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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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[1]

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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 F_2 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 F_2 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

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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

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Table 1. Descriptive statistics of the analyzed traits: BW and backfat thickness (BFT) recorded at different steps of the production cycle

Item	N	Mean	SD
IB × MS[1]			
BW1, kg	183	70.9	10.8
BFT1, mm	182	22.4	4.0
BW2, kg	243	98.1	18.3
BFT2, mm	232	28.4	5.2
BW3, kg	198	161.7	21.6
BFT3, mm	196	32.1	5.5
IB × LR[2]			
BW1, kg	310	84.4	11.4
BFT1, mm	310	16.8	4.3
BW2, kg	444	99.4	13.3
BFT2, mm	444	18.9	4.0

[1]Data for the 3 steps were recorded at 150, 198, and 357 d of mean age in the F_2 sows of the Iberian × Meishan cross.
[2]Data for the 2 steps were recorded at an average of 150 and 173 d of age in the F_2 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 F_2 population: an Iberian × Meishan (**IB × MS**) cross. The functional implication of a nonsynonymous *LEPR* gene SNP on body composition was previously considered in the IB × LR population (Ovilo et al., 2005). Thus, in this study, we analyzed such SNP for causality with respect to this QTL within both F_2 populations.

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 F_2 populations were used: IB × MS and IB × LR F_2 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 F_1 generation produced 282 F_2 sows. In the IB × LR cross, 6 sires and 72 dams of the F_1 generation produced 577 F_2 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 F_2 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 F_2 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 F_2 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, F_1, and F_2 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 m*M* Tris-HCl pH 9.0, 50 m*M* KCl, 20 m*M* $(NH_4)_2$ SO_4], a specific concentration of $MgCl_2$ for each primer pair (Table 2), 200 μ*M* deoxynucleoside triphosphate, 0.5 μ*M* 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

Table 2. Leptin receptor (*LEPR*) amplification conditions: forward (F) and reverse (R) PCR (rows 1 to 6) and pyrosequencing (Pyro4 and Pyro20, rows 7 to 12) primer sequences, amplicon sizes, annealing temperatures, and $MgCl_2$ concentrations

Exon	Primer sequence (5′ to 3′)	Size, bp	Annealing temperature, °C	$MgCl_2$, m*M*
4	F: 5′-GGCATATCCAATTACTCCTT-3′	223	50	2
	R: 5′-GTTTTTATCTTCCTCACTCCA-3′			
14	F: 5′-AGAGGACCTGAATTTTGGAGAA-3′	87	45	2.5
	R: 5′-AATTGGGAATACCTTCCAGA-3′			
20	F: 5′-CCGGAAACATTTGAGCATCTT-3′	824	52	2
	R: 5′-ACGGTTAGGTCATACATCTTG-3′			
Pyro4	F: 5′-TGCCTGCTGGAATCTCAAAG-3′	147	50	2
	R: 5′-biotin-TTCCTCACTCCAAAAGCAACAG-3′			
	P1: 5′-ATGAGGCAGTTGTTGAAA-3′			
	P2: 5′-TCAAACTTATCTTCTAAAACAACTT-3′			
Pyro20	F: 5′-ATTAAATATGCCACCCTGCTC-3′	180	50	1.5
	R: 5′-biotin-ATGCTGATCTGATAAAATAAAA-3′			
	P3: 5′-TCTAGCAGCAATTCCC-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.

Table 3. Single nucleotide polymorphisms detected in exons 4, 14, and 20 of the leptin receptor (*LEPR*) gene segregating in the Iberian × Meishan cross[1]

SNP	Exon	AA change
c.192A > *G	4	No
c.221C > *T	4	Thr69Met
c.232A > *T	4	Ile73Ser
c.233T > *C	4	Ile73Ser
c.275C > *T	4	Ser87Phe
c.2002C > *T	14	Leu663Phe
c.2769A > *G	20	No
c.3074T > *C	20	Leu1020Pro
c.3075A > *G	20	No
c.3345T > *C	20	No

[1]Positions are referred to the GenBank AF092422 sequence.
*Allele fixed in the Iberian population.

