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Genome-wide association study for intramuscular fatty acid composition in an Iberian × Landrace cross[1]

Y. Ramayo-Caldas,*[2] A. Mercadé,† A. Castelló,* B. Yang,*[3] C. Rodríguez,‡ E. Alves,‡ I. Díaz,# N. Ibáñez-Escriche,§ J.L. Noguera,§ M. Pérez-Enciso,*†‖ A.I. Fernández,‡ and J.M. Folch*†

*Centre for Research in Agricultural Genomics (CRAG), Consortium CSIC-IRTA-UAB-UB. Edifici CRAG, Campus Universitat Autonoma Barcelona. 08193 Bellaterra, Spain; †Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona. 08193 Bellaterra, Spain; ‡Departamento de Mejora Genética Animal, INIA, Ctra. De la Coruña km. 7. 28040 Madrid, Spain; §IRTA, Genètica i Millora Animal. 25198 Lleida, Spain; #IRTA, Tecnologia dels Aliments. 17121 Monells, Spain; and ‖ICREA, Passeig Lluis Companys. Barcelona, Spain.

ABSTRACT: The lipid content and fatty acid (FA) profile have an important impact in human health as well as in the technological transformation and nutritional and organoleptic quality of meat. A genome-wide association study (GWAS) on 144 backcross pigs (25% Iberian × 75% Landrace) was performed for 32 traits associated with intramuscular FA composition and indices of FA metabolism. The GWAS was carried out using Qxpak 5.0 and the genotyping information obtained from the Porcine SNP60K BeadChip (Illumina Inc., San Diego, CA). Signals of significant association considering a false-discovery rate (q-value < 0.05) were observed in 15 of the 32 analyzed traits, and a total of 813 trait-associated SNP (TAS), distributed in 43 chromosomal intervals on almost all autosomes, were annotated. According to the clustering analysis based on functional classification, several of the annotated genes are related to FA composition and lipid metabolism. Some interesting positional concordances among TAS and previously reported QTL for FA compositions and/or other lipid traits were also found. These common genomic regions for different traits suggest pleiotropic effects for FA composition and were found primarily on SSC4, SSC8, and SSC16. These results contribute to our understanding of the complex genetic basis of FA composition and FA metabolism.

Key words: candidate gene, fatty acid, genome-wide association study, intramuscular fat, meat quality

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INTRODUCTION

Pork production is an important source of human food and accounts for more than 40% of the meat produced worldwide (Rothschild and Ruvinsky, 2011). The lipid content and fatty acid (**FA**) profile have an important impact in the technological transformation and the nutritional and organoleptic quality of meat. In pigs, differences in FA composition of subcutaneous fat have an important effect on fat quality as defined in terms of firmness and the degree of cohesiveness between lean and fat tissues. In muscle, FA composition has a role in the tenderness and juiciness of cooked meat, affecting its oxidative stability during processing (Wood et al., 2008). The environmental and genetic

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[2] Corresponding author: yuliaxis.ramayo@uab.es

[3]Current address: Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University. Nanchang, China.

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effects on FA composition have been studied in pigs, showing moderate to high heritabilities in different tissues (Casellas et al., 2010; Ntawubizi et al., 2010).

Domestic animal populations have unique features that facilitate the genetic dissection of complex traits. Classical, family-based linkage analysis with microsatellites has been used for the detection of QTL. However, recently, new advances in high-throughput methods have allowed for the genotyping of a large number of SNP throughout the genome. Genome-wide association studies (**GWAS**) using dense marker maps are able to exploit linkage disequilibrium (**LD**) to map QTL. In comparison with the classical QTL mapping, GWAS has the advantage of using all recombination events after the mutations occurred and, therefore, increases the precision of the QTL position estimates and reduces their confidence intervals (Meuwissen and Goddard, 2000). Moreover, it considers the contribution of the variability within breeds or lines, whereas linkage analysis usually ignores it and, as a consequence, its power is decreased (Ledur et al., 2010).

Although several studies have identified QTL for FA composition and FA metabolism in pigs (Clop et al., 2003; Nii et al., 2006; Sanchez et al., 2007; Guo et al., 2009; Uemoto et al., 2009; Quintanilla et al., 2011), a GWAS analysis using genotypic information from the Porcine SNP60K BeadChip (Illumina Inc., San Diego, CA) has not been reported.

The goal of this study is to perform a GWAS to identify chromosomal regions and positional candidate genes associated with the profile of intramuscular FA composition and indices of FA metabolism in an Iberian × Landrace cross.

MATERIALS AND METHODS

As mentioned below, the experiments were performed in Europe following national and institutional guidelines for the ethical use and treatment of animals in experiments. In addition it was approved by the Ethical Committee of the Institution (IRTA- Institut de Recerca i Tecnologia Agroalimentàries)

Animal Material and Analyzed Traits

The population studied descends from the crossing of three Iberian (Guadyerbas line) boars with 31 Landrace sows (**IBMAP**) from the experimental farm of Nova Genètica S. A. in Lleida, Spain (Pérez-Enciso et al., 2000; Clop et al., 2003). Seventy-nine F1 individuals (6 males and 73 females) were obtained, and 5 of the 6 F1 males were backcrossed with 26 Landrace sows (**BC1_LD**). Here, we report results based on 144 BC1_LD (25% Iberian × 75% Landrace) pigs from 26 full-sib families. All pigs were raised and fed under the standard, intensive system in Europe; males were not castrated. After a suckling period of between 23 and 28 d, piglets were allocated in pens with 12 individuals in each pen and were given ad libitum access to a pelleted diet (13.4 MJ/kg of ME, 18.3% of CP, 1.2% of lysine). When the piglets were about 75 d old, they were moved to a fattening building. They were penned in groups of 10 to 12 animals separated by sex, and during the whole test period they had ad libitum access to a cereal-based commercial diet (13.4 MJ/kg of ME, 17.5% CP, 1% lysine). Pigs tested at the same time and in the same fattening building were considered as 1 contemporary group (batch). They were slaughtered according to national and institutional guidelines for the ethical use and treatment of animals in experiments at an average age of 179.8 ± 2.6 d. For all individuals, a 250 g sample of the LM was collected from the left half-carcass in the slaughter line, with 50 g of it quick-frozen in liquid nitrogen until genomic laboratory processing.

Meat analyses were performed on 200-g samples of the longissimus dorsi muscle at the IRTA-Centre of Food Technology (Lleita, Spain). The intramuscular percentage of FA (**IM FA**) was measured by Near Infrared Transmittance (**NIT**; Infratec 1625, Tecator, Hoganas, Sweden). A protocol based on gas chromatography of methyl esters (Mach et al., 2006) was employed to determine FA composition in the C:12 to C:22 range; subsequently, the percentage of each individual FA content was calculated, along with the global percentage of SFA, MUFA, and PUFA.

In total, 32 traits were analyzed: 15 for IM FA composition and 17 for indices of FA metabolism, including ratios of FA as indices for desaturase and elongase enzymes activities (Table 1).

Genotypying and Quality Control

A total of 197 animals, including the founder populations, were genotyped for 62,163 SNP with the Porcine SNP60K BeadChip (Ramos et al., 2009) and using the Infinium HD Assay Ultra protocol (Illumina Inc., San Diego, CA) and were visualized with the GenomeStudio software (Illumina). PLINK (Purcell et al., 2007) software was used to remove markers that showed minor allele frequency (**MAF**) < 5% and missing genotypes > 5%. The Pedstats program (Wigginton and Abecasis, 2005) was employed to check Mendelian inheritance errors. The SNP located in sex chromosomes and those not mapped in the Sscrofa10 assembly were also excluded. Therefore, a subset of 48,119 SNP was selected for subsequent data analysis.

