

Mapping of Quantitative Trait Loci on Porcine Chromosome 7 Using Combined Data Analysis*

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ABSTRACT : To further investigate the regions on porcine chromosome 7 that are responsible for economically important traits, phenotypic data from a total of 287 F2 individuals were collected and analyzed from 1998 to 2000. All animals were genotyped for eight microsatellite loci spanning the length of chromosome 7. QTL analysis was performed using interval mapping under the line-cross model. A permutation test was used to establish significance levels associated with QTL effects. Observed QTL effects were (chromosomewide significance, position of maximum significance in centimorgans): Birth weight (<0.01, 3); Carcass length (<0.05, 80); Longissimus muscle area (<0.01, 69); Skin percentage (<0.01, 69); Bone percentage (<0.01, 74); Fat depths at shoulder (<0.05, 54); Mean fat depth (<0.05, 81); Moisture in *m. Longissimus Dorsi* (<0.05, 88). Additional evidence was also found which suggested QTL for dressing percentage and fat depths at buttock. This study offers confirmation of several QTL affecting growth and carcass traits on SSC7 and provides an important step in the search for the actual major genes involved in the traits of economic interest. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 10 : 1350-1354)

Key Words : Pig, Quantitative Trait Loci (QTL), *Sus scrofa* Chromosome 7 (SSC7), Combined Data Analysis

INTRODUCTION

There is abundant evidence for quantitative trait loci (QTL) affecting economically important traits located on *Sus scrofa* chromosome 7 (SSC7). Such QTL have been found in many resource populations, such as wild boars×Large White (Andersson-Eklund et al., 1998), Chinese Meishan sires×Western commercial pigs (de Koning et al., 1999,2000,2001; Milan et al., 1998,2002; Sato et al., 2003), Berkshire sires×Yorkshire dams (Malek et al., 2001a,b), Iberian sires×Landrace dams (Olivo et al., 2000,2002), and Korean native sires×Landrace dams (Lee et al., 2003).

In order to locate QTL affecting economically important traits, an F2 cross between Large White and Meishan pigs was developed in our laboratory. The F2 offspring of the resource population were slaughtered in three contemporary groups between 1998 and 2000. Using genome scanning mapping approach with microsatellite markers, it was determined that there were some significant QTL influencing growth, carcass traits and meat quality on SSC7 in individual group analysis (Su et al., 2002a,b; Zuo et al., 2003a,b). It is of special necessity to confirm these QTL located on SSC7 so that fine mapping and positional

cloning in combination with comparative mapping can be performed in the future. The aim of this study was to further investigate the regions on SSC7 by increasing the sample size. To achieve this target, data from 1998 to 2000 were combined and analyzed to search for genomic QTL regions that control variation in our experimental studies.

MATERIALS AND METHODS

Resource population

Three Large White boars were crossed with seven Chinese Meishan sows. From the F1 offspring 5 boars were mated to 23 sows producing 287 offspring in 41 families. The pigs were slaughtered in three contemporary groups between 1998 and 2000 following a common protocol (Xiong and Deng, 1999). The average weight at slaughter was 87.9±6.14 kg. The growth and carcass traits analyzed were birth weight (BW) and carcass weight (CW), growth rate from birth to the end of test (GRBE), carcass length (CL), dressing percentage (DP), longissimus muscle area (LMA), skin percentage (SP) and bone percentage (BP). Fat depths at shoulder (BFS), loin (BFL), buttock (BFB) were measured as well as a mean fat depth (MF) over the 3 positions. Meat quality traits included Meat pH of *m. Semipinalis Capitis*, Moisture in *m. Longissimus Dorsi*, Water holding capacity (WHC), Intramuscular fat in *m. Longissimus Dorsi* (IMF).

