Single- and joint-population analyses of two experimental pig crosses to confirm quantitative trait loci on Sus scrofa chromosome 6 and leptin receptor effects on fatness and growth traits

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ABSTRACT: The primary goal of this study was to detect and confirm QTL on SSC6 for growth and fatness traits in 2 experimental F2 intercrosses: Iberian × Landrace (IB × LR) and Iberian × Meishan (IB × MS), which were used in this study for the first time in a QTL analysis related to productive traits. For this purpose, single- and joint-population analyses with single and bivariate trait models of both populations were performed. The presence of the SSC6 QTL for backfat thickness previously identified in the IB × LR cross was detected in this population with additional molecular information, but also was confirmed in the IB × MS cross. In addition, a QTL affecting BW was detected in both crosses in a similar position to the QTL detected for backfat thickness. This is the first study in which a QTL affecting BW is detected on SSC6 in the IB × LR cross, as well as in the IB × MS resource population. Furthermore, we analyzed a previously described nonsynonymous leptin receptor (LEPR) SNP located in exon 14 (c.2002C > T) for causality with respect to this QTL within both F2 populations. Our results supported the previously reported association between LEPR alleles and backfat thickness in the IB × LR cross, and this association was also confirmed within the IB × MS cross. An association not reported before between LEPR alleles and BW was identified in both populations.

Key words: backfat thickness, body weight, joint analysis, leptin receptor, pig, polymorphism

INTRODUCTION

Previous results obtained from several mapping populations have detected a highly significant QTL on SSC6 in the S0228-Sw1881 interval that affects fatness and meat quality traits (Ovilo et al., 2005; Mohrmann et al., 2006; Edwards et al., 2008). Moreover, fine-mapping and candidate gene analyses have suggested that a leptin receptor (LEPR) gene polymorphism could be responsible for the SSC6 QTL effects after a possible effect on food intake (Ovilo et al., 2005). Joint analysis of such data could lead to more power to increase precision of QTL location or could be used to confirm the presence of QTL detected in 1 population (Walling et al., 2000; Kim et al., 2005; Pérez-Enciso et al., 2005). Nevertheless, joint analysis cannot be easily performed, because commonly different parental populations, markers, environments, and phenotypic traits are analyzed in different studies. Identification of candidate gene(s) and causal mutation(s) that could be responsible for the observed QTL effects is the ideal situation; however, it is a complicated task to tackle. Due to the difficulty of the quantitative trait nucleotide(s) identification, the first step would be the confirmation of the previously detected QTL and association analysis results in different populations. Using this approach in...
### Table 1. Descriptive statistics of the analyzed traits: BW and backfat thickness (BFT) recorded at different steps of the production cycle

<table>
<thead>
<tr>
<th>Item</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB × MS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW1, kg</td>
<td>183</td>
<td>70.9</td>
<td>10.8</td>
</tr>
<tr>
<td>BFT1, mm</td>
<td>182</td>
<td>22.4</td>
<td>4.0</td>
</tr>
<tr>
<td>BW2, kg</td>
<td>243</td>
<td>98.1</td>
<td>18.3</td>
</tr>
<tr>
<td>BFT2, mm</td>
<td>232</td>
<td>28.4</td>
<td>5.2</td>
</tr>
<tr>
<td>BW3, kg</td>
<td>198</td>
<td>161.7</td>
<td>21.6</td>
</tr>
<tr>
<td>BFT3, mm</td>
<td>196</td>
<td>32.1</td>
<td>5.5</td>
</tr>
<tr>
<td>IB × LR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW1, kg</td>
<td>310</td>
<td>84.4</td>
<td>11.4</td>
</tr>
<tr>
<td>BFT1, mm</td>
<td>310</td>
<td>16.8</td>
<td>4.3</td>
</tr>
<tr>
<td>BW2, kg</td>
<td>444</td>
<td>99.4</td>
<td>13.3</td>
</tr>
<tr>
<td>BFT2, mm</td>
<td>444</td>
<td>18.9</td>
<td>4.0</td>
</tr>
</tbody>
</table>

1Data for the 3 steps were recorded at 150, 198, and 357 d of mean age in the F2 sows of the Iberian × Meishan cross.

2Data for the 2 steps were recorded at an average of 150 and 173 d of age in the F2 animals of the Iberian × Landrace cross.

This study, more evidence that supports LEPR as the basis for a QTL could be added.

