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Fine mapping of porcine chromosome 6 QTL and *LEPR* effects on body composition in multiple generations of an Iberian by Landrace intercross

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Summary

The leptin receptor gene (*LEPR*) is a candidate for traits related to growth and body composition, and is located on SSC6 in a region where fatness and meat composition quantitative trait loci (QTL) have previously been detected in several F_2 experimental designs. The aims of this work were: (i) to fine map these QTL on a larger sample of animals and generations (F_3 and backcross) of an Iberian × Landrace intercross and (ii) to examine the effects of *LEPR* alleles on body composition traits. Eleven single nucleotide polymorphisms (SNPs) were detected by sequencing *LEPR* coding regions in Iberian and Landrace pig samples. Three missense polymorphisms were genotyped by pyrosequencing in 33 F_0, 70 F_1, 418 F_2, 86 F_3 and 128 individuals coming from the backcross of four F_2 males with 24 Landrace females. Thirteen microsatellites and one SNP were also genotyped. Traits analysed were: backfat thickness at different locations (BF_T), intramuscular fat percentage (IMF_P), eye muscle area (EM_A), loin depth (LO_D), weight of shoulder (SH_W), weight of ribs (RIB_W) and weight of belly bacon (BB_W). Different statistical models were applied in order to evaluate the number and effects of QTL on chromosome 6 and the possible causality of the *LEPR* gene variants with respect to the QTL. The results support the presence of two QTL on SSC6. One, at position 60–100 cM, affects BF_T and RIB_W. The other and more significant maps in a narrow region (130–132 cM) and affects BF_T, IMF_P, EM_A, LO_D, SH_W, RIB_W and BB_W. Results also support the association between *LEPR* alleles and BF_T traits. The possible functional implications of the analysed polymorphisms are considered.

1. Introduction

Leptin is an adipocyte-derived signalling factor, whose role in food intake control and body weight regulation is well known as it has been demonstrated in different species including pigs (Houseknecht *et al*., 1998). An important role in regulating fat mobilization has been also suggested for this protein (Halaas *et al*., 1995). The satiety effects of leptin are mediated through the leptin receptor, a member of the class I cytokine receptor family. Due to the fact that the leptin receptor is an essential component in the genetic basis underlying energy homeostasis and body weight, the leptin receptor gene (*LEPR*) is considered a candidate for traits related to growth and body composition. Furthermore the porcine *LEPR* gene is located on SSC6 in a region where several quantitative trait loci (QTL) for fatness and body composition traits have been detected (de Koning *et al*., 2000; Óvilo *et al*., 2000; Bidanel *et al*. 2001; Varona *et al*. 2002; Szyda *et al*., 2003). These previous investigations into QTL detection usually analysed experimental F_2 populations between divergent lines and mapped QTL with confidence intervals of 20–30 cM. These QTL should be mapped at a finer scale and confirmed in different populations.

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Table 1. *Main statistics of the traits analysed*

Description	*n*	Trait	Mean	SD
Backfat thickness at first rib (cm)	599	$BF1_T$	4·5	0·7
Backfat thickness at last rib (cm)	599	$BF2_T$	2·6	0·6
Backfat thickness between third-fourth-last ribs (cm)	385	$BF3_T$	2·9	0·8
Intramuscular fat percentage (%)	508	IMF_P	1·4	0·6
Loin muscle area (cm^2)	373	EM_A	34·1	5·1
Loin depth at the third-ribs (mm)	385	LO_D	46·9	6·3
Mean weight of shoulders (kg)	598	SH_W	5·3	0·9
Weight of ribs with sternum (kg)	470	RIB_W	14·3	2·6
Weight of belly bacon (kg)	595	BB_W	3·2	1·8
Carcass weight (kg)	599	C_W	75·6	10·5

All traits were measured in the F_2, F_3 and backcross populations, except for $BF3_T$, EM_A and LO_D that were measured only in the F_2 poulation.

Knowledge of the molecular basis of QTL following QTL detection experiments is a complicated task due both to the low resolution of the maps actually used, in which QTL confidence intervals usually comprise several megabases, and to the linkage disequilibrium present in most of the populations used for gene mapping, which leads to spurious associations of closely linked candidate gene single nucleotide polymorphisms (SNPs) (Zhao *et al*., 2003). This problem was pointed out in a previous study with the Iberian × Landrace F_2 intercross material used in the present study (Óvilo *et al*., 2002*a*), in which *FABP3* and *LEPR* were evaluated as candidate genes. The polymorphisms used in that study were located in non-coding regions and show a low informativeness in our population, especially the *LEPR* polymorphism. Results indicated that the polymorphisms analysed by Óvilo *et al*. (2002*a*) were not responsible for the variation of fatness traits but were probably very closely linked to the causal mutation of the QTL.

Mutations in the rodent leptin receptors account for mouse and rat severe obese phenotypes, and the $Lepr^{db/db}$ mouse is one of the best known monogenetic forms of obesity in mice (Tartaglia *et al*., 1995; Chen *et al*., 1996; Chua *et al*., 1996; Lee *et al*., 1996). In humans there are also mutations described in this gene associated with body mass index and fat mass variability (Clement *et al*., 1998; Yiannakouris *et al*., 2001; Quinton *et al*., 2001), although severe obesity phenotypes in human beings due to loss of function mutations in genes identified as causal in monogenic rodent obesity are rather rare (Snyder *et al*., 2004). These observations in different species indicate that polymorphisms in the leptin receptor gene can lead to changes in traits related to fatness, some of which might be quite substantial. Also, DNA sequence variations in the leptin receptor gene are postulated to play a role in fat topography and may be involved in the predisposition to abdominal obesity (Wauters *et al*., 2001*a*). Recently many functional and expression studies have been performed that help us to understand the biological role and possible impact of changes in the structure or composition of this receptor (Zabeau *et al*., 2004; Balthasar *et al*., 2004). This information is very useful for the interpretation of results of association studies.

The first objective of this work was to fine map the previously detected QTL on chromosome 6 by using a larger sample of animals and generations (F_3 and backcross) and more informative markers. This objective comprised verification of the number of QTL, and improvement of estimation of the QTL location and effects for different traits. Secondly, we intended to detect polymorphisms in the porcine *LEPR* coding regions and examine their effects on fatness and body composition traits, in order to evaluate the causality of the *LEPR* polymorphisms analysed with respect to the QTL.

2. Material and methods

(i) *Animals and phenotypic information*

Three populations of pigs were used for our study: (1) an Iberian × Landrace F_2 experimental population which has been described previously (Óvilo *et al*., 2000); (2) an F_3 generation coming from the intercross of F_2 animals ($n=86$); and (3) a backcross population coming from the cross of 24 pure Landrace females with four F_2 boars ($n=128$). The parental lines differ substantially in growth, carcass and meat quality traits (Serra *et al*., 1998).

