Mol. Cells, Vol. 15, No. 1, pp. 62-67

Molecules and Cells

Quantitative Trait Loci Mapping for Fatty Acid Contents in the Backfat on Porcine Chromosomes 1, 13, and 18

Chaeyoung Lee*, Yeonseung Chung, and Jae-Hong Kim[1]

Laboratory of Statistical Genetics, Institute of Environment and Life Science, Hallym University, Chuncheon 200-702, Korea; [1] Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea.

(Received October 14, 2002; Accepted December 6, 2002)

A partial genome scan using microsatellite markers was conducted in order to detect quantitative trait loci (QTLs) for 10 fatty acid contents of the backfat in a pig reference population. Two QTLs were found by studying SSC1, SSC13, and SSC18, where QTLs had already been identified for backfat thickness. A QTL was located between marker loci *S0113* and *SW974* on chromosome 1; this QTL was only significantly detected ($P < 0.05$) for linoleic acid. The other QTL was discovered between markers *S0062* and *S0120* on chromosome 18, and its significance only showed ($P < 0.05$) for myristic acid. The two QTLs mapped to the same location as the backfat thickness QTL. A third of the phenotypic variation was explained for linoleic acid by the QTL on chromosome 1, and a quarter for myristic acid by the QTL on chromosome 18. Further studies on fine mapping and positional comparative candidate gene analyses will be the next step toward a better understanding of the genetic architecture of fatty acid contents.

Keywords: Fatty Acids; Genome Scan; Microsatellites; Quantitative Traits.

Introduction

QTLs that affect fatness traits have been found in swine. A QTL was found by Andersson *et al.* (1994) for backfat and abdominal fat on chromosome 4. A paternally-expressed QTL for fat deposition was mapped to the *IGF2* locus on chromosome 2 (Jeon *et al.*, 1999; Nezer *et al.*, 1999). On chromosome 13, a QTL affecting backfat was found near the *PIT1* gene (Yu *et al.*, 1999). On chromosome 6, a QTL was found for fatness traits in the location of a candidate gene, the heart fatty acid-binding protein gene (*H-FABP*) (Bidanel *et al.*, 2001; Gerbens *et al.*, 2000; Ovilo *et al.*, 2000). Additionally, QTLs for fatness were found on chromosomes 1, 7, and X (Bidanel *et al.*, 2001; Rohrer and Keele, 1998).

There has been a great concern about fatty acids as important nutrients for health. They especially help in maintaining the health of cell membranes, improve nutrient use, and establish and control cellular metabolism. However, QTL mapping that is related to fatty acid metabolism has only been studied on chromosome 4 (Perez-Enciso *et al.*, 2000).

The objective of this study was to search for QTLs that contribute to fatty acid contents by a partial genome scan in a pig reference population. The chromosomes that were examined in this study were 1, 13, and 18, on which QTLs for backfat thickness were found from the study by Wu *et al.* (2002). Priority was given to the 3 chromosomes to see the pleiotropic effects of QTLs on the 3 chromosomes, as well as to characterize the effect on the metabolism of fatty acids.

Materials and Methods

Reference population and trait measurement The pig reference population that was used in this study was initiated by mating a Landrace boar with a Yorkshire sow. The backcross and intercross progeny were produced by mating the animals from the founder and F1 generations. Subsequent generations were produced by advanced backcross and sib-mating. A total

* To whom correspondence should be addressed.
Tel: 82-31-380-1630; Fax: 82-31-388-3427
E-mail: clee@hallym.ac.kr

Abbreviations: dNTP, deoxyribonucleotide triphosphate; IBD, identity-by-descent; PCR, polymerase chain reaction; QTLs, quantitative trait loci; SOLAR, sequential oligogenic linkage analysis routines; SSC, *Sus scrofa* chromosome; VLDLR, very low density lipoprotein receptor gene.

Table 1. Mean and standard deviation (SD) of phenotypes for 10 fatty acid composition (%) used in QTL mapping.

Fatty acid composision	Mean	SD
Myristic acid	1.52	0.16
Palmitic acid	21.66	1.41
Stearic acid	9.93	1.01
Palmitoleic acid	2.37	0.31
Oleic acid	42.59	1.89
Octadecenoic acid	2.73	0.30
Eicosenoic acid	0.89	0.25
Linoleic acid	13.95	1.16
Eicosadienoic acid	0.78	0.04
Linolenic acid	1.18	0.20

Table 2. Number and heterozygosity of microsatellite markers used in QTL mapping.