Genotyping

The animals were genotyped for eight microsatellite loci (SWR1343, SW2155, SW1856, SW859, SW352, SW252,

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Table 1. Means and standard deviations for traits analyzed

Traits	N	Mean	Std dev
Birth weight, kg	206	1.45	0.26
Growth rate from birth to the end of test, kg/d	287	0.42	0.06
Carcass length, cm	221	91.3	4.39
Dressing percentage, %	221	70.92	3.88
Fat depths at shoulder, cm	221	3.40	0.81
Fat depths at loin, cm	221	2.06	0.64
Fat depths at buttock, cm	221	1.92	0.81
Mean fat depth, cm	221	2.46	0.69
Longissimus muscle area, cm ²	221	29.15	4.90
Skin percentage, %	221	10.16	1.80
Bone percentage, %	221	12.46	2.22
Meat pH of <i>m. Semipinalis capitis</i>	287	6.40	0.24
Moisture in <i>m. Longissimus dorsi</i> , %	287	73.66	0.86
Water holding capacity, %	287	90.95	6.46
Intramuscular fat in <i>m. Longissimus dorsi</i> , %	287	2.58	0.72

N is the number of individuals with records.

SW581, S0212). The procedure of genotyping was as described by Zuo et al. (2003b). All animals in the experiment were halothane tested. Of all animals, sixteen animals including one Large White boar, two F1 boars and thirteen F2 individuals were carrier of halothane negative.

Statistical methods

Linkage analysis was performed by using the CRIMAP version 2.4 software (Green et al., 1990). Web software (<http://qtl.cap.ed.ac.uk>) was used to carry out QTL analysis. The statistical model used was:

$$y = \text{sex} + \text{family} + \text{group} + \text{Hal} + \text{covariate} + c_a + c_d + e$$

Where y is the phenotype, family is the full-sib family (here 41 levels), group is the contemporary group (here 3 levels), Hal is the halothane gene. The covariate is carcass weight for carcass traits, the age at slaughter for meat quality traits. The coefficient c_a is the probability $P(QQ)-P(qq)$, and c_d is $P(Qq)$, at the chromosome position of interest. The dominance effect (d) and additive effect (a) are the parameters to be estimated. In this study, the additive

effects were estimated for Large White QTL allele, thus, positive values of the additive effects denote an increase of the trait due to the Large White QTL allele. The residuals are represented by e . The additive fraction of F2 phenotypic variance (σ_y^2) explained by a QTL was computed assuming that alternative alleles were fixed in each breed; i.e., $h^2_Q = a^2/2\sigma_y^2$ (Olivo et al., 2000). Chromosome-wise significance thresholds were calculated empirically by permutation tests. The 5% and 1% significance thresholds were $F=5.35$ and 7.20 , respectively, for the chromosome-wise test. The suggestive threshold was assumed to be 5.0 .

RESULTS

Table 1 shows the statistics for the traits analyzed. Linkage analysis found a marker order identical to that reported by the USDA-MARS (<http://www.genome.iastate.edu/pig>), but the distances were longer than those in the USDA map, which were similar to our previous results. There were minor differences in map lengths between sexes, with the female map being 13.8% longer on the average (Table 2). Results from the QTL analysis are presented in Table 3. A total of eight significant associations were detected, and two associations presented suggestive evidence for QTL.

QTL for growth

We found a QTL with highly significant effect on BW at position 3 cM, near the marker SWR1343. The QTL could account for about 5.52% of the variance in the F2 population, with an additive effect of 0.115 kg. The additive effect suggested that QTL effects were in the expected direction (i.e., the Large White alleles increased birth weight), but heterozygous pigs had the lowest birth weight (Table 3). The F -statistics for GRBE did not reach the significance level.

QTL for carcass traits

We located two QTL with impacts on CL and DP at positions of 80 cM and 138 cM, respectively. The QTL for CL could explain approximately 39.8% fraction of the

Table 2. Marker positions and statistics

Marker	Position (sex average)	Position (female)	Position (male)	#inf. mei.	#inf. mei. (phase known)
SWR1343	0 (12.2)	0	0	243	221
SW2155	31.6 (32.9)	32.0	30.6	553	503
SW1856	69.3 (61.5)	65.4	73.0	529	467
SW859	88.2 (75.3)	87.1	89.7	431	207
SW352	112.3 (87.7)	110.2	114.6	323	257
SW252	138.0 (99.4)	142.5	133.8	534	382
SW581	166.0 (123.8)	168.2	165.1	298	140
S0212	187.1 (141.2)	202.4	177.8	408	311

The data in brackets were cited from the USDA database; #inf. mei. represents the number of informative meioses.