The purpose of this study was to carry out single and joint analyses of 2 independent QTL mapping populations to confirm the presence of the SSC6 QTL previously detected for production traits. In this context, a refinement of a previously identified QTL mapping on SSC6 for backfat thickness (BFT) and a new analysis for BW in the Iberian × Landrace (IB × LR) cross was performed. In addition, existence of the SSC6 QTL and its effect on these traits were demonstrated using a new F2 population: an Iberian × Meishan (IB × MS) cross. The functional implication of a nonsynonymous SNP were detected previously (Mackowski et al., 2005; Ovilo et al., 2005). Exon 20 was selected for sequencing because it is the longest exon. The PCR reactions were performed in a 25-µL final volume containing 100 ng of DNA, standard PCR buffer [75 mM Tris-HCl pH 9.0, 50 mM KCl, 20 mM (NH4)2 SO4], a specific concentration of MgCl2 for each primer pair (Table 2), 200 µM deoxynucleoside triphosphate, 0.5 µM of each primer, and 1 U of Tth DNA polymerase (Biotools, Madrid, Spain). The PCR were carried out in an AB GeneAmp PCR System 9700 (Applied Biosystems, Warrington, UK), and the thermal profiles were 94°C for 5 min, followed by 35 cycles of 94°C (30 s), specific annealing temperature of each primer pair shown in Table 2, 30 s) and 72°C (30 s), with a final extension step of 10 min at 72°C. The PCR products were purified with the QIAquick Gel extraction kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer’s protocol and sequenced with both PCR primers using the BigDye Terminator Cycle Sequencing kit in an ABIPRISM 3100 Genetic Analyzer (Applied Biosystems, Warrington, UK) following the manufacturer’s protocol. Single nucleotide polymorphisms were detected by editing and aligning the sequences using the MegAlign software (Winstar package, DNASTAR Inc., Madison, WI).

### Materials and Methods

Research protocols followed the guidelines state in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

### Animals and Traits

Data from 2 F2 populations were used: IB × MS and IB × LR F2 crosses that were described previously (Ovilo et al., 2000; Rodríguez et al., 2005). Briefly, the 2 experimental populations consisted of 3 Iberian boars (Guadyerbas) that were mated to 18 Meishan and 31 Landrace sows, respectively. In the IB × MS cross, 8 sires and 97 dams of the F1 generation produced 282 F2 sows. In the IB × LR cross, 6 sires and 72 dams of the F1 generation produced 577 F2 animals. All of the animals were reared under normal intensive conditions on the experimental farm of Nova Genética (Lleida, Spain). Feeding was ad libitum, and males were not castrated. Records used for both F2 populations correspond to growth and fatness traits presented in Table 1. These traits included BW and ultrasonic BFT. Body weight and BFT measurements were recorded in the F2 females of the IB × MS intercross at 3 different times: a) at 150 d of mean age (BW1, BFT1), b) when the gilts were transferred to gestation pens to be inseminated with a mean BW close to 100 kg (BW2, BFT2), and c) 1 wk before the gilts gave birth to their first litter (BW3, BFT3). Equivalent records for BW1, BFT1, BW2, and BFT2 were available for the F2 males and females of the IB × LR population (Ovilo et al., 2000).

### Sequencing of LEPR

Three milliliters of blood was collected in 4.5-mL blood collection tubes containing 0.054 mL of EDTA (BD Vacutainer, Plymouth, UK). Blood samples were obtained from the parental, F1, and F2 animals of the IB × MS population. Deoxyribonucleic acid was extracted from blood samples according to a standard protocol (Sambrook et al., 1989). Genomic DNA from the parental samples was used for the amplification and sequencing of the LEPR exons 4, 14, and 20 using pig-specific primers designed from the porcine LEPR cDNA sequence available (GenBank Accession Number AF092422). Primers are listed in Table 2. Exons 4 and 14 were selected for sequencing because nonsynonymous SNP were detected previously (Mackowski et al., 2005; Ovilo et al., 2005). Exon 20 was selected for sequencing because it is the longest LEPR exon. The PCR reactions were performed in a 25-µL final volume containing 100 ng of DNA, standard PCR buffer [75 mM Tris-HCl pH 9.0, 50 mM KCl, 20 mM (NH4)2 SO4], a specific concentration of MgCl2 for each primer pair (Table 2), 200 µM deoxynucleoside triphosphate, 0.5 µM of each primer, and 1 U of Tth DNA polymerase (Biotools, Madrid, Spain). The PCR were carried out in an AB GeneAmp PCR System 9700 (Applied Biosystems, Warrington, UK), and the thermal profiles were 94°C for 5 min, followed by 35 cycles of 94°C (30 s), specific annealing temperature of each primer pair shown in Table 2, (30 s) and 72°C (30 s), with a final extension step of 10 min at 72°C. The PCR products were purified with the QIAquick Gel extraction kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer’s protocol and sequenced with both PCR primers using the BigDye Terminator Cycle Sequencing kit in an ABIPRISM 3100 Genetic Analyzer (Applied Biosystems, Warrington, UK) following the manufacturer’s protocol. Single nucleotide polymorphisms were detected by editing and aligning the sequences using the MegAlign software (Winstar package, DNASTAR Inc., Madison, WI).