Chromosome	SSC1	SSC13	SSC18
Number of markers			
Total	14	13	12
Polymorphic	9	10	7
Heterozygosity[1]			
Average	0.47	0.52	0.45
Range	0.30–0.59	0.34–0.62	0.33–0.64
Map length, cM	130.6	106.1	57.9

[1] Heterozygosity was calculated for each chromosome using only polymorphic markers. The range of heterozygosity refers to the means for each generation.

of 289 pigs were included in this study. The traits that were used in this study were the percentage of 10 different fatty acid contents in porcine backfat. They were myristic, palmitic, palmitoleic, stearic, oleic, octadecenoic, linoleic, linolenic, eicosenoic, and eicosadienoic acids. The fatty acid composition was measured from backfat samples by gas chromatography. Their phenotypic means and standard deviations are presented in Table 1.

Microsatellite analysis and genotyping Genomic DNAs were isolated following a conventional method (Sambrook *et al.*, 1989). Thirty-nine microsatellite markers on chromosomes 1, 13, and 18 were used in the PCR amplification, of which 26 were polymorphic in the founder generation (Table 2). These microsatellite markers gave a reasonable coverage of the 3 chromosomes, each belonging to one of the three types of swine chromosomes. Chromosome 1 was submetacentric; 13 and 18 were acrocentric.

The PCR analysis of microsatellites was carried out in an ABI PRISM™ 877-integrated thermal cycler (Perkin-Elmer, USA) using fluorescent-labeled PCR primers that were provided by the US Pig Genome Mapping Coordination Program. Thermocycling conditions were as follows: pre-denaturation for 10 min at 95°C, followed by 10 cycles of reaction with decreasing annealing temperatures (15 s at 95°C, 30 s at 64–55°C, 60 s at 72°C), 25 cycles of reaction at fixed-annealing temperatures (15 s at 89°C, 30 s at 55°C, 60 s at 72°C), and post-elongation of 1 h at 72°C. The reactions were made in a 10-μl final volume, each containing 50 ng genomic DNA, 1× PCR buffer, 2.5–3.5 mmol/L $MgCl_2$, 200 μmol each dNTP, 0.35 μmol/L each primer, and 0.25–0.35 U/ml TaqGold DNA polymerase (Perkin-Elmer, USA).

Each pooled sample, representing 0.4 μl of the PCR products, was heated to 95°C after adding 1.1 μl of the internal standard-dye-formamide mixture (0.25:0.25:0.60). The PCR products were loaded on 4.25% polyacrylamide denaturing-sequencing gel using an ABI PRISM™ 377 DNA sequencer (Perkin-Elmer, USA). The size of the PCR products was analyzed using GENSCAN and GEMMA software (Applied Biosystems, USA).

Statistical analysis The linkage maps of the frame markers were constructed using CRIMAP (Green *et al.*, 1990). First, the TWOPOINT option was used to find the linkage between markers with LOD scores that were larger than three. Then, the BUILD option was used to construct the framework map; the remaining markers were incorporated using the ALL option. Finally, the marker genotypes were checked using the CHROMPIC option. Parents with two or more double recombinants between the adjacent marker loci with an interval less than 20cM were re-examined. Unidentifiable recombinant genotypes were coded as "unknown". Marker genotypes that were inconsistent with the pedigree were also re-examined. Unidentifiable marker genotypes were coded as "unknown". The individuals with genotypes that were inconsistent with the pedigree information at two or more marker loci were all removed.

The QTL analysis was performed using SOLAR for the Linux system (Almasy and Blangero, 1998). The analytical model included sex and age effects. The Two-Point Analysis was implemented with a user-defined script to produce the LOD scores for each marker locus for 10 different fatty acids. The Marker-specific IBD matrix was computed by the Monte Carlo method. This IBD mapping was used to overcome the problem of multiple QTL detections that often confront QTL studies. For example, Kwon *et al.* (2001) detected two QTLs for plant regeneration ability on rice chromosome 3, which was contrary to the assumption of a single QTL with interval mapping. A genome-wide significance threshold was empirically obtained by the permutation test of Churchill and Doerge (1994). In order to obtain the threshold value at the significance level of 0.05, 5000 replicates were generated.

