIME PCR ANALYSIS

Longissimus dorsi samples were used to obtain total RNA using the RiboPure™RNA isolation kit (Ambion, Austin, TX, USA) following the manufacturer's recommendations. RNA obtained was quantified using NanoDrop equipment (NanoDrop Technologies, Wilmington, DE, USA) and RNA quality was assessed with an Agilent bioanalyzer device (Agilent Technologies, Palo Alto, CA, USA). The RNA integrity number (RIN) values obtained were in the range of 8.0–9.0, assuring the homogeneity and high quality of the samples.

Quantitative Real Time PCR was used to validate microarray expression data of *PCTP* gene. A subset of 28 individuals was

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[2]http://blast.ncbi.nlm.nih.gov/Blast.cgi
[3]http://www.bioconductor.org

[4]http://linux.nci.nih.gov/BRB-ArrayTools.html
[5]http://david.abcc.ncifcrf.gov

analyzed. First-strand cDNA synthesis was carried out with Super-script II (Invitrogen, Carlsbad, CA, USA) and random hexamers in a total volume of 20 μl containing 1 μg of total RNA and following the supplier's instructions. Quantitative Real Time PCR was run on an MX3000P sequence detector (Stratagene, La Jolla, CA, USA) using cycling parameters defined by the manufacturer. Reactions consisted of 1× SYBR Green PCR master mix (Takara), 0.15 mM of each primer (Forward 5′-GTTGGGGATGTTGAAGGGATAAT-3′; Reverse 5′-GAGAAAAGTAGGACACGGGAAGC-3′), 2.5 μl of 1/20 dilution cDNA, and 0.4 μl of ROXII reference dye in a reaction volume of 20 μl. A no-template control was also included. Cycling conditions were 95° for 10 min followed by 40 cycles of 95° (15 s) and 60° (1 min). Finally a dissociation curve to test PCR specificity was generated by one cycle at 95° (15 s), 60° (1 min), and was ramped up (0.01°/s) to 95°. Data were analyzed with MxPro software (Stratagene). All points and samples were run in triplicate as technical replicates and dissociation curves were carried out for each individual replicate. Specific amplification was confirmed by single peaks observation on dissociation curves. PCR efficiency of each gene was estimated by standard curve calculation using four points of 10-fold cDNA serial dilutions. All PCR efficiencies were greater than 95%. Ct values were transformed to quantities using the comparative Ct method, setting the highest relative quantities for each gene to 1 ($Qty = 10^{-\Delta Ct/slope}$). Data normalization was carried out using *Glyceraldehyde-3-Phosphate Dehydrogenase* (*GAPDH*) and *Beta-2 Microglobulin* (*B2M*) as reference genes. Gene expression relative measures obtained by RT-qPCR were analyzed conditional on genotypes of the *PCTP ASGA*0054039T > C and *ASGA*0054041A > G SNPs with a *t*-test.

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APPENDIX

Table A1 | Association analysis.

SNP	MAF	Position (Mb)	Gene	a_{SNP}	*P*-value
*M*1*GA*0016816 A > G	0.47	46.00	–	−0.049	0.000110
*ALGA*0065344 A > G	0.29	17.46	*C*17*orf*57	−0.057	0.000118
*ALGA*0065346 A > G	0.29	17.51	*ITGB*3	0.057	0.000118
*ASGA*0053496 C > T	0.29	17.55	*ITGB*3	−0.057	0.000118
*H3GA*0033448 C > T	0.46	11.41	–	−0.056	0.000124
*MARC*0112715 A > G	0.34	16.78	–	−0.053	0.000141
*ASGA*0055028 C > T	0.17	53.09	*PITPNM*3	−0.056	0.000151
*ALGA*0065461 C > T	0.38	19.41	*C*1*QL*1	0.046	0.000286
*ALGA*0066217 C > T	0.27	36.47	–	−0.039	0.000300
*ALGA*0066658 A > G	0.19	46.86	*NLK*	−0.040	0.000325
*MARC*0026665 A > G	0.22	20.74	*DHX*8	0.051	0.000442
*ALGA*0066707 C > T	0.16	50.29	*RPA*1	−0.090	0.009930
*H3GA*0034580 A > C	0.16	50.41	–	−0.090	0.009930
*CASI*0009186 C > T	0.17	49.89	–	−0.082	0.020300
*ASGA*0054824 A > G	0.35	47.70	–	−0.059	0.021500
*ALGA*0065841 C > T	0.26	29.86	–	0.057	0.028100
*ASGA*0054669 A > G	0.46	43.93	–	−0.049	0.028300
*ASGA*0052681 A > G	0.40	7.20	–	−0.060	0.040100
*ASGA*0098632 C > T	0.39	16.24	–	0.039	0.048600
*H3GA*0034686 G > T	0.40	53.14	–	−0.042	0.049000