### SNP Detection on LEPR

Ten SNP were detected by sequencing and alignment of LEPR exons 4, 14, and 20 from 21 parental pigs (3...
Iberian boars and 18 Meishan sows (Table 3). A total of 1,358 bp of the LEPR gene were sequenced, allowing the confirmation of 5 previously identified SNP within exons 4 (c.221C > T, c.232A > T, and c.233T > C), 14 (c.2002C > T), and 20 (c.2769A > G; Mackowski et al., 2005; Ovilo et al., 2005), and the detection of 5 new additional SNP within exons 4 (c.192A > G and c.275C > T) and 20 (c.3074T > C, c.3075A > G, and c.3345T > C). Of these 10 SNP, 6 were nonsynonymous polymorphisms located within exon 4: c.221C > T (p.Thr69Met), c.232A > T (p.Ile73Ser), c.233T > C (p.Ile73Ser), c.275C > T (p.Ser87Phe); exon 14: c.2002C > T (p.Leu663Phe); and exon 20: c.3074T > C (p.Leu1020Pro).

The LEPR c.221C > T (exon 4) and c.2002C > T (exon 14) polymorphisms showed the same segregation pattern and had alternative alleles fixed in parental populations. The c.232A > T and c.233T > C LEPR SNP within exon 4 co-segregated in the combination c.232A/c.233T and c.232T/c.233C. No co-segregation was observed between the c.275C > T (exon 4) or c.3074T > C (exon 20) polymorphisms. Thus, only c.221C > T, c.232A > T, and c.275C > T SNP located within exon 4, and c.3074T > C SNP within exon 20, were genotyped along the IB × MS pedigree.

**Genotyping**

The F1 and F2 IB × MS individuals were genotyped implementing a pyrosequencing protocol for the 4 nonsynonymous polymorphisms described above. Two porcine-specific PCR primers and 1 pyrosequencing primer were designed for assaying each SNP. A 147-bp fragment of exon 4 was amplified by PCR using Pyro4F and Pyro4R primers, and a 180-bp-long fragment of exon 20 was amplified by PCR using Pyro20F and Pyro20R primers (Table 2). Reverse primers were 5′-biotin-labeled for immobilization to streptavidin-coated magnetic beads. The PCR reactions were carried out in the same way as described above, but with 40 cycles of 94°C (30 s), specific annealing temperature of each primer pair shown in Table 2 (30 s), and 72°C (45 s), with a final extension step of 10 min at 72°C, and MgCl2 concentrations indicated in Table 2. Pyrosequencing was performed in a PSQ HS 96 System according to the pyrosequencing protocol (Pyrosequencing AB, Uppsala, Sweden) using Pyro4P1 as the sequencing primer for the c.221C > T and c.232A > T SNP, Pyro4P2 as the sequencing primer for the c.275C > T SNP, and Pyro20P3 as the sequencing primer for the c.3074T > C SNP (Table 2).

Animals were also genotyped for 8 microsatellites (Sw973, Sw1057, S0087, Sw316, S0228, Sw1881, Sw1328, and Sw2419) and 2 polymorphisms on the melanocortin 1 receptor (MC1R) and LH β polypeptide (LHB) genes. Microsatellite markers were individually amplified by standard PCR protocol in an AB GeneAmp PCR System 9700 (Applied Biosystems), and the products were analyzed by capillary electrophoresis with fluorescent detection in an ABI PRISM 3100 Ge-
linkage Analyzer (Applied Biosystems). Genotypes were
determined using the GeneScan Analysis software v3.7
(Applied Biosystems). Melacortin 1 receptor was geno-
typed by PCR-RFLP with digestion by NsI (Fernán-
dez et al., 2004) for the c.283G > A SNP (GenBank
Accession Number AF_326520). Luteinizing hormone β
polypeptide was genotyped by PCR-RFLP with diges-
tion by Tsp45I (Muñoz et al., 2005) for the c.1549C >
G SNP (GenBank Accession Number D00579).

Most of the genetic markers from the IB × LR ped-
igree were previously genotyped (Ovilo et al., 2005).
Briefly, these data consisted of 13 microsatellites
(S0035, Sw1329, Sw1057, Sw0087, Sw1976, Sw316, Sw71,
S0228, DG32, Sw1881, Sw1328, Sw2419, and Sw607),
1 SNP (c.2002C > T) within LEPR exon 14, and an
insertion of 2 cytosines at position 896 on the
MC1R gene (nt67insCC; Kijas et al., 2001). In the present
study, 2 additional microsatellites (S0121 and DG93)
were genotyped.

