Quantitative Trait Loci Mapping for Porcine Backfat Thickness**

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ABSTRACT : A partial genome scan using porcine microsatellites was carried out to detect quantitative trait loci (QTL) for backfat thickness (BFT) in a pig reference population. This population carried QTL on chromosomes 1, 13 and 18. The QTL on chromosome 1 was located between marker loci *S0113* and *SW1301*. The QTL corresponded to very low density lipoprotein receptor gene (*VLDLR*) in location and in biological effects, suggesting that *VLDLR* might be a candidate gene. The QTL found on chromosome 13 was found between marker loci *SWR1941* and *SW864*, but significance for the marker-trait association was inconsistent by using data with different generations. The QTL on chromosome 18 was discovered between markers *S0062* and *S0117*, and it was in proximity of the regions where *IGFBP3* and *GHRHR* were located. The porcine obese gene might be also a candidate gene for the QTL on chromosome 18. In order to understand genetic architecture of BFT better, fine mapping and positional comparative candidate gene analyses are necessary. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 7 : 932-937)***

Key Words : Backfat, Genome Scan, Microsatellites, QTL, Pigs

INTRODUCTION

Backfat thickness (BFT) is an important trait to the pig industry because of consumer demands for lean pork. The BFT is highly heritable (0.51-0.61) and genetically correlated with other fatness traits such as intramuscular fat content (Warris et al., 1990).

Since a comprehensive porcine map was constructed (Archibald et al., 1995; Rohrer et al., 1996), searching quantitative trait loci (QTL) for economically important traits in swine has been a great concern to animal geneticists. Andersson et al. (1994) found a QTL for backfat and for abdominal fat on chromosome 4. A QTL was also discovered on chromosome 4 for fatty acid metabolism (Perez-Enciso et al., 2000). A paternally expressed QTL for fat deposition was mapped to *IGF2* locus on chromosome 2 (Jeon et al., 1999; Nezer et al., 1999). On chromosome 13, a QTL affecting backfat was found near *PIT1* gene (Yu et al., 1999). On chromosome 6, a QTL was found by Ovilo et al. (2000), Gerbens et al. (2000) and Bidanel et al. (2001). The location of the QTL on chromosome 6 corresponded to that of a candidate gene for fatness trait, *H-FABP* (Gerbens et al., 1997). Additionally, QTL for fatness were found on chromosomes 1, 7, and X (Rohrer and Keele, 1998; Bidanel et al., 2001).

The objective of this study was to search for QTL that contributed to BFT by a partial genome scan in a pig reference population. Six chromosomes (1, 2, 11, 13, 17, and 18) were examined.

MATERIALS AND METHODS

Reference population

The pig reference population used in this study was initiated with two founder pigs. They were a Landrace boar and 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 of 253 pigs were included in this study. BFT was measured for each individual at slaughter.

Microsatellite analysis and genotyping

Genomic DNAs were isolated following a conventional method (Sambrook et al., 1989). Sixty five microsatellite markers on chromosomes 1, 2, 11, 13, 17 and 18 were used in the PCR amplification, of which 47 were polymorphic in the founder generation (table 1). These microsatellite markers gave a reasonable coverage of these 6 chromosomes, which belonged to one of three types of swine chromosomes. Chromosomes 1 and 2 were submetacentric, 11 was metacentric, and 13, 17 and 18 were acrocentric.

PCR analysis of microsatellites was carried out in an ABI PRISM^{TM} 877 integrated thermal cycler (Perkin-Elmer, USA) using fluorescently labeled PCR primers provided by

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US Pig Genome Mapping Coordination Program. Thermocycling conditions were: pre-denaturation for 10 min at 95°C, followed by 10 cycles of reaction with decreasing annealing temperatures (15 sec at 95°C, 30 sec at 64-55°C, 60 sec at 72°C), 25 cycles of reaction at fixed annealing temperature (15 sec at 89°C, 30 sec at 55°C, 60 sec at 72°C), and ending post-elongation of 1 h at 72°C. The reactions were made in 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 PCR products was heated to 95°C after adding 1.1 μl of internal standard-dye-formamide mixture (0.25:0.25:0.60). 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 the GENSCAN and GEMMA software (Applied Biosystems, USA).

