Identification of Quantitative Traits Loci (QTL) Affecting Growth Traits in Pigs

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ABSTRACT : Molecular genetic markers were used to detect chromosomal regions which contain economically important traits such as growth, carcass, and meat quality traits in pigs. A three generation resource population was constructed from a cross between Korean native boars and Landrace sows. A total of 240 F2 animals from intercross of F1 was produced. Phenotypic data on 17 traits, birth weight, body weights at 3, 5, 12, and 30 weeks of age, teat number, carcass weight, backfat thickness, body fat, backbone number, muscle pH, meat color, drip loss, cooking loss, water holding capacity, shear force, and intramuscular fat content were collected for F2 animals. Animals including grandparents (F0), parents (F1), and offspring (F2) were genotyped for 80 microsatellite markers covering from chromosome 1 to 10. Least squares regression interval mapping was used for quantitative trait loci (QTL) identification. Significance thresholds were determined by permutation tests. A total of 10 QTL were detected at 5% chromosome-wide significance levels for growth traits on SSCs 2, 4, 5, 6, and 8. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1524-1528)***

Key Words : QTL, Microsatellite Marker, Growth Traits, Pig

INTRODUCTION

Quantitative Trait Loci (QTL) refers to gene positions in genetic materials influencing quantitative characteristics which are determined by several genes and environmental factors, and interactions between them. Mapping QTL is a basic operation for positional cloning and application of marker-assisted selection or marker-assisted introgression in genetic improvement (Soller, 1994).

Comprehensive genetic maps of the porcine genome were developed during the last decade (Ellegren et al., 1994; Marklund et al., 1996; Rohrer et al., 1996). At present, more than 2,000 genes and markers have been mapped in the pig, with a majority of anonymous molecular markers (http://www.ri.bbsrc.ac.uk/pigmap, Malek et al., 2001a). These genetic maps have made it possible to perform a systematic search of individual loci affecting quantitative traits of economic importance. Experimental populations have been used for detection of QTL, such as the cross between European wild boar and Large White pigs described by Andersson et al. (1994), and several crosses between Meishan and Western pig breeds (e.g., Rothschild et al., 1995; Janss et al., 1997).

On the base of these linkage maps and data from F2 breed cross resource population, several reports have been published on genomic scans for quantitative trait loci in pigs. Andersson et al. (1994) conducted the first genome wide scan for growth and fat deposition traits in pigs based on a European wild Boar×Large White cross. They found the evidence of QTL on *Sus scrofa* chromosome 4 (SSC 4) with large effects on growth from birth to 70 kg, length of small intestine, and fat deposition. In addition, they found a QTL on SSC 13 affecting early growth. After that, using a genome wide scans, QTL for growth and fat deposition traits (Carsas-Carrillo et al., 1997; Knott et al., 1998; Paszek et al., 1999; Rohrer, 2000; Wada et al., 2000; Malek et al., 2001a; Bidanel et al., 2001), for carcass traits (Andersson-Eklund et al., 1998), for meat quality (Andersson-Eklund et al., 1998; De Koning et al., 1999, 2001; Malek et al., 2001b) and teat number (Kim et al., 2004).

This study conducted to map QTL for growth traits in pigs through interval mapping from SSC1 to 10, using Korean native pig×Landrace resource family that represents a cross between the two phenotypically divergent swine breeds.

MATERIALS AND METHODS

Family structure

The resource population was developed from a cross between Korean native boars and Landrace sows. Five boars of Korean native pig and eleven sows of Landrace were selected randomly from a herd at National Livestock Research Institute, Rural Development Administration in Korea. Each boar was mated naturally with two or more different sows to produce F_1 animals. Each F_1 sire was selected randomly from each litter, and mated naturally with all sows of same litter. Thus eleven sires and 36 dams were used to produce 240 F_2 animals.

Management

Piglets were weaned at 35 days of age, and moved to

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Table 1. Overall means and phenotypic standard deviations (SD) for the growth traits in F_2 animlas

Traits	No. of pigs	Mean	SD
Body weights (kg) at			
Birth day	240	1.30	0.18
3 weeks of age	240	4.90	1.01
5 weeks of age	240	7.10	1.61
12 week of age	240	23.90	5.65
30 weeks of age	240	88.60	14.19
Average daily gain (g d^{-1})			
Between birth to 5 weeks of age	240	0.18	0.05
Between 5 to 12 weeks of age	240	0.13	0.04
Between 12 to 30 weeks of age	240	0.57	0.10

indoor pens with a flush gutter.Performance test was conducted from 12 weeks to 30 weeks of age. When the pigs were being tested of their performance, they were placed in pens that allowed for an average of one square meters per pig. All diets were fortified with vitamins and minerals for the age of the pigs. Water was freely available. All conditions were consistent with proper animal care. The performance test was finished at 30 weeks of age.