SNPs with significant effects on linolenic fatty acid (P-value < 0.05).

Table A2 | Association analysis.

SNP	MAF	Position (Mb)	Gene	a_{SNP}	P-value
MARC0054687 A > G	0.37	29.03	–	−0.094	0.000698
MARC0008574 C > T	0.47	32.88	–	−0.077	0.001240
ASGA0054041 A > G	0.47	33.17	PCTP	−0.077	0.001240
ASGA0089300 G > T	0.46	33.64	–	−0.077	0.001240
MARC0044696 A > C	0.48	31.48	–	−0.078	0.001260
ASGA0054453 A > C	0.26	40.82	–	−0.084	0.001330
ALGA0065826 G > T	0.48	29.09	–	0.081	0.001560
DRGA0011659 C > T	0.30	22.74	–	−0.082	0.003150
ALGA0065995 A > C	0.18	33.73	ANKFN1	0.09	0.004470
CASI0006966 A > C	0.45	28.38	–	−0.073	0.004820
H3GA0034677 A > G	0.45	53.04	PITPNM	−0.074	0.005770
DIAS0001882 A > G	0.32	24.70	NPEPPS	0.072	0.005980
M1GA0017195 C > T	0.33	61.50	–	−0.071	0.007330
ALGA0104556 C > T	0.27	29.65	–	−0.079	0.008700
ALGA0065925 A > G	0.28	32.63	–	−0.078	0.009050
DRGA0011696 G > T	0.25	31.74	–	0.081	0.009270
ALGA0065904 A > G	0.25	32.08	–	0.081	0.009270
M1GA0017119 A > G	0.39	61.17	–	−0.063	0.009550
ALGA0065876 C > T	0.28	31.61	–	0.078	0.00960
H3GA0034902 A > T	0.31	58.50	–	−0.067	0.01080
ASGA0053778 A > G	0.25	24.99	–	−0.072	0.01160
MARC0021670 A > G	0.44	36.97	–	0.061	0.01220
ALGA0065773 A > G	0.26	27.79	MYCBPAP	−0.071	0.01670
H3GA0033984 C > T	0.26	27.81	SPATA20	−0.071	0.016700
H3GA0033980 A > G	0.26	27.85	CACNA1G	0.071	0.016700
ALGA0065691 A > G	0.32	25.90	–	−0.071	0.019300
MARC0093870 A > G	0.33	14.16	–	0.060	0.021100
ASGA0101438 C > T	0.26	59.22	–	0.055	0.022100
ALGA0065527 A > G	0.24	20.61	–	−0.065	0.024300
ASGA0053456 C > T	0.23	16.70	MARCH10	−0.068	0.026600
ASGA0053428 A > C	0.38	15.90	–	0.063	0.027200
ALGA0066610 C > T	0.30	45.55	–	0.088	0.028900
MARC0009546 C > T	0.25	59.17	–	0.066	0.030100
M1GA0016745 A > G	0.44	43.74	–	0.066	0.030400
DIAS0004710 A > G	0.46	15.87	–	−0.051	0.030800
MARC0037857 A > G	0.26	34.77	–	−0.061	0.030900
DIAS0003416 A > G	0.38	59.53	–	0.052	0.031200
ALGA0067326 C > T	0.18	63.85	–	0.069	0.032200
ASGA0053830 C > T	0.29	26.00	TTLL6	−0.070	0.032400
ASGA0055015 A > G	0.33	52.89	–	−0.067	0.032600
MARC0045984 C > T	0.37	34.38	–	−0.054	0.035100
SIRI0000304 A > C	0.48	13.47	–	0.055	0.035500
ASGA0054044 A > T	0.25	33.19	PCTP	−0.062	0.036500
MARC0055851 G > T	0.39	23.80	–	−0.055	0.036600
MARC0013841 C > T	0.30	15.85	–	−0.066	0.037300
ASGA0053899 A > G	0.34	27.97	–	0.059	0.038100
MARC0040976 A > G	0.21	16.67	MARCH10	0.066	0.039700
M1GA0016298 C > T	0.45	15.73	FTSJ3	0.049	0.039900
H3GA0033448 C > T	0.46	11.41	–	−0.060	0.042600
ASGA0088871 A > G	0.24	53.43	–	0.058	0.044300
H3GA0033801 A > G	0.33	20.20	–	−0.053	0.044500
ASGA0053416 A > G	0.43	15.65	SCN4A	0.052	0.044800