Genotyping

The F_1 and F_2 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 $MgCl_2$ 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 SNPs, 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 melacortin 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-

netic Analyzer (Applied Biosystems). Genotypes were determined using the GeneScan Analysis software v3.7 (Applied Biosystems). Melacortin 1 receptor was genotyped by PCR-RFLP with digestion by *Nsp*I (Fernández et al., 2004) for the c.283G > A SNP (GenBank Accession Number AF_326520). Luteinizing hormone β polypeptide was genotyped by PCR-RFLP with digestion by *Tsp45*I (Muñoz et al., 2005) for the c.1549C > G SNP (GenBank Accession Number D00579).

Most of the genetic markers from the IB × LR pedigree were previously genotyped (Ovilo et al., 2005). Briefly, these data consisted of 13 microsatellites (*S0035*, *Sw1329*, *Sw1057*, *S0087*, *Sw1376*, *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 option 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 analysis was performed with the QXPAK software (Pérez-Enciso and Misztal, 2004). The general univariate model for all traits was

$$y_i = \text{fixed\_effects}_i + \beta\ c_i + [P(g_i{\equiv}AA) - P(g_i{\equiv}BB)]\ a_{QTL} + P(g_i{\equiv}AB)\ d_{QTL} + u_i + e_i \quad [1]$$

where y_i = the ith individual record; fixed_effects = sex and batch in the IB × LR cross and batch in the IB × MS cross; β = 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 $P(g_i{\equiv}AB)$ are the probabilities, obtained via a Monte Carlo Markov chain algorithm, of the ith individual having alleles of breed origin A (Iberian) or B (Meishan/Landrace) at the position of interest (Pérez-Enciso 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 $\mathbf{A}\sigma_u^2, \mathbf{A}$ being the numerator relationship matrix.

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

$$y_{ij} = \text{cross}_j + \text{batch}_{ij} + \text{sex}_{ij} + \beta_j\ c_{ij} + \sum_{m=1}^{3} P_{ijm}\alpha_m + u_{ij} + e_{ij} \quad [2]$$

where y_{ij} = the ith observation within the jth cross (j = 1, 2); P_{ijm} = the probability of the ijth individual having an allele of breed origin m (at the position analyzed); α_m = the mth breed allelic effect; cross = the cross effect; and β = 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:

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

where λ_{ik} = an indicator variable with values 0, 1, and 2 depending on the number of copies of the kth allele at the *LEPR* gene and a_{LEPRk} = the corresponding substitution effect.

Likelihood ratio tests (**LRT**) were calculated comparing the appropriate decreased and full models. The nominal P-values were calculated assuming a χ^2 distribution of the LRT with the degrees of freedom given by the difference between the number of estimated parameters in the 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 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 polymorphisms located within exons 4, 14, and 20. The allelic frequencies of these SNP for the whole IB × MS population were as follows: c.221C/c.2002C = 0.52, c.232A/

c.233T = 0.45, c.275C = 0.16, and c.3074T = 0.35. Segregation analysis of these nonsynonymous SNP in the F_2 population revealed the existence of 6 different haplotypes. Haplotype 1 corresponded to the combination of alleles T-T-C-T-T-C for SNP within exons 4 (c.221C > T, c.232A > T, c.233T > C, c.275C > T), 14 (c.2002C > T), and 20 (c.3074T > C), respectively. This haplotype is fixed in the Iberian parental population and is not present in the Meishan population. Haplotype 2 corresponded to the combination C-A-T-T-C-T and was predominant in Meishan with a frequency of 0.47. Haplotypes 3 (C-A-T-C-C-T), 4 (C-T-C-T-C-C), 5 (C-A-T-C-C-C), and 6 (C-A-T-T-C-C) had restricted distribution with frequencies of 0.11, 0.14, 0.17, and 0.11, respectively. In the whole pedigree, frequencies of haplotypes 1 to 6 were 0.48, 0.24, 0.11, 0.07, 0.05, and 0.05, respectively. Partial coincidence was revealed with the *LEPR* haplotypes of the IB × LR population (Ovilo et al., 2005). Haplotype 1 was also fixed in the Iberian parental population of the IB × LR cross and revealed a very low frequency of 0.02 in the Landrace population, in which 3 more haplotypes were segregating. Haplotype 4 (C-T-C-T-C-C) had a frequency of 0.25, and 2 new haplotypes were detected. The major haplotype, named 7 (T-T-C-T-C-C), showed a frequency of 0.70; however, haplotype 8 (C-T-C-T-T-C) was scarce with a frequency of 0.03. In the whole IB × LR pedigree, frequencies of haplotypes 1, 4, 7, and 8 were 0.49, 0.11, 0.38, and 0.02, respectively.

