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Mapping of quantitative trait loci for cholesterol, LDL, HDL, and triglyceride serum concentrations in pigs

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1Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra; 2Genètica i Millora Animal, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Lleida; 3Selección Batallé S.A., Riudarenes; 4Tecnologia dels Aliments and 5Control i Avaluació de Porcí, IRTA, Monells; and 6Laboratori d’Anàlisi Clíniques, Hospital de Palamós, Palamós, Spain

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Gallardo D, Pena RN, Amills M, Varona L, Ramírez O, Reixach J, Díaz I, Tibau J, Soler J, Prat-Cuffi JM, Noguera JL, Quintanilla R. Mapping of quantitative trait loci for cholesterol, LDL, HDL and triglyceride serum concentrations in pigs. Physiol Genomics 35: 199–209., 2008. First published September 23, 2008; doi:10.1152/physiolgenomics.90249.2008.—The fine mapping of polymorphisms influencing cholesterol (CT), triglyceride (TG), and lipoprotein serum levels in human and mouse has provided a wealth of knowledge about the complex genetic architecture of these traits. The extension of these genetic analyses to pigs would be of utmost importance since they constitute a valuable biological and clinical model for the study of coronary artery disease and myocardial infarction. In the present work, we performed a whole genome scan for serum lipid traits in a half-sib Duroc pig population of 350 individuals. Phenotypic registers included total CT, TG, and low (LDL)- and high (HDL)-density lipoprotein serum concentrations at 45 and 190 days of age. This approach allowed us to identify two genomewide significant quantitative trait loci (QTL) for HDL-to-LDL ratio at 45 days (SSC6, 84 cm) and for TG at 190 days (SSC4, 23 cm) as well as a number of chromosomewide significant QTL. The comparison of QTL locations at 45 and 190 days revealed a notable lack of concordance at these two time points, suggesting that the effects of these QTL are age specific. Moreover, we have observed a considerable level of correspondence among the locations of the most significant porcine lipid QTL and those identified in humans. This finding might suggest that, in mammals, diverse polymorphisms located in a common set of genes are involved in the genetic variation of serum lipid levels.

Identification of the genetic factors regulating the concentrations of serum lipoproteins has been a major goal in the prevention and treatment of atherosclerosis. A tremendous effort has been made in this direction by using approaches that combine whole genome scans with information generated by gene expression and bioinformatic analyses (76, 81, 82). In humans, >100 quantitative trait loci (QTL) influencing high (HDL)- and low (LDL)-density lipoprotein levels as well as triglyceride (TG) concentrations have been found (82). Moreover, the comparison of mouse and human genomic data has shown that LDL, HDL, and TG QTL display a striking positional concordance in these two mammalian species (80–82).

The inclusion of additional mammalian genomes in this human-mouse QTL comparative framework would yield great benefits in the search of genes influencing susceptibility to atherosclerosis. Pigs are an interesting experimental model to tackle this issue for several reasons. First, pigs have been used as a biomedical model of human diseases for decades, with a special impact on those related to myocardial infarction, cerebral ischemia, and atherosclerosis (44). Familial hypercholesterolemia has been reported in pigs, and, unlike other model species, affected individuals develop atherosclerotic lesions in the coronary vasculature that closely match those observed in humans, suggesting that atherosclerosis shares a common pathogenesis in both species (31). Moreover, the existence of a relevant amount of additive genetic variability for serum lipid traits in pigs has been demonstrated by Rothschild and Chapman (67) and Pond et al. (56) and subsequently corroborated in several divergent selection experiments (29, 43, 85). Unfortunately, comprehensive mapping of cholesterol (CT) and lipoprotein metabolism traits in pig populations is still lacking, a feature that makes it difficult to establish a proper comparison with human and mouse. So far, only a partial genome scan exclusively devoted to mapping the locus responsible for porcine familial hypercholesterolemia has been performed (30). With regard to the analysis of pig candidate genes, mutations in the apolipoprotein B (APOB; Ref. 58) and cytochrome P-450, subfamily VIIa, polypeptide 1 (CYP7; Ref. 20) genes have been associated with familial hypercholesterolemia in certain pig lines selected for extreme total serum CT values, although the frequency of these mutant alleles in commercial lines remains to be assessed.

In the present study, we have performed a whole genome scan for CT, LDL, HDL, and TG serum levels at 45 and 190 days in a Duroc outbred population. Our main goal was to identify genomic regions influencing serum lipid traits at two time points that are relevant from a physiological point of view.

* D. Gallardo and R. N. Pena contributed equally to this work.

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view, since they coincide with the postweaning phase (45 days) and the end of the fattening period (190 days). Moreover, we have taken advantage of the chromosomal homology maps between human, mouse, and pig to compare our QTL results with those obtained in the two former species.

MATERIALS AND METHODS

Animal Population and Phenotypic Information

A high-intramuscular fat commercial Duroc line devoted to the production of fine-quality cured ham was used as a resource population. An experimental population of half-sib families was generated by mating 5 parental boars with 400 sows and taking at random 1 male offspring per litter. After a number of setbacks encountered during the generation of the experimental population (litters without weaned males, mortality and illness in fattening period, etc.), the analyzed population consisted of 350 castrated males born on 3 farms, belonging to 5 half-sib families (denoted as families 1–5), and distributed in 4 fattening batches. After weaning (3–4 wk of age), barrows were moved to the experimental test station (IRTA-CCP) and kept under normal intensive conditions, all being subjected to the same management procedures. During the first period of fattening (up to 90 kg of live weight, around 150 days of age) barrows were fed a standard diet oxidase-based method, in which cholesterol esterase, cholesterol oxidase, and peroxidase enzymes are employed, to determine CT levels.

Serum lipid concentrations were measured at two time points: (1) after weaning and before the initiation of fattening period (around 45 days of age) and (2) the day before slaughter (around 190 days of age and 122 kg of live weight). Blood samples taken at 45 and 190 days were used to measure CT, LDL, HDL, and TG serum concentrations with diverse enzymatic methods. We used a cholesterol oxidase-based method, in which cholesterol esterase, cholesterol oxidase, and peroxidase enzymes are employed, to determine CT levels (63). Serum HDL levels were measured with an immunoinhibition method (61), while TG concentrations were estimated with the glycercerol kinase reaction by a method reported by Fossati and Prencipe (26). Finally, serum LDL concentration was calculated according to the equation of Friedewald et al. (27). Some measurements from strongly hemolyzed blood samples or considered to be the result of inaccurate measurements were discarded from the data set, along with five outlier values of TG registers. The structure and basic statistics of the filtered data set analyzed in this study are shown in Table 1.