(Continued)

Table A2 | Continued

SNP	MAF	Position (Mb)	Gene	a_{SNP}	*P*-value
*DIAS*0004787 A > G	0.39	23.99	–	0.053	0.045800
*H3GA*0034675 A > G	0.38	52.96	*KIAA*0753	−0.060	0.046000
*ALGA*0065337 A > C	0.44	17.24	–	−0.050	0.047500
*MARC*0053916 C > T	0.44	17.28	*C17orf57*	−0.050	0.047500
*MARC*0053916 C > T	0.44	17.28	*C17orf57*	−0.050	0.047500
*MARC*0036876 A > C	0.44	17.53	*ITGB3*	0.050	0.047500
*ASGA*0053505 A > G	0.44	17.58	–	0.050	0.047500
*ALGA*0065366 A > G	0.44	17.59	*MYL4*	−0.050	0.047500
*ASGA*0053547 C > T	0.44	17.99	–	0.050	0.047500
*DRGA*0011754 G > T	0.46	39.92	*GGNBP2*	0.052	0.047900
*H3GA*0035092 A > G	0.16	63.77	–	0.064	0.048400
*ASGA*0055477 C > T	0.16	63.80	–	0.064	0.048400

SNPs with significant effects on palmitic fatty acid (P-value < 0.05).

Table A3 | Association analysis.