Statistical analysis

The linkage maps of frame markers were constructed using CRIMAP (Green et al., 1990). First, the TWOPOINT option was used to find linkage between markers with LOD scores larger than three. Then, the BUILD option was used to construct the framework map and 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 adjacent marker loci with an interval less than 20 cM were re-examined, and unidentifiable recombinant genotypes were coded as unknown. Marker genotypes inconsistent with the pedigree were also re-examined, and unidentifiable marker genotypes were coded as unknown. The individuals with genotypes inconsistent with the pedigree information at two or more marker loci were all removed.

The QTL analysis was carried out using Sequential Oligogenic Linkage Analysis Routines (SOLAR) for Linux system (Almasy and Blangero, 1998). The analytical model included sex and age effects. The Two-Point Analysis was implemented using a user-defined script to produce LOD score for each marker locus. Marker-specific identity-by-descent (IBD) matrix was computed using the Monte Carlo method. A genome-wide significance threshold was empirically obtained by permutation test (Churchill and Doerge, 1994). 5,000 replicates were used to get the threshold value at significance level of 0.05.

RESULTS AND DISCUSSION

Linkage map

The linkage analysis assigned the 47 polymorphic microsatellite markers into six linkage groups with the total length of 622.2cM (table 1). These linkage maps were basically in agreement with USDA-MARC pig map database (http://www.genome.iastate.edu/pig).

QTL detection

We found QTL segregated on porcine chromosomes 1 and 18 in this pig population (figure 1A and F), and they were referred as QTL1 and QTL3. Additional evidence for QTL affecting BFT was also found on chromosome 13 (figure 1D), but the marker-trait association was not consistently significant by using data with different generations. This was referred as QTL2. No QTL was significant in the other chromosomes (figure 1B, C and E). The QTL mapped on chromosome 2 by Jeon et al. (1999) and Nezer et al. (1999) was not found in the current study. The QTL might not be segregated in this pig population. On the other hand, no QTL for BFT on chromosome 17 discovered in the current study confirmed the results from Wu et al. (2000). They found a QTL on the chromosome for growth trait, but not for fatness trait.

QTL on chromosome 1

The QTL1 was located between the marker loci *S0113* and *SW1301* on chromosome 1, and it was a region of 58.8cM in the linkage length (table 2). The largest LOD score was found (9.39) at marker locus *S0113*, and the second largest was 8.10 at marker locus *SW974* (figure 1). This QTL1 concurred with QTL for BFT found in other

Table 1. Heterozygosity of microsatellite markers used in QTL mapping[1]

Chromosome	1	2	11	13	17	18
Number of markers						
Total	13	12	8	13	7	12
Polymorphic	8	9	7	10	6	7
Heterozygosity						
Average	0.45	0.53	0.46	0.52	0.43	0.43
Range	0.30-0.54	0.39-0.67	0.40-0.58	0.34-0.61	0.30-0.60	0.33-0.63
Map length, cM	130.2	164.6	87.3	106.5	86.4	47.2