Table 1. Descriptive statistics including mean, SD and estimated heritability (h^2) of fatty acid (FA) composition and FA indices

Item	Trait	Label	Mean	SD	h^2
SFA	C14:0	Myristic acid	1.18	0.15	0.54
	C16:0	Palmitic acid	22.60	1.20	0.57
	C17:0	Heptadecanoic acid	0.27	0.07	0.30
	C18:0	Stearic acid	14.18	1.03	0.24
	C20:0	Arachidic acid	0.25	0.08	0.42
MUFA	C16:1(n-7)	Palmitoleic acid	2.50	0.39	0.55
	C17:1	Heptadecenoic acid	0.27	0.11	0.41
	C18:1(n-9)	Oleic acid	40.08	2.76	0.40
	C18:1(n-7)	Octadecenoic acid	3.88	0.36	0.42
	C20:1(n-9)	Eicosenoic acid	0.85	0.11	0.47
PUFA	C18:2(n-6)	Linoleic acid	10.35	2.37	0.22
	C18:3(n-3)	α-Linolenic acid	0.65	0.29	0.18
	C20:2(n-6)	Eicosadienoic acid	0.54	0.12	0.26
	C20:3(n-6)	Eicosatrienoic acid	0.28	0.13	0.16
	C20:4(n-6)	Arachidonic acid	1.54	0.73	0.40
Metabolic ratios	ACL	Average Chain Length	17.47	0.05	0.41
	Σ SFA	SFA	38.47	1.63	0.53
	Σ MUFA	MUFA	47.96	3.06	0.19
	Σ PUFA	PUFA	13.36	3.29	0.18
	PI	Peroxidability indices	20.00	5.72	0.40
	DBI	Double-bond indices	0.78	0.09	0.41
	UI	Unsaturated indices	2.04	0.27	0.19
	PUFA/SFA	Ratio of PUFA to SFA	0.35	0.09	0.18
	MUFA/SFA	Ratio of MUFA to SFA	1.25	0.10	0.35
FA ratios					
Δ9-desaturase activity	C16:1(n-7)/C16:0		0.11	0.01	0.51
	C18:1(n-9)/C18:0		2.84	0.29	0.35
	C20:1/C20:0		3.64	1.06	0.42
Δ5-desaturase activity	C20:4(n-6)/C20:3(n-6)		5.86	3.02	0.37
Elongase activity	C18:1(n-7)/C16:1(n-7)		1.58	0.19	0.57
	C20:2(n-6)/C18:2(n-6)		0.05	0.01	0.39
Combined effect of desaturase and elongase	C20:3(n-6)/C18:2(n-6)		0.03	0.01	0.15
	C20:4/C18:2(n-6)		0.14	0.04	0.39

Genome-Wide Association Analysis

A GWAS on IM FA composition and indices of FA metabolism was performed in 144 BC1_LD animals. A mixed model (Henderson, 1984, 1975) accounting for additive effects (see below) was performed using Qxpak 5.0 (Pérez-Enciso and Misztal, 2011) using the formula:

$$y_{ijlkm} = Sex_i + Batch_j + \beta c_l + \lambda_l a_k + u_l + e_{iijlkm},$$

in which y_{ijlkm} is the lth individual record, sex (2 categories) and batch (5 categories) are fixed effects, β is a covariate coefficient with c being carcass weight, λ_l is a −1, 0, +1 indicator variable depending on the lth individual genotype for the kth SNP, a_k represents the additive effect associated with the SNP, u_l represents the infinitesimal genetic effect treated as random and distributed as N (0, $A\sigma_u$) where A is a numerator of kinship matrix, and e_{iijlkm} is the residual. The R package q-value (Storey and Tibshirani, 2003) was used to calculate the false-discovery rate (**FDR**), and the cut-off of the significant association at the whole-genome level was set at q-value ≤ 0.05.

To obtain, approximately, the variance explained by each SNP, we first fitted a model that included all associated SNP for each trait. This is important because, otherwise, SNP effect estimates are inflated by confounding effects (e.g., LD among SNP). The fraction of the phenotypic variance explained by SNP_t $\psi(_t)$ was calculated as:

$$\psi_t = \frac{2p_t(1-p_t)\hat{a}_t^2}{\hat{\sigma}_e^2 + \hat{\sigma}_a^2 + \sum_{n=1}^{n} 2p_n(1-p_n)\hat{a}_n^2}$$

in which p_t is the allele frequency of tth SNP in the population studied, with an estimated effect $\hat{a}_t$ and assuming there are n significant SNP associated with the

trait; and $\hat{\sigma}_e^2$ and $\hat{\sigma}_a^2$ are the estimated residual and the additive infinitesimal variances, respectively.

Version 2.12.1 of R (Ihaka and Gentleman, 1996; http://cran.r-project.org) was used to calculate the descriptive statistics for the 32 analyzed traits and the phenotypic correlations for traits that showed significantly associated SNP. Oleic FA was also included in the correlation analysis due to its importance in meat FA composition. Before analyzing the correlation, data were adjusted for fixed effect (sex, batch) and carcass weight as a covariate with the linear models procedure of R.

Gene Annotation and Functional Classification

The significantly associated SNP (q-value × 0.05) were initially mapped in the Sscrofa10 assembly. Then, the putative candidate chromosomal regions were defined by finding those genome locations comprising windows of at least 2 consecutive and significant SNP. The SNP positioned within the candidate chromosomal regions were also determined in Sscrofa9 assembly (Pig Ensembl Genome Browser, http://www.ensembl.org/Sus_scrofa/Info/index). Gene annotations were retrieved from the Ensembl Genes 62 Database using Biomart software (http://www.biomart.org; Flicek et al., 2010), assuming 1 Mb downstream/upstream around the candidate chromosomal regions. The functional classification of genes was performed using the DAVID online annotation database (http:// david.abcc.ncifcrf.gov; Huang et al., 2008, 2009). Those genes functionally related to lipid and FA metabolism were retained as positional candidate genes. The genomic coordinates of the annotated genes in the Ensembl Sscrofa10 dataset were validated by BLAST analysis (www.animalgenome.org).

RESULTS AND DISCUSSION

Phenotype Statistics and GWAS Results

The descriptive statistics of IMF FA composition and indices of FA metabolism are reported in Table 1. In accordance with previous reports (Yang et al., 2010; Quintanilla et al., 2011), moderate positive phenotype correlations were observed between myristic, palmitic and palmitoleic FA ($r_{C14:0,C16:0}$ = 0.68, $r_{C14:0,C16:1}$ = 0.61, $r_{C16:0,C16:1(n-7)}$ = 0.58). As expected, oleic acid showed a strong positive correlation with MUFA (r = 0.98) and a negative correlation with PUFA (r = −0.87). Conversely, oleic acid showed a clearly negative correlation with linoleic acid (r = −0.85), which also has been reported by Quintanilla et al. (2011) in gluteus medius and longissimus thoracis et lumborum muscles. The relationship between enzyme activity indices showed a moderate positive correlation for Δ^9–desaturase activity ($r_{C16:1(n-7)/C16:0,\ C18:1(n-9)/C18:0}$ = 0.70; Table 1), as previously reported in the LM of commercial pigs (Ntawubizi et al., 2010). In agreement with previous reports (Colman et al., 2008; Casellas et al., 2010; Ntawubizi et al., 2010; Sellier et al., 2010), the estimated heritabilities (h^2) were moderately large, ranging from 0.15 to 0.57 (Table 1).