SNP	MAF	Position (Mb)	Gene	a_{SNP}	*P*-value
*MARC*0054687 A > G	0.37	29.03	–	−0.023	0.000353
*MARC*0044696 A > C	0.48	31.48	–	−0.018	0.000912
*DRGA*0011659 C > T	0.30	22.74	–	−0.021	0.000937
*ALGA*0065826 G > T	0.48	29.09	–	0.019	0.000937
*MARC*0008574 C > T	0.47	32.88	–	−0.018	0.001030
*ASGA*0054041 A > G	0.47	33.17	*PCTP*	−0.018	0.001030
*ASGA*0089300 G > T	0.46	33.64	–	−0.018	0.001030
*M1GA*0017195 C > T	0.33	61.50	–	−0.020	0.002330
*ALGA*0065995 A > C	0.18	33.73	*ANKFN1*	0.021	0.003170
*DRGA*0011696 G > T	0.25	31.74	–	0.020	0.003180
*ALGA*0065904 A > G	0.25	32.08	–	0.020	0.003180
*H3GA*0034677 A > G	0.45	53.04	*PITPNM*	−0.017	0.005090
*M1GA*0017119 A > G	0.39	61.17	–	−0.016	0.007430
*DIAS*0001882 A > G	0.32	24.70	*NPEPPS*	0.019	0.007450
*ALGA*0104556 C > T	0.27	29.65	–	−0.018	0.007530
*CASI*0006966 A > C	0.45	28.38	–	−0.017	0.007870
*ALGA*0065691 A > G	0.32	25.90	–	−0.017	0.007970
*ASGA*0054453 A > C	0.26	40.82	–	−0.017	0.008250
*ALGA*0065876 C > T	0.28	31.61	–	0.017	0.008380
*ALGA*0065925 A > G	0.28	32.63	–	−0.017	0.008860
*ASGA*0053778 A > G	0.25	24.99	–	−0.016	0.009120
*ALGA*0067326 C > T	0.18	63.85	–	0.018	0.011200
*MARC*0055851 G > T	0.39	23.80	–	−0.014	0.011800
*MARC*0021670 A > G	0.44	36.97	–	0.015	0.012100
*H3GA*0033448 C > T	0.46	11.41	–	−0.016	0.012900
*ALGA*0065527 A > G	0.24	20.61	–	−0.015	0.013800
*H3GA*0035092 A > G	0.16	63.77	–	0.017	0.015500
*ASGA*0055477 C > T	0.16	63.80	–	0.017	0.015500
*DIAS*0004787 A > G	0.39	23.99	–	0.014	0.016000
*MARC*0037857 A > G	0.26	34.77	–	−0.014	0.019600
*MARC*0010590 G > T	0.28	25.89	–	−0.017	0.019900
*H3GA*0034902 A > T	0.31	58.50	*C17orf48*	−0.014	0.020600
*ASGA*0053830 C > T	0.29	26.00	*TTLL6*	−0.015	0.022100
*M1GA*0016745 A > G	0.44	43.74	–	0.015	0.022300
*ASGA*0055015 A > C	0.33	52.89	–	−0.014	0.023700
*ALGA*0065773 A > G	0.26	27.79	*MYCBPAP*	−0.016	0.026700
*H3GA*0033984 C > T	0.26	27.81	*SPATA20*	−0.016	0.026700
*H3GA*0033980 A > G	0.26	27.85	*CACNA1G*	0.016	0.026700
*ASGA*0054044 A > T	0.25	33.19	*PCTP*	−0.013	0.026700
*ASGA*0054864 A > G	0.31	50.31	*RPA1*	0.013	0.028100
*ASGA*0053715 A > C	0.26	22.80	*KRT20*	0.012	0.028300
*H3GA*0034675 A > G	0.38	52.96	*KIAA0753*	−0.013	0.028500
*H3GA*0033953 A > C	0.25	26.85	–	−0.012	0.030400
*ALGA*0066610 C > T	0.30	45.55	–	0.018	0.032600
*H3GA*0034044 C > T	0.21	32.32	–	0.016	0.035100
*ASGA*0101438 C > T	0.26	59.22	–	0.012	0.037400
*ALGA*0067033 C > T	0.36	56.94	*PI3R5*	−0.012	0.037700
*ASGA*0053428 A > C	0.38	15.90	*DDX42*	0.015	0.038500
*MARC*0093870 A > G	0.33	14.16	–	0.013	0.040800
*ASGA*0098632 C > T	0.39	16.24	–	0.009	0.041700
*SIRI*0000304 A > C	0.48	13.47	–	0.011	0.044000
*H3GA*0033801 C > T	0.33	20.20	–	−0.013	0.047700

SNPs with significant effects on DBI (P-value < 0.05).

Table A4 | Association analysis.