The pigs were slaughtered and each carcass was divided into standardized commercial joints. Records used in the current study correspond to the traits presented in Table 1. Backfat subcutaneous thickness was measured with a ruler at different carcass locations: first rib ($BF1_T$), last rib ($BF2_T$) and between the third- and fourth-last ribs ($BF3_T$). Intramuscular fat

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Table 2. *LEPR amplification conditions: primer sequences, fragment sizes, annealing temperatures* (*Anneal T*) *and $MgCl_2$ concentrations for the genomic DNA* (*rows 1–5*), *cDNA* (*rows 6–11*) *and pyrosequencing* (*rows 12–14*) *PCR reactions*

Exons	Forward (F) and reverse (R) PCR primers, and pyrosequencing (P) primers	Size (bp)	Anneal T	$MgCl_2$
4	F: 5′-GGCATATCCAATTACTCCTT-3′ R: 5′-GTTTTTATCTTCCTCACTCCA-3′	223	50	2
6	F: 5′-GCTCGAAGTGTTAGAAGGAT-3′ R: 5′-TTTATGGGCTGAACTGACA-3′	204	52	2
9	F: 5′-TTGGGTCTAACATTTCTTTTCACT-3′ R: 5′-ACTTTCCTCGAGGTTTGGTTG-3′	183	50	2
15	F: 5′-CATTGTGCAGCGTGAGAAGTT-3′ R: 5′-ATTAAAATTTGCGGAAGAAGC-3′	170	52	2
20	F: 5′-CCGGAAACATTTGAGCATCTT-3′ R: 5′-ACGGTTAGGTCATACATCTTG-3′	824	52	2
4–6	F: 5′-GGCATATCCAATTACTCCTT-3′ R: 5′-TTTATGGGCTGAACTGACA-3′	627	50	1·5
6–9	F: 5′-GCTCGAAGTGTTAGAAGGAT-3′ R: 5′-ACTTTCCTCGAGGTTTGGTTG-3′	718	50	1·5
9–12	F: 5′-CAGTATGATGTTGTGGGTGAC-3′ R: 5′-CATAGCGAATCTGGAACTGA-3′	586	52	1·5
12–15	F: 5′-TGCCTCCATCCAGTGTGA-3′ R: 5′-TTAAAATTTGCGGAAGAAGC-3′	571	53	1·5
15–17	F: 5′-CATTGTGCAGCGTGAGAAG-3′ R: 5′-ATGAATATGGGGTAAAGACTGAAT-3′	433	54	2
15–20	F: 5′-GGGAAACCACACTAAACT-3′ R: 5′-GAAGAGGGCCAAATGTCACTG-3′	647	52	1·5
Pyro 4	F: 5′-TGCCTGCTGGAATCTCAAAG-3′ R: 5′-biotin-TTCCTCACTCCAAAAGCAACAG-3′ P: 5′-ATGAGGCAGTTGTTGAAA-3′	143	50	2
Pyro 9	F: 5′-GAGAACAAGATCGTTTCCTCAAA-3′ R: 5′-biotin- TGCATTCATATTGGGAAAAGTG-3′ P: 5′-TTCCTCAAAGTCAGTATG-3′	126	55	1·5
Pyro 14	F: 5′-TAATTAATGAAGATGCCACTAAAA-3′ R: 5′-biotin-AATTGGGAATACCTTCCAGA-3′ P: 5′-AAAAAAGAGAGGAATATCACTCTG-3′	65	45	2·5

content was measured in a sample of the longissimus lumborum muscle at the level of the last rib with the NIT (near infrared transmittance) technique (Davies & Grant, 1987). Loin muscle area (EM_A) and loin depth (LO_D) were measured on the transverse cut of the longissimus thoracis muscle at the third–fourth last ribs. Carcass weight (C_W) was measured 30 min *post mortem*.

(ii) *LEPR sequencing*

(a) *Genomic DNA amplification*

DNA was obtained from blood samples of 3 Iberian (Guadyerbas strain) and 12 Landrace pigs, according to a standard protocol (Sambrook *et al.*, 1989). The largest *LEPR* exons 4, 6, 9, 15 and 20 were PCR-amplified with primers listed in Table 2. All the primers were designed from the porcine *LEPR* cDNA sequence available (genBank AF092422). PCR was performed in 25 μl volumes containing 70 ng of DNA, standard PCR buffer (75 mM Tris-HCl pH 9·0, 50 mM KCl, 20 mM $(NH_4)_2SO_4$), a concentration of $MgCl_2$ specific for each primer pair (shown in Table 2), 200 μM dNTPs, 0·5 μM of each primer and 0·5 U *Tth* polymerase (Biotools, Madrid, Spain). Amplification conditions were 94 °C for 5 min, followed by 40 cycles of 94 °C (30 s), the 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. PCR reactions were performed on a PTC-100 thermocycler (MJ Research, Watertown, MA).

(b) *cDNA amplification*

Total RNA was obtained from muscle and hypothalamus samples of four Iberian (Guadyerbas strain) and four Landrace pigs with Tri Reagent (Sigma-Aldrich Chemie, Germany). Reverse transcription (RT) was performed with Superscript II (Invitrogen, Life Technologies) and random hexamers following the supplier's instructions. Muscle cDNA was amplified on five overlapping fragments covering exons 4 to 17 (2376 bp). Hypothalamus cDNA was used to amplify one fragment covering exons 15 to 20. Primers used are listed on Table 2. PCR was performed in the

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Table 3. *LEPR single nucleotide polymorphism (SNP) information*

SNP (exon)	Location (AF092422)	Polymorphism	Amino acid change
4-1	221	C/T	Thr69met
4-2	355	G/A	No
6	673	G/A	No
9	1160	A/C	Asp382Ala
13	1878	T/C	No
14	2002	C/T	Leu663Phe
15	2187	C/T	No
16-1	2244	G/A	No
16-2	2385	C/G	No
18	2544	A/G	No
20	2769	A/G	No

same way as for genomic DNA but containing 2 μl cDNA.

(c) *Sequencing*

PCR and RT-PCR products were sequenced in both directions with the Dye-Terminator Cycle Sequencing 3·0 kit in an ABI 377 automatic sequencer (Applied Biosystems). Sequences obtained were edited and aligned with the Winstar package to search for SNPs.