A total of 144 BC1_LD animals passed quality control, and a subset of 48,119 SNP with high-genotyping quality (call rate > 0.99) was selected for GWAS. We were able to detect significant association signals (q-value < 0.05) in 15 out of the 32 traits analyzed. A total of 813 significant trait-associated SNP (**TAS**), located in 43 chromosomal regions distributed among almost all autosomes, were annotated according to the Sscrofa9 assembly (Table 2). Of the 813 TAS, 596 (73.3%) were mapped in intergenic regions, 189 (23.3%) were within genes, and 28 (3.4%) were unmapped in the Sscrofa9 assembly. Most of the gene-associated SNP were located in introns (93.6%): 7 were exonic and synonymous; and 5 were exonic, nonsynonymous SNPs. Annotation of the 5 genes containing nonsynonymous polymorphisms showed that 2 correspond to uncharacterized proteins (ENSSSCG00000004415 and ENSSSCG00000006022) and the rest to genes that were not directly related to the FA metabolism: Metadherin (MTDH, ENSSSCG00000006086), Serine incorporator 2 (SERINC2, ENSSSCG000000035959) and RAD17 homolog (*S. pombe*; RAD17, ENSSSCG00000016960). A more detailed description is presented in Table 2, including the 2 flanking genes around the most significant SNP for each trait.

A clustering analysis of the genes located in TAS regions revealed different functional categories related to lipid metabolism (Supplemental Table 3; http://journalofanimalscience.org). These positional candidate genes are discussed below.

Genomic regions associated with the composition of FA in LM were found for some SFA, MUFA, and PUFA. Significant association was found for the SFA, myristic (on SSC5 and SSC17), palmitic (on 8 different chromosomes, Table 2), and arachidic acids (SSC16). Within MUFA, 6 genomic regions (SSC4, SSC5, SSC6, SSC7, SSC8, and SSC15) were associated with palmitoleic acid amounts and 4 (SSC1, SSC4, SSC6, and SSC11) with oleic (Table 2). For PUFA, only linoleic acid composition was significant. However, this was the trait showing the largest number of associated positions along the pig genome, with significant association signals in 11 out of the 18 autosomes. Mammals lack the Δ12 and Δ15-desaturases required to synthesize FA of the n-6 and n-3 series. As a consequence, the polyunsaturated linoleic and α-linoleic acids must be provided by diet, and their tissue concentrations respond

Table 2. Description of the 43 putative candidate chromosomal intervals

Interval	Chromosome	Position Mb[1] Start-End	N_{SNP}	Most Significant SNP[3]	*P*-value	q-value	Ib[4]	Ld[5]	ψ [7]	Associated Traits
1	1	31.9-32.1	8	ALGA0002268	$6.0x10^{-6}$	$2.5x10^{-2}$	1	0.21	0.009	*SFA, UI
2	1	44.8-49.4	23	ASGA0002580	$3.4x10^{-5}$	$2.0x10^{-2}$	1	0.11	0.002	*C16:0, C18:2(n-6), SFA, UI
3	1	55.3-59.2	57	ASGA0003001	$1.9x10^{-5}$	$3.0x10^{-2}$	1	0.44	0.026	C16:0, SFA,*UI
4	1	72.7-78.3	97	H3GA0001996	$2.7x10^{-5}$	$4.0x10^{-2}$	1	0.15	0.019	*SFA, C16:0
5	1	126.6-130.8	46	DRGA0001507	$7.6x10^{-5}$	$3.0x10^{-2}$	0.83	0.32	0.026	*C18:2(n-6), MUFA/SFA
6	1	137.2-140.2	34	ALGA0006048	$6.9x10^{-5}$	$4.0x10^{-2}$	0.67	0.38	0.025	*C18:1(n-9), C18:2(n-6), PUFA,PUFA/SFA
7	2	19.1-23.8	6	DRGA0002840	$1.3x10^{-6}$	$6.1x10^{-3}$	1	0.06	0.010	C16:0
8	3	90.1-94.6	4	ASGA0015593	$1.4x10^{-5}$	$3.6x10^{-2}$	1	0.06	0.003	C18:1(n-7)/C16:1(n-7)
9	3	108.8-111.8	2	ASGA0101714	$5.3x10^{-5}$	$4.1x10^{-2}$	1	0.49	0.033	C16:1(n-7)/C16:0
10	4	10.9-14.5	24	ASGA0018461	$8.0x10^{-5}$	$3.0x10^{-2}$	0.67	0.23	0.031	*MUFA , C18:2(n-6), C18:1(n-9)
11	4	40.1-44.4	37	H3GA0012592	$3.0x10^{-6}$	$2.3x10^{-2}$	1	0.02	0.011	*PUFA, MUFA, PUFA/SFA
12	4	51.1-56.7	15	ASGA0019639	$2.8x10^{-5}$	$2.9x10^{-2}$	1	0.26	0.021	*C16:1(n-7), C18:2(n-6), C18:1(n-9)
13	4	78.2-80.2	13	ALGA0026066	$4.6x10^{-5}$	$4.6x10^{-2}$	1	0.13	0.004	MUFA/SFA
14	4	90.7-92.2	6	H3GA0013315	$5.7x10^{-6}$	$1.0x10^{-2}$	1	0.38	0.012	*C16:1(n-7), C18:2(n-6), C16:0, C18:1(n-7)/C16:1(n-7)
15	4	100.3-103.5	13	ALGA0027207	$6.4x10^{-5}$	$2.4x10^{-2}$	1	0.43	0.015	*C18:1(n-9), MUFA/SFA, MUFA
16	4	112.4-112.7	6	ASGA0021882	$5.4x10^{-5}$	$4.1x10^{-2}$	1	0.27	0.008	SFA
17	4	123.2-128.9	35	ALGA0029186	$8.7x10^{-7}$	$2.2x10^{-3}$	1	0.36	0.031	*C16:1(n-7), C16:0, C16:1(n-7)/C16:0, PUFA, PUFA/SFA
18	5	24.9-27.9	30	ALGA0031433	$1.1x10^{-5}$	$4.0x10^{-2}$	1	0.41	0.021	C14:0
19	5	70-72.1	2	ALGA0032946	$2.4x10^{-5}$	$2.7x10^{-2}$	1	0.38	0.019	C16:1(n-7)
20	6	55.6-56.9	18	ASGA0104276	$1.2x10^{-6}$	$1.3x10^{-2}$	1	0.3	0.018	*C18:1(n-9), MUFA, MUFA/SFA, C16:1(n-7), C18:2(n-6)
21	7	98.7-101.1	20	ASGA0035273	$7.9x10^{-5}$	$4.9x10^{-2}$	1	0.03	0.001	*C16:1(n-7), C20:1/C20:0
22	7	122.2-125.9	40	H3GA0023298	$1.6x10^{-4}$	$4.1x10^{-2}$	0.67	0.28	0.023	C18:2(n-6)
23	8	10.6-13.7	4	DRGA0008319	$7.2x10^{-7}$	$1.2x10^{-2}$	1	0.42	0.037	*SFA, UI
24	8	68.6-71.9	9	DRGA0008674	$7.8x10^{-5}$	$2.9x10^{-2}$	1	0.4	0.007	*C16:0, SFA
25	8	77.6-80.3	3	ALGA0048684	$3.5x10^{-7}$	$1.2x10^{-3}$	1	0.36	0.011	*C16:1(n-7), C18:1(n-7)/C16:1(n-7)
26	8	92.1-96.7	52	SIRI0000509	$3.7x10^{-9}$	$4.9x10^{-5}$	1	0.34	0.061	*C16:1(n-7), C16:0, C18:1(n-7)/C16:1(n-7), C16:1(n-7)/C16:0, SFA, UI
27	8	103.8-107.5	10	ASGA0039809	$1.2 x10^{-5}$	$1.1x10^{-2}$	1	0.4	0.025	C16:0
28	9	18.9-21.7	11	DRGA0009211	$4.2x10^{-5}$	$1.3x10^{-1}$	1	0.35	0.037	C20:1/C20:0
29	11	6.2-8.8	22	DRGA0010773	$3.9x10^{-6}$	$1.5x10^{-2}$	1	0.04	0.033	C18:1(n-9), C18:2(n-6), MUFA/SFA,*MUFA
30	13	78.4-83.3	10	DIAS0004396	$1.3x10^{-6}$	$1.4x10^{-2}$	1	0.47	0.044	PUFA/SFA, C16:0, C18:1(n-9), SFA,*UI
31	14	2.1-4.1	19	H3GA0038495	$1.9 x10^{-5}$	$2.5x10^{-2}$	1	0.38	0.032	*C18:2(n-6), PUFA, PUFA/SFA,UI
32	14	28.4-37	21	ASGA0062744	$1.8x10^{-5}$	$3.8x10^{-2}$	1	0.3	0.008	SFA
33	15	Unmapped [6]	2	MARC0042106	$1.4x10^{-5}$	$4.4 x10^{-2}$	1	0.22	0.027	C16:1(n-7)/C16:0
34	15	24.4-28	8	ALGA0111611	$4.1x10^{-6}$	$1.0x10^{-2}$	1	0.5	0.021	C16:0
35	15	33.4-34.5	13	ALGA0110309	$5.5x10^{-5}$	$4.2x10^{-2}$	1	0.12	0.003	*C16:1(n-7), MUFA/SFA
36	15	50-50.4	2	ASGA0069654	$2.6x10^{-4}$	$4.5x10^{-2}$	1	0.18	0.005	C16:0
37	15	104.2-105.6	9	ASGA0070398	$1.5x10^{-5}$	$1.1x10^{-2}$	0.5	0.28	0.004	C16:0
38	16	39.6-45.2	15	DRGA0016162	$1.5x10^{-6}$	$1.6x10^{-2}$	1	0.46	0.013	*C20:0, C20:1/C20:0
39	16	64.3-67.9	36	ASGA0085192	$1.5x10^{-4}$	$4.1x10^{-2}$	1	0.34	0.008	C18:2(n-6)
40	17	20.1-21.9	12	H3GA0048059	$8.0x10^{-5}$	$2.9x10^{-2}$	1	0.43	0.003	*C16:0, C20:1/C20:0,C14:0
41	17	34.5-34.7	4	ALGA0094522	$1.2x10^{-6}$	$2.3x10^{-2}$	0.83	0.38	0.040	C14:0
42	17	45.3-49.5	11	ALGA0095453	$1.6x10^{-6}$	$2.3x10^{-2}$	1	0.49	0.020	*C14:0,C16:1(n-7), C18:1(n-7)/C16:1(n-7)
43	18	10.2-15.8	4	ASGA0078979	$1.7x10^{-4}$	$3.9x10^{-2}$	1	0.26	0.025	C16:0