**Statistical Analysis**

Linkage-mapping was performed with the build op-
tion of the CriMap software version 2.4 (Green et al.,
1994) using all of the marker information described
above for both populations. The QTL detection anal-
ysis was performed with the QXPAK software (Pérez-
Enciso and Misztal, 2004). The general univariate mod-
el for all traits was

\[ y_i = \text{fixed\_effects}_i + \beta c_i + [P(g_i=AA) - P(g_i=BB)] \]

\[ + a_{QTL} + P(g_i=AB) \]  \[ d_{QTL} + u_i + e_i \]  \[ [1] \]

where \( y_i \) is the ith individual record; \text{fixed\_effects} = sex
and batch in the IB × LR cross and batch in the IB ×
MS cross; \( \beta = \) the covariate coefficient with \( c \) being age
(for growth) or BW (for fatness); \( a = \) the QTL additive
effect; \( d = \) the dominance effect; \( u = \) the infinitesimal
-genetic effect; and \( e = \) the residual. The coefficients
\( P(g_i=AB) \) are the probabilities, obtained via a Monte
Carlo Markov chain algorithm, of the ith individual
having alleles of breed origin A (Iberian) or B (Meish-
han/Landrace) at the position of interest (Pérez-Encisco
and Misztal, 2004). The Haldane mapping function is
assumed to obtain these probabilities. The dominance
QTL effect \( d \) was included only when it was significant
\( (P < 0.05) \). The infinitesimal genetic effect was treated
as random, with covariance \( \mathbf{A}_u^2 \mathbf{A} \) being the numerato-
-r relationship matrix.

In addition to single trait QTL analyses, we car-
yed out complementary analyses using models for 2 traits
(pleiotropy). Furthermore, the traits BW1, BFT1, BW2,
and BFT2 were included in a joint QTL analy-
sis for both populations, in which single and bivariate
traits were analyzed. Joint QTL analysis of data from
both experimental crosses was performed using the fol-
lowing model:

\[ y_i = \text{cross}_i + \text{batch}_i + \text{sex}_i + \beta_1 c_i \\
+ \sum_{m=1}^{3} P_{jm} \alpha_m + u_i + e_i \]  \[ [2] \]

where \( y_i \) = the ith observation within the jth cross
\( (j = 1, 2) \); \( P_{jm} \) = the probability of the ith individual
having an allele of breed origin \( m \) (at the position an-
alyzed); \( \alpha_m \) = the mth breed allelic effect; \( c \) = the
cross effect; and \( \beta \) = the covariate effect (nested within
cross). The covariate \( c \) was age for BW, or BW itself
for the BFT measures.

The effects of LEPR alleles (c.2002C > T SNP within
exon 14) were introduced in the candidate gene analysis
following the marker-assisted association test (MAAT)
proposed by Zhao et al. (2003), in which the marker
information is also included taking into account linkage
 disequilibrium (LD) between breed populations. This
test was performed separately for each experimental
cross with the following animal model:

\[ y_i = \text{batch}_i + \text{sex}_i + \beta c_i + [P(g_i=AA) - P(g_i=BB)] \\
+ a_{QTL} + P(g_i=AB) d_{QTL} + u_i + e_i \]  \[ [3] \]

where \( \lambda_{ik} \) = an indicator variable with values 0, 1, and
2 depending on the number of copies of the ith allele
at the LEPR gene and \( a_{LEPRk} \) = the corresponding sub-
stitution effect.

Likelihood ratio tests (LRT) were calculated compar-
ing the appropriate decreased and full models. The nomi-
nal P-values were calculated assuming a \( \chi^2 \) distri-
bution of the LRT with the degrees of freedom given
by the difference between the number of estimated pa-
rameters in the decreased and full models. Significance
thresholds cannot be calculated here by permutation
techniques, because randomization would destroy the
family structure, which is needed to estimate the in-
 infinitesimal genetic effect. Significance thresholds for
the interpretation of QTL detection results were calculated
using the procedure described by Nezer et al. (2002).
This approach yields chromosomewise critical values
of LRT with 1 df of 17.27, 12.92, 9.89, and 8.57, and 2 df
of 20.68, 16.06, 12.81, and 11.36 associated with type I
errors of 0.1, 1, 5, and 10%, respectively.

**RESULTS AND DISCUSSION**

**Polymorphisms and Haplotypes of LEPR**

Polymorphisms that cause AA changes could affect
the structure of the coded protein, thus affecting its
function with a possible repercussion on the phenotype.
In this study, from the 10 LEPR SNP detected, we were
interested in evaluating the 6 nonsynonymous polymor-
phisms located within exons 4, 14, and 20. The allelic
frequencies of these SNP for the whole IB × MS popu-
lation were as follows: c.221C/c.2002C = 0.52, c.232A/c.
Segregation analysis of these nonsynonymous SNP in c.233T = 0.45, c.275C = 0.16, and c.3074T = 0.35. Unfortunately, we could not perform a LEPR haplotype association study due to the restricted distribution of haplotypes 3, 4, 5, 6, and 8 and its low informativeness in these 2 crosses. For this reason, both linkage-mapping and QTL detection and candidate gene analyses on BW and BFT traits were performed using only the c.2002C > T LEPR SNP. We selected this substitution located within exon 14 (L663F) because it was the most informative one among the 6 nonsynonymous SNP detected; moreover, a functional implication for this SNP was proposed in a previous study, in which the IB × LR population was analyzed for traits related to body composition (Ovilo et al., 2005).

