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Porcine intramuscular fat content and composition are regulated by quantitative trait loci with muscle-specific effects[1]

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ABSTRACT: Intramuscular fat (IMF) storage is a biological process with a strong impact on nutritional and technological properties of meat and also with relevant consequences on human health. The genetic architecture of IMF content and composition phenotypes has been thoroughly studied in pigs through the identification of QTL and the estimation of genetic parameters. A question that has not been elucidated yet is if the genetic determinants of IMF-related phenotypes are muscle specific or, conversely, have broad effects on the whole skeletal muscle compartment. We have addressed this question by generating lipid QTL maps for 2 muscles with a high commercial value, gluteus medius (GM) and longissimus thoracis et lumborum (LTL), in a Duroc commercial population (n = 350). Our data support a lack of concordance between the GM and LTL QTL maps, suggesting that the effects of polymorphisms influencing IMF, cholesterol, and fatty acid contents are modulated to some extent by complex spatial factors related to muscle location, metabolism, and function. These results have important implications on the implementation of genomic selection schemes aimed to improve the lipid profile of swine meat.

Key words: fatty acid profile, genetics of muscle lipids, intramuscular fat, pig, quantitative trait locus

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INTRODUCTION

From a nutritional perspective, lipid deposition in skeletal muscle has a strong impact on sensory, nutritional, and technological properties of swine meat (Wood et al., 2008). Remarkably, several genome scans for the proportion and composition of intramuscular fat (**IMF**) have been performed in pigs (Nii et al., 2006; Sanchez et al., 2007; Guo et al., 2009), mainly because of the strong impact of these traits on meat quality. These QTL studies have delineated the genetic architecture of muscle lipid phenotypes, confirming pigs as a valuable model to analyze their inheritance and genetic variability. However, we do not know yet if the genetic mechanisms involved in IMF storage vary according to function, metabolic profile, and location of muscles. The main goal of this study was to determine if polymorphisms influencing IMF-related phenotypes act in a muscle-specific way or if they have broader effects on the skeletal muscle compartment. We have addressed this issue by comparing the lipid QTL landscapes of the gluteus medius (**GM**) and longissimus thoracis et lumborum (**LTL**) muscles of Duroc pigs with registers for multiple traits, including IMF. These 2 muscles are particularly suitable to our purposes because of their strong differences in IMF content (Casellas et al., 2010) and also because of their high commercial value.

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MATERIALS AND METHODS

The experimental procedures, traits recording, and blood sampling were approved by the Ethical Committee of the Institut de Recerca i Tecnologia Agroalimentàries (**IRTA**).

Animal Material and Phenotypic Information

We have employed as animal material a Duroc commercial line with increased IMF content. The resource population included 350 barrows distributed in 5 half-sib families. Barrows were kept under intensive standard conditions at the IRTA-Pig Control Centre facilities. Pigs were fed ad libitum with a standard diet containing a NE concentration of 2,450 kcal/kg that was replaced 30 to 40 d before slaughter with another diet of 2,375 kcal/kg. Body weight and backfat thickness were measured on a regular basis during the fattening period. A thorough description of the experimental population and management conditions can be found in Gallardo et al. (2008, 2009).

Pigs were slaughtered at around 190 d of age (approximately 122 kg of BW) following a commercial protocol. Several carcass traits were measured as described previously by Gallardo et al. (2008, 2009). Meat analyses were performed at IRTA–Centre of Food Technology on 200-g samples of the GM and LTL muscles. We measured IMF percentage by Near Infrared Transmittance (NIT, Infratec 1625, Tecator Hoganas, Sweden). Muscle cholesterol content was measured following Cayuela et al. (2003). 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, PUFA, n-3, and n-6 fatty acids (**FA**). The structure and basic statistics of the phenotypic measurements related to IMF percentage and composition are shown in Table 1.

Microsatellite Genotyping

A total of 116 informative microsatellites covering the 18 porcine autosomes were employed to genotype the 5 parental boars and their offspring. The list and location of these microsatellites are shown in Supplemental Table 1 (http://jas.fass.org/content/vol89/issue10/). Microsatellites were considered to be informative when at least 2 sires harbored heterozygous genotypes. The number of markers on each chromosome varied from 3 on SSC18 to 11 on SSC7, with a total length of 1,863.9 cM and an average interval space between markers of 19.2 cM. Genomic DNA was isolated from blood samples as described in Vidal et al. (2005). Genotyping procedures are fully detailed in Gallardo et al. (2008).