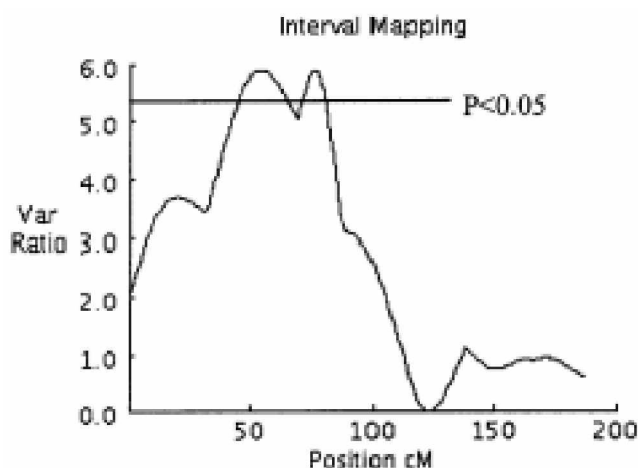


Figure 1. F ratio curve for fat depths at shoulder on chromosome 7. The X-axis indicates the relative positions on the linkage map. The Y-axis represents the F-ratio. The line indicates the chromosome-wide significance level ($p < 0.05$).

phenotypic variation. Individuals homozygous for the Meishan alleles were on the average 3.722 cm longer than those homozygous for Large White alleles, and Meishan alleles caused an increase in CL. The QTL for DP had a dominance effect of 1.561% and no significant additive effect. The QTL affecting fat depths mapped to different positions on SSC7. The significant QTL for MF mapped to the same location as the suggestive QTL affecting BFB. Another significant QTL for BFS was detected at about the 54 cM position with a twin peak (Figure 1), similar to the results reported by Rohrer and Keele (1998a) and Wada et al. (2000). The positive additive estimate of 0.221–0.274 cm indicated higher fat depths for Large White allele compared to an allele of the Meishan breed. We found strong evidence for a QTL affecting LMA at 69 cM. The additive effect of

the Large White allele was 1.279 cm², and there was also an indication of a dominance effect at this QTL. The QTL affecting SP mapped to the same region as that detected for LMA. The most convincing result in the analysis was the evidence for a QTL affecting BP, for which a F -statistics of 11.76 was obtained. The highest probability for QTL position was found to be 74 cM, between the marker SW1856 and SW859.

QTL for meat quality

At 88 cM there were indications of QTL affecting moisture in *m. Longissimus Dorsi*, and these effects reached statistical significance at 5% chromosome-wise level. Increased proportion of Meishan alleles yielded meat with more moisture. No significant QTL was observed for other meat quality traits.

DISCUSSION

The ultimate mapping resolution of QTL mapping studies is limited by the number of meioses (Darvasi et al., 1993). This number can be increased by increasing the sample size or by accumulating meioses over a number of generations (Haley, 1999). By a combined analysis of samples from three contemporary groups, the present study detected more significant QTL than the individual group analysis reported earlier (Su et al., 2002a,b; Zuo et al., 2003a,b), demonstrating that combining data can substantially improve the power of QTL mapping.

In the present study, a total of eight chromosome-wise significant QTL and two suggestive QTL were detected, confirming that SSC7 contains QTL for growth, carcass traits and meat quality. The QTL effects for birth weight may be caused by *tumour necrosis factor α* (*TNF α*) gene, as the QTL has been shown to be located near *TNF α* locus