(iii) *Polymorphisms in LEPR*

Eleven SNPs were detected by sequencing 3424 bp of the *LEPR* coding region. A total of 1604 bp of the *LEPR* gene, corresponding to the largest exons, were sequenced from genomic DNA of the porcine breeds Iberian and Landrace, and allowed the identification of three SNPs at exons 4 (named 4-1), 9 and 20 (Table 3). Subsequently, cDNA comprising exons 4 to 20 was also sequenced from muscle and hypothalamus samples of the same breeds, allowing the confirmation of the three detected SNPs and the identification of eight additional SNPs. Four of them, located on exons 4 (4-2), 6, 15 and 16 (16-1), showed the new allele at low frequency. The other four SNPs, on exons 13, 14, 16 (16-2) and 18, were more informative, with different frequencies on Iberian and Landrace samples.

Exons 4 to 17 were sequenced from muscle cDNA, but the PCR fragment covering exons 15 to 20 could not be amplified from this source in many different amplification conditions. Two new primer pairs were designed in order to amplify this region, covering from exons 15 and 16 to exon 20 (sequences not shown), with the same negative results. Lack of amplification of all the PCR reactions including primers contained on exons 18 to 20 from muscle cDNA suggests that a short isoform is being predominantly transcribed in this tissue (Tartaglia, 1997). This fact was confirmed when the same primers (exons 15 to 20) allowed the amplification when we used hypothalamus cDNA in the RT-PCR, without the need for any optimization.

(iv) *Genotyping*

Three missense polymorphisms, located on exons 4 (T69M), 9 (D382A) and 14 (L663F), were selected due to their potential effects on protein structure and function and were genotyped by pyrosequencing in the Landrace × Iberian resource population comprising 33 F_0, 70 F_1, 418 F_2, 86 F_3 and 128 backcross individuals. Pyrosequencing was performed in a PSQ96 system (Pyrosequencing AB). A PCR primer pair and a pyrosequencing primer were designed for each SNP and are listed on Table 2. One of the PCR primers was 5′-biotin labelled for immobilization to streptavidin-coated magnetic beads. PCR reactions were carried out in the same way as described for the genomic DNA amplification but with extension times of 30 s, and annealing temperatures and $MgCl_2$ concentrations as indicated in Table 2.

Haplotypes were derived from the three SNP genotypes. We found five alleles segregating in the experimental population and nine different genotypes resulting from their combinations. Haplotype 1 corresponds to the combination of alleles T-A-T for SNPs on exons 4, 9 and 14 respectively. This allele is fixed in the Iberian parental population and has a very low frequency in the Landrace population (0·02). Haplotype 2 corresponds to the combination T-A-C and is predominant in Landrace (frequency 0·7). Haplotypes 3 (C-A-T), 4 (C-A-C) and 5 (C-C-C) are scarce (frequencies 0·03, 0·02 and 0·23 respectively). In the whole population the frequencies of haplotypes 1 to 5 were: 0·427, 0·440, 0·033, 0·017 and 0·083 respectively.

Thirteen microsatellites and one polymorphism at the *MC1R* gene were also genotyped. Microsatellites were: *S0035*, *Sw1329*, *Sw1057*, *S0087*, *Sw1376*, *Sw316*, *Sw71*, *S0228*, *DG32*, *Sw1881*, *Sw1328*, *Sw2419* and *Sw607*. These microsatellites were PCR-amplified and the products were analysed with Genescan software on capillary electrophoresis equipment with fluorescent detection (ABI PRISM 3100 genetic analyzer). *MC1R* gene was genotyped for the insertion of two cytosines at position 896 of this gene (nt67insCC; Kijas *et al*., 2001), which is totally informative in our cross (alternative alleles fixed in the two parental populations) and allows a better coverage of the chromosome as this gene is located at the end of the SSC6 p arm. This insertion was genotyped by the amplification of a 203–205 bp fragment, using primers MC1RA: 5′-CCAGCCAGGGCGAGTGTG-3′ and MC1RB: 5′-CTGGGGGCCCGTCTGGTTGGTC TG-3′ (labelled with 6-FAM), and the analysis of the

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size of the amplification products with Genescan software on capillary electrophoresis equipment (ABI PRISM 3100 genetic analyzer).

(v) *Statistical analysis*

Linkage mapping was performed using the 'Build' option of the CriMap software version 2·4 (Green *et al*., 1990). The QTL detection analysis was performed with QXPAK software (Pérez-Enciso & Misztal, 2004). Three statistical models were used for the QTL detection. The first was the mixed model:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{w}_1\mathbf{a} + \mathbf{w}_2\mathbf{d} + \mathbf{Z}\mathbf{u}_i + \mathbf{e}$$

Where $\mathbf{y}$ is the $n \times 1$ vector of observations, $\boldsymbol{\beta}$ the fixed effects (sex and batch) and covariates (carcass weight), $\mathbf{u} \approx \mathrm{N}(0, \mathbf{A}\sigma^2_{\mathrm{u}})$ is the $n \times 1$ vector of the additive polygenic effects with relationship matrix $\mathbf{A}$, and $\mathbf{w_1}$ and $\mathbf{w_2}$ are $n \times 1$ vectors of the c_a and c_d coefficients for the additive (a) and the dominance (d) effects respectively. The coefficients c_a and c_d were calculated as

$$C_a = \mathrm{Pr(QQ)} - \mathrm{Pr(qq)}$$

$$C_a = \mathrm{Pr(Qq)}$$

where Pr(QQ), Pr(Qq) and Pr(qq) are the probabilities of being homozygous for the allele of Iberian origin, or heterozygous or homozygous for the Landrace origin, respectively. These probabilities were computed using all markers and animals in the five-generation pedigree simultaneously.

In the second statistical model two traits were included in the analysis. The multitrait model was applied for the following pairs of traits: $BF1_T$–$BF2_T$, $BF1_T$–$BF3_T$, $BF2_T$–$BF3_T$, $BF1_T$–IMF_P and EM_A–LO_D. In the third model single-trait screening was done for two QTL.

For the candidate gene analysis the genotypic information of the *LEPR* was introduced in the analysis following the marker-assisted association test proposed by Zhao *et al*. (2003), in which the marker information is also included to account for between-breed linkage disequilibrium. This test was performed with an animal model with the sex and batch as fixed effects and the carcass weight as a covariate.

Likelihood ratio (LR) tests were calculated comparing the appropriate reduced and full models. The nominal P values were calculated assuming a chi-square distribution of the LR test with the degrees of freedom given by the difference between the number of estimated parameters in the reduced and full models. Significance thresholds cannot be calculated here by permutation techniques because we included an infinitesimal genetic value and randomization of the data would break the family structure. Then, significance thresholds for the interpretation of QTL detection results were calculated using the procedure described by Nezer *et al*. (2002), assuming one chromosome of 178 cM and an average distance between adjacent markers of 12·7 cM. This approach yields chromosome-wise critical values of LR tests with 2 d.f. of 19·60, 14·68, 12·07 and 10·69 associated with type I errors of 0·1%, 1%, 5% and 10%, respectively. The 95% confidence intervals for QTL locations were calculated according to Mangin *et al*. (1994).

