Confirmed quantitative trait loci for fatness and growth on pig chromosome 4

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Quantitative trait loci (QTLs) with large effects on fatness and growth have been identified previously on pig chromosome 4 in an intercross between the European wild pig and Large White domestic pigs. Two F_2 sows, heterozygous for the actual chromosome region, were backcrossed to a Large White boar, and two backcross (BC_1) boars were in turn backcrossed to Large White/Landrace sows. One of the boars was heterozygous for an intact wild pig-derived QTL region, whereas the other carried a recombinant haplotype. A total of 85 BC_2 animals were produced. Phenotypical measurements included daily weight gain, ultrasonic measurements of fat depth at 70 and 90 kg and several carcass traits. QTL segregation was deduced using 15 markers previously assigned to chromosome 4. Highly significant QTL effects were observed on all fatness traits and on the length of the carcass. A small but significant effect on growth was also observed. The results confirm the presence of one or more QTLs on chromosome 4 affecting fatness and growth. There was a good agreement between the estimates of QTL effects in the F_2 and BC_2 generations. The results from the recombinant sire family allowed us to map the major QTL effect distal to the recombination breakpoint. We propose that this confirmed QTL with a major effect on fatness is designated *FAT1*.

Keywords: abdominal visceral fat, growth, pig, quantitative trait loci, subcutaneous fat.

Introduction

Genetic dissection of complex polygenic traits is a topical subject in humans, plants and animals (Lander & Schork, 1994; Paterson, 1995; Georges & Andersson, 1996). We have previously identified quantitative trait loci (QTLs) for growth and fatness traits in an intercross between two divergent populations of pigs, the European wild pig and the Large White domestic pig (Andersson *et al.*, 1994; Knott *et al.*, 1998). The study was the first genome-wide screening for QTLs in outbred animals. The QTLs with the largest effect were located on chromosome 4 and explained about 12% and 18% of the phenotypical variance in the F_2 generation for growth and fatness traits respectively. The wild pig alleles at these loci were associated with reduced growth and higher fat deposition, as expected because of the intense selection for lean growth in modern domestic pigs.

It is essential that the presence and location of QTLs are confirmed before attempts are made towards their cloning or exploitation in animal breeding by marker-assisted selection. There are, in fact, several reasons why one may fail to confirm the QTL in a subsequent experiment: e.g. (i) the original observation may be a type I error (see Lander & Kruglyak, 1995); (ii) the QTL effect may depend on epistasis (Frankel & Schork, 1996); (iii) a large QTL effect may be caused by several linked QTLs each with a small effect, and the linkage may break up in subsequent generations; (iv) the estimated QTL effect may be seriously inflated when the power of QTL detection is low (Georges *et al.*, 1995); (v) segregation at the QTL in the recipient population may make some BC animals homozygous at the QTL or make the difference between QTL alleles too small to detect.

The objective of this study was to confirm the presence of QTLs with major effects on growth and fatness on pig chromosome 4 and to improve the

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map localization by backcrossing F_2 animals to domestic pigs.

Materials and methods

Animals

All F_2 males from the intercross between European wild pig and Large White (Swedish Yorkshire) domestic pigs (Andersson *et al.*, 1994) had been castrated in order to minimize the cost of producing the F_2 generation and could not be used for further breeding. Therefore, two F_2 females were selected for breeding on the basis of marker information for the chromosome 4 region harbouring the fatness QTL. Marker data indicated that they were both heterozygous at the fatness QTL. One sow carried an intact wild pig-derived segment, whereas the other sow carried a recombinant wild pig/Large White haplotype (Fig. 1). The sows were backcrossed to a Large White boar. Two backcross (BC_1) males with QTL genotypes corresponding to the two mothers (Fig. 1) were identified and backcrossed to six Large White and four Large White/Landrace crossbred sows generating a total of 85 BC_2 progenies. After weaning, the progenies were placed in groups of eight per pen according to QTL genotype (as deduced using marker *S0175*), weight at 9 weeks of age and sex (gilts and boars separated). All 12 groups were fed a commercial diet (12.3 MJ, 16.8% crude protein) *ad libitum*. Phenotypical measurements included daily weight gain, ultrasonic measurements of fat depth at 70 and 90 kg and

several carcass traits (Lundström *et al.*, 1995) as compiled in Table 1.

Markers

The segregation was studied using 15 chromosome 4 markers (Table 2), 14 of which were anonymous microsatellites and one random fragment length polymorphism (RFLP) (*ATP1B1*). The references for markers are given in Marklund *et al.* (1996) except for *S0023* (Coppieters *et al.*, 1993), *S0214* (Robic *et al.*, 1995), *Sw445* and *Sw856* (Rohrer *et al.*, 1994).