*Indicates the most significant trait for each chromosomal interval. The *P*- and q-values refer to this trait. All of the traits shown in the table are significant (q-value ≤ 0.05).

[1]The genomic coordinates are expressed in Mb and are relative to the Sscrofa April 2009 genome sequence assembly (Sscrofa9).

[2]Number of significant SNP in the interval.

[3]The most significant SNP within the chromosomal interval.

[4]Ib = allelic frequency in the founder Iberian boars.

[5]Ld = allelic frequency in the founder Landrace sows.

[6]The SNP that defined the interval were mapped in the Sscrofa10 assembly, but not in the Sscrofa9 (in which gene annotation is available).

[7]ψ = proportion of the phenotypic variance explained by the most significant SNP.

rapidly to dietary changes. Therefore, loci associated with linoleic acid content may have an effect on its absorption or transformation into longer-chain PUFA rather than on its biosynthesis.

Concordance of TAS with QTL

It was investigated herein whether the chromosomal regions delimited by the significant SNP described in the present study overlap with QTL previously reported in the IBMAP cross (Pérez-Enciso et al., 2000; Clop et al., 2003). In addition, the concordance between TAS and QTL for the profile of FA or other lipid traits deposited in the pig QTL database (http://www.animalgenome.org/cgi-bin/QTLdb/SS/index) was analyzed. Twenty-three of the 43 chromosomal intervals detected in this study (53.5%) showed positional concordance with QTL for FA composition and/or other lipid traits reported in the pig QTL database (Supplemental Table 4; http://journalofanimalscience.org). The chromosomal regions in which concordant QTL have been reported for fatty acid metabolism are discussed in detail below; a full description is in Supplementary Table 4 (http://journalofanimalscience.org).

SSC1

The 44.8–49.4 Mb region of SSC1 was associated with the percentages of palmitic acid, linoleic acid, SFA, and unsaturated indices (**UI**). Single nucleotide polymorphism ASGA0002580 showed the greatest significance for palmitic acid (P value = 3.4×10^{-5}; Table 2). This region overlaps with QTL reported for total lipid content in LM (Malek et al., 2001; Pig QTLdb Id: 79) and low density lipoprotein (**LDL**) cholesterol concentrations (Gallardo et al., 2008; Pig QTLdb Id: 7709).

SSC4

In SSC4, the 51.1–56.7 Mb region was associated with percentages of palmitoleic, oleic, and linoleic acids (Table 2), SNP ASGA0019639 being the most significantly associated for palmitoleic acid (P value= 2.8×10^{-5}), which explained 2.1% of the phenotypic variance of this trait (Table 2). In previous works, an F2-cross between Iberian (Guadyerbas) boars and Landrace sows was analyzed and QTL for backfat thickness (Varona et al., 2002), the percentage of oleic and linoleic acids, the double-bond index (**DBI**) and the peroxidability index (**PI**; Pérez-Enciso et al., 2000; Clop et al., 2003) were reported. Several positional candidate genes were analyzed for this QTL, including 2,4-dienoyl-CoA reductase (**DECR1**; Clop et al., 2003; Amills et al., 2005), FA binding protein 4 (**FABP4**; Mercadé et al., 2006), and FABP5 (Estellé et al., 2006; Ojeda et al., 2008). However, these studies indicated that the SNP analyzed in candidate genes are not the causative mutation of the detected QTL. An association analysis with FABP4–FABP5 haplotypes suggested that the QTL for backfat thickness is likely located between these 2 genes. Here, we have analyzed the IMF FA composition in BC1_LD animals (a backcross of 25% Iberian and 75% Landrace). Remarkably, a SNP (H3GA0012720; P value = 5.2×10^{-5}) within the significant chromosomal interval mapped at intron 1 of the FABP5 gene was only at 1.8 kb of distance from the SNP FABP5:g.3000T>G reported by Estellé et al. (2006). The observed concordance suggests a pleiotropic effect of this locus on both fat deposition and FA composition, which may affect both intramuscular fat and backfat thickness.