Microsatellite Genotyping

The five parental boars and their offspring were genotyped for 109 informative microsatellites covering the whole genome (Table 2). Primer sequences were obtained from the US Department of Agriculture (USDA) pig resources web page (http://www.annualgenome.org/pigs/resources/expandmarc2001.html) and the UnistS database from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/sites/entrez). Microsatellites were considered to be informative when at least two sires harbored heterozygous genotypes. Genomic DNA was isolated from blood samples as described by Vidal et al. (77). Amplification reactions were carried out in either an ABI Prism 877 Integrated Thermal Cycler (Applied Biosystems, Foster City, CA) or a Perkin Elmer 9700 Thermal Cycler (Applied Biosystems). Microsatellite genotyping was performed in capillary electrophoresis ABI Prism 3730 equipment (Applied Biosystems) and analyzed with the Gene Mapper software (Applied Biosystems). Marker distances from the USDA/MARC linkage map (64), available at http://www.ncbi.nlm.nih.gov/genome/guide/pig/, were taken into account to carry out QTL analyses.

Selected microsatellites provided coverage for the 18 autosomes with intervals between adjacent markers, whenever possible, ≤20 cM. Although 34 intervals exceeded this distance and 4 intervals were higher than 50 cM, the average marker interval was 20.5 cM.

Statistical Analyses

Exploratory analyses. Before the genome scan was carried out, several exploratory analyses were done in order to investigate the distribution of raw data and the best-fitting model. The effects of nongenetic discrete effects (farm of origin, batch and box of fattening, and date of slaughter) and covariates (age at blood extraction, live weight, or backfat thickness) on these traits were tested by means of the GLM procedure of SAS (SAS Inst., Cary, NC). Normal probability plots and Kolmogorov-Smirnov tests were performed to investigate the goodness of fit of the residuals with the normal distribution.

Serum lipid concentrations at 45 and 190 days of age

Table 1. Serum lipid concentrations at 45 and 190 days of age

<table>
<thead>
<tr>
<th>Phenotypic Correlations</th>
<th>Postweaning (45 ± 8 days)</th>
<th>At Slaughter (190 ± 8 days)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HDL</td>
<td>LDL</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Postweaning (45 ± 8 days)</td>
<td>+0.76‡‡‡</td>
<td>+0.81‡‡‡</td>
</tr>
<tr>
<td>CT 77.46±13.27</td>
<td>+0.45‡‡‡</td>
<td>+0.48‡‡‡</td>
</tr>
<tr>
<td>HDL 30.60±6.73</td>
<td>+0.33‡‡‡</td>
<td>+0.23‡‡‡</td>
</tr>
<tr>
<td>LDL 38.19±8.99</td>
<td>+0.18‡‡</td>
<td>+0.51‡‡‡</td>
</tr>
<tr>
<td>TG 43.25±16.43</td>
<td>+0.08 ns</td>
<td>+0.03 ns</td>
</tr>
<tr>
<td>HDL/LDL 0.839±0.266</td>
<td>+0.05 ns</td>
<td>+0.07 ns</td>
</tr>
</tbody>
</table>

At slaughter (190 ± 8 days)

| CT 125.81±26.77          | +0.62‡‡                                | +0.85‡‡                                | +0.37‡‡                                | -0.35‡‡                  |
| HDL 51.95±10.34          | +0.23‡‡                                | +0.04 ns                                | +0.47‡‡                                | -0.74‡‡                  |
| LDL 63.42±20.70          | +0.20‡‡                                | -0.15‡                                  | +0.04 ns                                | -0.74‡‡                  |
| TG 50.04±20.21           |                                         |                                         |                                         |                         |
| HDL/LDL 0.880±0.269      |                                         |                                         |                                         |                         |

Values are means ± SD (in mg/dl) and phenotypic correlations of raw data for serum total cholesterol (CT), serum low-density lipoprotein (LDL), serum high-density lipoprotein (HDL), and serum triglyceride (TG) concentrations; n = 44, 86, 69, 74, 60 and 43, 85, 72, 75, 60 records belonging to families 1–5 for 45 and 190 days, respectively. ns, Nonsignificant. *P < 0.05, †P < 0.01, ‡P < 0.001.
Data in raw form and their residuals were quite skewed to the right. The box-whisker plots highlighted the presence of outlier phenotypes (lying at 3 SD from the population mean) for TG levels at both ages and for LDL at 190 days. In general, the log-transformation of data was employed to perform QTL scan analyses. To correct for these departures from normality, so a filtered data set (Table 1) was used, plus the removal of the most extreme outlier observations. Log-transformed data was employed to perform QTL scan analyses. In addition to these exploratory analyses, correlations among all analyzed log-transformed phenotypes were computed after adjusting for several significant environmental factors, which are described in the next section.

**Genome scan for QTL affecting serum CT, HDL, LDL, and TG concentrations.** QTL analyses for CT, HDL, LDL, TG, and HDL/LDL concentrations across the 18 autosomes were performed with the regression approach described by Knott et al. (38) for the analysis of outbred half-sib families. These analyses were performed 1) in the whole population and 2) for each half-sib family separately, in order to detect QTL with different phases of linkage disequilibrium across families.