Results and Discussion

Linkage map The linkage analysis assigned the 26 polymorphic microsatellite markers into three linkage groups with a total length of 294.6cM (Table 2). These

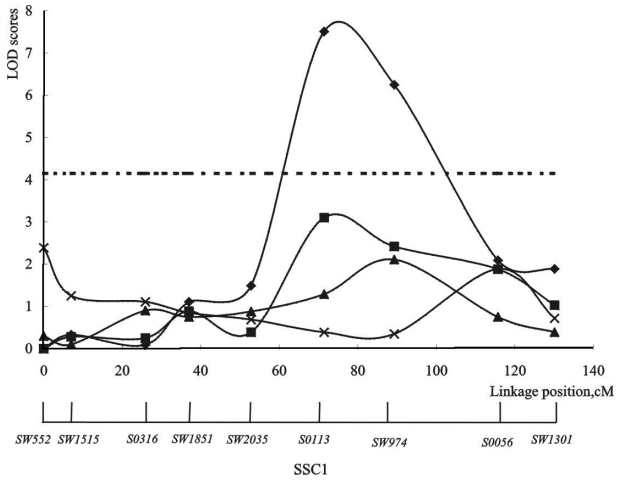

Fig. 1. LOD scores for fatty acids (myristic, palmitoleic, stearic, and linoleic acids) in the backfat on porcine chromosome SSC1 by IBD mapping for QTL. The dotted line (ST0.05) indicates the genome-wide significance threshold at $\alpha = 0.05$.

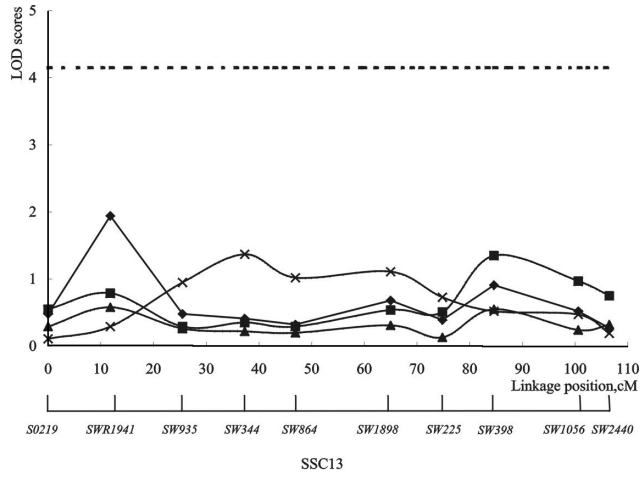

Fig. 2. LOD scores for fatty acids (myristic, palmitoleic, stearic, and linoleic acids) in the backfat on porcine chromosome SSC13 by IBD mapping for QTL. The dotted line (ST0.05) indicates the genome-wide significance threshold at $\alpha = 0.05$.

linkage maps basically agreed with the USDA-MARC pig map database (http://www.genome.iastate.edu/pig). The maximum difference of 6.5cM on SSC1 was observed at SW974; the linkage position was 93.8cM in the USDA database, and 87.3cM in the current study. On SSC13, the maximum difference was 6.9cM at SW398; the linkage position was 77.7cM in the USDA database and 84.6cM in this study. The linkage map particularly corresponded to each other on SSC18, and the maximum difference was only 1.7cM, observed at S0120. The linkage position was 45.2cM in the USDA database, and 46.9cM in the current study.

QTL detection We found QTLs that segregated on SSC1 and SSC18 for fatty acid contents in this pig population (Figs. 1 and 3). The four fatty acids that were most influenced were chosen to be presented in Figs. 1, 2, and 3. No QTL was observed ($P > 0.05$) on SSC13 (Fig. 2, only the most significant profiles are shown in this figure). The QTLs that were mapped for backfat on SSC13 by Yu *et al.* (1999) and Wu *et al.* (2002) were not found for fatty acid contents in the current study. In fact, the QTL that was observed by Wu *et al.* (2002) was significant at $\alpha = 0.1$, but not at $\alpha = 0.05$. Furthermore, even the significance at $\alpha = 0.1$ was observed using a partial family, but not using the whole family. Perhaps, the QTL was not segregated in this pig population.

QTL on SSC1 The QTL that was discovered on SSC1 was only significant ($P < 0.05$) for linoleic acid, but not ($P > 0.05$) for the other fatty acids (Fig. 1). The QTL was located between the marker loci *S0113* and *SW974*, and its region was 60.7cM in the linkage length (Table 3). It was mapped on the same position as the backfat QTL that was found in Wu *et al.* (2002). The region in the current study was nested within the region of the backfat QTL. This might be due to a negative correlation between the linoleic acid content and fat deposition in animals (Nurnberg *et al.*, 1998). The largest LOD score was found at marker locus *S0113*; the second largest score was obtained at marker locus *SW974* (Fig. 1). The region for the QTL in this study corresponded to that of a backfat thickness QTL that was suggested by Rohrer and Keele (1998). It also showed the proximity to the region of a backfat QTL that was reported by de Koning *et al.* (1999). This region was homologous to a QTL on HSA9q of human chromosome 9 (Goureau *et al.*, 1996).