Statistical Analyses

Previous to the QTL genome scan, several exploratory analyses for the phenotypes reported in Table 1 were carried out. Nongenetic discrete effects (farm of origin, batch and box of fattening, and slaughter date) and covariates (BW, backfat thickness, and IMF) were tested by means of the GLM procedure (SAS Inst. Inc., Cary, NC). Additionally, residual correlations among all analyzed phenotypes were computed after adjusting for significant environmental effects, using the CORR procedure of SAS.

Quantitative trait loci analyses for the 10 phenotypes depicted in Table 1 were performed across the 18 autosomes using the regression approach for outbred half-sib families described by Knott et al. (1996). These analyses were performed in the whole population and taking into consideration 5 within-sire allele substitution effects at each position of the genome. Subsequently, a second analysis was performed for each half-sib family separately, to detect QTL with different phases of linkage disequilibrium across families. The models assumed in the QTL scan, on the basis of significant effects previously detected, were

in the whole-population analyses:

$$y_{ij} = \mu + b_i + \beta\,\mathrm{cov}_{ij} + \sum_{\mathrm{sire}=1}^{5} \alpha_{sire}\, p_{ij(sire)} + e_{ij},$$

in the within-family analyses:

$$y_{ij} = \mu + b_i + \beta\,\mathrm{cov}_{ij} + \alpha\, p_{ij} + e_{ij},$$

where y_{ij} represents the phenotypic observation of individual j; μ is the common mean; b_i is the systematic effect of ith batch of fattening (4 levels); β and cov_{ij} are, respectively, the regression coefficient and a covariate that changes depending on the trait under consideration: backfat thickness (or BW) for IMF, or IMF for FA content; α is the regression coefficient of phenotypes onto the probability of having inherited a given allele from the common parent (in the case of whole-population analyses, nested within sire); p_{ij} is the probability of individual k inheriting a given allele from its common parent, calculated at any putative location in the genome by the multipoint approach developed by Knott (2005); and e_{ij} represents the residual effect.

Analyses were performed by means of the QTL express software (Seaton et al., 2002), available at http://qtl.cap.ed.ac.uk/. An F-ratio with 5 (whole-population analyses) or 1 (within-family analyses) df in the numerator was computed at each cM by comparing the QTL model to an equivalent model without any linked QTL.

Chromosome-wide significance thresholds were determined empirically by data permutation (Churchill and

Table 1. Descriptive statistics of lipid traits recorded in 2 muscles from a Duroc commercial population

	Gluteus medius		Longissimus thoracis et lumborum	
Trait[1]	Mean	SD	Mean	SD
Intramuscular fat, %	5.21	2.05	3.91	1.53
Cholesterol content, mg/g	64.65	11.06	58.60	9.45
Myristic FA, %	1.39	0.23	1.37	0.28
Palmitic FA, %	23.22	1.42	23.47	1.63
Palmitoleic FA, %	2.82	0.49	2.98	0.59
Stearic FA, %	11.21	1.12	11.71	1.21
Oleic FA, %	35.10	4.48	34.92	5.18
cis-Vaccenic FA, %	4.06	0.31	4.28	0.34
Linoleic FA, %	14.95	4.10	14.16	5.11
Arachidonic FA, %	3.20	1.55	3.53	1.81

[1]FA: fatty acid.

Doerge, 1994), performing a total of 10,000 permutations to obtain the distribution under the null hypothesis (no linked QTL). As in Gallardo et al. (2008), common *F*-values (2.6 and 7.5 in the whole-population and within-family analyses, respectively) were taken as the suggestive threshold ($P < 0.05$) for all chromosome-trait analyses. Genome-wide significance thresholds were approximated from Bonferroni correction as described in Gallardo et al. (2008). The resulting nominal *P*-values corresponding to 95 and 99% genome-wide significance levels were $P = 0.0009$ and $P = 0.0002$, respectively.