A different SNP-defined region (90.7–92.2 Mb) on SSC4 was associated with palmitic, palmitoleic, and linoleic acids and the C18:1(n-7)/C16:1(n-7) ratio. Many QTL have been reported in this region of SSC4. In the IBMAP cross, Pérez-Enciso et al. (2000) identified QTL for palmitic and linoleic acids and UI (Pig QTLdb Id: 530, 535, 541); Grindflek et al. (2001) for heptadecanoic acid; and Guo et al., (2009) for linoleic, MUFA, PUFA, and linolenic acids (Pig QTLdb Ids: 6392 ,6393, 6394, 6395). The most significant SNP within this interval was H3GA0013315 (P value = 5.7×10^{-6}), which is located at ≈1.1Mb of the apolipoprotein A-II gene (**APOA2**) and explained 1.2% of the phenotypic variance for this trait. This gene encodes the second most abundant protein of the high-density lipoprotein (**HDL**) particles and is one of the target genes of the PPAR signaling pathway (Blanco-Vaca et al., 2001). In addition, a polymorphism in the promoter region of the gene interacts with the saturated fat intake to influence body mass index in humans (Corella et al., 2009). The APOA2 gene has been linked to the plasma concentrations of the APOA2 protein and FFA in mice and humans (Warden et al., 1993). Upstream transcription factor 1 (**USF1**) is located within the same chromosomal interval. The protein encoded by this gene is one of the major components of the complexes that bind to the insulin response sequence of the FA synthase (**FAS**) promoter. Hence, it may play an important role in the regulation of FAS (Wong and Sul, 2010).

SSC8

A strong association signal was found in SSC8 (92.1–96.7 Mb) with palmitic acid, palmitoleic acid, SFA, and C16:1(n-7)/C16:0 and C18:1(n-7)/C16:1(n-7) ratios (Figure 1, Table 2). These results suggest the presence of

genes that regulate both the desaturation and elongation processes of FA. In this chromosomal region, SNP SIRI0000509 was the most significant for palmitic acid (P-value = 8.2×10^{-8}), palmitoleic acid (P-value = 3.7×10^{-9}) and the C18:1(n-7)/C16:1(n-7) ratio (P-value = $1.8x10^{-8}$). Conversely, the most significant SNP for the C16:1(n-7)/C16:0 ratio was ALGA0049269 (P-value = 8.3×10^{-7}), located at 1.8 Mb of SIRI0000509, according to Sscrofa9 assembly. It should be noted that SNP SIRI0000509 showed the greatest contribution to the phenotypic variance, explaining 6.1% of the palmitoleic acid variation (Table 2). A positional concordance was observed between this region and QTL for palmitic (Pig QTLdb Id: 469) and palmitoleic (Pig QTLdb Id: 470) acids reported in backfat (Clop et al., 2003). Recently, Yang et al. (2011), using a casual phenotype network, suggested that this QTL has a direct effect on palmitic and palmitoleic acids but only an indirect association with oleic acid in both the IBMAP and Duroc × Erhualian crosses. It is worth noting that a nonsynonymous polymorphism in the microsomal triglyceride transfer protein gene (**MTTP**) was associated with palmitic, palmitoleic, and oleic acids and also to the lipid transfer activity of the MTTP protein (Estellé et al., 2009). This gene is located within the QTL region and is involved in the transport of triglycerides into endoplasmic reticulum to form lipid droplets. However, it should be noted that 3 additional candidate genes are located proximal to the markers mentioned. The cytochrome P450 family 2 subfamily U polypeptide 1 gene (**CYP2U1**) is located ≈334 kb from SNP ALGA0049269 and ≈2.1 Mb from SNP SIRI0000509. This gene is involved in the arachidonic acid signaling pathway and catalyzes the hydroxylation of arachidonic, docosahexaenoic, and other long-chain FA (Chuang et al., 2004). In addition, the phospholipase A2 group XIIA (**PLA2G12A**), a gene involved in the same pathway, is located ≈863 kb from SNP ALGA0049269 and ≈935 kb from SNP SIRI0000509; PLA2G12A is a member of the phospholipase A2 (**PLA2**) family, which catalyzes the hydrolysis of the sn-2 position of membrane glycerophospholipids to liberate FFA and lysophospholipids (Murakami et al., 2010). Finally, the hydroxyacyl-CoA dehydrogenase gene (**HADH**) was located ≈278 kb from SNP ALGA0049269 and ≈2.1 Mb from SNP SIRI0000509. This gene is involved in the mitochondrial FA beta-oxidation pathway and has an elevated activity with medium-chain-length FA (Safran et al., 2010).

SSC14

The 28.4–37 Mb genomic region on SSC14 was associated with the SFA content, ASGA0062744 being the SNP showing the strongest association signal (P-value = 1.8×10^{-5}) and explaining 0.8% of the phenotypic variance (Table 2). In this position, QTL for PUFA (Pig QTLdb Id: 7446) and the PUFA:SFA ratio (Pig QTLdb Id: 7447) have previously been reported in LM (Sanchez et al., 2007). One of the most interesting candidate genes within this interval is the acetoacetyl-CoA synthetase gene (**AACS**), a member of the acyl-CoA synthetases that catalyze the activation of FA by esterification with CoA. This initial activation reaction is fundamental for both the anabolic and catabolic pathways of FA (Watkins et al., 2007).

SSC16

In SSC16 (39.6–45.2 Mb), a strong association signal for arachidic acid and the C20:1/C20:0 ratio was found (Figure 2, Table 2). A QTL for arachidic acid has been reported in the same region, both in LM and abdominal fat (Guo et al., 2009). In addition, a QTL for myristic acid was reported by Uemoto et al, (2009) in the same interval. The most significant SNP were DRGA0016176 for arachidic acid (P-value = 1.5×10^{-6}) and ALGA0090471 for the ratio of C20:1/C20:0 (P-value = 5.5×10^{-5}). Interestingly, within this interval, ≈221 kb from SNP ALGA0090471 and ≈2.9 Mb from SNP DRGA0016176, the ELOVL family member 7 elongation of long-chain FA (**ELOVL7**) gene is positioned. This gene is a member of the elongation of the very-long-chain FA gene family and mainly uses SFA and MUFA as substrates (Guillou et al., 2010).

It should be noted that full concordance is not expected between the chromosomal regions identified in this study and the QTL previously reported in the IBMAP cross. Previous FA QTL were measured in the backfat of F2 cross animals, whereas here IM FA in backcross animals have been analyzed. The method of analysis used was also different; previously reported results were obtained with linkage analyses and microsatellite markers, whereas here an association method was employed. Nevertheless, linkage disequilibrium and linkage signals are partly confounded because of the material analyzed (a backcross). This is particularly true if allelic frequencies are very different among founders, and this was observed for a large amount of the most significant SNP (Table 2).