The models assumed in the QTL scan, on the basis of significant effects detected in the exploratory analyses, were 1) in the whole population analyses for measures at 45 and 190 days, respectively

\[ y_{ijk} = \mu + b_i + f_j + \sum_{s=1}^{S} \alpha_{sire} \beta_{sire} + e_{ijk} \]

and 2) in the within-family analyses for measures at 45 and 190 days, respectively

\[ y_{ijk} = \mu + b_i + f_j + d_{j(i)} + \beta \text{cov}_{j(i)} + \sum_{s=1}^{S} \alpha_{sire} \beta_{sire} + e_{ijk} \]

where \( y_{ijk} \) represents the transformed phenotypic observations (logarithm of serum CT, LDL, HDL, and TG concentrations and of HDL-to-LDL ratio at both 45 and 190 days of age) of individual \( k \) in the \( i \)th and \( j \)th levels of corresponding systematic effects; \( b_i \) is the systematic effect of the \( i \)th batch of fattening (4 levels); \( f_j \) is the systematic effect of the \( j \)th farm of origin (3 levels); \( d_{j(i)} \) is the fixed effect of the \( j \)th date of blood extraction, nested within the \( i \)th fattening batch (2 dates of extraction at 190 days per batch); \( \beta \) and \( \text{cov}_{j(i)} \) are, respectively, the regression coefficient and a covariate that varies according to the trait: backfat thickness (or live weight) for CT, HDL, and LDL or age at blood collection for TG (an additional analysis without covariates was also carried out in order to evaluate the effect of these covariates on QTL detection); \( p_{j(i)} \) is the probability of individual \( k \) inheriting a given allele from its common parent (in the case of whole population analyses, nested within sire), calculated at any putative location in the genome by the multipoint approach developed by Knott et al. (38); and \( \alpha \) is the regression coefficient of phenotypes onto the probability of having inherited a given allele from the common parent, i.e., the allele substitution effect (within sire).

Analyses were performed by means of QTL express software (70), available at http://qtl.cap.ed.ac.uk/. For each combination of trait*family, a whole genome scan was performed. At each centimorgan, an F-ratio with five (whole population analyses) or one (within-family analyses) degrees of freedom in the numerator was computed comparing the QTL model to an equivalent model without any linked QTL.

Chromosomewide significance thresholds were determined empirically by data permutation (15). A total of 10,000 permutations were performed to obtain the distribution under the null hypothesis (no linked QTL) for each independent analysis (chromosome*trait in whole population analyses and chromosome*family*trait in within-family analyses). Estimated chromosomewide significance thresholds did not differ much among traits. However, they varied according to chromosome marker coverage and informativity, and to family size (in the within-family analyses). In the whole population analyses, chromosomewide significance thresholds \( P < 0.05 \) ranged between 2.6 and 3.2, depending on the chromosome under consideration. In the within-family analyses, chromosomewide significance thresholds for the different chromosome*family combinations ranged from 5.6 to 7.6 \( (P < 0.05) \); the most conservative criteria \( (F > 7.6) \) was taken as the suggestive F-threshold for all within-family analyses.

**Table 2. Pig microsatellites employed in whole genome scan**

<table>
<thead>
<tr>
<th>Pig Chromosome</th>
<th>Microsatellites (position in cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC1</td>
<td>SW1515 (0), CGA (28.6), S0122 (43.8), S0313 (62.3), S0155 (77.5)</td>
</tr>
<tr>
<td>SSC2</td>
<td>SW2623 (0), SW395 (56.3), S0226 (64.3), S0010 (68.1), SW2192 (106.4), S0036 (122.3)</td>
</tr>
<tr>
<td>SSC3</td>
<td>SW72 (0), S0206 (24.5), SW2618 (33.0), S0164 (42.7), SW2408 (76.4), S0002 (84.4), SW349 (94.8), SW590 (111.5)</td>
</tr>
<tr>
<td>SSC4</td>
<td>S0301 (0), S0001 (14.7), SW317 (26.4), SW839 (35.2), S0073 (47.3), S0214 (52.2), S0097 (92.9)</td>
</tr>
<tr>
<td>SSC5</td>
<td>ACR (0), SW413 (8.4), SW425 (72.3), S0005 (88.2), IGF1 (118.7), SWR112 (130.3)</td>
</tr>
<tr>
<td>SSC6</td>
<td>SW2535 (0), SW1057 (37.6), S0087 (53.1), SW1129 (70.7), SW1053 (77.0), SW1473 (84.4), S0003 (92.5), S0121 (106.5), SW2419 (151.9)</td>
</tr>
<tr>
<td>SSC7</td>
<td>S0025 (0), SW1873 (7.2), S0064 (26.5), SW1409 (53.4), SW1856 (57.8), SW1701 (69.7), SW2036 (74.5), SW255 (81.9), SW632 (100.7), S0101 (131.2), S0212 (137.5)</td>
</tr>
<tr>
<td>SSC8</td>
<td>SW2410 (0), SW905 (19.5), S0017 (59.1), S0069 (72.7), S0225 (81.5), SW61 (111.0)</td>
</tr>
<tr>
<td>SSC9</td>
<td>SW668 (0), SW911 (32.8), SW940 (57.0), SW2571 (69.3), S0295 (96.5), SW1349 (138.5)</td>
</tr>
<tr>
<td>SSC10</td>
<td>SW830 (0), S0038 (4.3), S0070 (62.1), SW920 (86.3), SW951 (101.0), SW2067 (128.0)</td>
</tr>
<tr>
<td>SSC11</td>
<td>SW391 (0), S0071 (38.7), S0230 (51.4), SW703 (71.2)</td>
</tr>
<tr>
<td>SSC12</td>
<td>S0143 (0), SW874 (56.1), S0090 (73.6), S0106 (89.2)</td>
</tr>
<tr>
<td>SSC13</td>
<td>S0219 (0), S0076 (26.3), SWR1008 (51.4), S0068 (60.6), SW398 (77.7), SW2440 (100.6), S0291 (124.6)</td>
</tr>
<tr>
<td>SSC14</td>
<td>SW957 (0), SW1125 (14.8), SW210 (38.9), SW2504 (52.6), SW1557 (80.5), SW2515 (101.3)</td>
</tr>
<tr>
<td>SSC15</td>
<td>S0555 (0), S0004 (15.1), SW1111 (26.0), S0149 (66.3), SW936 (74.7), SW1339 (108.2)</td>
</tr>
<tr>
<td>SSC16</td>
<td>SW742 (0), SW2517 (18.3), S0061 (37.7), SW403 (46.4), S0026 (82.7)</td>
</tr>
<tr>
<td>SSC17</td>
<td>SW24 (0), SW2142 (15.1), SW1920 (33.1), S0359 (44.7)</td>
</tr>
<tr>
<td>SSC18</td>
<td>SW1023 (0), SW787 (27.0), S0120 (40.6)</td>
</tr>
</tbody>
</table>

SSC, Sus scrofa chromosome.
Finally, empirical confidence intervals (CI) of the significant QTL locations were calculated with the bootstrap resampling method (79), which is more appropriate than the logarithm of odds (LOD) drop-off procedure (42) when dealing with small or medium-sized populations. A total of 10,000 bootstrap samples were performed to obtain the 95% CI for each QTL.