Search for Candidate Genes

A search for candidate genes mapping to the detected QTL was carried out with the BioMart tool of Ensembl Genome Browser (http://www.ensembl.org/biomart/index.html). We used the Ensembl Genes 62 database for *Sus scrofa* genes. Additionally, we used the *Homo sapiens* database GRCh37.p3 to look for other genes mapping to human genome regions orthologous to the QTL. Those genes functionally related to lipid metabolism and fat deposition were retained as functional and positional candidate genes.

RESULTS

Phenotypic Means and Correlations of Lipid Traits in the GM and LTL Muscles

Mean IMF percentage, muscle cholesterol content, and FA composition of GM and LTL muscles are reported in Table 1. Lipid deposition was clearly different in both muscles, with the GM muscle displaying a significantly greater IMF percentage than LTL (GM: 5.21% vs. LTL: 3.91%; $P < 0.0001$ after a *t*-test), accordingly with results reported in previous studies (e.g., Kim et al., 2008). Cholesterol content was also remarkably greater in GM than in LTL (64.65 vs. 58.60 mg/g; $P < 0.0001$ after a *t*-test). Conversely, no relevant differences between muscles were observed for FA composition. As expected, oleic acid was the major FA (35.10 and 34.92% in GM and LTL, respectively), followed by palmitic (23.22 and 23.47%) and linoleic (14.95 and 14.16%) FA.

Correlation coefficients between muscle lipid traits followed the same qualitative and quantitative trends in both muscles (Supplemental Table 2; http://jas.fass.org/content/vol89/issue10/). In this way, IMF content correlated significantly with the percentages of most relevant SFA and MUFA. In general, these correlations took moderate positive values (from 0.37 to 0.46) for SFA (myristic, palmitic, and stearic) and MUFA (oleic and palmitoleic) percentages. In contrast, correlations between IMF and PUFA (linoleic and arachidonic) were negative (between −0.44 and −0.47). Relationships between FA percentages were consistent with these trends. Hence, very strong and negative correlations were found between oleic and PUFA [e.g., linoleic ($r = -0.93, -0.96$) and arachidonic ($r = -0.91, -0.94$)], whereas oleic showed positive correlations with SFA (e.g., myristic, palmitic, and stearic). A remarkable result was the absence of significant correlations between muscle cholesterol content and other lipid content and composition traits (only a few weak and probably spurious correlations with myristic, palmitic, and palmitoleic FA were detected in the LTL muscle). This result is very relevant because it suggests that selection for decreasing muscle cholesterol content would not be expected to have any impact on IMF percentage and composition.

Correlation analysis of lipid composition traits between GM and LTL muscles (Supplemental Table 2; http://jas.fass.org/content/vol89/issue10/) evidenced a highly significant and positive ($r = 0.71$; $P < 0.0001$) relationship between IMF content of both muscles. Correlations between LTL and GM FA percentages were always positive and highly significant, but took moderate values (from 0.28 to 0.58). In strong contrast, GM and LTL cholesterol contents did not correlate significantly.

Table 2. Most significant QTL detected in the whole population analyses for intramuscular fat (IMF), muscle cholesterol content, and percentage of fatty acids in 2 muscles

Chromosome	Trait	Position, cM	*F*-value[1]	Nominal *P*-value	Within-family substitution effect[2]				
					Family 1	Family 2	Family 3	Family 4	Family 5
Gluteus medius									
SSC7	IMF_{covBW}[3]	133	3.75†	0.0025	−2.787**	0.411	−0.627	−0.254	−0.850
SSC7	*cis*-Vaccenic	82	3.83†	0.0022	−0.111	0.125	0.254**	0.059	0.106
Longissimus thoracis et lumborum									
SSC3	IMF_{covBFT}[3]	86	4.72*	0.0003	0.624	−0.868**	−0.012	−0.218	−1.276**
SSC11	CHOL[4]	64	4.47*	0.0006	12.129**	3.344	−3.687	0.766	−9.324**
SSC12	SFA[4]	73	4.29*	0.0008	−1.889*	1.646*	0.250	−1.635**	0.314
SSC14	*cis*-Vaccenic	91	3.98†	0.0016	−0.318*	0.276**	0.182	0.194	0.022

[1]*F*-value column: *$P < 0.05$ at genome-wide level; †$P < 0.01$ at chromosome-wide level.

