Mapping a Quantitative Trait Locus for Growth and Backfat on Porcine Chromosome 18**

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ABSTRACT : A QTL was localized near *S0120* on porcine chromosome 18. The QTL was significant ($p<0.05$) for average daily gain (ADG) of body weight and backfat thickness (BFT). The estimates of additive and dominance effects for the QTL were 0.0135 kg/day ($p<0.001$) and 0.0138 kg/day ($p>0.5$) for ADG and 1.6115 mm ($p<0.001$) and 0.9281 mm ($p>0.05$) for BFT. The location of this QTL coincided with a few growth hormone pathway genes. This study suggested that a QTL allele probably resulted from a mutation responsible for physiological lipase deficiency favoring obesity. This QTL might be important to obesity as well as growth in pigs. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 12 : 1665-1669)*

Key Words : Genome Scan, Mixed Models, Backfat, Obesity, Pigs

INTRODUCTION

Mapping quantitative trait loci (QTL) has been a concern to swine geneticists because that is a major step toward positional cloning of causative genes affecting quantitative traits. A clear picture of the genome architecture of quantitative trait genes is important for effective introgression and marker-assisted breeding (Zeng et al., 1999). Genome scans using anonymous molecular markers serve as an important tool for mapping QTL. A successful search for QTL in pigs by a genome-wide scan was reported by Andersson et al. (1994). They found a QTL on chromosome 4 that accounted for 20% of the phenotypic variance for average backfat and abdominal fat. Since a comprehensive porcine map became available (Rohrer et al., 1996), genome scans have been increasingly used to search for QTL in swine (Wilkie et al., 1996; Cassas-Carillo et al., 1997; Rothje et al., 1997; Rohrer and Keele, 1998a, 1998b; Wilkie et al., 1999; Paszek et al., 1999; de Koning et al., 2000; Rattink et al., 2000).

The objective of this study was to identify and map QTL for growth and backfat in a reference pig family using a genome scan. In this paper, we present the results from analyses of chromosome 18.

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MATERIALS AND METHODS

Animals and traits

The pig reference family used in this study was initiated by mating a Landrace boar to a Yorkshire sow. The daughters in the F1 generation were mated back to their boar, producing the backcross progeny. Four more subsequent generations were produced by advanced backcross and sib-mating. A total of 203 pigs in this pig family were used.

The pigs were weighed individually at slaughter, and average daily gain (ADG) was calculated for each individual as ADG=(body weight)/(days from birth to slaughter). Back-fat thickness (BFT) was also measured at slaughter.

Microsatellite amplification and genotyping

Twelve microsatellite markers derived from pig chromosome 18 were used in the preliminary scan and seven were used in the formal PCR. These 7 markers were polymorphic in the founder generation and gave a reasonable coverage of this chromosome (table 1).

PCR of the microsatellites was carried out using fluorescent labelled PCR primers on an ABI PRISMTM 877 intergrated thermal cycler (for markers *SW1080*, *SW1984* and *S0120*) or a PTC-100 programmable thermal controller (for other markers). The reaction volume of each PCR was 10 μl containing 50 ng genomic DNA, 1×PCR buffer, 2.5 mM $MgCl_2$, 200 μM of each dNTP, 0.35 μM of each primer, and 0.25 U HotStarTaq DNA polymerase (Qiagen, Gemany). The thermocycling conditions were: pre-denaturation for 10 min at 95°C, followed by 10 cycles of reaction at decreasing annealing temperatures (15 sec at 95°C, 30 sec at 64-55°C, 60 sec at 72°C), 25 cycles of

Table 1. Characteristics of the marker loci[1]

Marker	Allele size, bp	Number of alleles	Linkage position, cM	T_A, C
SW1808	134/142/152 (106-147)	3 (6)	0 (0)	60
SW1023	93/109/111/133 (94-117)	4 (5)	5.5 (5.0)	60
SW1984	132/142 (99-152)	2 (8)	28.8 (29.4)	55
SW787	155/161 (153-161)	2 (5)	31.1 (31.6)	60
S0062	156/188/190 (146-196)	3 (7)	44.6 (43.5)	55
S0120	153/161/165/171 (154-176)	4 (7)	46.0 (45.2)	58
S0177	166/168 (143-173)	2 (8)	56.5 (55.3)	65

[1] The data in brackets were cited from the USDA database.

reaction at a fixed annealing temperature (15 sec at 89°C, 30 sec at 55°C, 60 sec at 72°C), and ending at 72°C for 1 h to maximize the "plus A".