Linkage and QTL analysis

The linkage analysis was performed using CRI-MAP version 2.4 (Green *et al.*, 1990) with genotype information from all five generations. The order of markers obtained was in complete agreement with previously published maps for pig chromosome 4 (Archibald *et al.*, 1995; Marklund *et al.*, 1996; Rohrer *et al.*, 1996). The CHROMPIC option in CRI-MAP was used to identify every recombination event among the animals and also to trace allelic origin (wild pig or domestic pig) through the pedigree.

The QTL analysis was carried out as single-marker analysis using the SAS package (procedure MIXED and procedure GLM; SAS, 1992). Preliminary statistical analyses revealed significant differences between sires and between sexes for the fatness traits; on average, females had higher fat deposition

Fig. 1 Summary of previous analysis using data from the F_2 generation of a wild pig intercross showing the presence of one or more fatness QTLs on pig chromosome 4; genetic markers used in the linkage analysis are shown below the x -axis. The wild pig segments (marked in black) carried by the selected BC_1 sires of the present study are shown below. The dotted horizontal line represents the 5% genome-wide significance threshold.

Table 1 Least-square means for different traits according to QTL genotype on pig chromosome 4

Trait	Heterozygote wild/domestic	Homozygote domestic	<i>P</i> -value	% of phenotypical variance
Fatness traits				
Abdominal visceral fat (kg)	1.69 ± 0.08	1.30 ± 0.07	<0.0001	15.4
Subcutaneous fat depth (mm)	21.8 ± 0.65	19.5 ± 0.62	<0.0001	7.3
Percentage body fat of carcass (% BF)	22.6 ± 0.61	20.3 ± 0.58	<0.0001	9.7
BMI (kg m ⁻²)	77.0 ± 0.84	76.0 ± 0.80	0.21	—
Growth traits				
Weight at slaughter (kg)	107 ± 0.98	109 ± 0.98	0.038	5.0
Fat-free weight (%)	57.8 ± 0.73	60.4 ± 0.72	0.0002	11.9
Daily weight gain (g day ⁻¹)	610 ± 9.8	634 ± 9.7	0.015	5.8
Length of carcass (cm)	97.4 ± 0.4	98.9 ± 0.4	0.001	11.4
Length of small intestine (m)	18.3 ± 0.41	18.1 ± 0.41	0.61	—
Age at slaughter (days)	174 ± 1.9	171 ± 1.9	0.05	—

than males. Therefore, the effects of sire and sex were adjusted for in the statistical analysis. A mixed model was used including sire, sex and marker as fixed effects and dam nested within sire and residual error as random effects. Interaction was tested at

the marker position with the largest QTL effect on each trait. No significant interaction was found between marker and sire, marker and sex or sire and sex. Therefore, interactions were not included in further analysis.

Table 2 Pig chromosome 4 markers used in QTL analysis with frequencies of alleles originating from the wild (W) and domestic (D) founders in two BC₁ sire families (W782 and W859)

Locus	No. of progeny*				Map position (cM)
	Sire W782 <i>n</i> = 50		Sire W859 <i>n</i> = 35		
	W	D	W	D	
<i>S0011</i>	—	—	14	21	1.6
<i>S0301</i>	—	—	14	21	12.6
<i>Sw835</i>	—	—	16	19	16.6
<i>S0001</i>	—	—	17	18	27.6
<i>Sw871</i>	—	—	16	19	35.0
<i>S0175</i>	25	25	15	20	52.9
<i>S0107</i>	25	25	—	—	54.5
<i>S0023</i>	25	25	12	23	57.6
<i>ATP1B1</i>	26	24	13	22	61.8
<i>S0214</i>	27	23	17	18	71.0
<i>S0067</i>	—	—	15	20	93.3
<i>Sw445</i>	—	—	15	20	98.4
<i>S0097</i>	32	18	16	19	119.2
<i>S0161</i>	32	18	—	—	124.3
<i>Sw856</i>	31	19	—	—	130.5

* — Not informative because homozygous for Large White-derived segment (see Fig. 1) or homozygous for marker.

The analysis of combined data involved only the markers, which were informative in both sires. In the separate analysis for each sire, the effect of sire was not included and dam (sire) was replaced by the effect of dam. Procedure MIXED was used to calculate all F -statistics. The proportion of the phenotypic variance explained by the QTL was estimated using procedure GLM as the differences in r^2 -values between a model with and without the marker information. A nominal significance threshold was used, as the design of this experiment was to test for the presence of a QTL at a defined location in the genome.