Analysis of Positional Concordance with Human Serum Lipid QTL

The remarkable positional concordance observed for serum lipid QTL between human and mouse (81) led us to investigate whether the pig QTL identified in the present work had any “orthologous” QTL in human. Pig QTL intervals defined according to their flanking markers were aligned with their orthologous human chromosomal regions with the Align tool of the Pig QTL database (http://www.animalgenome.org/QTLdb/pig.html). Subsequently we investigated whether these human chromosomal regions contained any serum lipid QTL by performing a bibliographic search at PubMed (http://www.ncbi.nlm.nih.gov).

RESULTS

Phenotypic Variability of Cholesterol, HDL- and LDL-Cholesterol, and Triglyceride Serum Levels

The mean values of CT, HDL, LDL, and TG concentrations at 45 days and 190 days in the analyzed Duroc barrows are shown in Table 1. Despite all Duroc pigs being fed with the same diet, we observed a remarkable degree of phenotypic variability at both ages. This statement was particularly applicable to TG serum levels (coefficient of variation of 38% and 40% at 45 and 190 days, respectively) and LDL concentrations at 190 days (coefficient of variation of 33%).

Phenotypic correlations among serum lipid concentrations at 45 and 190 days are shown in Table 1. In general, there were positive correlations among serum lipid levels within time periods. Serum CT concentration was positively and highly correlated with both LDL and HDL levels, particularly with LDL, whereas HDL and LDL showed moderate positive correlations at both time points. Serum TG levels were positively correlated with CT measures at both ages, although correlation with HDL levels at 190 days was not significant. A relevant result was the absence of significant correlations between serum lipid levels at 45 days vs. 190 days, with only a few exceptions where weak positive correlations were found (Table 1).

Also, significant (P < 0.0001) and positive phenotypic correlations were found between serum CT concentrations at 190 days and backfat thickness measured at the same age (the correlation coefficients with CT, HDL, and LDL took values of 0.34, 0.34 and 0.27, respectively). Similarly, significant (P < 0.01, 0.001, and 0.05) but lower phenotypic correlations were found between live weight and serum CT, HDL, and LDL at 190 days (0.20, 0.23 and 0.18, respectively). Conversely, no relevant associations of serum TG concentrations with backfat thickness or with live weight were found. Both backfat thickness and live weight were considered as alternative covariates in the QTL analyses of CT, HDL, and LDL at 190 days.

Identification of QTL for Serum Total Cholesterol, HDL-and LDL-Cholesterol, and Triglyceride Levels in Pigs

We have identified QTL for serum lipid traits by performing two separate analyses at the whole population (Table 3) and within-family (Table 4) levels. The whole population genome scan allowed us to identify several chromosomewide significant QTL. The most significant QTL affected TG (SSC4, 2 cM) and LDL (SSC13, 75 cM) levels at 190 days. Mean substitution effects on analyzed traits at the most likely posi-

Table 3. Significant results from whole population QTL genome scan analysis for serum total CT, HDL- and LDL-cholesterol, and TG concentrations (in logarithmic scale) after weaning (45 days) and at slaughter (190 days)

<table>
<thead>
<tr>
<th>SSC</th>
<th>Trait</th>
<th>Position, cM</th>
<th>95% CI, cM</th>
<th>F-Ratio</th>
<th>Nominal P Value</th>
<th>Within-Family Mean Substitution Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fam 1</td>
</tr>
<tr>
<td>6</td>
<td>HDL</td>
<td>85</td>
<td>0–151</td>
<td>2.63*</td>
<td>0.0238</td>
<td>0.337*</td>
</tr>
<tr>
<td>6</td>
<td>HDL/LDL</td>
<td>84</td>
<td>3.5–151</td>
<td>3.47*</td>
<td>0.0046</td>
<td>0.499*</td>
</tr>
<tr>
<td>7</td>
<td>CT</td>
<td>101</td>
<td>2–137</td>
<td>2.60*</td>
<td>0.0245</td>
<td>–0.206†</td>
</tr>
<tr>
<td>7</td>
<td>HDL</td>
<td>100</td>
<td>40–134</td>
<td>3.07*</td>
<td>0.0101</td>
<td>–0.294†</td>
</tr>
<tr>
<td>9</td>
<td>HDL</td>
<td>33</td>
<td>0–128</td>
<td>3.29*</td>
<td>0.0065</td>
<td>0.152 ns</td>
</tr>
<tr>
<td>9</td>
<td>TG</td>
<td>32</td>
<td>0–138</td>
<td>3.45*</td>
<td>0.0048</td>
<td>0.298*</td>
</tr>
<tr>
<td>9</td>
<td>HDL/LDL</td>
<td>39</td>
<td>9–108</td>
<td>2.79*</td>
<td>0.0176</td>
<td>–0.061 ns</td>
</tr>
<tr>
<td>12</td>
<td>TG</td>
<td>17</td>
<td>0–89</td>
<td>2.91*</td>
<td>0.0139</td>
<td>0.301 ns</td>
</tr>
<tr>
<td>13</td>
<td>HDL</td>
<td>78</td>
<td>9–119</td>
<td>2.90*</td>
<td>0.0141</td>
<td>–0.088 ns</td>
</tr>
<tr>
<td>13</td>
<td>HDL/LDL</td>
<td>78</td>
<td>3–121</td>
<td>2.85*</td>
<td>0.0157</td>
<td>0.0080 ns</td>
</tr>
</tbody>
</table>

QTL, quantitative trait locus; CI, confidence interval. F-ratio: +P < 0.05, ++P < 0.01 at chromosomewide level. Significance of estimated mean allele effects: *P < 0.05, †P < 0.01, ‡P < 0.001.
Table 4. Significant results from within-family QTL genome scan for serum total CT, HDL- and LDL-cholesterol, and TG concentrations (in logarithmic scale) after weaning (45 days) and at slaughter (190 days)