Each pooled sample representing 0.4 μl of the PCR product and 1.1 μl of internal size standard-formamide mixture was heated to 95°C for 5 min and then kept on ice. These samples were latter loaded on 4.25% polyacrylamide denaturing sequencing gels, and run on a ABI PRISMTM 377 DNA sequencer. The allele size of the amplification products was analyzed using the GeneScan software (Applied Biosystems, USA).

Linkage mapping

Linkage analysis was performed using CRIMAP version 2.4 (Green et al., 1990). Initially, the option TWOPOINT was used to find linkage between markers with a lod score larger than three. Subsequently, the option BUILD was used to construct the framework map and the remaining markers were incorporated using the option ALL. Finally, the genotypes were checked using the option CHROMPIC.

QTL analysis

Marker-QTL association was examined using the following mixed model:

$$y = X\beta + Z\theta + \varepsilon \qquad (1)$$

where y was the vector of observations, β was the vector of unknown fixed effects for sex, slaughter age, and full-sib litter, θ was the vector of unknown random effects for marker genotypes, X and Z were the design matrices relating the elements in y to those in β and θ, respectively, and ε was the vector of random residuals.

Marker genotype means were calculated at the locus where the largest marker-trait association was observed. The QTL effects were then estimated using a weighted least square (WLS) method (Lee and Wu, 2001). Since the linked marker locus of interest on chromosome 18 had multiple alleles, a putative multi-allelic QTL model was used to determine the number of QTL alleles and the marker-QTL phases before the final QTL analysis was performed. The model assumed that each marker allele was linked to a QTL allele with different additive effect. Under this assumption, the marker means were partitioned into additive and dominance effects by the following model:

$$\theta_{ij} = 0.5a_i + 0.5a_j + d_{ij} + r_{ij} \qquad (2)$$

where θ_{ij} was an element of θ, a_i and a_j were the additive QTL effects associated with the two marker alleles, d_{ij} was the dominance QTL effect associated with the marker alleles, and r_{ij} was the residual that was not explained by the QTL.

RESULTS

Linkage analysis and map construction

PCR for the 7 microsatellites amplified fragments that mostly fell in between the corresponding size ranges in the USDA database (table 1). However, new alleles were found for two microsatellite loci, the third allele (152 bp) at locus *SW1808* and the forth allele (133 bp) at locus *SW1023*. The linkage map of the markers with accumulated Kosambi cM distance was shown in table 1. This marker map was in agreement with the USDA-MARC pig linkage map, and was used as the frame marker map in the following QTL mapping.

QTL analysis

Single marker analysis indicated that the reference pig family carried a QTL on chromosome 18. A significant marker-trait association was observed around the marker region *S0062-S0117* for both ADG and BFT. The smallest P-value was estimated at marker loci *S0062* and *S0120*. This marker region represented the most possible location for a QTL (figure 1). This QTL seemed to be of greater importance to BFT than ADG by judging from the F ratio test (figure 1). Estimates of QTL variance at S0120 were significant for both ADG ($p<0.05$) and BFT ($p<0.01$) (table 2).

A putative multi-allelic QTL model was used to examine the QTL effect. In other words, the founder pigs were assumed to be heterozygous (*Q2Q4* vs *Q1Q3*) at the QTL locus. This was because the heterozygosity of the markers on chromosome 18 for this pig family was large in the founder generation where 5 out of the 7 marker loci were heterozygous and 4 of them were multi-allelic loci.