[2]Within-family substitution effect columns: *$P < 0.05$; **$P < 0.01$ after a *t*-test.

[3]IMF_{covBW} and IMF_{covBFT}: when the covariates BW or backfat thickness are included in the QTL analyses for IMF, respectively.

[4]CHOL = muscle cholesterol content (mg/g); SFA = total percentage of SFA.

Identification of QTL for GM and LTL Muscle Lipid Content and Composition Traits

The most significant QTL (either at genome-wide level or $P < 0.01$ at the chromosome-wide level) in the whole-population and the within-family genome scans are shown in Tables 2 and 3, respectively. Supplemental Tables 3 and 4 (http://jas.fass.org/content/vol89/issue10/) gather the rest of the QTL that were significant ($P < 0.05$) at the chromosome-wide level.

The whole-population genome scan allowed us to identify 3 and 2 significant QTL (Table 2 and Supplemental Table 3; http://jas.fass.org/content/vol89/issue10/) affecting IMF of GM (in SSC3, SSC6, and SSC7) and LTL (in SSC3 and SSC13) muscles, respectively. All these QTL happened to be significant, irrespective of the covariate used in the analysis model (BW or backfat thickness), but only the SSC3 QTL for IMF in the LTL muscle reached the genome-wide significance level (Table 2). One QTL at SSC1 influencing IMF of the GM muscle was also detected (Supplemental Table 3; http://jas.fass.org/content/vol89/issue10/), but this QTL disappeared when the covariate backfat thickness was included in the analysis. Besides, 1 genome-wide (SSC11, 64 cM; Table 2) and 5 chromosome-wide (SSC1, SSC6, SSC8, SSC14, and SSC18; Supplemental Table 3; http://jas.fass.org/content/vol89/issue10/) significant QTL were found for muscle cholesterol content of LTL, whereas for GM we only detected a single chromosome-wide significant QTL at SSC11. With regard to FA composition, a total of 21 and 11 suggestive QTL affecting the percentages of several FA (or related indexes) were found for GM and LTL, respectively (Table 2 and Supplemental Table 3; http://jas.fass.org/content/vol89/issue10/). Among these, only the *cis*-vaccenic QTL found on SSC6 (Supplemental Table 3; http://jas.fass.org/content/vol89/issue10/) and SSC7 (GM: Table 2, LTL: Supplemental Table 3; http://jas.fass.org/content/vol89/issue10/) showed some level of positional concordance between the 2 analyzed muscles. Finally, it is worth mentioning 1 genome-wide significant SSC12 QTL for SFA content at LTL (73 cM, Table 2).

Whole-population analyses of mean substitution QTL effects showed important differences among paternal families. In most cases, only 1 or 2 of the 5 half-sib families showed a mean allelic effect significantly different from zero, and the magnitude or direction or both of these effects was not always concordant across families. This suggested the existence of a differential segregation of the causal mutations associated with these QTL in each family. In light of these results, we decided to perform a within-family QTL analysis.

This second approach resulted in the identification of 4 QTL highly significant at the genome-wide level ($P < 0.01$) as shown in Table 3. These QTL had effects on IMF percentage (SSC7), *cis*-vaccenic (SSC7, Figure 1) and palmitic (SSC18) contents of GM, and cholesterol content of LTL (SSC6). Moreover, genome-wide significant ($P < 0.05$) QTL were found for IMF and *cis*-vaccenic contents (both at SSC3, 15 cM) as well as for myristic percentage (SSC5) of GM, *cis*-vaccenic and stearic contents of LTL (SSC6 and 14, respectively), and SFA percentages in GM (SSC18) and LTL (SSC12). Another genome-wide significant QTL affecting LTL IMF at SSC1 was only identified when BW was considered as a covariate (Table 3). The location of this QTL coincided with a chromosome-wide significant QTL affecting IMF of GM. Both QTL lost their statistical significance when backfat thickness, instead of BW, was considered as a covariate in the model. Additionally, IMF QTL significant at the chromosome-wide level were identified for GM (SSC6, SSC11, and SSC15; Supplemental Table 4; http://jas.fass.org/content/vol89/issue10/) and for LTL (SSC3, Table 3; SSC13 and SSC17, Supplemental Table 4; http://jas.fass.org/content/vol89/issue10/) muscles.