3. Results

(i) *Linkage analysis*

The information on genotypes for all the markers (13 microsatellites, the 2 bases insertion in the *MC1R* gene and the haplotype of the *LEPR* gene) was used to construct an SSC6 linkage map, which was in agreement with previous evidence regarding the order and distances between markers (http://www.genome.iastate.edu/maps/marcmap.html). The map obtained was: *MC1R*–14·0–*S0035*–16·3–*Sw1329*–32·2–*Sw1057*–14·4–*S0087*–16·6–*Sw1376*–9·8–*Sw316*–8·1–*Sw71*–8·5–*S0228*–3·3–*DG32*–8·9–*LEPR*–5·1–*Sw1881*–28·9–*Sw1328*–7·6–*Sw2419*–4·3–*Sw607*, with a total length of 178 cM in the sex-averaged map. The mean distance between markers was 12·7 cM. The *LEPR* gene was located at 132·1 cM.

(ii) *Fine-mapping of QTL on chromosome 6*

The main statistics of the phenotypic records of the analysed traits are presented in Table 1. Results of single QTL analyses are shown in Table 4, and LR test profiles across the chromosome for the different traits are shown in Figure 1. When we applied the single QTL model and used all the polymorphisms as markers (including *LEPR* haplotype), the results confirmed previous findings, indicating the existence of one QTL located around the positions 125–132 cM (region defined by the markers *DG32* and *LEPR*), which affects all the traits analysed in the present work, except RIB_W. The statistical significance and the additive effect of the QTL are very high. The Iberian allele of the QTL increases fatness traits (BF_T, IMF_P and BB_W) and reduces muscle traits (EM_A, LO_D and SH_W). For the trait RIB_W another QTL was detected at a different location (103 cM). The LR profile across the chromosome (Fig. 1) shows two peaks of similar significance for this trait, located at positions 103 (maximum LR) and 130.

The multitrait model including records for pairs of fatness measures ($BF1_T$–$BF2_T$, $BF1_T$–$BF3_T$, $BF2_T$–$BF3_T$ and $BF1_T$–IMF_P) and two muscle traits (EM_A–LO_D) yields similar results to the single-trait

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Table 4. *Results of the single QTL analyses*

Trait	LR	S	Position	a (SE)	d (SE)
$BF1_T$	60·1	***	131 (128–134)	0·331 (0·042)	−0·086 (0·057)
$BF2_T$	69·8	***	129 (126–132)	0·283 (0·033)	−0·075 (0·046)
$BF3_T$	81·0	***	130 (127–132)	0·448 (0·049)	−0·205 (0·068)
IMF_P	49·0	***	132 (130–136)	0·254 (0·039)	−0·185 (0·052)
BB_W	29·2	***	129 (126–134)	0·299 (0·055)	−0·095 (0·076)
EM_A	32·4	***	131 (128–135)	−1·465 (0·337)	1·710 (0·456)
LO_D	18·3	**	130 (126–135)	−1·581 (0·438)	1·420 (0·603)
SH_W	19·3	**	125 (114–129)	−0·118 (0·030)	0·095 (0·041)
RIB_W	25·4	***	103 (98–109)	0·428 (0·094)	0·299 (0·136)

LR, likelihood ratio test; S, significance level $P<0·01$ (**) and $P<0·001$ (***); a, additive effect; d, dominance effect. Position is given in cM, confidence intervals in parentheses.

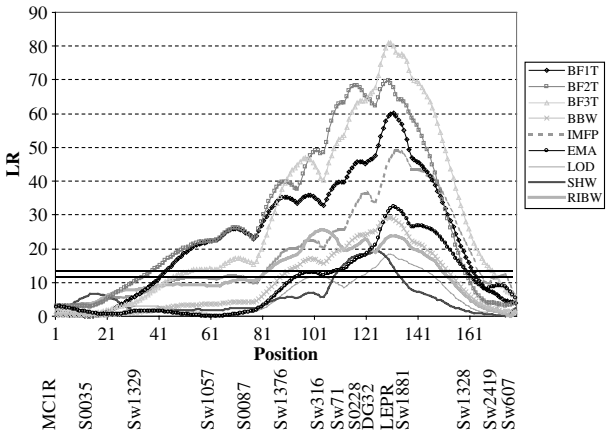

Fig. 1. Likelihood-ratio test profiles across chromosome 6 for the standard single QTL model. Horizontal continuous lines indicate the 5% and 1% significance thresholds.

analysis, detecting one extremely significant QTL at position 129–132 (results not shown).

The shape of the LR profile across the chromosome for the traits $BF1_T$, $BF2_T$ and $BF3_T$ shows secondary peaks at 60–90 cM; and the LR graph for RIB_W shows two peaks of similar significance around the positions 100 and 130 (Fig. 1). A single-trait/two-QTL model was applied for fatness traits ($BF1_T$, $BF2_T$, $BF3_T$, IMF_P and BB_W) and RIB_W. Relevant results obtained from the comparison of this two-QTL model against a reduced model including a single QTL at locations 125–135 cM are shown in Table 5. For the backfat measures $BF1_T$ and $BF2_T$, the results suggest the presence of two QTL ($P<0·10$). The first is the previously detected QTL at position 129–131 cM, and the second would be located at 64 and 84 cM for $BF1_T$ and $BF2_T$ traits, respectively (marker brackets *Sw1057–S0087* and *S0087–Sw1376*). For RIB_W the model assuming the presence of two QTL is also more likely than the model of one QTL ($P<0·05$; positions 106 and 129 cM). For $BF3_T$, IMF_P and BB_W the two QTL model did not provide significant results.

(iii) *LEPR candidate gene association analysis*

In the marker-assisted association test, a full model including the effects of both QTL and *LEPR* alleles (alternative hypothesis) was compared with a reduced model fitting QTL but no *LEPR* effects (null hypothesis). The significance of the effects of *LEPR* alleles was tested by a LR test between both models. Since this involves only a single test, *P* values were obtained from standard tables of the χ^2 distribution. The effects of four *LEPR* alleles were estimated as the difference relative to the effect of haplotype 5 (Table 6). Significant associations of *LEPR* gene variants with backfat traits, BB_W and RIB_W were found. The Iberian *LEPR* haplotype (allele 1) increases fatness and weight of ribs. No evidence of significant effects of *LEPR* alleles was found for the other traits (IMF_P, EM_A, LO_D and SH_W) for which QTL were mapped in the same region.