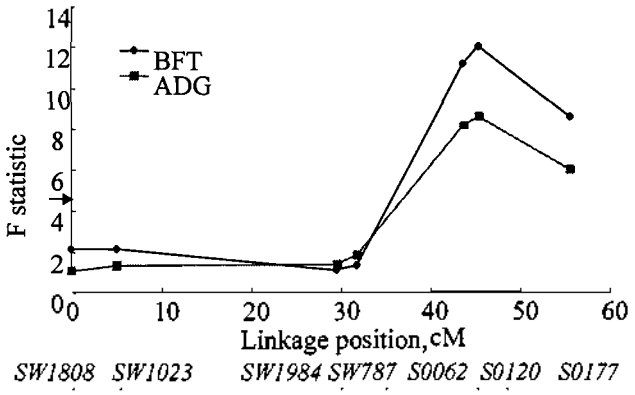

Figure 1. A QTL was localized around the marker *S0120* on porcine chromosome 18. The QTL exhibited a significant effect on backfat thickness (BFT) and average daily gain (ADG). The arrow indicated a 0.05 chromosome-wide significance threshold obtained by permutation with 1,000 replicates

Using the putative multi-allelic QTL model, the allele additive and dominance effects associated with the linked marker (*S0120*) were estimated in tables 3 and 4. Adjusted P-values were obtained using the step-down Bonferroni method (Holm, 1979) in order to control "family-wise" error rate in multiple tests.

Neither the allele substitution effect nor the dominance effect was significant for ADG ($p>0.05$). However, the estimates of allele substitution effects for BFT were significant ($p<0.05$) for, *A-D*, *C-B* and *C-D*. The estimate of dominance effect for BFT was significant for only *B-D* ($p<0.05$).

The estimates of marker allele additive and dominance effects indicated that marker alleles *B* and *D* at locus *S0120* were linked to the QTL allele favoring less backfat and faster growth (referred to as *Q*). The marker alleles *A* and *C* were linked to the alleles (referred to as *q*) responsible for more backfat and slower growth. The additive and dominance effects of the QTL were estimated in table 5. The additive effects of the QTL for the two traits were all significant ($p<0.001$). However, none of the dominance effects was significant ($p>0.05$).

DISCUSSION

The present research found a large QTL on pig chromosome 18 which affected backfat and body weight. This QTL was localized to the region where some important

Table 2. Estimate of QTL variance at *S0120*

Traits	Var (QTL)	StdDey	Z	Pr > |Z|
Average daily gain	0.0045	0.0022	2.01	0.0442
Backfat thickness	2.7356**	0.9545	2.87	0.0042

** $p<0.01$.

Table 3. Estimates of allele substitution effects at *S0120* using a putative multi-allelic QTL model[1]

Allele pairs	Estimate	Std error	T for H0	Pr > |T| Raw	Pr > |T| StepBon
	-------- Average daily gain ---------				
A-B	0.016+	0.0056	1.87	0.0634	0.3306
A-C	-0.0060	0.0060	-1.00	0.3198	0.6396
A-D	0.0104+	0.0054	1.94	0.0551	0.3306
B-C	-0.0161+	0.0088	-1.83	0.0691	0.3306
B-D	0.0016	0.0066	0.25	0.8046	0.8046
C-D	0.0168+	0.0094	1.79	0.0760	0.3306
	-------- Backfat thickness ---------				
A-B	1.5986*	0.7366	2.17	0.0316	0.0948
A-C	-0.1745	0.5055	-0.35	0.7300	1.0000
A-D	1.4497**	0.5214	2.78	0.0063	0.0252
B-C	-1.7733**	0.6086	-2.91	0.0042	0.0252
B-D	-0.1491	0.4813	-0.31	0.7573	1.0000
C-D	1.6242**	0.5679	2.86	0.0049	0.0252

+ $p<0.10$, * $p<0.05$, ** $p<0.01$.

[1] Raw=raw p-value; StepBon=adjusted p-values using the stepdown Bonferroni method of Holm (1979).