SNP	MAF	Position (Mb)	Gene	a_{SNP}	P-value
MARC0054687 A > G	0.37	29.03	–	−0.094	0.000698
MARC0008574 C > T	0.47	32.88	–	−0.077	0.001240
ASGA0054041 A > G	0.47	33.17	PCTP	−0.077	0.001240
ASGA0089300 G > T	0.46	33.64	–	−0.077	0.001240
MARC0044696 A > C	0.48	31.48	–	−0.078	0.001260
ASGA0054453 A > C	0.26	40.82	–	−0.084	0.001330
ALGA0065826 G > T	0.48	29.09	–	0.081	0.001560
DRGA0011659 C > T	0.30	22.74	–	−0.082	0.003150
ALGA0065995 A > C	0.18	33.73	ANKFN1	0.09	0.004470
CASI0006966 A > C	0.45	28.38	–	−0.073	0.004820
H3GA0034677 A > G	0.45	53.04	PITPNM	−0.074	0.005770
DIAS0001882 A > G	0.32	24.70	NPEPPS	0.072	0.005980
M1GA0017195 C > T	0.33	61.50	–	−0.071	0.007330
ALGA0104556 C > T	0.27	29.65	–	−0.079	0.008700
ALGA0065925 A > G	0.28	32.63	–	−0.078	0.009050
DRGA0011696 G > T	0.25	31.74	–	0.081	0.009270
ALGA0065904 A > G	0.25	32.08	–	0.081	0.009270
M1GA0017119 A > G	0.39	61.17	–	−0.063	0.009550
ALGA0065876 C > T	0.28	31.61	–	0.078	0.00960
H3GA0034902 A > T	0.31	58.50	–	−0.067	0.01080
ASGA0053778 A > G	0.25	24.99	–	−0.072	0.01160
MARC0021670 A > G	0.44	36.97	–	0.061	0.01220
ALGA0065773 A > G	0.26	27.79	MYCBPAP	−0.071	0.01670
H3GA0033984 C > T	0.26	27.81	SPATA20	−0.071	0.016700
H3GA0033980 A > G	0.26	27.85	CACNA1G	0.071	0.016700
ALGA0065691 A > G	0.32	25.90	–	−0.071	0.019300
MARC0093870 A > G	0.33	14.16	–	0.060	0.021100
ASGA0101438 C > T	0.26	59.22	–	0.055	0.022100
ALGA0065527 A > G	0.24	20.61	–	−0.065	0.024300
ASGA0053456 C > T	0.23	16.70	MARCH10	−0.068	0.026600
ASGA0053428 A > C	0.38	15.90	–	0.063	0.027200
ALGA0066610 C > T	0.30	45.55	–	0.088	0.028900
MARC0009546 C > T	0.25	59.17	–	0.066	0.030100
M1GA0016745 A > G	0.44	43.74	–	0.066	0.030400
DIAS0004710 A > G	0.46	15.87	–	−0.051	0.030800
MARC0037857 A > G	0.26	34.77	–	−0.061	0.030900
DIAS0003416 A > G	0.38	59.53	–	0.052	0.031200
ALGA0067326 C > T	0.18	63.85	–	0.069	0.032200
ASGA0053830 C > T	0.29	26.00	TTLL6	−0.070	0.032400
ASGA0055015 A > G	0.33	52.89	–	−0.067	0.032600
MARC0045984 C > T	0.37	34.38	–	−0.054	0.035100
SIRI0000304 A > C	0.48	13.47	–	0.055	0.035500
ASGA0054044 A > T	0.25	33.19	PCTP	−0.062	0.036500
MARC0055851 G > T	0.39	23.80	–	−0.055	0.036600
MARC0013841 C > T	0.30	15.85	–	−0.066	0.037300
ASGA0053899 A > G	0.34	27.97	–	0.059	0.038100
MARC0040976 A > G	0.21	16.67	MARCH10	0.066	0.039700
M1GA0016298 C > T	0.45	15.73	FTSJ3	0.049	0.039900
H3GA0033448 C > T	0.46	11.41	–	−0.060	0.042600
ASGA0088871 A > G	0.24	53.43	–	0.058	0.044300
H3GA0033801 A > G	0.33	20.20	–	−0.053	0.044500

(Continued)