In this study we carried out single and joint statistical analyses for both IB × LR and IB × MS crosses. 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. These 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/pigs/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 *Sw316* 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

Table 4. Single-population analyses[1]

	IB × MS					IB × LR			
Trait[2]	LRT[3] value	S[4]	Position, cM	a[5] ± SE	d[6] ± SE	LRT value	S	Position, cM	a ± SE
Single trait analyses									
BW1, kg	26.69	***	127	5.59 ± 1.02	—	6.32	NS	97	2.25 ± 0.89
BFT1, mm	20.16	**	127	1.55 ± 0.36	−1.11 ± 0.48	16.77	***	126	1.19 ± 0.28
BW2, kg	42.33	***	128	7.56 ± 1.15	−3.90 ± 1.61	12.05	*	100	2.69 ± 0.79
BFT2, mm	11.82	*	121	1.56 ± 0.46	—	40.14	***	120	1.37 ± 0.21
BW3, kg	32.72	***	125	10.71 ± 1.71	—	—	—	—	—
BFT3, mm	10.05	*	127	1.91 ± 0.60	—	—	—	—	—
Bivariate analyses of growth and fatness traits									
BW1, kg	39.26	***	124	5.57 ± 1.04	—	28.41	***	128	1.45 ± 0.95
BFT1, mm				2.71 ± 0.45	—				1.45 ± 0.33
BW2, kg	44.53	***	124	7.74 ± 1.23	—	48.34	***	112	2.36 ± 0.80
BFT2, mm				2.03 ± 0.46	—				1.61 ± 0.23
BW3, kg	41.99	***	127	10.77 ± 1.80	—	—	—	—	—
BFT3, mm				2.55 ± 0.61	—				—

[1]Quantitative trait loci detected on SSC6, significance level, position, and additive and dominance effects within both experimental pig crosses.
[2]BFT = backfat thickness.
[3]Likelihood ratio test.
[4]Significance level: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[5]Additive effect: effect of the Iberian line (IB) vs. the Meishan (MS) or Landrace line (LR).
[6]Dominance effect.

genetic background with different effects on BW. This fact could be influencing the dissimilar results detected for BW traits between IB × MS and IB × LR crosses in the single trait analysis, regarding significance and position of the BW QTL.

The bivariate analysis assumed that there is a QTL effect on both BW and BFT traits. For the IB × MS population, this analysis indicated evidence of a highly significant QTL ($P < 0.001$) for all of the pairs of traits analyzed (BW1-BFT1, BW2-BFT2, and BW3-BFT3). The QTL was located at 124–127 cM in the *S0121-Sw1881* interval. The same analysis for the IB × LR data showed a highly significant QTL ($P < 0.001$) within the *LEPR-Sw1881* marker interval at 128 cM for BW1-BFT1. This effect might be due to the highly significant QTL for BFT1 detected in the single trait analysis, because there was not a significant QTL detected for BW1. The bivariate analysis revealed another QTL within the *Sw71-S0228* marker interval at 112 cM affecting BW2-BFT2 traits. In this case, the result could be due to the combined effects of the QTL detected in the single trait analysis for both BW2 and BFT2 traits at 100 and 120 cM, respectively. With the available data, we cannot confirm if there is a QTL affecting 2 different traits (BFT and BW) or if there are 2 different QTL located around the same region affecting 1 trait each. In general, BW and BFT bivariate analyses demonstrated a greater statistical significance of the detected QTL than single trait analyses.