The within-family analyses also revealed the existence of 1 (Supplemental Table 4; http://jas.fass.org/content/vol89/issue10/) and 6 (Table 3 and Supplemental Table 4; http://jas.fass.org/content/vol89/issue10/) QTL for

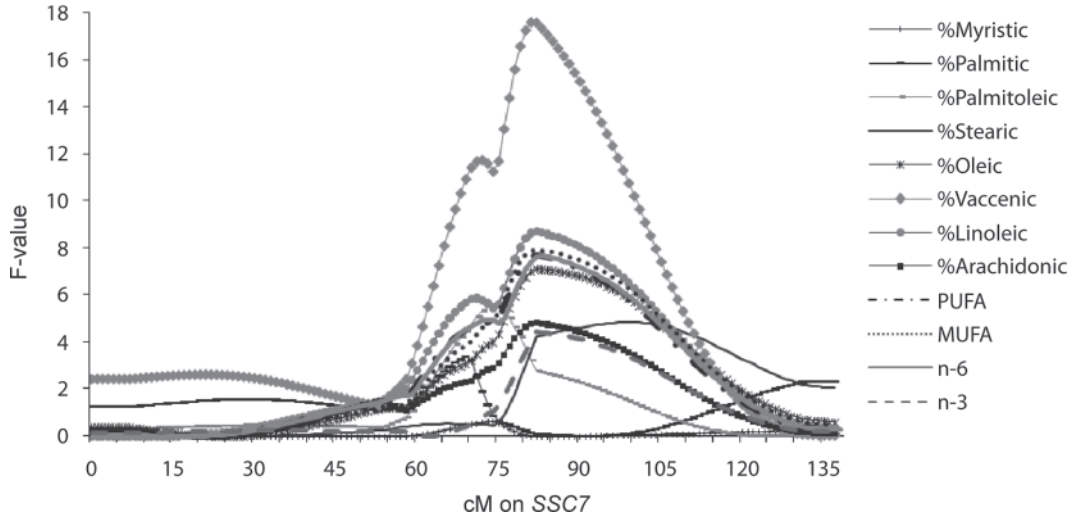

Figure 1. Profile of *F*-ratios when a QTL is fitted (model with QTL vs. no QTL model) throughout SSC7 for fatty acids profile of gluteus medius muscle (family 3).

Table 3. Most significant QTL detected in the within-family analyses for intramuscular fat (IMF), muscle cholesterol content, and percentage of fatty acids in 2 muscles

Chromosome	Trait	Family	Position, cM	*F*-value	Nominal *P*-value	Mean effect (SD)
Gluteus medius						
SSC1	IMF_{covBW}[1]	4	63	10.10†	0.0022	−1.553 (0.489)
SSC3	IMF_{covBW}	3	28	12.90*	0.0006	2.035 (0.567)
	IMF_{covBFT}[1]		15	12.64*	0.0007	1.721 (0.484)
SSC6	IMF_{covBFT}	1	92	11.70†	0.0014	2.653 (0.776)
SSC7	IMF_{covBW}	1	133	16.11**	0.0002	−2.750 (0.685)
	IMF_{covBFT}		133	16.48**	0.0002	−2.798 (0.689)
SSC3	*cis*-Vaccenic	2	15	12.95*	0.0005	0.207 (0.058)
SSC5	Myristic	4	94	13.98*	0.0004	0.346 (0.092)
SSC7	*cis*-Vaccenic	3	81	17.60**	0.0001	0.250 (0.060)
SSC13	Stearic	5	73	11.82†	0.0011	−0.758 (0.220)
SSC18	Palmitic	3	39	15.67**	0.0002	0.912 (0.230)
	SFA[2]	3	40	14.46*	0.0004	1.302 (0.342)
Longissimus thoracis et lumborum						
SSC1	IMF_{covBW}	4	66	13.31*	0.0005	−1.025 (0.281)
SSC3	IMF_{covBW}	2	33	11.57†	0.0010	−1.123 (0.330)
	IMF_{covBFT}		53	10.24†	0.0019	−1.149 (0.359)
	IMF_{covBFT}	5	85	10.73†	0.0018	−1.227 (0.374)
SSC6	CHOL[2]	2	35	16.57**	0.0001	14.063 (3.455)
SSC11	CHOL	5	71	11.25†	0.0014	−10.020 (2.987)
SSC14	CHOL	4	40	11.42†	0.0012	13.095 (3.874)
SSC6	*cis*-Vaccenic	2	95	14.56*	0.0003	−0.275 (0.072)
SSC7	*cis*-Vaccenic	2	75	11.19†	0.0012	0.230 (0.069)
SSC12	SFA	4	74	13.31*	0.0005	−1.534 (0.421)
SSC13	Stearic	5	56	11.76†	0.0011	−1.028 (0.300)
SSC14	*cis*-Vaccenic	2	68	11.24†	0.0012	0.410 (0.122)
	Stearic	5	85	12.28*	0.0009	−1.110 (0.317)
SSC16	Myristic	1	18	11.24†	0.0017	0.185 (0.055)
	Myristic	3	43	11.48†	0.0012	−0.178 (0.053)