<table>
<thead>
<tr>
<th>SSC</th>
<th>Trait</th>
<th>Position, cM</th>
<th>95% CI, cM</th>
<th>Family</th>
<th>F-Ratio</th>
<th>Nominal P Value</th>
<th>Mean Effect (SD)</th>
</tr>
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<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td></td>
<td>Serum concentrations at 45 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>LDL</td>
<td>33</td>
<td>4–59</td>
<td>4</td>
<td>11.88**</td>
<td>0.0010</td>
<td>–0.180 (0.052)</td>
</tr>
<tr>
<td>1</td>
<td>HDL/LDL</td>
<td>50</td>
<td>0–77</td>
<td>4</td>
<td>8.30*</td>
<td>0.0053</td>
<td>0.215 (0.075)</td>
</tr>
<tr>
<td>3</td>
<td>CT</td>
<td>15</td>
<td>0–84</td>
<td>4</td>
<td>8.46*</td>
<td>0.0049</td>
<td>0.108 (0.037)</td>
</tr>
<tr>
<td>6</td>
<td>HDL/LDL</td>
<td>84</td>
<td>9–106</td>
<td>1</td>
<td>14.22*</td>
<td>0.0005</td>
<td>0.397 (0.105)</td>
</tr>
<tr>
<td>7</td>
<td>CT</td>
<td>101</td>
<td>0–117</td>
<td>1</td>
<td>8.44</td>
<td>0.0060</td>
<td>–0.203 (0.070)</td>
</tr>
<tr>
<td>7</td>
<td>HDL</td>
<td>101</td>
<td>0–137</td>
<td>1</td>
<td>11.41**</td>
<td>0.0016</td>
<td>–0.277 (0.082)</td>
</tr>
<tr>
<td>9</td>
<td>HDL</td>
<td>33</td>
<td>26–67</td>
<td>4</td>
<td>11.70**</td>
<td>0.0010</td>
<td>0.182 (0.053)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>9</td>
<td>TG</td>
<td>33</td>
<td>15–130</td>
<td>3</td>
<td>10.14**</td>
<td>0.0022</td>
<td>–0.249 (0.078)</td>
</tr>
<tr>
<td>13</td>
<td>HDL</td>
<td>79</td>
<td>52–99</td>
<td>5</td>
<td>11.96**</td>
<td>0.0010</td>
<td>–0.333 (0.099)</td>
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<tr>
<td>13</td>
<td>LDL</td>
<td>104</td>
<td>14–124</td>
<td>1</td>
<td>9.07*</td>
<td>0.0045</td>
<td>–0.252 (0.084)</td>
</tr>
<tr>
<td></td>
<td>Serum concentrations at 190 days</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>HDL</td>
<td>8</td>
<td>0–72</td>
<td>3</td>
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<td>0.0038</td>
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</tr>
<tr>
<td>3</td>
<td>CT</td>
<td>72</td>
<td>35–82</td>
<td>5</td>
<td>11.12**</td>
<td>0.0015</td>
<td>–0.189 (0.057)</td>
</tr>
<tr>
<td>3</td>
<td>LDL</td>
<td>68</td>
<td>5–79</td>
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<td>0.190 (0.069)</td>
</tr>
<tr>
<td>6</td>
<td>HDL/LDL</td>
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<td>43–84</td>
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<td>11.99**</td>
<td>0.0010</td>
<td>–0.296 (0.085)</td>
</tr>
<tr>
<td>6</td>
<td>HDL</td>
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<td>3–76</td>
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</tr>
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<td>29–88</td>
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<td>0.0069</td>
<td>0.249 (0.088)</td>
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<tr>
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<td>TG</td>
<td>23</td>
<td>0–36</td>
<td>2</td>
<td>19.15**</td>
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<td>0.434 (0.099)</td>
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<td>0.0018</td>
<td>0.366 (0.109)</td>
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<tr>
<td>13</td>
<td>CT</td>
<td>72</td>
<td>61–109</td>
<td>3</td>
<td>7.95*</td>
<td>0.0063</td>
<td>–0.178 (0.063)</td>
</tr>
<tr>
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<td>LDL</td>
<td>74</td>
<td>61–124</td>
<td>3</td>
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<td>0.0059</td>
<td>–0.293 (0.103)</td>
</tr>
<tr>
<td>13</td>
<td>TG</td>
<td>5</td>
<td>0–83</td>
<td>1</td>
<td>10.65**</td>
<td>0.0023</td>
<td>0.364 (0.112)</td>
</tr>
</tbody>
</table>

+P < 0.05, ++P < 0.01 at chromosomewide level; *P < 0.05, **P < 0.01 at genomewide level.

In the light of these results, we decided to perform a within-family QTL analysis (Table 4). This second approach resulted in the identification of two QTL significant at the genomewide level. The most significant QTL (P < 0.01 at genomewide level in within-family analysis) was detected at SSC4 and affected TG levels at 190 days exclusively in family 1 (Tables 3 and 4). The profile of F-ratios throughout SSC4 when the TG QTL at 190 days is fitted in the five paternal half-sib families is shown in Fig. 1. We would like to emphasize the robustness of this QTL result, which happened to be highly significant in all statistical analyses (e.g., with weight as covariate instead of backfat thickness). Moreover, we found a second genomewide significant QTL on SSC6 for HDL-to-LDL ratio at 45 days. Six and five QTL highly significant at the chromosome-wide level were also identified for 45 day and 190 day measures, respectively. In addition, several genomic regions displayed associations with multiple serum lipid traits. For instance, we found a SSC3 region (58–78 cM) containing several QTL for CT, LDL, and HDL/LDL at 190 days. These QTL exclusively segregated in families 4 and 5. The comparison of the F-ratio profiles throughout SSC3 when these QTL are fitted in both families is shown in Fig. 2. This result indicates that, for a given trait and when both families are compared, QTL peaks coincide in their location but not in their significance. In addition, a TG at 190 days (TG190) QTL segregating in family 1 was detected close to the aforementioned QTL on SSC3 (at 83 cM). Another region where multiple associations were found was SSC13 (72–104 cM interval), with suggestive effects on HDL45, LDL45, CT190, and LDL190.