Furthermore, this QTL corresponded to a QTL for growth that was found by Paszek *et al.* (1999). They discovered the QTL for post-weaning average daily gain between markers *SW373* and *SW1301*. Considering that linoleic acid is an important fatty acid for growth, the correspondence might suggest that they were the same QTL; however, further investigation is needed. The influence of the QTL on growth and fatness might contribute to the phenotypic correlation of those traits that were observed in the field data. The QTL for growth and body composition possibly affected metabolism or energy partitioning. It implied the pleiotropic effects of the QTL. Understanding the pleiotropic effects of a QTL may suggest an indication to the biochemical pathway, and would provide valuable insights into the selection of positional candidate genes.

Recently, *VLDLR* was physically mapped to 1q26 on porcine chromosome 1 (Pinton *et al.*, 2000). The QTL might be influenced by the *VLDLR*. Or, the *VLDLR* might be a candidate gene for this QTL, because the QTL corresponded to *VLDLR* in both location and biological effects.

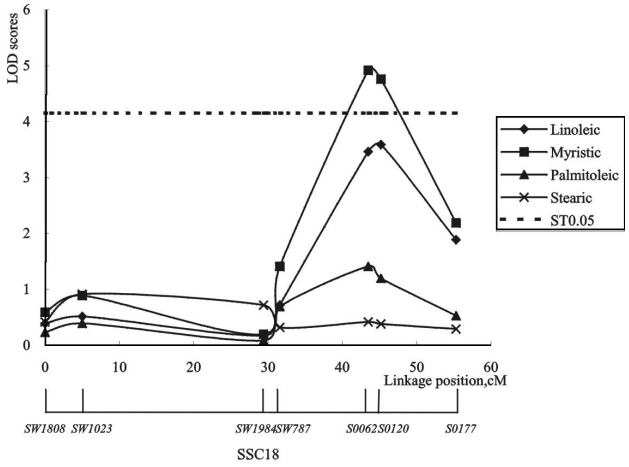

Fig. 3. LOD scores for fatty acids (myristic, palmitoleic, stearic, and linoleic acids) in the backfat on porcine chromosome SSC18 by IBD mapping for QTL. The dotted line (ST0.05) indicates the genome-wide significance threshold at $\alpha = 0.05$.

This linoleic acid QTL explained 33% of the phenotypic variance in this pig family (Table 3). If the QTL on SSC1 that was found in this study, and the QTL on SSC4 that was found by Perez-Enciso *et al.* (2000), additively influence the linoleic acid content, then 58% of its phenotypic variance could be explained by these two QTLs.

QTL on SSC18 The QTL that was found on chromosome 18 was only significant ($P < 0.05$) for myristic acid, but not ($P > 0.05$) for the other fatty acids (Fig. 3). Myristic acid is considered to be the most potent elevator of plasma cholesterol. A candidate gene that is involved in cholesterol concentration might influence the QTL that was observed in this study. This assumption is supported by a previous study, where a QTL was found for the cholesterol percentage of longissimus dorsi on porcine chromosome 18 (Malek *et al.*, 2001). Another important function of myristic acid is protein N-myristoylation, which is catalyzed by myristoyl-CoA:protein N-myristoyltransferase (Nmt). Nineteen Nmts and their encoding genes were identified across 15 species, including three mammalian species (human, mouse, Bos Taurus) (Farazi *et al.*, 2001). The QTL of this study might imply the location of an Nmt gene on porcine chromosome 18. The QTL was located between the marker loci *S0062* and *S0120* (Table 3). This region concurred with the location of the backfat thickness QTL that was found in the study by Wu *et al.* (2002). The QTL was in the proximity of the regions where some growth hormone pathway genes were located. One was the insulin-like growth factor binding protein 3 (*IGFBP3*) that was mapped with the regional assignment of *SSC18q24* (Lahbib-Mansais *et al.*, 1996). Another was the growth hormone releasing hormone re-ceptors (*GHRHR*) that were mapped on the same region

Table 3. QTLs for fatty acids in backfat of pigs.