Table A4 | Continued

SNP	MAF	Position (Mb)	Gene	a_{SNP}	*P*-value
*ASGA*0053416 A > G	0.43	15.65	*SCN4A*	0.052	0.044800
*DIAS*0004787 A > G	0.39	23.99	–	0.053	0.045800
*H3GA*0034675 A > G	0.38	52.96	*KIAA*0753	−0.060	0.046000
*ALGA*0065337 A > C	0.44	17.24	–	−0.050	0.047500
*MARC*0053916 C > T	0.44	17.28	*C17orf57*	−0.050	0.047500
*MARC*0036876 A > C	0.44	17.53	*ITGB3*	0.050	0.047500
*ASGA*0053505 A > G	0.44	17.58	–	0.050	0.047500
*ALGA*0065366 A > G	0.44	17.59	*MYL4*	−0.050	0.047500
*ASGA*0053547 C > T	0.44	17.99	–	0.050	0.047500
*DRGA*0011754 G > T	0.46	39.92	*GGNBP2*	0.052	0.047900
*H3GA*0035092 A > G	0.16	63.77	–	0.064	0.048400
*ASGA*0055477 C > T	0.16	63.80	–	0.064	0.048400

SNPs with significant effects on UI (P-value < 0.05).

Table A5 | Association analysis.

SNP	MAF	Position (Mb)	Gene	a_{SNP}	P-value
*MARC*0054687 A > G	0.37	29.03	–	−0.040	0.000204
*DRGA*0011659 C > T	0.30	22.74	–	−0.036	0.001040
*ALGA*0065826 G > T	0.48	29.09	–	0.033	0.001140
*MARC*0044696 A > C	0.48	31.48	–	−0.030	0.001670
*MARC*0008574 C > T	0.47	32.88	–	−0.029	0.001840
*ASGA*0054041 A > G	0.47	33.17	*PCTP*	−0.029	0.001840
*ASGA*0089300 G > T	0.46	33.64	–	−0.029	0.001840
*DIAS*0001882 A > G	0.32	24.70	*NPEPPS*	0.032	0.003450
*M1GA*0017195 C > T	0.33	61.50	–	−0.028	0.006550
*CASI*0006966 A > C	0.45	28.38	–	−0.027	0.006580
*MARC*0021670 A > G	0.44	36.97	–	0.025	0.006740
*ALGA*0065995 A > C	0.18	33.73	*ANKFN*1	0.032	0.009190
*M1GA*0017119 A > G	0.39	61.17	–	−0.025	0.009980
*ASGA*0053778 A > G	0.25	24.99	–	−0.026	0.015200
*H3GA*0033801 A > G	0.33	20.20	–	−0.024	0.017800
*ASGA*0054453 A > C	0.26	40.82	–	−0.025	0.018200
*H3GA*0034677 A > G	0.45	53.04	*PITPNM*	−0.025	0.019600
*ALGA*0065691 A > G	0.32	25.90	–	−0.027	0.020400
*ASGA*0098632 C > T	0.39	16.24	–	0.020	0.021700
*ALGA*0104556 C > T	0.27	29.65	–	−0.027	0.022700
*DIAS*0004787 A > G	0.39	23.99	–	0.023	0.023900
*ALGA*0065773 A > G	0.26	27.79	*MYCBPAP*	−0.026	0.024000
*H3GA*0033984 C > T	0.26	27.81	*SPATA*20	−0.026	0.024000
*H3GA*0033980 A > G	0.26	27.85	*CACNA*1*G*	0.026	0.024000
*ALGA*0065876 C > T	0.28	31.61	–	0.026	0.024000
*H3GA*0033448 C > T	0.46	11.41	–	−0.027	0.024700
*MARC*0037857 *A > G*	0.26	34.77	–	−0.025	0.025200
*DRGA*0011696 G > T	0.25	31.74	–	0.027	0.025300
*DRGA*0011696 G > T	0.25	31.74	–	0.027	0.025300
*ALGA*0065904 A > G	0.25	32.08	–	0.027	0.025300
*ALGA*0065925 A > G	0.28	32.63	–	−0.026	0.025900
*ASGA*0053830 C > T	0.29	26.00	*TTLL*6	−0.026	0.032000
*MARC*0040976 A > G	0.21	16.67	*MARCH*10	0.027	0.033800
*ASGA*0054160 A > G	0.22	34.81	–	0.025	0.036400
*MARC*0093870 A > G	0.33	14.16	–	0.021	0.040000
*MARC*0010590 G > T	0.28	25.89	–	−0.027	0.045500
*ASGA*0053715 A > C	0.26	22.80	*KRT*20	0.022	0.045900
*DIAS*0004274 A > G	0.39	13.36	–	0.022	0.047300
*ALGA*0066043 A > G	0.18	34.13	–	−0.029	0.047500
*ALGA*0065337 A > C	0.44	17.24	–	−0.020	0.048200
*MARC*0053916 C > T	0.44	17.28	*C*17*orf*57	−0.020	0.048200
*MARC*0036876 A > C	0.44	17.53	*ITGB*3	0.020	0.048200
*ASGA*0053505 A > G	0.44	17.58	–	0.020	0.048200
*ALGA*0065366 A > G	0.44	17.59	*MYL*4	−0.020	0.048200
*ASGA*0053547 C > T	0.44	17.99	–	0.020	0.048200