Numerous SSC6 QTL have been reported for fatness, meat quality, and body composition in different experimental populations (Malek et al., 2001a; Ovilo et al., 2002a; Varona et al., 2002; Szyda 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 *Sw316* and *Sw 1881* (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. Bidanel 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 estimat-

Table 5. Joint-population analyses[1]

Trait[2]	LRT[3] value	S[4]	Position, cM	a[5] ± SE	
				IB × MS	IB × LR
Single trait analyses					
BW1, kg	25.83	***	127	5.45 ± 1.10	1.36 ± 0.91
BFT1, mm	35.17	***	127	1.57 ± 0.35	1.17 ± 0.28
BW2, kg	55.47	***	128	7.31 ± 1.05	2.68 ± 0.85
BFT2, mm	43.06	***	120	1.54 ± 0.37	1.37 ± 0.26
Bivariate analyses of growth and fatness traits					
BW1, kg	45.31	***	125	6.13 ± 1.24	1.79 ± 1.18
BFT1, mm				1.85 ± 0.40	1.23 ± 0.28
BW2, kg	93.04	***	124	7.75 ± 1.13	2.30 ± 0.85
BFT2, mm				2.33 ± 0.37	1.60 ± 0.27

[1]Quantitative trait loci detected on SSC6, significance level, position, and additive effects.
[2]BFT = backfat thickness.
[3]Likelihood ratio test.
[4]Significance level: ***$P < 0.001$.
[5]Additive effect: effect of the Iberian line (IB) vs. the Meishan (MS) or Landrace line (LR).

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 ($P < 0.001$) for both 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 F_2 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 BFT within both crosses. Results from the IB × MS cross have confirmed the previous results from IB × LR (Ovilo et al., 2005). In addition, our analysis has detected at least a highly significant QTL at 125–128 cM for BW in the resource IB × MS cross population. Moreover, we have performed a joint-population analysis offering more power to detect the QTL at 120–128 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

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

		Effects of c.2002C > T *LEPR* SNP				QTL effects				
Cross	Trait[1]	a^2 ± SE	d^3 ± SE	LRT[4] value	S[5]	Position	a ± SE	d ± SE	LRT value	S[6]
IB × MS	BW1, kg	5.37 ± 0.93	—	12.01	5×10^{-4}	100	−1.53 ± 0.93	—	2.05	NS
	BFT1, mm	1.52 ± 0.33	−0.98 ± 0.42	17.69	1×10^{-4}	18	−0.66 ± 0.35	−1.07 ± 0.57	6.61	NS
	BW2, kg	7.27 ± 1.12	−3.52 ± 1.52	8.58	0.014	36	2.30 ± 1.25	−3.41 ± 2.08	5.90	NS
	BFT2, mm	1.51 ± 0.44	—	1.42	0.233	171	−0.80 ± 0.44	—	3.33	NS
	BW3, kg	8.11 ± 1.88	—	15.90	7×10^{-5}	97	4.48 ± 2.16	—	4.27	NS
	BFT3, mm	1.69 ± 0.56	—	6.56	0.010	57	1.19 ± 0.52	—	5.57	NS
IB × LR	BW1, kg	4.53 ± 1.46	—	9.33	2×10^{-3}	123	−3.12 ± 1.48	—	4.41	NS
	BFT1, mm	1.04 ± 0.25	—	3.81	0.051	66	−0.69 ± 0.29	—	5.48	NS
	BW2, kg	4.96 ± 1.34	—	12.55	4×10^{-4}	134	−2.65 ± 1.51	—	8.37	NS
	BFT2, mm	0.81 ± 0.30	—	4.20	0.040	112	0.76 ± 0.30	—	6.26	NS

[1]BFT = backfat thickness.
[2]Additive effect: effect of the Iberian line (IB) vs. the Meishan (MS) or Landrace line (LR).
[3]Dominance effect.
[4]Likelihood ratio test.
[5]Nominal significance level ($P < 0.05$).
[6]Chromosome-wise significance level, NS (QTL not significant).

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 become 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 (Farooqi 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 F_2 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

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.

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