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

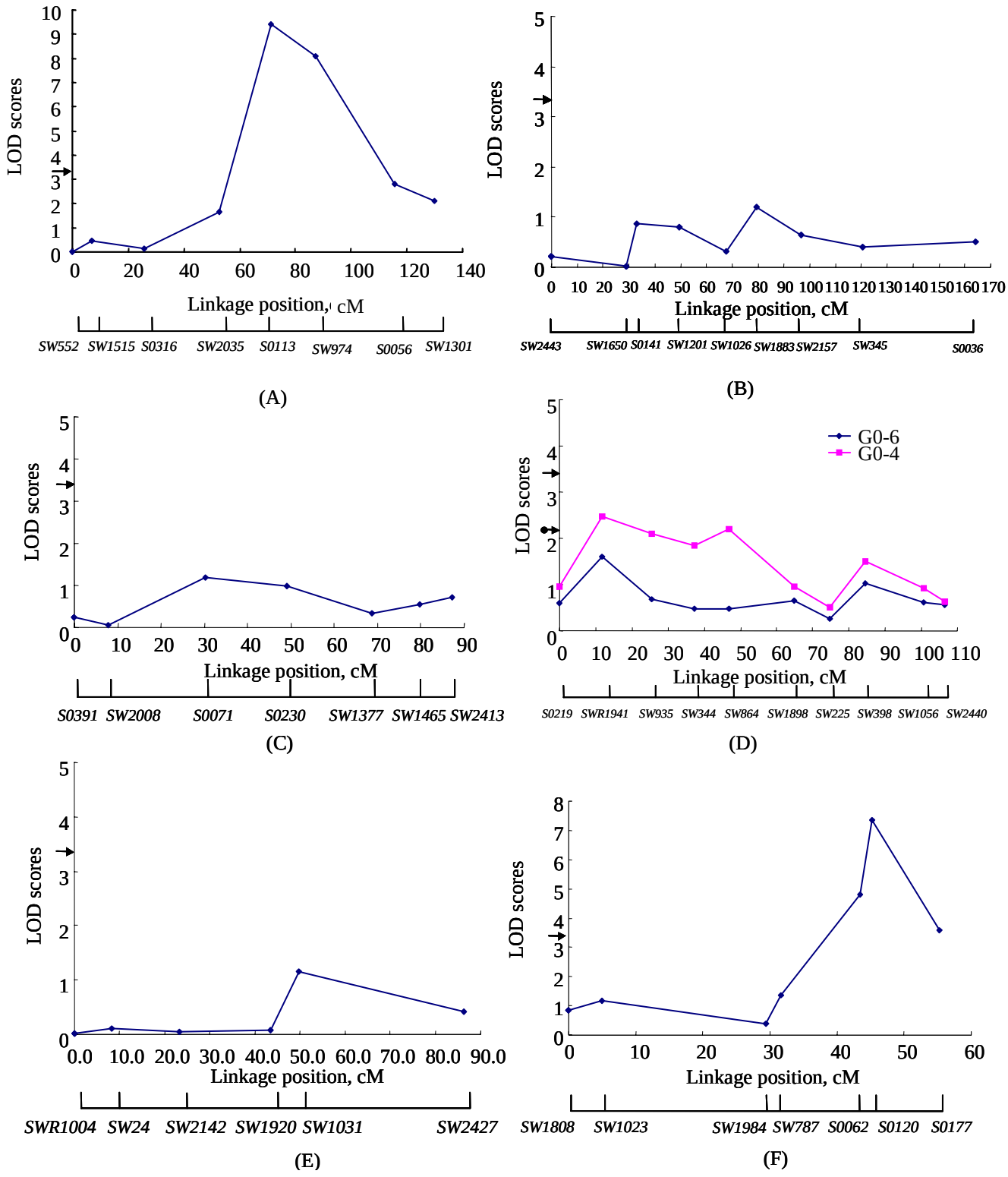

Figure 1. LOD scores for backfat thickness on porcine chromosomes 1 (A), 2 (B), 11 (C), 13 (D), 17 (E), and 18 (F) by IBD mapping for QTL. The arrow indicates genome-wide significance threshold at α=0.05, and indicates the threshold at α=0.1. D0-6 represents the result for generations 0 through 6 and D0-4 represents that for generations 0 through 4.

studies. The region between the loci *S0113* and *SW974* included the region of a QTL suggested by Rohrer and Keele (1998), and it was close to a QTL reported by de Koning et al. (1999).

Table 2. QTL for backfat thickness in pigs[1]

	QTL1	QTL2	QTL3
Chromosome	1	13	18
Marker region	SO113-SW1301	SWR1941-SW864	S0062-SO117
Linkage interval, cM	58.8	35.1	35.6
Maximum LOD	9.39	1.60 (2.47)	7.34
Heritability			
QTL	0.48	0.16 (0.27)	0.37
Residual	0.30	0.23 (0.21)	0.25

[1] The data in the brackets was obtained using the first five generation.

Furthermore, this QTL1 corresponded with a QTL for growth found by Paszek et al. (1999). He discovered the QTL for post-weaning average daily gain between markers *SW373* and *SW1301*. This correspondence might suggest that they were the same QTL, yet further investigation was in order. The influence of this QTL1 on growth and fatness might contribute to the phenotypic correlation of those traits observed in the field data. The QTL for growth and body composition possibly affected metabolism or energy partitioning. It implied pleiotropic effects of the QTL. Understanding the pleiotropic effects of a QTL ought to be a clue to the biochemical pathway, and would provide valuable insights into selection of positional candidate genes. This region was homologous to a QTL on HSA9q of human chromosome 9 (Goureau et al., 1996).