Results

Development of backcross generations, BC₁ and BC₂

Two BC₁ males were selected for this study on the basis of marker analysis. W859 carries a wild pig-derived segment, which covers almost the entire chromosome 4, whereas W782 carries a recombinant chromosome 4 (Fig. 1). By contrasting the observed QTL effects in the progeny groups of these sires, we should be able to evaluate whether the major QTL effect on fatness is located proximal or distal to the recombination breakpoint or if the recombination event will break up a QTL haplotype so that the QTL effect is markedly reduced in the W782 family. The wild pig chromosome segments carried by W782 and W859 originate from the same founder boar. This boar was homozygous for all markers in the interval *S0107*–*S0067* (38.8 cM), but flanking markers indicated that the wild pig segment of W782 and W859 originated from different chromosome 4 homologues.

The two BC₁ males were backcrossed to Large White or Large White/Landrace females, and a total of 85 BC₂ progenies were used in the present study. Growth and fatness data were collected on all animals, and 15 chromosome 4 markers were used for segregation analysis (Table 2); the observed deviations from the expected 1:1 ratio for some markers were not statistically significant.

QTL analysis

The results for four major traits are depicted in Fig. 2 for each sire family and for combined data; it should be noted that these graphs do not represent the results of interval mapping but single point analyses for each marker, one at a time. Unequivocal evidence for QTL effects on fatness and growth traits was obtained. Although the QTL graphs

differed considerably between sires, there was no significant heterogeneity in QTL effects between sires (P varies between 0.37 and 0.99).

Least-square means for different traits according to deduced QTL genotype are given in Table 1; the data were combined over sire families and represent estimates obtained with the most significant marker for each trait. The progenies that had received a wild pig chromosome segment were associated with markedly higher fat deposition ($P < 0.0001$), lower fat-free weight ($P < 0.0002$), reduced growth ($P = 0.015$) and shorter length of carcass ($P = 0.001$). There was no significant difference between QTL genotypes in body mass index (BMI), a frequently used parameter for obesity in humans; BMI was measured as (weight at slaughter)/(length of carcass)². The result suggests that the reduced fatness in animals homozygous for a domestic pig segment was accompanied by a higher muscularity. Finally, it was not possible to confirm a chromosome 4 effect on the length of the small intestine, as found in the F₂ generation (Andersson *et al.*, 1994; Knott *et al.*, 1998).

Discussion

The results of this study confirm the presence of one or more QTLs on pig chromosome 4 affecting fatness and growth. To the best of our knowledge, this is the first time the segregation of QTLs over several generations has been documented in outbred populations of domestic animals. We propose that this confirmed pig QTL with a major effect on fatness is designated *FAT1*. The results imply that the chromosome 4 QTLs are not strongly dependent on epistasis, because the wild pig contribution to the BC₂ population was only 12.5%. There was a good agreement between the results in the F₂ and BC₂ populations, as regards both the location and the estimated effects of QTLs for growth and fatness. (Table 3). However, the data only allow a rough comparison of the QTL effects over generations, as there are some notable differences between the two data sets. First, the F₂ data represent the additive effect (i.e. half the difference between the two homozygous classes), whereas the BC₂ data represent the difference between the heterozygote and one of the homozygotes. Moreover, the average growth rate differs markedly between the two populations, as the F₂ generation and BC₂ generations have 50% and 12.5% wild pig origin respectively. Consequently, the age and weight at slaughter differed significantly between the two generations. Finally, the BC₂ animals were fed *ad libitum*,

Fig. 2 Single-marker QTL analysis on pig chromosome 4 with *F*-values for (a) abdominal visceral fat, (b) subcutaneous fat depth, (c) length of carcass and (d) daily weight gain based on the BC₂ generation. Results for individual sire families are shown together with combined data. The locations of the markers used in the QTL analysis are indicated by the symbols ●, ■ and ▲. The map locations in cM of some chromosome 4 markers are indicated on the x-axis.

Table 3 Estimated QTL effects on pig chromosome 4 using F₂ and BC₂ animals from a wild pig–Large White intercross

Trait	F ₂ animals* (<i>n</i> = 191)	BC ₂ animals† (<i>n</i> = 85)
Average subcutaneous fat (mm)	2.30 ± 0.42	2.33 ± 0.58
Abdominal visceral fat (kg)	0.38 ± 0.06	0.39 ± 0.07
Length of carcass (mm)	−8.0 ± 0.23	−14.9 ± 4.4
Growth (g day ^{−1})	−23.5 ± 4.9	−24.3 ± 9.7

*Additive effect estimated as half the difference between animals homozygous for wild pig allele and domestic pig allele respectively.

†Differences between animals heterozygous and homozygous for the domestic pig allele.

whereas the F_2 animals were subjected to restricted feeding because of their low growth capacity. The only chromosome 4 QTL that we could not confirm was the one affecting the length of the small intestine. As pointed out above, there are many possible causes for a failure to confirm a QTL in a subsequent generation, and we do not have any indication which is the most plausible in this case.

The QTL analysis was carried out as a single marker analysis, as no software is yet available for interval mapping in backcross pedigrees. However, the result of a preliminary analysis using a stochastic method for interval mapping was in good agreement with the results presented here (Nyström *et al.*, 1998).