Table 3. QTL analysis for the traits analyzed

Traits	Position (cM)	a \pm SE	d \pm SE	h^2_Q	F_{Max}	P_c
BW	3	0.115 \pm 0.039	-0.196 \pm 0.076	5.52	7.23	<0.01
GRBE	167	-0.0005 \pm 0.0006	0.002 \pm 0.001	0.00	1.62	No
CL	80	-1.861 \pm 0.526	-0.604 \pm 0.828	39.80	6.25	<0.05
DP	138	0.0001 \pm 0.381	1.561 \pm 0.504	0.00	5.24	Suggestive
BFS	54	0.274 \pm 0.089	0.233 \pm 0.153	5.40	5.90	<0.05
BFL	94	0.201 \pm 0.071	0.116 \pm 0.109	3.49	4.20	No
BFB	80	0.25 \pm 0.077	0.101 \pm 0.121	4.89	5.23	Suggestive
MF	81	0.221 \pm 0.067	0.132 \pm 0.105	4.41	5.62	<0.05
LMA	69	1.279 \pm 0.477	-1.596 \pm 0.642	17.48	8.12	<0.01
SP	69	-0.604 \pm 0.185	0.348 \pm 0.25	10.00	7.29	<0.01
BP	74	-1.254 \pm 0.268	0.111 \pm 0.398	33.9	11.76	<0.01
pH	186	-0.043 \pm 0.025	0.06 \pm 0.043	0.46	2.90	No
Moisture	88	-0.236 \pm 0.081	0.09 \pm 0.116	4.11	5.51	<0.05
WHC	98	-0.275 \pm 0.783	-2.64 \pm 1.311	0.66	2.03	No
IMF	105	-0.13 \pm 0.082	-0.178 \pm 0.141	1.44	2.48	No

Position in centimorgans corresponding to F_{Max} : a, additive effect; d, dominance effect; h^2_Q , fraction of phenotypic variance explained by the QTL. P_c , p-value for the chromosome-wise test; Negative values of the additive effects denote a decrease of the trait due to the Meishan alleles.

in the USDA linkage map, which was found to be significantly associated with birth weight (Rothschild et al., 1995). The QTL for carcass length had a highly significant effect and explained 39.8% of the phenotypic variance. Meishan alleles were additive and presented positive and desirable effects, which was not consistent with breed differences. These results confirmed QTL that have been detected in Meishan×Large White population (Rohrer and Keele, 1998b) and Meishan×Duroc population (Sato et al., 2003). As carcass weight can reflect an indirect measure of dressing percentage, several studies identified significant QTL for carcass weight on SSC7 (Andersson-Eklund et al., 1998; Rohrer and Keele, 1998b; Sato et al., 2003), in accordance with the mapping results of dressing percentage in this study.

It is interesting to note that the regions containing the QTL for fat depths are in good agreement with the many results reported for Meishan-derived populations earlier (Milan et al., 1998; Moser et al., 1998; Rohrer and Keele, 1998; de Koning et al., 1999, 2001; Rattink et al., 2000). Nearly all the QTL were located in the region where the swine lymphocyte antigen (SLA) complex resided, and the allele with higher fat depths originated from the commercial breed and not from the Meishan pigs. Therefore, there is a strong possibility that the same cryptic QTL exist in the Meishan population and more research should be conducted to explore this possibility. Improving the resolution of the pig-human comparative map in this QTL region can facilitate the search for QTL candidate genes. To help in the identification of the responsible genes, a higher density radiation hybrid map of the swine MHC region was constructed. This showed that the gene order was conserved between the two MHC-containing regions (Genêt et al., 2001). Recent studies also showed that a rearranged fragment of about 3.7 Mb was found in the pig approximately 20 Mb upstream from the expected location on the basis of the human map. The rearranged fragment contains two microsatellites found at the most likely QTL location in the INRA QTL experiment. It also contains the *BMP5* gene, which, together with *CLPS*, could be considered as a possible candidate (Olivier et al., 2003).

IMPLICATIONS

This work has demonstrated that combining data can improve the power of QTL detection. The finding of the present study also confirmed the existence of QTL affecting economically important traits on SSC7. Further studies will be aimed at fine mapping of the chromosome regions of interest and comparative candidate gene analysis so that the possible QTL actual gene could be isolated and characterized. Eventual identification of the gene may have an important economic impact on pig breeding.

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