**Marker Linkage Map**

Seven microsatellite markers and the LEPR SNP in exon 14 were common to both populations. This markers plus the individual genotype marker information from each cross population (9 microsatellite markers and 2 MC1R and LHB SNP) allowed the construction of a joint SSC6 linkage map. The joint map obtained was: MC1R – 12.6 – S0035 – 15.0 – Sw1329 – 0.0 – Sw973 – 29.5 – Sw1057 – 14.5 – S0087 – 15.6 – Sw1376 – 0.0 – LHB – 9.7 – Sw316 – 8.4 – Sw71 – 8.3 – S0228 – 3.7 – DG32 – 5.1 – S0121 – 3.9 – LEPR – 2.8 – Sw1881 – 3.8 – DG93 – 27.8 – Sw1328 – 7.0 – Sw2419 – 4.4 – Sw607. The complete sex-average map included 19 markers spanning 172.2 cM. Average marker spacing on the composite map was 9.1 cM. Marker order and relative locations were in agreement with previous map studies available in the public database (http://www.animalgenome.org/maps/index.html#map), and LEPR was located at 126.4 cM.

**QTL Mapping**

**Single-Population Analysis.** The results from the QTL detection study are summarized in Table 4, including both single and bivariate growth and fatness traits analyses. For each trait, in each cross, the table provides the estimated position of a QTL. The LRT value, the significance level, the additive effect of the Iberian allele, and the dominance effect are given for the estimated position.

The IB × MS results from single trait analyses revealed at least 1 QTL located around the position 121–128 cM (region defined by markers DG32 and Sw1881, which includes LEPR), which affects all of the traits analyzed in the present work. This QTL was highly significant (P < 0.001) for all of the BW traits and significant for BFT1 (P < 0.01) and for BFT2 and BFT3 (P < 0.05). Positive additive effects of the Iberian QTL allele were observed for both BW and fatness. The same QTL also had dominance effects for BFT1 and BW2.

The IB × LR results from single trait analysis indicated the existence of a highly significant QTL (P < 0.001) located in an estimated position around 120–126 cM, and affecting both fatness traits. This result was in agreement with prior analyses in which a highly significant QTL located on SSC6 that affected fatness traits was detected (de Koning et al., 2000; Ovilo et al., 2000, 2005; Grindflek et al., 2001; Mohrmann et al., 2006; Edwards et al., 2008). In our study, the QTL showed an additive effect, and, as expected, the Iberian allele increased BFT. A QTL for BW2 was identified in a different position (100 cM, between markers Sw816 and Sw71) than the QTL detected for the same trait in the IB × MS cross at 128 cM. This second QTL showed a low significance (P < 0.05) and revealed cryptic additive effects, because we expected that a Landrace allele would increase growth. Although not significant, the BW1 trait produced a LRT maximum peak with an estimated position at 97 cM and an additive effect similar to that of the BW2 trait. The existence of 2 different QTL for BW located around the same chromosome region (100 cM in IB × LR and 127–128 cM in IB × MS) is not possible to validate in this material with the available information. A possible explanation for these results could be the different genetic background among the parental populations in both crosses. Iberian and Meishan lines have similar growth characteristics, whereas the Landrace population has a greater growth capacity than Iberian or Meishan populations. In the IB × LR cross, the favorable QTL effect on growth of the Iberian allele might be attenuated by the Landrace
Several SSC6 QTL have been reported for fatness traits in different experimental populations (Malek et al., 2001a; Ovilo et al., 2002a; Varona et al., 2002; Szpyda et al., 2003; Nii et al., 2005; Mohrmann et al., 2006; Edwards et al., 2008). Mohrmann et al. (2006) detected QTL for several carcass fat characteristics within the S0228-Sw1881 interval, around the same region of the BFT QTL detected in the present study. Moreover, in our analysis, the results detected concerning fatness traits for both populations are in agreement with previous findings, in which QTL affecting fat deposition and other body composition traits were detected in SSC6, between markers Sw116 and Sw1881 (Varona et al., 2002) and markers DG32 and LEPR (Ovilo et al., 2005) within the IB × LR cross. Although Varona et al. (2002) detected significant QTL in SSC2, 4, 5, 8, and 17 for BW, QTL were not identified for this trait on SSC6. A possible explanation for these differences with the results in our study could be that, in the analysis of Varona et al. (2002), a whole-genome scan was developed in which just 7 markers were utilized for the SSC6 linkage map construction; however, in our work, the linkage map of SSC6 was refined, increasing the number of markers and leading to a greater power of QTL detection. Bidal et al. (2001) developed a QTL analysis of growth and fatness data from a cross between Meishan and Large White pig breeds. Body weight, ADG, and BFT were analyzed at different periods. Results revealed significant gene effects on SSC6 for BW at 13 wk close to the S0121 marker at 134 cM. This location is coincident with the QTL detected in our work for BW between S0121 and Sw1881 markers within the IB × MS population. These authors also identified significant QTL for BW at 17 wk and for BFT at 13 and 17 wk of age, and at 40 kg between S0087-S0059 markers at 60–74 cM.