The design of the experiment allowed us to conclude that a major QTL is located distal to the recombination breakpoint present in the wild pig-derived haplotype of sire W782 (compare Figs 1 and 2). This conclusion is based on the fact that the QTL effect was also significant in tests within the recombinant sire family only and that there was no significant interaction between sire families and QTL effects (P varies between 0.37 and 0.99). However, the fatness QTL graph for the 'non-recombinant' sire W859 (Fig. 2a and b) suggested the possible presence of a QTL located proximal to the recombination breakpoint as well. However, it must be emphasized that the precision in QTL location is poor with such small progeny groups as those used in this study. The shape of the QTL graph in the two sires may also differ because of genetic heterogeneity contributed by the founder boars or by the domestic sows. Thus, we conclude that there is clear evidence for a QTL located distal to the recombination breakpoint, and we cannot exclude the presence of additional QTL(s) proximal to the breakpoint. This illustrates the difficulties of QTL mapping in outbred populations.

The large effect of the fatness QTL and the fact that we have been able to follow Mendelian transmission of this QTL over four generations opens up possibilities for high-resolution QTL mapping using marker-assisted backcrossing. This implies that genetic markers are used to identify informative recombinants such as sire W782 in this study. The QTL genotype for each selected parent is then determined by progeny testing. In our case, about 25 progenies are needed to be 90% sure of detecting QTL segregation in a heterozygous parent, given the magnitude of the effects and residual variances estimated in this backcross. The map resolution obtained in such an undertaking should be a few centiMorgans if 5–10 different recombinants can be

identified and tested. Such an experiment will reveal whether the large QTL effect on chromosome 4 is consistent with a single locus or several linked QTLs, each with a smaller effect.

Is it possible to clone a QTL such as the one affecting fatness on pig chromosome 4? This will depend on whether the QTL is a single gene effect or caused by mutations at several linked loci. In the latter case, it will be exceedingly difficult, if not impossible, with existing technology. Presently, the most reasonable strategy for approaching a QTL in a farm animal is by comparative positional candidate cloning. This strategy has recently been used successfully to reveal that pigs with the dominant white phenotype carry a duplication of the *KIT* gene (Johansson Moller *et al.*, 1996) and that hypercholesterolaemia in one line of pigs is caused by a missense mutation in the *LDLR* gene (Hasler-Rapacz *et al.*, 1998). We are convinced that marker-assisted backcrossing will allow us to define the map location of the chromosome 4 QTL at a resolution (a few centiMorgans) capable of making a comparative screen for candidate genes meaningful.

Pig chromosome 4 shares homology with human chromosomes 1 and 8 (Rettenberger *et al.*, 1995; Fröncke *et al.*, 1996; Goureau *et al.*, 1996). Unfortunately, the fatness QTL is located close to the border between these two regions of conserved synteny to human chromosomes. The pig comparative map of this region needs to be improved to facilitate the identification of potential candidate genes. A number of genes causing obesity in mice or rats have been identified (Bouchard, 1995). However, none of these is likely to be homologous to the fatness QTL on pig chromosome 4. The obese gene (*OB*) encoding leptin is located on pig chromosome 18 (Neuenschwander *et al.*, 1996), and the leptin receptor gene (*LEPR*) maps to the distal region of pig chromosome 6q (Ernst *et al.*, 1997). The other mouse obesity loci map to regions that are unlikely to share homology with the region of interest on pig chromosome 4.

The genetics of fat metabolism is of great agricultural significance, as manipulating the amount and distribution of fat in animal food products is a major objective in animal breeding. The genetic control of fat metabolism is also a central topic in human medicine, as obesity is a major health problem. In particular, abdominal visceral fat (AVF) obesity has a strong genetic component and is associated with severe diseases such as atherosclerosis, diabetes and hypertension (Bouchard, 1995; Pérusse *et al.*, 1996). We have proposed previously (Andersson, 1996) that normal obesity (in contrast to morbid obesity) is

not caused by a major biochemical defect but rather reflects an adaptive storage of energy when there is excess of food available; the pathological consequences may be secondary effects caused by a burden of fat tissue accumulated over many years. In this context, it should be noted that our data from the F₂ and BC₂ generations show that the wild pig in general has a higher fat deposition than purebred Large White domestic pigs and that the wild pig chromosome 4 QTL allele in particular was associated with higher fat deposition (Table 1). This ability to store fat is most certainly a selective advantage in the wild pig to survive periods of food shortage. The further characterization of the fatness QTL on pig chromosome 4 may therefore be an important animal model for the genetics of normal obesity in humans. The line of pigs carrying this wild pig QTL allele may also be useful when evaluating new drugs for the treatment of obesity.

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