Comparison between TAS and QTL of the pig QTL database showed common genomic regions for different, although related, traits, suggesting pleiotropic effects. Conversely, other studies proposed that the genetic architecture of FA composition is mainly different between tissues (Nii et al., 2006; Guo et al., 2009; Quintanilla et al., 2011), with the exception of the QTL on SSC16 for arachidic acid in abdominal fat and LM (Guo et al., 2009) and QTL on SSC6 and SSC7

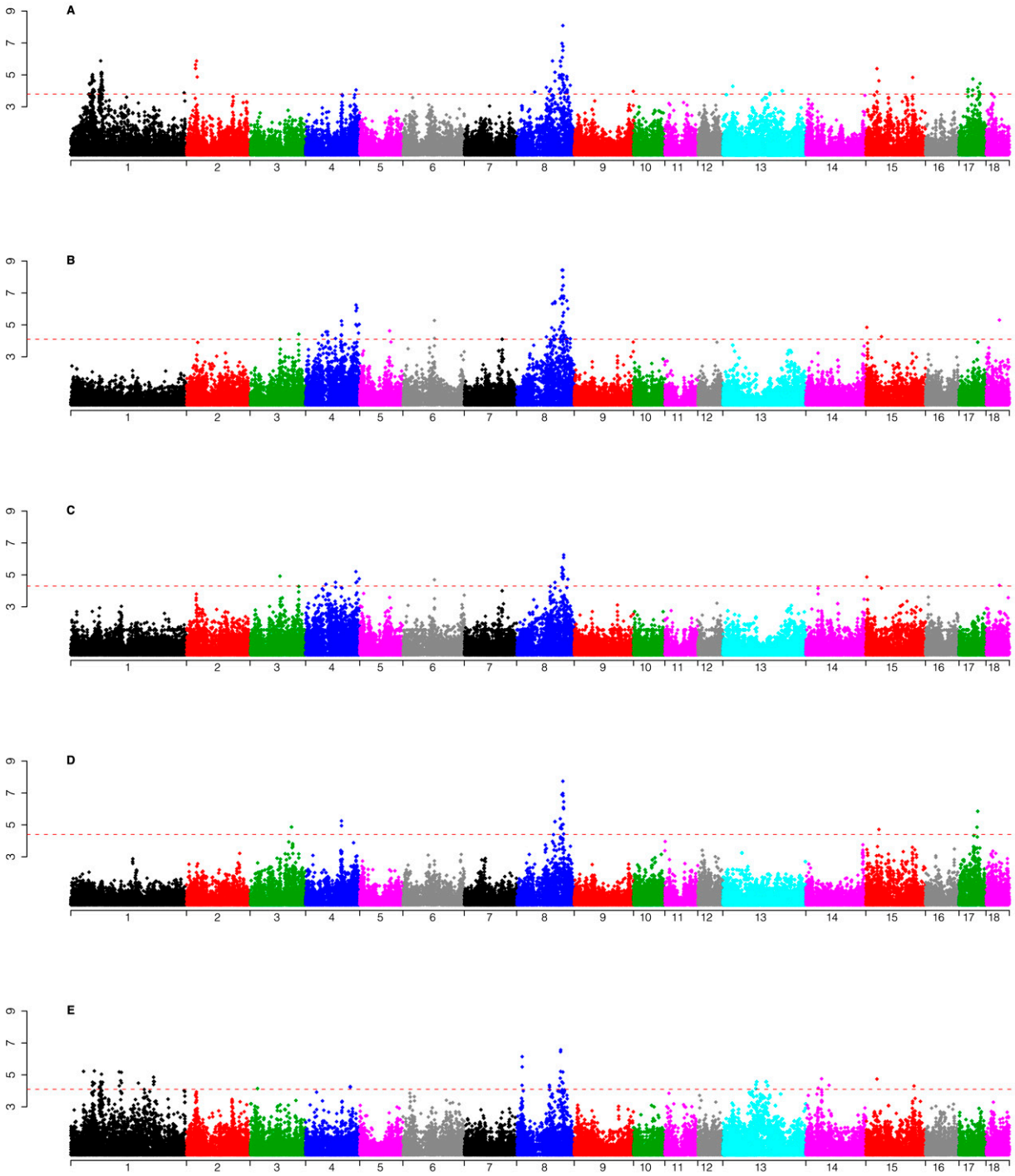

Figure 1. Manhattan plot of the genome-wide association study (GWAS) result for A) palmitic acid, B) palmitoleic acid, C) ratios of C16:1(n-7)/C16:0, D) ratios of C18:1(n-7)/C16:1(n-7), and E) SFA. The X-axis represents the chromosomes, and the Y-axis shows the –log10 (*P*-value). The horizontal, dashed line represents the cut-off of the significant association at the whole-genome stage (q-value < 0.05). See online version for figure in color.

(Quintanilla et al., 2011) for cis-vaccenic in two different muscles (gluteus medius and longissimus thoracis).

The results obtained here will enhance our knowledge of the genetic basis of FA composition and metabolism. This information can be used in the selection of commercial pig breeds through marker-assisted selection to improve meat quality without affecting production yield. In addition, pig is an excellent biomedical model, and the identification of genes and polymorphisms that regulate lipid metabolism in pigs will likely be important for the study of diseases such as obesity, diabetes, and atherosclerosis in humans.