Comparison of Tables 3 and 4 revealed differences between the whole population and within-family results. For instance, while we found significant CT190 and HDL190 QTL mapping...
at the SSC3 27–28 cM interval in the whole population approach (with effects in families 1 and 3), these two QTL were not detected in the within-family analyses. Conversely, several QTL segregating in certain half-sib families were not detected in the whole population analysis. This is the case of multiple cholesterol-related QTL found at the SSC3 58–78 cM interval (families 4 and 5) as well as of the SSC1 QTL linked to LDL at 45 days in family 4.

Mean QTL allele substitution effects (on a logarithmic scale) for serum lipid traits in the within-family analysis are shown in Table 4. According to these estimates, the substitution of one QTL allele by the alternative one might lead to important variations on serum lipid levels. This is particularly true for the QTL influencing TG levels, which is in fact the most variable phenotype. For instance, the TG190 QTL on SSC4 (23 cM) displays a mean substitution effect of 0.434, more than 1 SD of the trait in a logarithmic scale. Other important substitution effects were observed for serum HDL concentration and HDL-to-LDL ratio at SSC12 and SSC6.

The empirical 95% CI for QTL locations in the within-family analyses (Table 4) ranged from 36 to 137 cM. The CI generally decreased with increase in QTL significance but also in family size, and only some of most significant QTL showed CI covering <40% of the chromosome. In this way, the narrowest CI (36 cM) corresponded to the TG190 QTL on SSC4 detected in family 2, followed by the CI of LDL190 QTL on SSC3 and HDL45 QTL on SSC9 (41 cM in both cases) detected in families 5 and 4, and the CI of HDL45 QTL on SSC13 detected in family 3 (47 cM). For the aforementioned QTL, the CI spanned 30–39% of the chromosome length. In the whole population analyses (Table 3) the CI were notably wider, ranging from 76 to 151 cM and spanning from 68% to 100% of the corresponding chromosomes.

Finally, it is worth mentioning that chromosomal locations of the detected QTL for serum cholesterol levels at 190 days did not vary substantially when serum lipid levels at 190 days were adjusted for live weight (results not shown) instead of backfat thickness, but when no covariate was considered in the model new suggestive QTL for LDL and CT at 190 days appeared on chromosomes 14 and 18.

**Positional Concordance Between Human and Pig Serum Lipid QTL**

Results of the comparative positional analyses with human serum lipid QTL are shown in Table 5, where positive matches between pig and human QTL are displayed. Since pig QTL had large CI, in some instances they have correspondences with two human chromosomes. Nevertheless, several correspondences between human and pigs can be observed. For instance, pig SSC4 QTL for TG at 190 days has a clear orthologous QTL on human 8q12.1 (8q11.23–21.3), which was identified by analyzing 501 American three-generation families (82). Pig QTL for LDL at 45 days at SSC1 (peak: 33 cM) corresponds to two human regions (6q12–q25 and 18q21) both containing QTL for LDL levels according to studies performed in United States and French Canadian populations, respectively (82). With regard to pig HDL QTL, we have observed, among others, an interesting correspondence between pig SSC7 QTL for HDL at 45 days (peak at 101 cM) and a human 15q14–q21.3 region where several HDL QTL have been mapped in African and Caucasian populations (66). Comparison with mouse showed that many of these orthologous human-pig QTL pairs display positional concordance with murine serum lipid QTL (for a detailed comparison see Refs. 66, 80–82), evidencing that these QTL locations are “conserved” across distinct mammalian species.

**DISCUSSION**

Despite being an excellent animal model for CT-related human diseases (44), the genetic architecture of serum lipid metabolism is not well understood in pigs. We have dissected the genetic component of serum CT, HDL, LDL, and TG concentrations in this species by performing a QTL genome scan in a commercial Duroc population. Serum CT, HDL, and TG levels at 45 days (Table 1) were similar to values reported by Pond et al. (57) in 56-day-old boars belonging to lines selected for high and low serum CT concentrations. In contrast, values at 190 days were slightly higher than those reported by Lu et al. (43) and Pond et al. (57) in 105- and 56 day-old boars, respectively. The analysis of phenotypic correlations among CT, LDL, HDL, and TG levels within age showed that these parameters usually increase their levels concomitantly (Table 1). Previously, Hasler-Rapacz et al. (31) analyzed serum lipid levels in 4-mo-old pigs and found that both CT and TG levels were positively and negatively correlated with LDL and HDL concentrations, respectively. In any case, relationships among cholesterol measures (CT, HDL- and LDL-bound) observed in the present work are consistent with the fact that LDL constitutes the largest fraction of total serum CT and HDL the next-largest fraction (55). Accordingly, positive genetic correlations between CT vs. LDL (16) and HDL (23) concentrations have also been found in humans. On the other hand, we have found that most of the phenotypic correlations between serum lipid levels at 45 and 190 days of age happen to be nonsignificant (Table 1). These two time points, which correspond to the...
Table 5. Comparative positional analysis of CT, LDL, HDL, and TG QTL in human and mouse with most significant porcine lipid QTL found in analyzed Duroc population

<table>
<thead>
<tr>
<th>SSC</th>
<th>QTL Trait</th>
<th>Position, cM</th>
<th>Significance</th>
<th>PCB</th>
<th>HCHR</th>
<th>QTL in Human</th>
<th>HCBP (CI)</th>
<th>References</th>
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<td>1</td>
<td>LDL 45d</td>
<td>33</td>
<td>++</td>
<td>1p2.5-q1.2</td>
<td>6q12-q25</td>
<td>LDL 6q25.2 (q24.2-27)</td>
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<tr>
<td>3</td>
<td>LDL 190d</td>
<td>74</td>
<td>++</td>
<td>3q1.1-q2.6</td>
<td>2p25-p11</td>
<td>LDL 18q21, q21.1-22.2</td>
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</tr>
<tr>
<td>3</td>
<td>CT 190d</td>
<td>72</td>
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<td>3q1.1-q2.6</td>
<td>2p25-p11</td>
<td>LDL 2p23 (p25.2-21)</td>
<td>54</td>
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<tr>
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<td>LDL 190d</td>
<td>74</td>
<td>++</td>
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<td>ATH 2p33.2 (p12-11.2)</td>
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</tr>
<tr>
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<td>HDL/LDL 190d</td>
<td>78</td>
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<td>ATH 2p33.2 (p12-11.2)</td>
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<tr>
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<td>TG 190d</td>
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<td>++</td>
<td>3q2.5-q2.6</td>
<td>2p25-p23</td>
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<td>CT 45d</td>
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<td>3p25-p22</td>
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<td>33</td>
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</table>