A complementary analysis was carried out to check the consequences on the QTL mapping of fitting *LEPR* effects in the statistical model. The above full model was compared with a reduced model fitting only the *LEPR* covariates. Results of this comparison are shown in Fig. 2 for the traits $BF1_T$, $BF2_T$, $BF3_T$, BB_W and RIB_W (for which the *LEPR* alleles have significant effects) and IMF_P (as a reference trait not affected by *LEPR* alleles). By comparing Figs.1 and 2, it can be observed that the inclusion in the model of effects of *LEPR* alleles produces important changes in the QTL detected. For the traits affected by the *LEPR* alleles, the QTL now detected does not correspond to the one detected in the standard single-QTL analysis, mapping to a different location. For $BF1_T$, $BF2_T$ and RIB_W, in which two QTL have been detected in this chromosome, these new locations correspond to the region of the second QTL (68–109 cM). For the traits $BF3_T$ and BB_W, the QTL detection results are not significant in this new contrast. For IMF_P, the QTL at location 132 cM is maintained in this analysis, although its significance is reduced compared with the results of standard single-QTL analysis.

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Table 5. *Relevant results of the QTL analyses with the two-QTL model*

Trait	LR	S	QTL	Position	a (SE)	d (SE)
$BF1_T$	11·8	+	1	131	0·300 (0·042)	−0·083 (0·056)
			2	64 (*Sw1057–S0087*)	0·145 (0·042)	−0·028 (0·056)
$BF2_T$	11·5	+	1	129	0·253 (0·036)	−0·043 (0·046)
			2	84 (*S0087–Sw1376*)	0·083 (0·038)	−0·142 (0·052)
RIB_W	12·8	*	1	129	0·279 (0·130)	−0·417 (0·155)
			2	106 (*Sw316–Sw71*)	0·247 (0·119)	0·449 (0·144)

LR, likelihood ratio test from the comparison of the models fitting two or one QTL; S, significance level $P<0{\cdot}1$ (+) and $P<0{\cdot}05$ (*); a, additive effect; d, dominance effect. Position is given in cM, with flanking markers in parentheses.

Table 6. *Effects of the LEPR haplotypes estimated fitting the marker-assisted association test*

	Additive effects (SE) relative to allele 5					
Trait	Allele 1	Allele 2	Allele 3	Allele 4	LR	*P* value
$BF1_T$	0·178 (0·072)	−0·104 (0·073)	−0·136 (0·124)	0·029 (0·161)	17·6	$0{\cdot}150\times10^{-2}$
$BF2_T$	0·137 (0·059)	−0·067 (0·056)	−0·039 (0·093)	0·010 (0·120)	26·6	$0{\cdot}241\times10^{-4}$
$BF3_T$	0·324 (0·092)	−0·063 (0·097)	−0·067 (0·221)	0·051 (0·201)	13·8	$0{\cdot}806\times10^{-2}$
IMF_P	0·204 (0·107)	0·114 (0·073)	0·116 (0·122)	0·258 (0·144)	5·5	0·238
BB_W	0·382 (0·104)	−0·001 (0·109)	0·369 (0·202)	0·150 (0·225)	17·1	$0{\cdot}183\times10^{-2}$
EM_A	−1·203 (0·888)	0·848 (0·640)	1·115 (1·404)	1·287 (1·271)	8·0	$0{\cdot}932\times10^{-1}$
LO_D	−0·949 (1·136)	0·191 (0·847)	1·023 (1·897)	−2·058 (1·718)	3·4	0·497
SH_W	0·003 (0·062)	0·032 (0·054)	0·083 (0·091)	−0·098 (0·119)	2·4	0·665
RIB_W	0·430 (0·186)	0·044 (0·188)	0·485 (0·356)	0·118 (0·391)	14·1	$0{\cdot}696\times10^{-2}$

LR, likelihood ratio test from the comparison of the models fitting the *LEPR* alleles and one-QTL effects against a reduced model fitting the QTL.

4. Discussion

(i) *Fine mapping of QTL on chromosome 6*

Our previous study on an experimental cross between Landrace and Iberian pigs allowed us to detect a very significant QTL on SSC6 affecting fatness and carcass composition traits (Ovilo *et al*., 2000; Varona *et al*., 2002), which was also detected and confirmed in other QTL experiments based on different breeds (de Koning *et al*., 2000; Bidanel *et al*., 2001; Szyda *et al*., 2003). In the present study fine mapping of SSC6 QTL has been performed, using more animals and populations (F_3 + backcross) and more markers. The statistical methodology has also been refined. Although simple regression is a powerful method for QTL detection, polygenic effects cannot be accommodated and this constitutes a drawback when dealing with crosses between outbred lines. In such situations using a mixed model (animal model) will show advantages (Nagamine & Haley, 2001). We used a flexible recently developed software for QTL modelling (Pérez-Enciso & Misztal, 2004) which allows the analysis of a variety of populations including F_3 and backcross. This software allows analysis of multitrait and multi-QTL models obtaining maximum likelihood estimates via an EM algorithm.

In the single-QTL analysis, the presence of the QTL on SSC6 is confirmed, with a high significance level and effect on all the traits considered in this study. For most of the traits, the location of the LR test peak corresponds to the 8·9 cM bracket between the markers *DG32* and *LEPR* ($BF1_T$, $BF2_T$, $BF3_T$, IMF_P, BB_W, EM_A, LO_D and SH_W). The trait RIB_W presents a more distant peak, located in the interval *Sw316–Sw71*. Confidence intervals obtained in the present study are smaller than with only the F_2 experimental population (Óvilo *et al*., 2002*a*; Varona *et al*., 2002). A direct comparison of the confidence intervals is not appropriate due to the different coverage and length of the map in the previous and present studies. Nevertheless, the former analysis showed confidence intervals of 10–25 cM for most of the traits, while the inclusion of the F_3 and backcross populations reduces them below 10 cM in most cases (i.e. for IMF_P the confidence interval has been reduced from 12 to 6 cM and for EM_A from 24 to 7 cM). Moreover, all the confidence intervals overlap in a 2 cM region (130–132 cM) when the results for all the fatness traits are examined simultaneously.