Table 4. Estimates of marker dominance effects at *S0120* using a putative multi-allelic QTL model[1]

Allele pairs	Estimate	Std error	T for H0	Pr > |T| Raw	Pr > |T| StepBon
	-------- Average daily gain ---------				
A-B	0.0107	0.0104	1.03	0.3051	0.5075
A-C	0.0048	0.0265	0.18	0.8578	0.8587
A-D	0.0078	0.0099	0.78	0.4343	0.5212
B-C	0.0186	0.0175	1.06	0.2890	0.5075
B-D	0.0169	0.0161	1.05	0.2926	0.5075
C-D	0.0179	0.0187	0.96	0.3383	0.5075
	---------- Backfat thickness ---------				
A-B	0.2919	0.7990	0.37	0.7154	0.7154
A-C	1.2807	2.0341	0.63	0.5301	0.7154
A-D	0.3284	0.7649	0.43	0.6684	0.7154
B-C	-0.8701	1.3394	-0.65	0.5171	0.7154
B-D	2.2218**	0.7818	2.84	0.0052	0.0312
C-D	0.2525	0.6746	0.37	0.7088	0.7154

** $p<0.01$.

[1] Raw=raw p-value; StepBon=adjusted p-values using the stepdown Bonferroni method of Holm (1979).

Table 5. Estimates of the additive and dominance effect at *S0120* using a bi-allelic QTL model

Trait	Average daily gain, kg	Backfat thickness, mm
a	0.0135***±0.0045	1.6115***±0.4497
d	0.0138±0.0078	0.9281±0.6021

*** $p<0.001$

genes were previously located. The insulin-like growth factor binding protein 3 (*IGFBP3*) was mapped to pig chromosome 18 by Lahbib-Mansais et al. (1996), and the regional assignment was SSC18q24. *IGF*-binding proteins prolonged the half-life of the *IGF*s and was shown either to inhibit or to stimulate the growth promoting effects of the *IGF*s on cell culture. They altered the interaction of *IGF*s with their cell surface receptors. The growth hormone releasing hormone receptors (*GHRHR*) was also mapped to the same region of the pig chromosome 18 with strong linkage to markers *S0062* and *S0120* (Sun et al., 1997). This was a receptor for *GRF*. The activity of this receptor was mediated by G proteins that activated adenylyl cyclase. Additionally, *GHRHR* and *IGFBP3* in human chromsome 7 (*HSA7*) were mapped near to each other (Gaylinn et al., 1994), and they were localized in the homologous region of pig chromosome 18. These were in fact growth hormone (*GH*) pathway genes. Effective and regulated expression of the growth hormone pathway is essential for growth in stature as well as homeostasis of carbohydrate, protein, and fat metabolism (Cogan and Phillips, 1998).

Furthermore, the porcine obese (leptin) gene was assigned to chromosome 18 (Sasaki, 1996). Previously identified homologs were mouse *ob* mapped to the proximal end of chromosome 6 (Zhang et al., 1994) and human *OBS* mapped to chromosome 7q32 (Geffroy, 1995). The genes were located in the homologous region where we localized the QTL in the current study. Genetically obese (*ob/ob*) mice exhibited hyperphagia, hyperglycemia, and severe obesity (Bray and York, 1979). Mutations in mouse *ob* resulted in either the absence of *ob* mRNA or an incomplete protein (Zhang et al., 1994). The protein encoded by the *ob* gene reduced feed intake and body fat in both *ob/ob* and wild-type mice (Pelleymounter et al., 1995; Halaas et al., 1995).

These previous results supported the results from the current study that the QTL found in chromosome 18 affected backfat and body weight. However, it was still not clear if this QTL was one of these previously reported genes or a new locus linked to the genes. Nevertheless, the large additive effect on BFT suggested that they might be responsible for or related to obesity in pigs. A mutation model for this QTL was therefore suggested to give a reasonable explanation of this situation. In other words, we assumed that the allele *Q* (linked to marker alleles *B* and *D*) was the normal form of gene that increased the growth and body weight. This normal gene was responsible for a normal regulation of growth and fat metabolism. As a contrast, the allele *q* (linked to marker alleles *A* and *C*) was a mutant isoform and responsible for physiological lipase deficiency favoring obesity. This mutation increased backfat deposition and led to decreased growth. As a result, the homozygotes (*qq*) were observed with significantly increased fat deposition due to physiological lipase deficiency. However, a genotype carrying two normal alleles or one normal allele and one mutant allele would favor normal growth because the normal allele was able to code for the normal mRNAs responsible for synthesizing the normal proteins (lipase). This hypothesis was in agreement with the observations that the expression of monogenic obesity was mostly recessive (Taylor and Philips, 1997).

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