SNPs with significant effects on PUFA/SFA (P-value < 0.05).

Table A6 | Differentially expressed probes between *Qq* and *qq* QTL genotypes with effect on C16:0.

Affymetrix probe ID	Annotation	Chromosome	Position (Mb)	*P*-value	Ratio[a]
Ssc.24800.2.A1_at	LOC100514982	SSC12	27.53	1.10×10^{-6}	1.21
Ssc.18579.1.A1_at	CDK5RAP3	SSC12	–	5.00×10^{-6}	1.31
Ssc.9883.1.A1_at	ADRBK2	SSC14	47.16	8.07×10^{-6}	0.74
Ssc.6484.1.S1_at	PNPO	SSC12	25.02	2.56×10^{-5}	0.70
Ssc.3964.1.S1_at	CUEDC1	SSC12	–	4.30×10^{-5}	1.36
Ssc.877.1.S1_at	SKA2	SSC12	–	4.41×10^{-5}	1.24
Ssc.22988.1.S1_at	PCTP	SSC12	33.17	9.93×10^{-5}	0.77
Ssc.30925.1.A1_at	DKK2	SSC8	120.79	0.00016	0.74
Ssc.1286.1.A1_at	SUZ12	SSC12	45.18	0.00016	1.21
Ssc.24930.1.S1_at	LTV1	SSC1	22.45	0.00020	1.18
Ssc.13777.2.S1_at	TMEM97	SSC12	–	0.00027	1.26
Ssc.21326.1.S1_at	NEO1	SSC7	–	0.00035	1.23
Ssc.7149.3.S1_at	CYB5D1	SSC12	–	0.00046	1.15
Ssc.11077.1.S1_at	POU2F1	SSC4	87.45	0.00050	1.24
Ssc.12255.1.A1_at	FSTL5	SSC8	–	0.00061	1.15
Ssc.24711.1.S1_at	SMYD4	SSC12	–	0.00070	1.15
Ssc.428.5.S1_at	# NA	–	–	0.00074	0.83
Ssc.29805.1.A1_at	SKA2	SSC12	–	0.00083	1.22

In red probes with FDR < 10%, #NA probes that could not be annotated. Position in Mb, when available, according with Sscrofa10.
[a]Expression ratio between Qq and qq genotypes in the untransformed scale.

Table A7 | Differentially expressed probes conditional on copy number of *ACACA* ASGA0066302A allele.

Affymetrix probe ID	Annotation	Chromosome	Position (Mb)	*P*-value	Ratio[b]
Ssc.9782.1.A1_at	KRT10	SSC12	–	6.82×10^{-6}	1.19
Ssc.29848.1.A1_at	PDE4D	SSC16	–	2.76×10^{-5}	1.16
Ssc.3709.1.S1_at	MRPL10	SSC12	–	2.81×10^{-5}	1.17
Ssc.1286.1.A1_at	SUZ12	SSC12	45.18	0.00024	0.86
Ssc.19416.1.A1_at	IL21R	SSC3	–	0.00027	0.67
Ssc.23484.1.A1_a_at	SCARB2	SSC8	75.07	0.00028	0.86
Ssc.19050.1.S1_at	DHRS7C	SSC12	57.63	0.00038	1.21
Ssc.24698.1.S1_at	GCHFR	SSC1	–	0.00071	1.17
Ssc.4246.3.S1_at	ARHGEF10L	SSC6	–	0.00085	1.23
Ssc.15565.1.S1_at	LCP2	SSC16	57.11	0.00090	1.16
Ssc.14311.1.A1_at	KIAA1407	SSC13	15.14	0.00096	0.87
Ssc.8528.2.S1_at	CTSC	SSC9	–	0.00097	1.31
Ssc.8528.1.A1_at	CTSC	SSC9	–	0.00099	1.27