[1]IMF_{covBW} and IMF_{covBFT}: when the covariates BW or backfat thickness are included in the QTL analyses for IMF, respectively.

[2]CHOL = muscle cholesterol content (mg/g); SFA = total percentage of SFA.

†$P < 0.01$ at chromosome-wide level; *$P < 0.05$ at genome-wide level; **$P < 0.01$ at genome-wide level.

muscle cholesterol contents of GM and LTL, respectively. Moreover, 28 and 17 chromosome-wide significant QTL for GM and LTL FA composition traits were found, respectively (Table 3 and Supplemental Table 4; http://jas.fass.org/content/vol89/issue10/). Although most of these suggestive QTL corresponded to QTL formerly reported in the whole-population analyses, the within-family approach allowed us to find new loci segregating exclusively in several of the analyzed families. A list of positional and functional candidate genes for the QTL identified in the current work, together with the metabolic processes in which these genes are involved, is presented in Supplemental Table 5 (http://jas.fass.org/content/vol89/issue10/).

DISCUSSION

Lack of Concordance Between QTL Maps for GM and LTL Muscles Suggests the Existence of Muscle-Specific Genetic Factors Regulating IMF Content and Composition

In the current work, we have identified several genome-wide significant QTL for IMF, FA composition, and cholesterol content of 2 muscles using a Duroc commercial line, along with many suggestive QTL. Certain discrepancies between the whole-population and within-family analyses have been observed, probably because of differences in family sizes, linkage phases, parent informativeness, and systematic effect estimates. This statistical issue has been thoroughly discussed by Gallardo et al. (2008) and, on a broader perspective, by Knott (2005). As a whole, the comparative QTL analysis between our study and previous reports has highlighted several coincidences of interest, and the number and magnitude of muscle lipid QTL we have found are similar to those reported in several F_2 divergent crosses (Malek et al., 2001; Clop et al., 2003; Nii et al., 2006; Muñoz et al., 2007; Sanchez et al., 2007; Guo et al., 2009). These results combined with the medium-to-high heritabilities estimated in the same population by Casellas et al. (2010) suggest that this commercial Duroc line still retains a significant fraction of genetic variability for muscle lipid content and composition.

In contrast with other studies performed in pigs, we have generated lipid QTL maps, in the same resource population, for 2 muscles with different IMF contents. This approach enabled us to investigate if polymorphisms with effects on IMF content and composition act in a muscle-specific manner or not. Of notice, residual phenotypic correlations between IMF composition traits of both muscles (i.e., LTL vs. GM), happened to be just moderate (although very significant), suggesting that GM and LTL lipid phenotypes are not identically modulated by the same combination of genetic and environmental factors. Consistently, genome scans clearly showed that GM and LTL lipid QTL maps are dramatically different. This result suggests that the sets of genetic polymorphisms influencing lipid storage in both muscles are not coincident. Only 3 QTL shared a similar location in GM and LTL: 2 located on SSC1 (63 to 66 cM) and SSC3 (28 to 33 cM) with effects on IMF content, and 1 mapping to SSC7 (75 to 81 cM) influencing *cis*-vaccenic FA content. The remaining QTL showed striking differences among muscles with regard to their genomic location and significance. For instance, the most significant QTL affecting IMF of GM mapped to SSC7 (133 cM, Table 2) in a genomic region where many IMF QTL had been previously found in other populations (de Koning et al., 2000; Szyda et al., 2002; Sato et al., 2003). In contrast, none of the LTL QTL with chromosome- or genome-wide effects on IMF mapped to SSC7. Interestingly, investigations carried out in pigs have also highlighted the existence of differences in the genetic determinism of adipose tissue lipid composition. In this way, Nii et al. (2006) showed that lipid QTL in backfat (SSC1p, 1q, 4, 5, 9, 15, and 17) and perirenal fat (SSC2, 3, 4, 5, 6, 14, 16, and X) do not coincide. Similarly, Guo et al. (2009) demonstrated a clear lack of correspondence between QTL influencing FA composition in abdominal fat vs. LTL intramuscular fat. In mouse, discrepancies (of varying magnitude) between QTL locations have been detected when comparing the genetic architecture of gonadal vs. retroperitoneal fat (Reed et al., 2006), epididymal vs. mesenteric fat (Kobayashi et al., 2010), and weight of 5 different muscles (Lionikas et al., 2010).