**Joint-POPulation Analysis.** In a second approach, data from both populations were analyzed together. Results from the joint analyses are presented in Table 5, including both single and bivariate growth and fatness traits analyses. The table provides the estima-
ed position of a QTL, the LRT value, the significance level, and the additive effects of the Iberian allele for the estimated position.

Results from single trait analyses confirmed previous findings, indicating the existence of a highly significant QTL ($P < 0.001$) at 120–128 cM within the DG32-Sw1881 marker interval including LEPR, which affected all of the common traits analyzed within the joint-population analysis (BW1, BFT1, BW2, and BFT2). Although the QTL for BW1 did not reach statistical significance in the IB × LR single-population analysis, a highly significant QTL with additive effects was detected for this trait in the joint-population analysis. Additionally, the LRT and significance level for BFT1, BW2, and BFT2 were greater in the joint analysis than in the single-population analysis. The Iberian allele of the QTL increased both BW and fatness traits, and again the QTL affecting BW traits revealed cryptic additive effects in the IB × LR cross as occurred in the single-population analysis.

The bivariate analysis of growth and fatness traits revealed the presence of a highly significant QTL ($P < 0.001$) at 120–128 cM for BW1–BFT1 and BW2–BFT2, located at 124–125 cM in the same marker interval as the QTL detected in the single-population analysis and close to the LEPR gene. The QTL also showed an additive effect, and the Iberian allele again increased BW and fatness traits in both crosses. The LRT and significance level for the traits analyzed in common for both crosses (BW1-BFT1 and BW2-BFT2) were greater in the joint-population analysis than in the single-population analysis; this is consistent with more individuals leading to greater evidence of QTL effects and mapping resolution. Walling et al. (2000) demonstrated the potential of joint analysis in a QTL study of 7 different F2 crosses between a Western commercial breed and either the Meishan or European wild boar. Significant QTL for BW, BFT, and growth rate on chromosome 4 were detected. Other studies reported joint multibreed-multitrait QTL analyses in diverse breed cross populations that allowed the detection of more significant and additional numbers of QTL for several production traits than in a single-experiment analysis (Kim et al., 2005; Pérez-Enciso et al., 2005).

Several QTL analyses for different trait types have been reported independently in both IB × MS and IB × LR experimental crosses. These studies focused on analyzing fatness, meat quality, and fat composition traits in the IB × LR cross (Ovilo et al., 2002a,b; Varona et al., 2002; Clop et al., 2003; Mercade et al., 2005); however, the IB × MS studies have been mainly related to reproductive traits (Rodríguez et al., 2005). Our analysis is the first report wherein the IB × MS cross is studied for production traits. The single-population analysis has detected a QTL at 120–127 cM for BW1–BFT1 and BW2–BFT2, located at 124–125 cM in the single trait analysis and at 124–125 cM in the bivariate trait analysis for both BW and BFT traits.

### LEPR Association Analysis

The leptin gene product is an important circulating signal for regulation of BW. Leptin decreases food intake and stimulates energy expenditure by activating its receptor in specific hypothalamic nuclei. It has been found that LEPR, a single-transmembrane-domain receptor family, modulates feed intake and LH and GH secretion; it may also serve as a neural link between nutritional status and the reproductive and growth axis in the pig (Bell et al., 2005; Barb et al., 2006). A functional implication for the SNP located in the LEPR exon 14 that produces a L663F transition has

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**Table 5. Joint-population analyses**