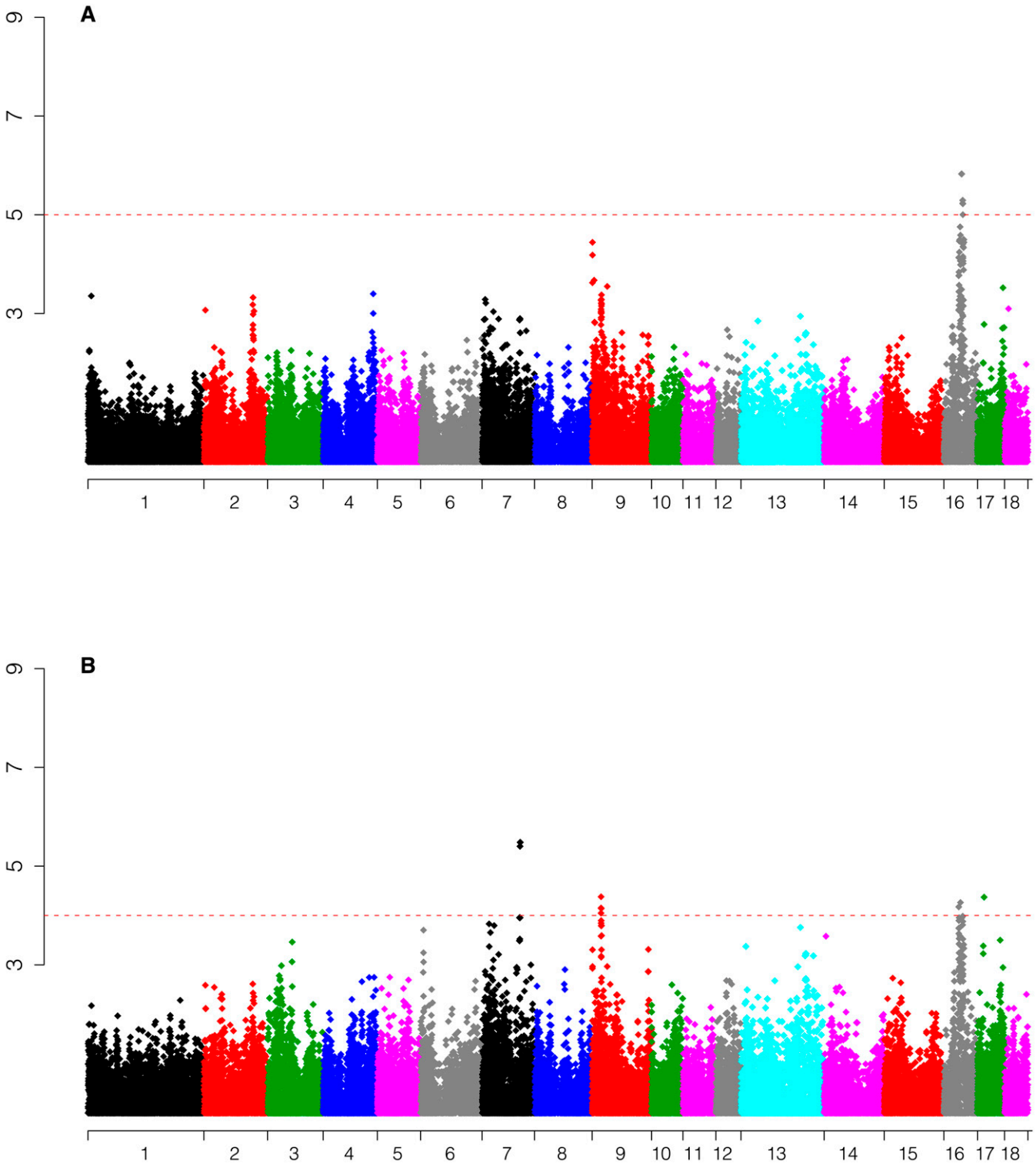

Figure 2. Manhattan plot of the genome-wide association study (GWAS) result for A) arachidic acid and B) ratios of C20:1/C20:0. The X-axis represents the chromosomes, and the Y-axis shows the –log10 (*P*-value). The horizontal, dashed line represents the cut-off of the significant association at the whole-genome stage (q-value < 0.05). See online version for figure in color.

Conclusions

A GWAS was performed using the Porcine SNP60K BeadChip genotypes and the profile of intramuscular FA composition and indices of FA metabolism in a backcross of Iberian × Landrace pigs. A total of 43 chromosomal regions containing 813 TAS were identified at a q-value < 0.05. The annotation of these genomic regions revealed genes that are related to FA metabolism. A relevant concordance was detected between associated genomic regions and previously reported QTL for FA and different lipid traits, mainly in SSC4, SSC8 and

SSC16. These results suggest pleiotropic effects of these QTL and contribute to our understanding of the genetic basis of FA composition.

LITERATURE CITED

Amills, M., O. Vidal, L. Varona, A. Tomás, M. Gil, A. Sánchez, and J. L. Noguera. 2005. Polymorphism of the pig 2,4-dienoyl CoA reductase 1 gene (DECR1) and its association with carcass and meat quality traits. J. Anim. Sci. 83:493−498.

Blanco-Vaca, F., J. C. Escola-Gil, J. M. Martín-Campos, and J. Julve. 2001. Role of apoA-II in lipid metabolism and atherosclerosis: Advances in the study of an enigmatic protein. J. Lipid Res. 42:1727−1739.

Casellas, J., J. L. Noguera, J. Reixach, I. Díaz, M. Amills, and R. Quintanilla. 2010. Bayes factor analyses of heritability for serum and muscle lipid traits in Duroc pigs. J. Anim. Sci. 88:2246−2254.

Chuang, S. S., C. Helvig, M. Taimi, H. A. Ramshaw, A. H. Collop, M. Amad, J. A. White, M. Petkovich, G. Jones, and B. Korczak. 2004. CYP2U1, a novel human thymus- and brain-specific cytochrome P450, catalyzes ω- and (ω-1)-hydroxylation of fatty acids. J. Biol. Chem. 279:6305−6314.

Clop, A., C. Ovilo, M. Pérez-Enciso, A. Cercos, A. Tomás, A. Fernandez, A. Coll, J. M. Folch, C. Barragan, I. Díaz, M. A. Oliver, L. Varona, L. Silio, A. Sánchez, and J. L. Noguera. 2003. Detection of QTL affecting fatty acid composition in the pig. Mamm. Genome 14:650−656.

Colman, E., S. Janssens, M. Ntawubizi, K. Raes, N. Buys, and S. De Smet. 2008. Genetic parameters for intramuscular fatty acid composition and metabolism in pork. 4 p. In: 54th International Congress of Meat Science and Technology, Cape Town, South Africa.

Corella, D., G. Peloso, D. K. Arnett, S. Demissie, L. A. Cupples, K. Tucker, C.-Q. Lai, L. D. Parnell, O. Coltell, Y.-C. Lee, and J. M. Ordovas. 2009. APOA2, dietary fat, and body mass index: Replication of a gene-diet interaction in 3 independent populations. Arch. Intern. Med. 169:1897−1906.

Estellé, J., A. I. Fernández, M. Pérez-Enciso, A. Fernández, C. Rodríguez, A. Sánchez, J. L. Noguera, and J. M. Folch. 2009. A non-synonymous mutation in a conserved site of the mttp gene is strongly associated with protein activity and fatty acid profile in pigs. Anim. Genet. 40:813−820.

Estellé, J., M. Pérez-Enciso, A. Mercadé, L. Varona, E. Alves, A. Sánchez, and J. M. Folch. 2006. Characterization of the porcine fabp5 gene and its association with the fat1 qtl in an iberian by landrace cross. Anim. Genet. 37:589−591.

Flicek, P., M. R. Amode, D. Barrell, K. Beal, S. Brent, Y. Chen, P. Clapham, G. Coates, S. Fairley, S. Fitzgerald, L. Gordon, M. Hendrix, T. Hourlier, N. Johnson, A. Kahari, D. Keefe, S. Keenan, R. Kinsella, F. Kokocinski, E. Kulesha, P. Larsson, I. Longden, W. McLaren, B. Overduin, B. Pritchard, H. S. Riat, D. Rios, G. Ritchie, M. Ruffier, M. Schuster, D. Sobral, G. Spudich, Y. A. Tang, S. Trevanion, J. Vandrovcova, A. Vilella, S. White, S. P. Wilder, A. Zadissa, J. Zamora, B. L. Aken, E. Birney, F. Cunningham, I. Dunham, R. Durbin, X. M. Fernandez-Suarez, J. Herrero, T. J. Hubbard, A. Parker, G. Proctor, J. Vogel, and S. M. J. Searle. 2010. Ensembl 2011. Nucleic Acids Res. 39:D800−806.

Gallardo, D., R. N. Pena, M. Amills, L. Varona, O. Ramirez, J. Reixach, I. Díaz, J. Tibau, J. Soler, J. M. Prat-Cuffi, J. L. Noguera, and R. Quintanilla. 2008. Mapping of quantitative trait loci for cholesterol, LDL, HDL, and triglyceride serum concentrations in pigs. Physiol. Genomics 35:199−209.

Grindflek, E., J. Szyda, Z. Liu, and S. Lien. 2001. Detection of quantitative trait loci for meat quality in a commercial slaughter pig cross. Mammalian Genome 12:299–304.

Guillou, H., D. Zadravec, P. G. Martin, and A. Jacobsson. 2010. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. Prog. Lipid Res. 49:186−199.

Guo, T., J. Ren, K. Yang, J. Ma, Z. Zhang, and L. Huang. 2009. Quantitative trait loci for fatty acid composition in longissimus dorsi and abdominal fat: Results from a White Duroc × Erhualian intercross F2 population. Anim. Genet. 40:185−191.

Henderson, C. R. 1975. Best linear unbiased estimation and prediction under a selection model. Biometrics 31:423−447.

Henderson, C. R. 1984. Applications of Linear Models in Animal Breeding. 462 p. University of Guelph, Guelph, Ontario, Canada.

Huang, D. W., B. T. Sherman, and R. A. Lempicki. 2008. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4:44−57.

Huang, D. W., B. T. Sherman, and R. A. Lempicki. 2009. Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 37:1−13.

Ihaka, R., and R. Gentleman. 1996. R: A language for data analysis and graphics. J. Comput. Graph. Stat. 5:299−314.