Iberian alleles increase all fatness traits and RIB_W, and have a negative effect on the muscle traits EM_A, LO_D and SH_W. The QTL effect is important, with a

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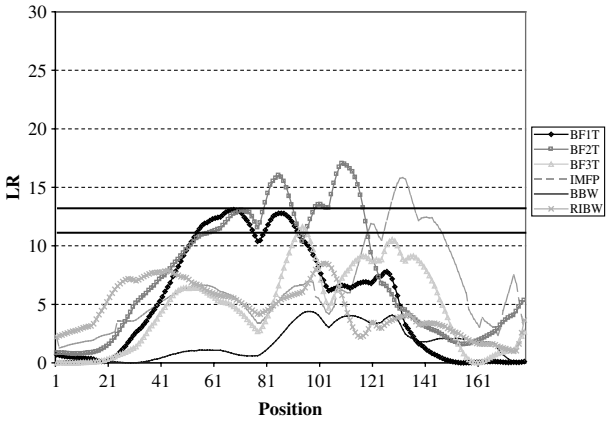

Fig. 2. Likelihood-ratio test profiles across chromosome 6 for the single-QTL detection model fitting the effects of *LEPR* alleles. Horizontal continuous lines indicate the 5% and 1% significance thresholds.

difference between homozygotes of 6–9 mm on backfat, depending on the location of the measurement; 0·51% on IMF_P (0·87 SD), 0·6 kg on BB_W (0·34 SD), 2·9 cm^2 on EM_A (0·57 SD), 3 mm on LO_D (0·48 SD) and 0·24 kg on SH_W (0·28 SD). One of the previous studies reporting the chromosome 6 QTL described parent-of-origin effects (de Koning *et al*., 2000); but the QTL detected in our population did not show significant imprinting (Varona *et al*., 2001), and thus this type of effect was not tested in the present study.

Multitrait analysis were performed for the single-QTL model using information on backfat and muscle traits simultaneously. The results obtained were concordant with the univariate analysis, showing one QTL with an increased statistical significance, at similar positions as in the single-trait/single-QTL analyses. In theory, QTL detection by multivariate analysis should give increased power and higher accuracy of gene localization when the traits analysed are highly correlated. However, in our hands the estimation of effects and position of the QTL was not improved by the inclusion of information on several traits, probably due to the high amount of information already available in our experiment giving very precise results with the univariate model, or due to the limited marker definition which does not allow a better approximation.

The two-QTL scan performed suggests the presence of a second QTL for $BF1_T$ and $BF2_T$ that matches with the region of a QTL previously described with effect on fatness and fatty acid composition traits (de Koning *et al*., 2000; Ovilo *et al*., 2002*b*). For RIB_W the two-QTL model is also more likely than the single-QTL model, suggesting the presence of two QTL for this trait located at 106 (*Sw316–Sw71*) and 129 cM. As the secondary QTL is not included in the same marker bracket, we performed tests for the two pairs of traits – $BF1_T$–$BF2_T$ and $BF1_T$–RIB_W – to check whether they could be considered different loci, by comparing the model of two linked QTL (at locations 40–110 cM) with the null hypothesis of pleiotropy. Both models included the effects of the *LEPR* alleles. The results did not allow the pleiotropy model to be rejected (results not shown). Thus, the joint QTL detection results support the presence of at least two QTL on SSC6, one affecting BF_T and RIB_W (position 64–106 cM), and the other and more significant one affecting fatness and all the carcass traits analysed here (position 129–132 cM).

(ii) *LEPR candidate gene association analysis*

The *LEPR* gene was evaluated in this work as a positional candidate gene for the QTL located at 129–132 cM. Positional candidate gene analyses are intended to examine whether a candidate gene is a QTL or, at least, is closely linked to the QTL. Association studies yield significant results when the candidate gene is the causal mutation and also when it is in linkage disequilibrium (LD) with the causal mutation. Analysis of positional candidate gene loci in QTL mapping populations such as breed crosses is then complicated by the extensive LD that is created in the cross, and thus significant effects on phenotype are detected even for candidate genes that are located at considerable distances from the real QTL. Zhao *et al*. (2003) used computer simulations to evaluate candidate gene tests in QTL mapping populations and concluded that, in a marker-assisted association test, the inclusion of QTL effects associated with markers could remove at least in part the impact of the between-breed LD that exists at the F_2 level, and thus improves the analysis of positional candidate genes in such populations. A similar conclusion has been reached by Varona *et al*. (2005), who show that it is not possible to discriminate between the causal mutation and any other neutral polymorphism when both are located in the same region and the analysed polymorphism is fixed or nearly fixed in the parental populations, as in our case.

Our results with the marker-assisted association test suggest that *LEPR* gene variants could be responsible for some of the QTL effects ($BF1_T$, $BF2_T$, $BF3_T$, BB_W and RIB_W), but there must be other QTL in the region that influence the other traits (IMF_P, EM_A, LO_D and SH_W). These results are concordant with those of Szyda *et al*. (2003) who, using multivariate mixed inheritance models, found that the chromosome 6 QTL affecting IMF_P is different from that affecting meat quality and body composition traits, supporting the hypothesis of several closely linked loci in the QTL region.

The influence of *LEPR* variants on fatness traits is consistent, as all the correlated backfat measures ($BF1_T$, $BF2_T$ and $BF3_T$) and BB_W are involved. These

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associations could, however, easily be ascribed to differences in appetite, due to the known role of leptin in feeding behaviour and energy expenditure and the modulating role of its receptor, and the direct relation of food consumption level and fat accumulation. However, the effect of this gene on the weight of the bone trait, ribs and sternum, is not so clear, as it is the only carcass composition trait related to *LEPR* variants. Moreover, when the same analysis was carried out for other correlated available carcass traits excluded from this study (i.e. length of the carcass), we did not obtain significant results (results not shown). A possible explanation could be that the effect of *LEPR* variants is produced in the early stages of development, coinciding with the period of maximum bone growth, although a false positive can not be discarded.

Observation of the allele distribution in the Landrace parental population shows that the fatness-associated allele (1) is present in this commercial population, although its frequency is very low. This population has been selected for lean growth, but the applied selection has not totally eliminated this fatness-promoting allele and so there may be a practical application of the molecular *LEPR* information for selection programmes in commercial pig populations.

Using the Simple Modular Architecture Research Tool (SMART; http://smart.embl-heidelberg.de) a prediction of the protein structure was performed for the five different alleles found, and found no differences between them in the distribution of regions and domains. The three studied polymorphisms correspond to the extracellular region of the coded protein, and one L663F (exon 14) is inside the third fibronectin type III domain. The fibronectin type III repeat regions are involved in a number of important functions, including binding with DNA. In humans, Fong *et al*. (1998) localized the leptin binding domain in the leptin receptor to the second segment of cytokine receptor domain/fibronectin type 3 domain (residues 428–635). These authors further provided evidence that the two following membrane proximal FN3 domains have no affinity for the ligand, but nevertheless are essential for receptor activation. In human, a polymorphism in the *LEPR* gene located inside this third fibronectin type III domain (K656N) was found to be associated in obese women with glucose and insulin metabolism and abdominal fat accumulation (Wauters *et al*., 2001*a*, *b*), and in a recent work the same SNP was associated with lean mass and fat mass in Caucasian nuclear families (Liu *et al*., 2004).