<table>
<thead>
<tr>
<th>Trait</th>
<th>LRT value</th>
<th>S</th>
<th>Position, cM</th>
<th>IB × MS</th>
<th>IB × LR</th>
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</tr>
<tr>
<td>BW1, kg</td>
<td>25.83</td>
<td>***</td>
<td>127</td>
<td>5.45 ± 1.10</td>
<td>1.36 ± 0.91</td>
</tr>
<tr>
<td>BFT1, mm</td>
<td>35.17</td>
<td>***</td>
<td>127</td>
<td>1.57 ± 0.35</td>
<td>1.17 ± 0.28</td>
</tr>
<tr>
<td>BW2, kg</td>
<td>55.47</td>
<td>***</td>
<td>128</td>
<td>7.31 ± 1.05</td>
<td>2.68 ± 0.85</td>
</tr>
<tr>
<td>BFT2, mm</td>
<td>43.06</td>
<td>***</td>
<td>120</td>
<td>1.54 ± 0.37</td>
<td>1.37 ± 0.26</td>
</tr>
<tr>
<td>Bivariate analyses of growth and fatness traits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW1, kg</td>
<td>45.31</td>
<td>***</td>
<td>125</td>
<td>6.13 ± 1.24</td>
<td>1.79 ± 1.18</td>
</tr>
<tr>
<td>BFT1, mm</td>
<td>1.85 ± 0.40</td>
<td>1.23 ± 0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW2, kg</td>
<td>93.04</td>
<td>***</td>
<td>124</td>
<td>7.75 ± 1.13</td>
<td>2.30 ± 0.85</td>
</tr>
<tr>
<td>BFT2, mm</td>
<td>2.33 ± 0.37</td>
<td>1.60 ± 0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Quantitative trait loci detected on SSC6, significance level, position, and additive effects.
2BFT = backfat thickness.
3Likelihood ratio test.
4Significance level: ***$P < 0.001$.
5Additive effect: effect of the Iberian line (IB) vs. the Meishan (MS) or Landrace line (LR).
been proposed in a previous work (Ovilo et al., 2005) based on its location in a region coding for a domain, which binds to a variety of substances including DNA and fibronectin receptors on cell surfaces, and the large degree of conservation of the residue coded. In rodents, homozygous mutations in LEPR were found causing early onset morbid obesity, hyperphagia, and decreased energy expenditure (Lee et al., 1996). A SNP in the leptin receptor of type 1 diabetes-prone NOD/LtJ mice was detected producing a G640V transversion in the extracellular domain. All mutant mice became obese and hyperinsulinemic at weaning, with 70 to 80% developing early onset hyperglycemia (Lee et al., 2006). In humans, the K656N polymorphism was associated with resting metabolic rate, which is considered an intermediate phenotype of energy balance and body composition (Loos et al., 2006). Furthermore W664R and H684P polymorphisms, which resulted in impaired receptor signaling, were detected in subjects affected by hyperphagia, severe obesity, alterations in immune function, and delayed puberty (Faruqui et al., 2007). These polymorphisms are located in the extracellular domain of the protein and occurred in the third fibronectin III domain, as did the LEPR porcine L663F variant that we analyzed in this study.

Quantitative trait loci for daily feed consumption have been detected within the Sw1881-Sw322 marker interval at 121–150 cM (Mohrmann et al., 2006). This interval is coincident with the region of the BFT and BW QTL detected in the present study and moreover with the LEPR gene location. Consequently, we evaluated the LEPR gene as a physiological and also positional candidate gene for the QTL located at 120–128 cM. Positional candidate gene analysis was used to investigate whether the candidate gene locus is the QTL or the candidate gene polymorphism is linked to the QTL. In a standard association study, a significant result suggests that the candidate gene polymorphism analyzed is either the causal mutation or is in LD with the causative gene. Analysis of candidate gene loci in F2 populations is, however, influenced by extensive between-breed LD. Zhao et al. (2003) concluded that using a MAAT with inclusion of the candidate gene loci effects in a QTL mapping analysis is expected to decrease part of the between-breed LD. Varona et al. (2005) similarly concluded that it is unlikely that we can distinguish between the causative polymorphism and any other neutral polymorphism when both are located in the same genomic region, in particular if this polymorphism has alternative fixed alleles or very dissimilar frequencies in both parental populations, as occurs in our experiment.

In this context, we carried out a MAAT for the LEPR c.2002C > T SNP in both crosses, implementing a full model that included the effects of both QTL and LEPR alleles. In a first step, the full model was compared with a decreased model fitting QTL but no LEPR effects. The results of the first contrast shown in Table 6 (effects of c.2002C > T LEPR SNP) demonstrated significant association of LEPR with all of the traits in both cross populations, except for BFT2 within the IB × MS cross. The results suggested that the LEPR polymorphism might be responsible for the effects of the QTL located at 120–128 cM for at least BW1, BFT1, BW2, BW3, and BFT3 in the IB × MS population and for BW1, BFT1, BW2, and BFT2 in the IB × LR population. The Iberian LEPR allele increased BFT and BW in both experimental crosses.

In a second step, a complementary analysis was performed, in which the full model was compared with a decreased model, fitting only the LEPR effects to check out the consequences for QTL mapping. Results of these comparisons are shown in Table 6 (QTL effects). The second contrast showed relevant alterations in the QTL detected. Inclusion of the LEPR gene as a fixed effect in the QTL analysis for single traits strongly decreased

### Table 6. Results of the marker-assisted association test for the leptin receptor (LEPR) c.2002C > T SNP in both cross populations, implementing a full model that included the effects of both QTL and LEPR alleles