	QTL1	QTL2
Traits	Linoleic acid	Myristic acid
Chromosome	1	8
Marker region	S0113-SW974	S0062-S0120
Linkage position, cM	60.7	42.8
Maximum LOD	7.51	4.92
Heritability		
QTL	0.33	0.25
Residual	0.24	0.19

with strong linkage to markers *S0062* and *S0120* (Sun *et al.*, 1997). Perhaps the effective and regulated expression of the growth hormone pathway is essential to maintain homeostasis of fat metabolism (Cogan and Phillips, 1998). Furthermore, the QTL that was obtained in this study might be influenced by the porcine obese (leptin) gene on SSC18 (Sasaki *et al.*, 1996). We could not exclude the possibility that the obese gene is a candidate gene for the QTL. In the human chromosome 7 (HSA7), *GHRHR* and *IGFBP3* were close to each other (Gaylinn *et al.*, 1994); they were localized in the homologous region of porcine chromosome 18.

Future directions QTL mapping is a major step toward the identification and positional cloning of causative genes that affect quantitative traits. It is also important for effective introgression and marker-assisted selection, and ultimately for understanding the genetic architecture of such quantitative traits. Genetic architecture is a moving target that changes, depending on the genetic and environmental variances of a certain population, implying that cautions should be addressed for false positives or negatives from QTL mapping (Lee, 2002). The current QTL study rendered an additional picture to understand the genetic architecture of porcine fatty acid contents. The large genetic effect of the QTL on SSC1 suggested that it might be of great importance in the genetic improvement of pigs. The dominance effect of this QTL was suspected, because the phenotypic mean for heterozygous genotype at the marker locus with a maximum likelihood ratio estimate was smaller than those for the homozygous marker genotypes. These dominance effects need to be studied. Chromosome 18 seemed to be also important for fat metabolism, considering the results from the current and previous studies. A dilemma in the study of SSC18 was the wide interval (24.4cM) between the markers of *SW1023* and *SW1984*, which might lead to failure in detecting another QTL in that site. This problem was inevitable because polymorphic microsatellite markers were unavailable. However, we did investigate this chromosome, because of its potential influence on fat traits. Therefore, in the near future, there needs to be a search

for other QTLs with newly emerging-informative markers. Further research will be aimed at the fine mapping of the regions that are found in this experiment and positional comparative candidate gene analysis.

Acknowledgments This research was supported by the Hallym Academy of Sciences at Hallym University, Korea, 2002-3.

References

Almasy, L. and Blangero, J. (1998) Multipoint quantitative-trait linkage analysis in general pedigrees. *Am. J. Hum. Genet.* **62**, 1198–1211.

Andersson, L., Haley, C. S., Ellegren, H., Knott, S. A., Johansson, M., Andersson, K., Andersson-Eklund, L., Edfors-Lilja, I., Fredholm, M., Hansson, I., Hakansson, J., and Lundstrom, K. (1994) Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* **263**, 1771–1774.

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., and Chevalet, C. (2001) Detection of quantitative trait loci for growth and fatness in pigs. *Genet. Sel. Evol.* **33**, 289– 309.

Churchill, G. A. and Doerge, R. W. (1994) Empirical threshold values for quantitative trait mapping. *Genetics* **138**, 963–971.

Cogan, J. D. and Phillips 3rd, J. A. (1998) Growth disorders caused by genetic defects in the growth hormone pathway. *Adv. Pediatr.* **45**, 337–361.

de Koning, D. J., Janss, L. L., Rattink, A. P., van Oers, P. A., de Vries, B. J., Groenen, M. A., van der Poel, J. J., de Groot, P. N., Brascamp, E. W., and van Arendonk, J. A. (1999) Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs (*Sus scrofa*). *Genetics* **152**, 1679–1690.

Farazi, T. A., Waksman, G., and Gordon, J. I. (2001) The biology and enzymology of protein N-myristoylation. *J. Biol. Chem.* **275**, 23516–23522.

Gaylinn, B. D., von Kap-Herr, C., Golden, W. L., and Thorner, M. O. (1994) Assignment of the human growth hormone-releasing hormone receptor gene (*GHRHR*) to 7p14 by in situ hybridization. *Genomics* **19**, 193–195.

Gerbens, F., de Koning, D. J., Harders, F. L., Meuwissen, T. H., Janss, L. L., Groenen, M. A., Veerkamp, J. H., Van Arendonk, J. A., and te Pas, M. F. (2000) The effect of adipocyte and heart fatty acid-binding protein genes on intramuscular fat and backfat content in Meishan crossbred pigs. *J. Anim. Sci.* **78**, 552–559.