Ledur, M. C., N. Navarro, and M. Pérez-Enciso. 2010. Large-scale SNP genotyping in crosses between outbred lines: How useful is it? Heredity 105:173−182.

Mach, N., M. Devant, I. Díaz, M. Font-Furnols, M. A. Oliver, J. A. Garcia, and A. Bach. 2006. Increasing the amount of n-3 fatty acid in meat from young Holstein bulls through nutrition. J. Anim. Sci. 84:3039−3048.

Malek, M., J. C. M. Dekkers, H. K. Lee, T. J. Baas, K. Prusa, E. Huff-Lonergan, and M. F. Rothschild. 2001. A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig II. Meat and muscle composition. Mamm. Genome 12:637−645.

Meuwissen, T. H., and M. E. Goddard. 2000. Fine mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. Genetics 155:421−430.

Murakami, M., Y. Taketomi, Y. Miki, H. Sato, T. Hirabayashi, and K. Yamamoto. 2011. Recent progress in phospholipase A2 research: From cells to animals to humans. Prog. Lipid Res. 50:152−192.

Nii, M., T. Hayashi, F. Tani, A. Niki, N. Mori, N. Fujishima-Kanaya, M. Komatsu, K. Aikawa, T. Awata, and S. Mikawa. 2006. Quantitative trait loci mapping for fatty acid composition traits in perirenal and back fat using a Japanese wild boar × Large White intercross. Anim. Genet. 37:342−347.

Ntawubizi, M., E. Colman, S. Janssens, K. Raes, N. Buys, and S. De Smet. 2010. Genetic parameters for intramuscular fatty acid composition and metabolism in pigs. J. Anim. Sci. 88:1286−294.

Ojeda, A., J. Estellé, J. M. Folch, and M. Pérez-Enciso. 2008. Nucleotide variability and linkage disequilibrium patterns at the porcine FABP5 gene. Anim. Genet. 39:468−473.

Pérez-Enciso, M., A. Clop, J. L. Noguera, C. Ovilo, A. Coll, J. M. Folch, D. Babot, J. Estany, M. A. Oliver, I. Díaz, and A. Sánchez. 2000. A QTL on pig chromosome 4 affects fatty acid metabolism: Evidence from an Iberian × Landrace intercross. J. Anim. Sci. 78:2525−2531.

Pérez-Enciso, M., and I. Misztal. 2011. Qxpak.5: Old mixed model solutions for new genomics problems. BMC Bioinformatics 12:202.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. de Bakker, M. J. Daly, and P. C. Sham. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81:559−575.

Quintanilla, R., R. N. Pena, D. Gallardo, A. Cánovas, O. Ramirez, I. Díaz, J. L. Noguera, and M. Amills. 2011. Porcine intramuscular fat content and composition are regulated by quantitative trait loci with muscle-specific effects. J. Anim. Sci. 89:2963−2971.

Ramos, A. M., R. P. M. A. Crooijmans, N. A. Affara, A. J. Amaral, A. L. Archibald, J. E. Beever, C. Bendixen, C. Churcher, R. Clark, P. Dehais, M. S. Hansen, J. Hedegaard, Z.-L. Hu, H. H. Kerstens, A. S. Law, H.-J. Megens, D. Milan, D. J. Nonneman, G. A. Rohrer, M. F. Rothschild, T. P. L. Smith, R. D. Schnabel, C. P. Van Tassell, J. F. Taylor, R. T. Wiedmann, L. B. Schook, and M. A. M. Groenen. 2009. Design of a high density SNP genotyping assay in the pig using SNPs identified and characterized by next generation sequencing technology. PLoS One 4:e6524

Rothschild, M. F., and A. Ruvinsky, editors. 2011. The Genetics of the Pig, 2nd ed. CABI, Wallingford, Oxfordshire, UK.

Safran, M., I. Dalah, J. Alexander, N. Rosen, T. Iny Stein, M. Shmoish, N. Nativ, I. Bahir, T. Doniger, H. Krug, A. Sirota-Madi, T. Olender, Y. Golan, G. Stelzer, A. Harel, and D. Lancet. 2010. GeneCards version 3: The human gene integrator. Database (Oxford) 2010.

Sanchez, M. P., N. Iannuccelli, B. Basso, J. P. Bidanel, Y. Billon, G. Gandemer, H. Gilbert, C. Larzul, C. Legault, J. Riquet, D. Milan, and P. Le Roy. 2007. Identification of QTL with effects on intramuscular fat content and fatty acid composition in a Duroc × Large White cross. BMC Genet.8:55.

Sellier, P., L. Maignel, and J. Bidanel. 2010. Genetic parameters for tissue and fatty acid composition of backfat, perirenal fat and longissimus muscle in Large White and Landrace pigs. Animal 4:497–504.

Storey, J. D., and R. Tibshirani. 2003. Statistical significance for genomewide studies. Proc. Natl. Acad. Sci. USA 100:9440−9445.

Uemoto, Y., S. Sato, C. Ohnishi, S. Terai, A. Komatsuda, and E. Kobayashi. 2009. The effects of single and epistatic quantitative trait loci for fatty acid composition in a Meishan × Duroc crossbred population. J. Anim. Sci. 87:3470−3476.

Varona, L., C. Ovilo, A. Clop, J. L. Noguera, M. Pérez-Enciso, A. Coll, J. M. Folch, C. Barragán, M. A. Toro, D. Babot, and A. Sánchez. 2002. QTL mapping for growth and carcass traits in an Iberian × Landrace pig intercross: Additive, dominant and epistatic effects. Genet. Res. 80:145−154.

Warden, C. H., A. Daluiski, X. Bu, D. A. Purcell-Huynh, C. De Meester, B. H. Shieh, D. L. Puppione, R. M. Gray, G. M. Reaven, Y. D. Chen, and et al. 1993. Evidence for linkage of the apolipoprotein A-II locus to plasma apolipoprotein A-II and free fatty acid levels in mice and humans. Proc. Natl. Acad. Sci. USA 90:10886−10890.

Watkins, P. A., D. Maiguel, Z. Jia, and J. Pevsner. 2007. Evidence for 26 distinct acyl-coenzyme A synthetase genes in the human genome. J. Lipid Res. 48:2736−2750.

Wigginton, J. E., and G. R. Abecasis. 2005. PEDSTATS: Descriptive statistics, graphics and quality assessment for gene mapping data. Bioinformatics 21:3445−3447.

Wong, R. H. F., and H. S. Sul. 2010. Insulin signaling in fatty acid and fat synthesis: A transcriptional perspective. Curr. Opin. Pharmacol. 10:684−691.

Wood, J. D., M. Enser, A. V. Fisher, G. R. Nute, P. R. Sheard, R. I. Richardson, S. I. Hughes, and F. M. Whittington. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. 78:343−358.

Yang, B., N. Navarro, J. L. Noguera, M. Muñoz, T. F. Guo, K. X. Yang, J. W. Ma, J. M. Folch, L. S. Huang, and M. Pérez-Enciso. 2011. Building phenotype networks to improve QTL detection: A comparative analysis of fatty acid and fat traits in pigs. Journal of Anim. Breed. Genet. 128:329−343.

Yang, K. X., J. W. Ma, Y. M. Guo, T. F. Guo, Y. G. Zhao, N. S. Ding, M. Betti, G. S. Plastow, and L. S. Huang. 2010. Correlations between fat depot traits and fatty acid composition in abdominal subcutaneous adipose tissue and longissimus muscle: Results from a White Duroc × Erhualian intercross F2 population. J. Anim. Sci. 88:3538−3545.