<table>
<thead>
<tr>
<th>Cross</th>
<th>Trait</th>
<th>$a^2 \pm SE$</th>
<th>$d^2 \pm SE$</th>
<th>LRT$^4$ value</th>
<th>$S^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB × MS</td>
<td>BW1, kg</td>
<td>5.37 ± 0.93</td>
<td>—</td>
<td>12.01</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>BFT1, mm</td>
<td>1.52 ± 0.33</td>
<td>−0.98 ± 0.42</td>
<td>17.69</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>BW2, kg</td>
<td>7.27 ± 1.12</td>
<td>−3.52 ± 1.52</td>
<td>8.58</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>BFT2, mm</td>
<td>1.51 ± 0.44</td>
<td>—</td>
<td>1.42</td>
<td>0.233</td>
</tr>
<tr>
<td></td>
<td>BW3, kg</td>
<td>8.11 ± 1.88</td>
<td>—</td>
<td>15.90</td>
<td>$7 \times 10^{-5}$</td>
</tr>
<tr>
<td></td>
<td>BFT3, mm</td>
<td>1.69 ± 0.56</td>
<td>—</td>
<td>6.56</td>
<td>0.010</td>
</tr>
<tr>
<td>IB × LR</td>
<td>BW1, kg</td>
<td>4.53 ± 1.46</td>
<td>—</td>
<td>9.33</td>
<td>$2 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>BFT1, mm</td>
<td>1.04 ± 0.25</td>
<td>—</td>
<td>3.81</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>BW2, kg</td>
<td>4.96 ± 1.34</td>
<td>—</td>
<td>12.55</td>
<td>$4 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>BFT2, mm</td>
<td>0.81 ± 0.30</td>
<td>—</td>
<td>4.20</td>
<td>0.040</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Position</th>
<th>$a \pm SE$</th>
<th>$d \pm SE$</th>
<th>LRT value</th>
<th>$S^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>−1.53 ± 0.93</td>
<td>—</td>
<td>2.05</td>
<td>NS</td>
</tr>
<tr>
<td>18</td>
<td>−0.66 ± 0.35</td>
<td>−1.07 ± 0.57</td>
<td>6.61</td>
<td>NS</td>
</tr>
<tr>
<td>36</td>
<td>2.30 ± 1.12</td>
<td>−3.41 ± 2.08</td>
<td>5.90</td>
<td>NS</td>
</tr>
<tr>
<td>171</td>
<td>−0.80 ± 0.44</td>
<td>—</td>
<td>3.33</td>
<td>NS</td>
</tr>
<tr>
<td>97</td>
<td>4.48 ± 2.16</td>
<td>—</td>
<td>4.27</td>
<td>NS</td>
</tr>
<tr>
<td>57</td>
<td>1.19 ± 0.52</td>
<td>—</td>
<td>5.57</td>
<td>NS</td>
</tr>
<tr>
<td>123</td>
<td>−3.12 ± 1.48</td>
<td>—</td>
<td>4.41</td>
<td>NS</td>
</tr>
<tr>
<td>66</td>
<td>−0.69 ± 0.29</td>
<td>—</td>
<td>5.48</td>
<td>NS</td>
</tr>
<tr>
<td>134</td>
<td>−2.65 ± 1.51</td>
<td>—</td>
<td>8.37</td>
<td>NS</td>
</tr>
<tr>
<td>112</td>
<td>0.76 ± 0.30</td>
<td>—</td>
<td>6.26</td>
<td>NS</td>
</tr>
</tbody>
</table>

1BFT = backfat thickness.
2Additive effect: effect of the Iberian line (IB) vs. the Meishan (MS) or Landrace line (LR).
3Dominance effect.
4Likelihood ratio test.
5Nominal significance level ($P < 0.05$).
6Chromosome-wise significance level, NS (QTL not significant).
the LRT value for the QTL previously detected for BW and BFT in both populations. Moreover, inclusion of LEPR SNP effects in the model leads to a large change in the location of the QTL peaks detected for most of the traits, even for BFT2 in the IB × MS cross. A possible explanation for the differences found for BFT2 in the first contrast could be the presence of other gene(s) that could be located close to LEPR and that might be responsible for the QTL effect on fatness at different periods of the life of a pig. Our results support the hypothesis that the polymorphism of the pig LEPR gene analyzed in this experiment might be responsible for the QTL detected for BFT and BW in both experimental crosses, even though LD with a closely linked causal mutation cannot be discarded.

In conclusion, this experiment verifies the usefulness of joint analysis within QTL mapping studies in terms of increase in power to detect QTL for productive traits, even when pig crosses involved have very different growth and fatness characteristics. This study supports earlier results regarding the association between LEPR alleles and BFT in the IB × LR cross, and also confirms this association in the IB × MS resource population. Moreover, this study detects an association not reported before between LEPR alleles and BW in both crosses. The QTL and LEPR effects identified in this study for BFT and growth traits could have important economic effects on pork production, particularly on the control of feed intake and body condition. It would require further validation of these effects in commercial populations and ideally the quantitative trait nucleotide confirmation by functional assays.

**LITERATURE CITED**