This residue 663 is conserved across species, showing a leucine at this position in all the sequences available at GenBank (human, rhesus monkey, rat, mouse and chicken). Degree of conservation is an indicator of the importance of each amino acid position for the protein function and, based on this fact, the software SIFT (Sorting Intolerant From Tolerant, http://blocks.fhcrc.org/sift/SIFT.html) sorts intolerant from tolerant amino acid substitutions and predicts whether an amino acid substitution in a protein will have a phenotypic effect. We applied this sequence-homology-based tool to study of the three missense SNPs analysed in this work. When we used the standard parameters, the program classified the three SNPs as 'tolerated' with scores of 0·12, 0·71 and 0·06 for the SNPs on exons 4, 9 and 14 respectively, the usual threshold being 0·05.

As this tool was designed to detect mutations that contribute to disease (Ng & Henikoff, 2001, 2002), the polymorphisms classified as deleterious are expected to produce total loss-of-function phenotypes. Then, the cut-off established is probably too demanding if we try to identify non-synonymous SNPs that would only modify the protein function (as we do not observe loss-of-function phenotypes in our population). In fact, the authors advise that scores slightly above the 0·05 cut-off could be considered as affecting protein function, depending on the purpose or characteristics of the study. Based on this information we can postulate that substitution L663F (score 0·06) would have a phenotypic effect on protein function that could explain the effect of the *LEPR* gene on the productive traits analysed.

Due to the possible phenotypic effect of the L663F substitution and the high informativeness of this mutation in our material, an independent analysis was performed for this SNP (exon 14). A marker-assisted association test was applied for the traits $BF1_T$, $BF2_T$, $BF3_T$, BB_W, and RIB_W, affected by *LEPR* haplotypes, comparing a full model with the QTL effects and a covariate indicating the number of copies of the allele T (fixed in Iberian and at low frequency in Landrace), against a reduced model fitting only the QTL effects. Results are included in Table 7 and are significant for the five traits analysed, with similar effects to the ones obtained in the haplotype analysis for allele 1, but with lower standard errors. Although this study validates the usefulness of association tests

Table 7. *Effects of the T/C polymorphism on LEPR exon 14 estimated fitting the marker-assisted association test*

Trait	Allele T effect (SE)	LR	*P* value
$BF1_T$	0·239 (0·035)	10·53	$0{\cdot}117 \times 10^{-2}$
$BF2_T$	0·169 (0·032)	21·38	$0{\cdot}376 \times 10^{-5}$
$BF3_T$	0·295 (0·086)	10·11	$0{\cdot}147 \times 10^{-2}$
BB_W	0·232 (0·052)	5·57	$0{\cdot}182 \times 10^{-1}$
RIB_W	0·369 (0·104)	12·18	$0{\cdot}480 \times 10^{-3}$

LR, likelihood ratio test from the comparison of the models fitting the *LEPR* alleles and one-QTL effects against a reduced model fitting the QTL.

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of intragenic haplotypes to identify quantitative trait genes, functional studies of this L663F substitution are required to confirm the hypothesis of its phenotypic effect.

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References

Balthasar, N., Coppari, R., McMinn, J., Liu, S. M., Lee, C. E., Tang, V., Kenny, C. D., McGovern, R. A., Chua, S. C. Jr, Elmquist, J. K. & Lowell, B. B. (2004). Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. *Neuron* **42**, 983–991.

Bidanel, J. P., Milan, D., Iannuccelli, N., Amigues, Y., Boscher, M. Y., Bourgeois, F., Caritez, J. C., Gruand, J., Le Roy, P., Lagant, H., Quintanilla, R., Renard, C., Gellin, J., Ollivier, L. & Chevalet, C. (2001). Detection of quantitative trait loci for growth and fatness in pigs. *Genetics, Selection, Evolution* **33**, 289–309.

Chen, H., Charlat, O., Tartaglia, L. D., Woolf, EA., Weng, X., Ellis, S. J., Lakey, N. D., Culpepper, J., Moore, K. J., Breitbart, R. E., Duyk, G. M., Tepper, R. I. & Morgenstern, J. P. (1996). Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* **84**, 491–495.

Chua, S. C., Jr, Chung, W. K., Wu-Peng, S., Zhang, Y., Liu, S.-M., Tartaglia, L. & Leibel, R. L. (1996). Phenotypes of mouse *diabetes* and rat *fatty* due to mutations in the OB (leptin) receptor. *Science* **271**, 994–996.

Clement, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gourmelen, M., Dina, C., Chambaz, J., Lacorte, J.-M., Basdevant, A., Bougneres, P., Lebouc, Y., Froguel, P. & Guy-Grand, B. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* **392**, 398–401.

Davies, A. M. C. & Grant, A. (1987). Near infrared analysis of food. *International Journal of Food, Science and Technology* **22**, 191–207.

De Koning, D. J., Rattink, A. P., Harlizius, B., Van Arendonk, J. A., Brascamp, E. W. & Groenen, M. A. (2000). Genome-wide scan for body composition in pigs reveals important role of imprinting. *Proceedings of the National Academy of Sciences of the USA* **97**, 7947–7950.

Fong, T. M., Huang, R. C., Tota, M. R., Mao, C., Smith, T., Varnerin, J., Karpitskiy, V. V., Krause, J. E. & Van der Ploeg, L. H. T. (1998). Localization of leptin binding domain in the leptin receptor. *Molecular Pharmacology* **53**, 234–240.

Green, P., Falls, K. & Crooks, S. (1990) Documentation of CRIMAP. Unpublished mimeo (http:// Biobase.dk. Embnetut/crimap).

Halaas, J. L., Gajiwala, K. S., Maffei, M., Cohen, S. L., Chait, B. T., Rabinowitz, D., Lallone, R. L., Burley, S. K. & Friedman, J. M. (1995). Weight reducing effects of the plasma protein encoded by the obese gene. *Science* **269**, 543–546.

Houseknecht, K. L., Baile, C. A., Matteri, R. L. & Spurlock, M. E. (1998). The biology of leptin: A review. *Journal of Animal Science* **76**, 1405–1420.

Kijas, J. M., Moller, M., Plastow, G. & Andersson, L. (2001). A frameshift mutation in MC1R and a high frequency of somatic reversions cause black spotting in pigs. *Genetics* **158**, 779–785.

Lee, G. H., Proenca, R., Montez, J. M., Carroll, K. M., Darvishzadeh, J. G., Lee, J. I. & Friedman, J. M. (1996). Abnormal splicing of the leptin receptor in diabetic mice. *Nature* **379**, 632–635.