Significance: + P < 0.05, ++ P < 0.01 at chromosomewide level; **P < 0.01 at genomewide level. Human homologous regions of the pig QTLs were identified with the pig QTL database.

d, Days; PCB, pig cytogenetic band; HCHR, human chromosome homologous regions; HCBP, human cytogenetic band; ATH, human atherosclerosis.

postweaning phase and the end of the fattening period, are remarkably different from a physiological and metabolic perspective. The alimentary stress that piglets undergo at 45 days, a time when they begin to ingest solid food, conceivably involves changes in gene expression profiles devoted to facilitate piglet adaptation to the new nourishing source. In the fattening period, and compared with lactation and transition, pigs experience a marked increase in their average daily gain although they become less efficient at converting feed to lean primarily because of the decreasing ratio of lean to fat gain (68). Moreover, and of paramount importance, the feeding regime at these two ages is markedly different.

The genomic landscape that has emerged from our analysis consists of two genomewide highly significant QTL for TG at 190 days (SSC4) and HDL-to-LDL ratio at 45 days (SSC6). This number is fairly high, taking into account that we have used an outbred pig population and not a divergent cross. Our data reinforce the notion that genetic variance is an important component of the total variance of serum lipid concentrations and agree well with the moderate to high heritabilities estimated for these traits in pigs (e.g., Refs. 29, 43, 56, 67, 85), notwithstanding limitations in statistical power. QTL reported in the present experiment have, however, large empirical CI. On the other hand, although there was a general agreement between whole population and within-family results, several discrepancies were also found between results from different analyses (Tables 3 and 4). These two major issues need to be taken into account when interpreting the data. With regard to empirical CI, they spanned >36 cM and covered >30% of the corresponding chromosomes. According to some authors (e.g., Refs. 73, 69), this situation is not far from other QTL experiments dealing with outbred animal populations, where QTL CI usually cover >20 cM and sometimes a whole chromosome. As reported by Darvasi and Soller (19) in the context of backcross and F2 designs, later corroborated in other population designs (78), QTL CI width is inversely related to sample size and QTL gene effects. In consequence, this drawback could be solved by analyzing larger populations. The second point that needs to be discussed makes reference to the discrepancies found between whole population and within-family analyses. Knott (37) pointed out that QTL scans in outbred populations suffer from several drawbacks that explain why
QTL associations are not always found across different analyses. First, parents are only informative if they harbor heterozygous genotypes for both the analyzed marker and the QTL. Second, the phase of linkage between the marker and the QTL alleles may vary among families. It must be taken into account that whole population analyses assume a common maximum for all families whereas within-family analyses do not, so different maxima can be obtained when differences in marker informativity or in segregation patterns exist across families. Consistent with this argument, most QTL reported in the present work were detected in certain families but not in others, which can in turn be related to differences in family size. Finally, differences in estimates of fixed effects and covariates could also play a role in discrepancies between whole population and within-family analyses, but additional within-family analyses carried out with data previously adjusted for environmental effects estimated in the whole population yielded similar results (data not shown). In summary, the aforementioned discrepancies should be interpreted with special caution because it is difficult to disentangle whether they have been produced by genetic or experimental factors. More powerful experiments with a larger number of animals would be also helpful in solving these inconsistencies. However, the considerable management and economic requirements involved in the performance of this kind of studies in commercial pig populations constitutes an obstacle not easy to overcome.

We have investigated whether the serum lipid QTL described in the present study coincide with QTL reported for other lipid traits. Obviously, the large CI we have usually found when defining QTL locations need to be kept in mind when establishing this and other comparisons among studies. First we would like to mention that the performance of a genome scan for fatty acid composition of intramuscular fat in our Duroc populations has evidenced that several QTL for these traits, located on SSC3, SSC6, and SSC7 (60), show a positional concordance with serum lipid QTL reported in the present work. In addition, most serum lipid QTL coincide or lie close to pig QTL with effects on growth and fatness traits reported in other experiments (e.g., Refs. 9, 10, 22, 65, 87). Moreover, the most significant QTL detected in this study, i.e., the QTL for TG190 on SSC4, coincides with a QTL for backfat thickness, the FAT1 locus, that has been reported in many unrelated pig crosses and populations (5, 10, 39, 47). Consistent with these facts, significant positive correlations were found in our Duroc population between cholesterol measures at 190 days and other fatness and morphological traits, specifically backfat thickness (correlation coefficients from 0.27 to 0.34) and live weight (correlation coefficients between 0.18 and 0.23), but no significant correlations were found with TG levels. Other authors have shown the existence of positive correlations between body weight and plasma cholesterol concentration (55, 85). However, Young et al. (85) did not find significant differences in backfat thickness at slaughter between lines produced in the context of a three-generation divergent selection experiment for serum CT concentration at 56 days. Besides these considerations, the chromosomal coincidence between fatness and lipid serum concentration QTL, even after adjusting data for backfat thickness (in the case of measures at 190 days), suggests the existence of common genes regulating these traits whose polymorphisms would have multiple effects on lipid metabolism. Consistent with this, we have identified several genomic regions with multiple effects on CT, TG, and lipoprotein concentrations. For instance, the SSC3 region (58–83 cM interval) contains QTL peaks with suggestive effects on CT, LDL, TG, and HDL/LDL at 190 days, and SSC13 encompasses HDL45, LDL45, CT190, and LDL190 QTL whose most likely positions map to the 72–104 cM interval. These results, which obviously should be confirmed by analyzing a larger population, might be attributable to the existence of pleiotropic genes, whose expression simultaneously modulates the concentration of diverse serum lipid traits, or, conversely, to the existence of several closely linked QTL. In this sense, studies performed in human and mouse have demonstrated the existence of diverse chromosomal regions with simultaneous effects on diverse plasma lipid traits (82).