Recently, Musunuru et al. (2010) elucidated one of the possible mechanisms by which spatial factors (such as organ, tissue, and body location) shape genetic determinism. These authors identified the causal polymorphism (rs12740374) of 2 1p13 QTL for plasma LDL concentration and myocardial infarction, and demonstrated that its minor allele created a new CCAAT/enhancer binding protein site between genes *CELSR2* and *PSRC1*. This transcription factor, in turn, altered the expression of *SORT1*, which modulates the hepatic secretion of VLDL. Importantly, this causal mutation influenced *SORT1* mRNA abundance in the liver but not in other tissues where this gene is also expressed. In the specific context of muscle physiology, Hamelin et al. (2006) demonstrated that a 3′UTR mutation in the myostatin gene of Texel sheep leads to the hypertrophy of the semimembranosus and tensor fasciae latae muscles, but not of the vastus medialis. Similarly, in double-muscled cattle, the myostatin genotype is associated with hypertrophy of semimembranosus and hypotrophy of vastus medialis (Dumont, 1980).

A plausible hypothesis that arises from our investigation is that the lack of concordance between GM and LTL QTL maps might be partly explained by genetic factors related with mRNA expression. Previous studies performed in rats indicated the existence of differences in the gene expression profiles of certain muscles types (Donsmark et al., 2002; Janovská et al., 2010). Moreover, transcriptomic analyses performed in pigs are closely aligned with these results. In this way, Li et

al. (2010) compared the mRNA expression profiles of red and white skeletal muscles of Meishan pigs and detected differential expression of genes that participate in multiple signaling cascades related to embryogenesis, morphogenesis, cell growth and differentiation, extracellular matrix, or insulin metabolism. Other studies focused on individual genes directly related to lipid metabolism and fat deposition have also shown gene expression differences between porcine skeletal muscles. This is the case of *H-FABP* and *LEPR* genes, whose mRNA abundance differed in the LM and semimembranosus muscles of several pig breeds (Tyra et al., 2011). Stachowiak et al. (2010) also found mRNA expression differences between these muscles for the *ADIPOR1* gene in Polish Large White and Landrace pigs, whereas differences were negligible in the Duroc breed. Altogether, these results suggest that the penetrance of causal mutations (and hence QTL) with effects on growth or metabolism or both is modulated by spatial factors related with tissue and body location. As previously discussed, these factors may likely be the underlying cause of the differences we have observed between the LTL and GM lipid QTL maps. A crucial point to analyze this issue from a molecular perspective would be to find out if there is some level of interaction between muscle mRNA expression patterns and the phenotypic expression of QTL (i.e., to elucidate if QTL penetrance is influenced by the specific transcriptomic landscape of each muscle and vice versa). Obviously, this landscape would be integrated not only by mRNA but also by any other type of RNA with regulatory functions, a feature that is expected to substantially increase the complexity of the gene QTL expression network of interactions.