[b]Effect of the SD of the SNP allele copy number in the untransformed scale (SD = 0.58 copies).

Table A8 | Differentially expressed probes conditional on copy number of *PCTP* ASGA0054039C.

Affymetrix probe ID	Annotation	Chromosome	Position (Mb)	*P*-value	Ratio[b]
Ssc.22988.1.S1_at	PCTP	SSC12	33.17	3.34×10^{-11}	1.21
Ssc.5902.1.A1_at	COX11	SSC12	–	1.16×10^{-8}	0.83
Ssc.17449.1.S1_at	OLFM1	SSC1	–	1.63×10^{-8}	0.73
Ssc.6525.2.A1_at	PPP1R12A	SSC5	105.55	1.07×10^{-5}	1.78
Ssc.29609.2.A1_at	SVIP	SSC2	–	2.55×10^{-5}	0.78

[b]Effect of the SD of the SNP allele copy number in the untransformed scale (SD = 0.51 copies).

Table A9 | Differentially expressed probes conditional on copy number of *PCTP* ASGA0054041G.

Affymetrix probe ID	Annotation	Chromosome	Position (Mb)	*P*-value	Ratiob
Ssc.22988.1.S1_at	*PCTP*	SSC12	33.17	4.25×10^{-14}	1.34
Ssc.5902.1.A1_at	*COX*11	SSC12	–	7.4×10^{-9}	0.78
Ssc.6484.1.S1_at	*PNPO*	SSC12	25.02	2.33×10^{-5}	1.27
Ssc.1023.1.S1_at	*RBM*35*B*	SSC6	–	3.21×10^{-5}	1.21
Ssc.30718.1.A1_at	*KCNH*2	SSC18	–	4.98×10^{-5}	1.30
Ssc.10391.1.A1_at	*MCAM*	SSC9	–	7.34×10^{-5}	0.85
Ssc.19050.1.S1_at	*DHRS*7*C*	SSC12	57.63	7.48×10^{-5}	1.19
Ssc.2847.1.S1_at	*ANKRD*9	SSC7	–	0.00010	1.25
Ssc.25859.1.S1_at	*ANKRD*40	SSC12	27.89	0.00029	1.16
Ssc.6978.1.A1_at	*MAX*	SSC1	–	0.00030	1.16
Ssc.6382.1.A1_at	*PPP*1*R*3*B*	SSC8	88.60	0.00031	1.37
Ssc.10549.1.A1_at	*BOC*	SSC13	152.01	0.00051	1.18
Ssc.21383.1.A1_at	*PHOSPHO*1	SSC12	58.49	0.00063	1.15
Ssc.16679.1.S1_at	*BMP*1	SSC14	7.29	0.00069	0.78
Ssc.13476.1.A1_at	*PEG*10	SSC9	81.94	0.00074	0.75
Ssc.4895.2.S1_at	*FBXO*25	SSC15	–	0.00075	1.16
Ssc.10675.1.A1_at	*LNX*1	SSC8	–	0.00085	1.16
Ssc.29510.1.A1_at	*ZNF*780*A*	SSC6	–	0.00090	1.17
Ssc.8295.1.A1_at	*AGXT*2*L*1	SSC8	–	0.00094	1.36
Ssc.27429.1.A1_at	*CPEB*2	SSC8	8.30	0.00094	0.81
Ssc.22197.1.S1_at	*FANCG*	SSC1	240.61	0.00097	0.84

[b]*Effect of the SD of the SNP allele copy number in the untransformed scale (SD = 0.66 copies).*