We have also investigated the positional concordance of QTL through time by measuring serum lipid traits at two ages, 45 days and 190 days (Tables 3 and 4). These results agreed well with the measurement of phenotypic correlations among serum lipid levels at 45 days and 190 days (Table 1). In this way, a clear lack of positional concordance among QTL affecting serum lipid levels at 45 days and 190 days was detected, corroborating the existence of either genetic or environmental age-linked effects. While QTL on SSC1, SSC6, SSC7, SSC9, and SSC13 had the most significant effects on serum lipids after weaning, at the end of the fattening period QTL on SSC3, SSC4, SSC12, and SSC13 happened to be the most significant. This result coincides with what has been observed in humans (71), where serum lipid and apolipoprotein levels vary considerably with age, with four extremely critical periods: first years of life, puberty, menopause in women, and old age. Nance et al. (51) made a longitudinal study of HDL concentrations in 11-, 12.5-, and 14-yr-old twins and found that 19–40% of total variation of HDL levels was attributable to the expression of new genes. Similarly, Snieder et al. (72) found that 46% (TG), 66% (HDL), 76% (LDL), and 80% (CT) of the genetic variance of these lipid traits were common for middle-aged and adolescent groups, a feature that convincingly demonstrates the existence of age-related genetic effects probably due to the action of newly expressed genes. Our results are compatible with a scenario in which a significant part of the polymorphisms underlying serum lipid QTL do not have effects throughout all the productive life of pigs, likely because the pattern of gene expression is also different at weaning and at maturity. These fluctuations in gene expression might be explained by genetic and environmental age-linked effects. As mentioned above, diet composition is very different in piglets and adult pigs, and it is expected to have a major impact on the transcriptome. Of note, this pattern of temporal variation of QTL effects has been observed in a wide variety of traits and species, including growth in mice (49), fecundity and longevity in Drosophila (36), and body mass index in humans (8).

An intriguing and suggestive finding in the search for serum lipid genetic determinants in model organisms was the demonstration of a close positional concordance between human and mouse QTL (81, 82). These results suggested the existence of a common set of genes with polymorphisms regulating the expression of lipid traits in two species that diverged ~75 million years ago (81, 82). We have extended this comparison to pigs, another mammalian species taxonomically classified in a superorder (Laurasiatheria) different from primates and ro-
dents (Euarchontoglires). This analysis needs to be further refined before meaningful comparisons can be established, since QTL CI are remarkably wide in our study. However, initial results are encouraging since they reveal a considerable level of positional concordance between pig vs. human and mouse (Table 5). Since Euarchontoglires and Laurasiatheria likely split in the Cretaceous period, ~85–95 million years ago (50), we might hypothesize that this common set of polymorphic genes has been conserved throughout an extended evolutionary period. Of course, this statement does not imply that the same polymorphisms are conserved in such distantly related species. Most likely, the polymorphisms that explain CT, HDL, LDL, and TG QTL found in mice, humans, and pigs are different in nature and position, although with similar effects at the functional level. This is best exemplified by the case of familial hypercholesterolemia in humans and swine. Despite the fact that as many as 64 mutations with effects on human LDL receptor function are documented in the OMIM database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM), none of these correspond to the R84C amino acid replacement described in pigs (30).

These regions that consistently show QTL effects on serum lipid levels in different mammalian species contain genes of functional importance in lipid metabolism. The endothelial lipase gene (LIPG) maps to the LDL45 QTL on SSC1, and its overexpression leads to a reduction of plasma HDL levels (35). In the most likely region of the SSC3 QTL for LDL190 and CT190 (72–74 cM) we have identified the ATP-binding cassette, subfamily G, members 5 and 8 (ABCG5 and ABCG8) genes, which limit the intestinal absorption of CT by excreting excess of this metabolite back to the intestinal lumen (32). In this way, knockout mice for ABCG5 and ABCG8 show an enhanced intestinal absorption of CT (32). We would like to highlight the SSC4 QTL for TG190, which maps to the FAT1 locus influencing backfat thickness, reported in several pig populations (5, 10, 39, 47, 53). The CI of this QTL coincides with the location of genes encoding fatty acid binding proteins 4 and 5 (FABP4 and FABP5; Ref. 48), which are highly expressed in adipocytes and play a key role in the binding and transportation of fatty acids (45). Associations between polymorphisms in these loci and backfat thickness have been reported in pigs (25, 28, 48). Moreover, serum FABP4 and TG levels are strongly correlated in humans (75). In humans, genetic variation in the APOA1/C3/A4/A5 cluster, which maps to the SSC9 QTL for HDL45 and TG45 at 33 cM, shows consistent associations with TG and HDL levels (18, 52, 59, 84). Pig SSC9 contains another interesting region with one QTL for HDL45. Gene content of this region in humans includes the fatty acid translocase gene (CD36), which is involved in the intestinal uptake of fatty acids and CT; the sterol o-acyltransferase 1 (SOAT1) locus that forms cholesterol esters from CT (46); and apolipoprotein-A-II, a major constituent of HDL particles (40). Finally, the SSC13 QTL (72–79 cM) with pleiotropic effects on serum lipid traits might contain several loci of interest, such as the phosphate cytidyltransferase 1, choline, α-isofom (PCYT1A) and the UDP-gal-β-GlcNAc β-1,4-galactosyltransferase, polypeptide 4 (B4GALT4) genes, with known effects on HDL levels (34, 83), whereas the SSC13 region (5 cM) shares associations with TG levels at 190 days mapped to the peroxisome proliferator-activated receptor-γ (PPARG) gene, a nuclear transcription factor with allelic variants showing associations with plasma TG levels (13, 14).

As a whole, the results obtained in the present study provide the foundations for understanding the genetic architecture of serum lipid traits in pigs and argue for the convenience of performing additional genome scans in a broad diversity of porcine populations with the aim of capturing a relevant fraction of the allelic variation affecting total CT homeostasis. This line of action should be accompanied by the subsequent refinement of the linkage map of the most significant and consistent QTL. The main benefit derived from this kind of studies would be the narrowing of the detected QTL by interspecies comparison, a tool that would pave the way to the subsequent identification of the underlying mutations.

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