We have generated a list of candidate genes related to lipid metabolism that are located within the confidence intervals of the IMF and FA composition QTL (Supplemental Table 5; http://jas.fass.org/content/vol89/issue10/). This might serve as a source of information to fine map and identify the causal mutations explaining the segregation of these QTL. However, as stated by Switonski et al. (2010), the candidate gene approach has not been very successful in identifying polymorphisms with consistent effects on pig phenotypes. Several reasons explain this negative outcome (Switonski et al., 2010). First, the resolution of QTL studies is very low, yielding confidence intervals that span hundreds of centimorgans. This drawback might be overcome by employing high-throughput genotyping platforms yielding genotypes of thousands of closely spaced markers. Second, causal mutations might be located at noncoding regions that are difficult to characterize at the molecular level because the pig genome is currently in a draft state. Although many SNP have been detected at exonic regions of lipid metabolism genes (Switonski et al., 2010), there is scarce information about the variability of intronic, promoter, and intergenic regions. These regions might contain regulatory mutations affecting gene expression and phenotypic variation of traits of economic interest, as demonstrated by Van Laere et al. (2003). Finally, the phenotypic effects of mutations, as we have discussed previously, might be relatively small and strongly determined by the interaction with genetic (i.e., epistasis) and environmental factors (including the transcriptomic landscape). In the light of the above, complementing the candidate gene approach with other methods such as ultra-dense mapping with SNP chips and massive sequencing of target QTL regions as well as gene expression analysis will be essential to disentangle the genetic factors that modulate IMF content and composition.

The Genetic Architecture of Lipid Traits Differs Among Muscles: Implications on Human Health and Pig Production

Deciphering the genetic architecture of muscle lipid deposition in pigs might have interesting implications in human health because excessive intramuscular triglyceride accumulation has been associated with the development of insulin resistance (Kiens, 2006). This association is probably due to the noxious effects of certain lipid metabolites, such as diacylglycerol and long-chain acyl-CoA, which impair the insulin-stimulated glucose transport (Kiens, 2006). Our data provide strong evidence that polymorphisms regulating IMF content and composition in pigs have different effects depending on the muscle under consideration, suggesting that their penetrance might be modulated in a complex way. Similar results have been obtained in other tissues (Nii et al., 2006; Guo et al., 2009) and species (Reed et al., 2006; Kobayashi et al., 2010; Lionikas et al., 2010), implying that this is a very general phenomenon. This spatial source of variability should be taken into account when searching for causal mutations conferring susceptibility to phenotypes of clinical interest (e.g., insulin resistance) in human populations and model organisms.

The dissection of the genetic determinants influencing IMF content and composition might also be fundamental to improve the organoleptic and nutritional properties of swine meat (Wood et al., 2008). The selection of appropriate breeding objectives for these traits, however, is hindered by the existence of a certain conflict between nutritional and technological goals. From a nutritional perspective, increased concentrations of long-chain n-3 PUFA combined with a concomitant reduction in IMF and SFA would be beneficial to pig meat consumers because unsaturated FA have cardiovascular protective effects (McDonald, 1991; Harris et al., 2007). Reducing meat cholesterol content might be also beneficial in this regard. However, IMF content is positively associated with an increased tenderness and juiciness of cooked meat, although the strength of this association varies among studies (Wood et al., 2008). The proportion and characteristics of IMF are also key factors affecting water migration, ripening, storage stability, and flavor of fresh meat and dry-cured prod-

ucts (Chizzolini et al., 1998, López-Bote, 1998). With regard to IMF composition, PUFA are unfavorably associated with meat quality because they decrease fat melting point, and more important, they can be easily oxidized, producing a rancid odor and taste (Wood et al., 2008). Conversely, MUFA and SFA have a positive influence on the sensory properties of pork such as flavor and firmness (Chizzolini et al., 1998; Carrapiso et al., 2003).

In summary, selection aimed to simultaneously improve sensorial, technological, and nutritional properties of pig meat is very far from being a simple matter, and multiple, and often opposing, factors need to be reconciled when planning selection schemes. Even more, our work highlights the existence of an additional layer of complexity by showing that many QTL with effects on IMF content and composition are muscle-specific. These findings pose unresolved questions about the consequences of implementing genomic selection to improve meat lipid traits in pigs because polymorphisms might have different effects depending on the muscle under consideration. In this way, selecting a given mutation might have beneficial effects in certain muscles but not in others. To overcome this problem, genomic selection should be mostly focused on those QTL that have consistent and important effects across muscles.

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