The dominance effect of this QTL was suspected because the phenotypic mean (12.67 mm) for heterozygous genotype at the marker *S0113* was smaller than those (14.10 mm and 12.90 mm) for homozygous marker genotypes. A large effect of the QTL1 for BFT suggested that it might be of great importance in genetic improvement of pigs.

Recently, very low density lipoprotein receptor gene (*VLDLR*) was physically mapped to 1q26 on porcine chromosome 1 (Pinton et al., 2000). The *VLDLR* might be a candidate gene for this QTL because the QTL1 corresponded to *VLDLR* in both location and biological effects.

QTL on chromosome 13

The QTL2 was found between marker loci *SWR1941* and *SW864* (table 2). The LOD score for the marker region ranged from 1.85 to 2.47 ($p<0.05$) using the first five generations of the pig population, but it decreased to below 1.60 ($p>0.05$) using the whole population. The lack of significance with later generations might be due to the bias caused by inbreeding depression. The accumulative inbreeding in later generations tended to change mean and variance for each genotype. As the result, it might shrink or enlarge the QTL effect. Yu et al. (1999) reported a QTL affecting backfat in proximity of *PIT1*, an essential transcriptional regulatory factor of growth hormone, prolactin, and thyrotropin b subunit (Ingraham et al., 1990; Radovick et al., 1992). The QTL2 was located 20-60 cM apart from the QTL found by Yu et al. (1999).

QTL on chromosome 18

The QTL3 was located between the marker loci *S0062* and *S0117* on chromosome 18 (table 2). LOD scores ranged from 4.80 to 7.34 ($p<0.05$) at the region (figure 1). This concurred with the study of Wu et al. (2001) where estimates were obtained by a single marker analysis using a mixed model. The QTL3 was in proximity of the regions where some growth hormone pathway genes were located. One was the insulin-like growth factor binding protein 3 (*IGFBP3*) mapped with the regional assignment of *SSC18q24* (Lahbib-Mansais et al., 1996). Another was the growth hormone releasing hormone receptors (*GHRHR*) mapped to the same region with strong linkage to markers *S0062* and *S0120* (Sun et al., 1997). Effective and regulated expression of the growth hormone pathway is essential to maintain homeostasis of fat metabolism (Cogan and Phillips, 1998). Furthermore, the porcine obese (leptin) gene on chromosome 18 (Sasaki et al., 1996) might be a candidate gene for QTL3. In human chromosome 7 (HSA7), *GHRHR* and *IGHBP3* were close to each other (Gaylinn et al., 1994), and they were localized in the homologous region of porcine chromosome 18.

A hypothesis for the effect of QTL3 on porcine chromosome 18 was established with a mutation model. The allele Q linked to marker alleles B and D was normal, and the allele q linked to marker alleles A and C was a mutant responsible for physiological lipase deficiency favoring obesity. The A, B, C, and D referred to the 4 alleles at *S0120*, and their numbers of amplified base pairs were 153 bp, 161 bp, 165 bp, and 171 bp, respectively. Individuals carrying qq were observed with increased fat deposition. On the other hand, the individuals carrying QQ or Qq were normal because the normal allele was able to code the normal mRNAs which in turn were responsible for synthesizing the normal lipase. This hypothesis agreed with the study of Taylor and Phillips (1997) where the expression of monogenic obesity was mostly recessive. From this hypothesis, although performance records were not available for the founder animals, the founder sow carrying the allele A and C was suspected to have a high content of fat.

Future directions

QTL mapping is a major step toward identifying and positional cloning of causative genes affecting quantitative traits. It is also important for effective introgression and marker-assisted breeding. The current results of QTL would add to understanding genetic architecture of porcine BFT.

Further research will be aimed at fine mapping of the regions found in this experiment and positional comparative candidate gene analysis. Eventually, it will lead to the characterization and isolation of the genes.

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