Liu, Y. J., Rocha-Sanchez, S. M. S., Liu, P. Y., Long, J. R., Lu, Y., Elze, L., Recker, R. R. & Deng, H. W. (2004). Tests of linkage and/or association of the *LEPR* gene polymorphisms with obesity phenotypes in Caucasian nuclear families. *Physiology Genomics* **17**, 101–106.

Mangin, B., Goffinet, B. & Rebai, A. (1994). Constructing confidence intervals for QTL location. *Genetics* **138**, 1301–1308.

Nagamine, Y. & Haley, C. S. (2001). Using the mixed model for interval mapping of quantitative trait loci in outbred line crosses. *Genetical Research* **77**, 199–207.

Nezer, C., Moreau, L., Wagenaar, D. & Georges, M. (2002). Results of a whole genome scan targeting QTL for growth and carcass traits in a Pietrain × Large White intercross. *Genetics, Selection, Evolution* **34**, 371–387.

Ng, P. C. & Henikoff, S. (2001). Predicting deleterious amino acid substitutions. *Genome Research* **11**, 863–874.

Ng, P. C. & Henikoff, S. (2002). Accounting for human polymorphisms predicted to affect protein function. *Genome Research* **12**, 436–446.

Ovilo, C., Perez-Enciso, M., Barragan, C., Clop, A., Rodriquez, C., Oliver, M. A., Toro, M. A. & Noguera, J. L. (2000). A QTL for intramuscular fat and backfat thickness is located on porcine chromosome 6. *Mammalian Genome* **11**, 344–346.

Ovilo, C., Oliver, A., Noguera, J. L., Clop, A., Barragan, C., Varona, L., Rodriguez, C., Toro, M., Sanchez, A., Perez-Enciso, M. & Silio, L. (2002*a*). Test for positional candidate genes for body composition on pig chromosome 6. *Genetics, Selection, Evolution* **34**, 465–479.

Ovilo, C., Varona, L., Barragán, C., Clop, A., Rodriguez, C., Toro, M., Silió, L., Sánchez, A. & Noguera, J. L. (2002*b*). Discrimination between linked and pleiotropic QTL on pig chromosome 6 by multitrait least squares analysis. *Proceedings of the 7th World Congress on Genetics Applied to Livestock Production* (Montpellier) **30**, 51–54.

Pérez-Enciso, M. & Misztal, I. (2004). Qxpak: a versatile mixed model application for genetical genomics and QTL analyses. *Bioinformatics* **20**, 2792–2798.

Quinton, N. D., Lee, A. J., Ross, R. J. M., Eastell, R. & Blakemore, A. I. F. (2001). A single nucleotide polymorphism (SNP) in the leptin receptor is associated with BMI, fat mass and leptin levels in postmenopausal Caucasian women. *Human Genetics* **108**, 233–236.

Sambrook, J., Fritsh, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Serra, X., Gil, F., Pérez-Enciso, M., Oliver, M. A., Vázquez, J. M., Gispert, M., Díaz, I., Moreno, F., Latorre, R. & Noguera, J. L. (1998). A comparison of carcass, meat quality and histochemical characteristics of Iberian (Guadyerbas line) and Landrace pigs. *Livestock Production Science* **56**, 215–223.

Snyder, E. E., Walts, B., Perusse, L., Chagnon, Y. C., Weisnagel, S. J., Rankinen, T. & Bouchard, C. (2004). The human obesity gene map: the 2003 update. *Obesity Research* **12**, 369–439.

Szyda, J., Grindflek, E., Liu, Z. & Lien, S. (2003). Multivariate mixed inheritance models for QTL detection on porcine chromosome 6. *Genetical Research* **81**, 65–73.

Tartaglia, L. A. (1997). The leptin receptor. *Journal of Biological Chemistry* **272**, 6093–6096.

Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G. J., Campfield, L. A., Clark, F. T., Deeds, J. Muir, C., Sanker, S., Moriarty, A., Moore, K. J., Smutko, J. S., Mays, G. G., Woolf, E. A., Monroe, C. A. & Tepper, R. I. (1995). Identification and expression cloning of a leptin receptor, OB-R. *Cell* **83**, 1263–1271.

Varona, L., Sánchez, A., Rodríguez, M. C., Clop, A., Ovilo, C., Coll, A., Barragán, C., Oliver, M. A., Babot, D., Diaz, I., Toro, M., Folch, J., Perez-Enciso, M., Silió, L. & Noguera, J. L. (2001). Análisis de imprinting en caracteres de calidad de canal, carne y ácidos grasos en un cruce F_2 Ibérico × Landrace. *Información Técnica Económica Agraria* **22**, 115–117.

Varona, L., Ovilo, C., Clop, A., Noguera, J. L., Pérez-Enciso, M., Coll, A., Folch, J. M., Barragán, C., Toro, M. A., Babot, D. & Sánchez, A. (2002). QTL mapping for growth and carcass traits in an Iberian by Landrace pig intercross: additive, dominant and epistatic effects. *Genetical Research* **80**, 145–154.

Varona, L., Gómez-Raya, L., Rauw, W. M. & Noguera, J. L. (2005). A simulation study on the detection of causal mutations from F_2 experiments. *Journal of Animal Breeding and Genetics* **122**, 30–36.

Wauters, M., Mertens, I., Chagnon, M., Rankinen, T., Considine, R. V., Chagnon, Y. C., Van Gaal, L. F. & Bouchard, C. (2001*a*). Polymorphisms in the leptin receptor gene, body composition and fat distribution in overweight and obese women. *International Journal of Obesity and Related Metabolic Disorders* **25**, 714–720.

Wauters, M., Mertens, I., Rankinen, T., Chagnon, M., Bouchard, C. & Van Gaal, L. (2001*b*). Leptin receptor gene polymorphisms are associated with insulin in obese women with impaired glucose tolerance. *Journal of Clinical Endocrinology and Metabolism* **86**, 3227–3232.

Yiannakouris, N., Yannakoulia, M., Melistas, L., Chan, J. L., Klimis-Zacas, D. & Mantzoros, C. S. (2001). The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability. *Journal of Clinical Endocrinology and Metabolism* **86**, 4434–4439.

Zabeau, L., Defeau, D., Van der Heyden, J., Iserentant, H., Vandekerckhove, J. & Tavernier, J. (2004). Functional analysis of leptin receptor activation using a Janus kinase/signal transducer and activator of transcription complementation assay. *Molecular Endocrinology* **18**, 150–61.

Zhao, H., Rothschild, M. F., Fernando, R. L. & Dekkers, J. C. M. (2003). Tests of candidate genes in breed cross populations for QTL mapping in livestock. *Mammalian Genome* **14**, 472–482.