Goureau, A., Yerle, M., Schmitz, A., Riquet, J., Milan, D., Pinton, P., Frelat, G., and Gellin, J. (1996) Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. *Genomics* **36**, 252–262.

Green, P., Falls, K., and Crooks, S. (1990) *Documentation for CRIMAP*, Version 2.4, Washington University School of Medicine, USA.

Jeon, J. T., Carlborg, O., Tornsten, A., Giuffra, E., Amarger, V., Chardon, P., Andersson-Eklund, L., Andersson, K., Hansson, I., Lundstrom, K., and Andersson, L. (1999) A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. *Nat. Genet.* **21**, 157–158.

Kwon, Y. S., Kim, K. M., Eun, M. Y., and Sohn, J. K. (2001) Quantitative trait loci mapping associated with plant regeneration ability from seed derived calli in rice (*Oryza sativa* L.). *Mol. Cells* **11**, 64–67.

Lahbib-Mansais, Y., Yerle, M., Pinton, P., and Gellin, J. (1996) Chromosomal localization of homeobox genes and associated markers on porcine chromosomes 3, 5, 12, 15, 16, and 18: comparative mapping study with human and mouse. *Mamm. Genome* **7**, 174–179.

Lee, C. (2002) What holds the future of quantitative genetics? - A review. *Asian-Aust. J. Anim. Sci.* **15**, 303–308.

Malek, M., Dekkers, J. C. M., Lee, H. K., Baas, T. J., Prusa, K., Huff-Lonergan, E., and Rothschild, M. F. (2001) A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle composition. *Mamm. Genome* **12**, 637–645.

Nezer, C., Moreau, L., Brouwers, B., Coppieters, W., Detilleux, J., Hanset, R., Karim, L., Kvasz, A., Leroy, P., and Georges, M. (1999) An imprinted QTL with major effect on muscle mass and fat deposition maps to the *IGF2* locus in pigs. *Nat. Genet.* **21**, 155–156.

Nurnberg, K., Wegner, J., and Ender, K. (1998) Factors influencing fat composition in muscle and adipose tissue of farm animals. *Livest. Prod. Sci.* **56**, 145–156.

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

Paszek, A. A., Wilkie, P. J., Flickinger, G. H., Rohrer, G. A., Alexander, L. J., Beattie, C. W., and Schook, L. B. (1999) Interval mapping of growth in divergent swine cross. *Mamm. Genome* **10**, 117–122.

Perez-Enciso, M., Clop, A., Noguera, J. L., Ovilo, C., Coll, A., Folch, J. M., Babot, D., Estany, J., Oliver, M. A., Diaz, I., and Sanchez, A. (2000) A QTL on pig chromosome 4 affects fatty acid metabolism: evidence from an Iberian by Landrace intercross. *J. Anim. Sci.* **78**, 2525–2531.

Pinton, P., Schibler, L., Cribiu, E., Gellin, J., and Yerle, M. (2000) Localization of 113 anchor loci in pigs: improvement of the comparative map for humans, pigs, and goats. *Mamm. Genome* **11**, 306–315.

Rohrer, G. A. and Keele, J. W. (1998) Identification of quantitative trait loci affecting carcass composition in swine: I. Fat deposition traits. *J. Anim. Sci.* **76**, 2247–2254.

Sambrook, J., Fritish, E. F., and Maniatis, T. (1989) *Molecular Cloning*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sasaki, S., Clutter, A. C., and Pomp, D. (1996) Assignment of the porcine obese (leptin) gene to chromosome 18 by linkage analysis of a new PCR-based polymorphism. *Mamm. Genome* **7**, 471–472.

Sun, H. S., Taylor, C., Robic, A., Wang, L., Rothschild, M. F., and Tuggle, C. K. (1997) Mapping of growth hormone releasing hormone receptor to swine chromosome 18. *Anim. Genet.* **28**, 351–353.

Wu, X. L., Lee, C., Jiang, J., Peng, Y. L., Yan, H. F., Yang, S. L., Xiao, B. N., Liu, X. C., and Shi, Q. S. (2002) Quantitative trait loci mapping for porcine backfat thickness. *Asian-Aust.*

J. Anim. Sci. **15**, 932–937.
Yu, T. P., Wang, L., Tuggle, C. K., and Rothschild, M. F. (1999) Mapping genes for fatness and growth on pig chromosome 13: a search in the region close to the pig *PIT1* gene. *J. Anim. Breed. Genet.* **116**, 269–280.