Traits measured

Body weight of piglets was measured at birth day, 3 weeks, and 5 weeks of age. Pigs were weighed and transferred to performance testing pens at 12 weeks of age. At 12 weeks of age the number of nipples was recorded. They were weighed and slaughtered at 210 days of age. Average daily gain was calculated from birth to 5 weeks of age, from 5 to 12 weeks of age, and from 12 to 30 weeks of age. The number of records and overall means and standard deviations of the growth traits studied are shown in Table 1.

DNA isolation, marker selection and genotyping

Blood samples were collected from all F2 animals and their parents (F1) and grandparents (F0), and DNA was isolated with Wizzard Genomic DNA Purification Kit (Promega, USA). Microsatellite markers used were selected from markers distributed from U.S. Pig Genome Coordination Program (Coordinator; Max F. Rothschild). Initially, a total of 509 markers was screened to determine polymorphic marker in our resource family. Markers were selected based on ease of scoring, informativeness, and location in the genome. Finally, eighty markers from chromosome 1 to 10 were selected to genotype our resource population. Intervals between adjacent markers were less than 20 cM whenever possible, and average marker interval was approximately 19 cM base don USDA-MARC map (Rohrer et al., 1996). Microsatellite markers were amplified by PCR using 10 ng pig genomic DNA as a template. PCR was performed in 10 μl reactions with 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP, 3 pmole each primer, 0.5 units Taq DNA polymerase (TaKaRa Shuzo Co., Shiga, Japan). Thermal cycling conditions included an initial denaturation for 5 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at optimum temperature depends on markers and 1 min at 72°C, and a final extension of 10 min at 72°C in GeneAmp PCR System 9600 (Perkin-Elmer Co., USA), TPC 100 thermal cycler (MJ research, USA), or Multi-block thermal cycler (MWG Co., Germany).

PCR products of up to 9 markers were combined, and analyzed simultaneously on an automated DNA sequencer (ABI 377 or 310, Perkin-Elmer Co., USA). Fragment length of the PCR products was determined with Genescan software version 2.1 (Perkin-Elmer Co., USA), and marker genotypes were assigned to the animals using Genotyper software version 2.5 (Perkin-Elmer Co., USA).

Statistical analysis

Linkage analysis was performed using CRIMAP software version 2.4 (Green et al., 1990), using the FLIPS and all options to get the best order of the markers and the fixed option to obtain the map distances. The maps were then used for QTL analysis of the 10 autosomes from SSC 1 to 10. For QTL analysis, the F_2 QTL Analysis Servlet of QTL express which is a web-based QTL mapping tool was used (http://qtl.cap.ed.ac.uk/).

Table 2. QTL for growth traits in pigs

SSC	Traits	Loc[f]. (cM)	F[g]	Additive		Dominance		Var.[i]
				Estimate	SE[h]	Estimate	SE	
2	B0W [a]	25	4.88*	-0.084	0.036	-0.119	0.067	16.26
2	ADG[b] (12 to 30wks)	155	6.03*	0.016	0.020	0.098	0.029	26.15
4	ADG (12 to 30wks)	56	5.09*	-0.012	0.014	-0.057	0.019	9.14
5	B12W[c]	77	4.90*	-1.500	0.510	-0.765	0.805	6.24
6	B5W[d]	77	5.58*	-0.037	0.245	1.228	0.373	14.36
6	ADG (birth to 5 wks)	76	5.07*	0.001	0.001	0.033	0.01	12.50
6	B12W	78	6.31*	-1.213	0.861	4.410	1.337	14.76
8	B30W[e]	0	6.57*	-5.055	2.122	6.080	2.895	8.02
8	B12W	0	5.88*	-1.235	0.792	2.818	1.081	7.25
8	B0W	140	4.75*	0.078	0.035	-0.142	0.070	18.68

[a] Birth weight (kg). [b] Average daily gain (g). [c] Body weight at 12 weeks of age (kg). [d] Body weight at 5 weeks of age (kg).
[e] Body weight at 30 weeks of age (kg). [f] Location. [g] F-values. [h] Standard error. [i] Variance (%).* $p<0.05$.

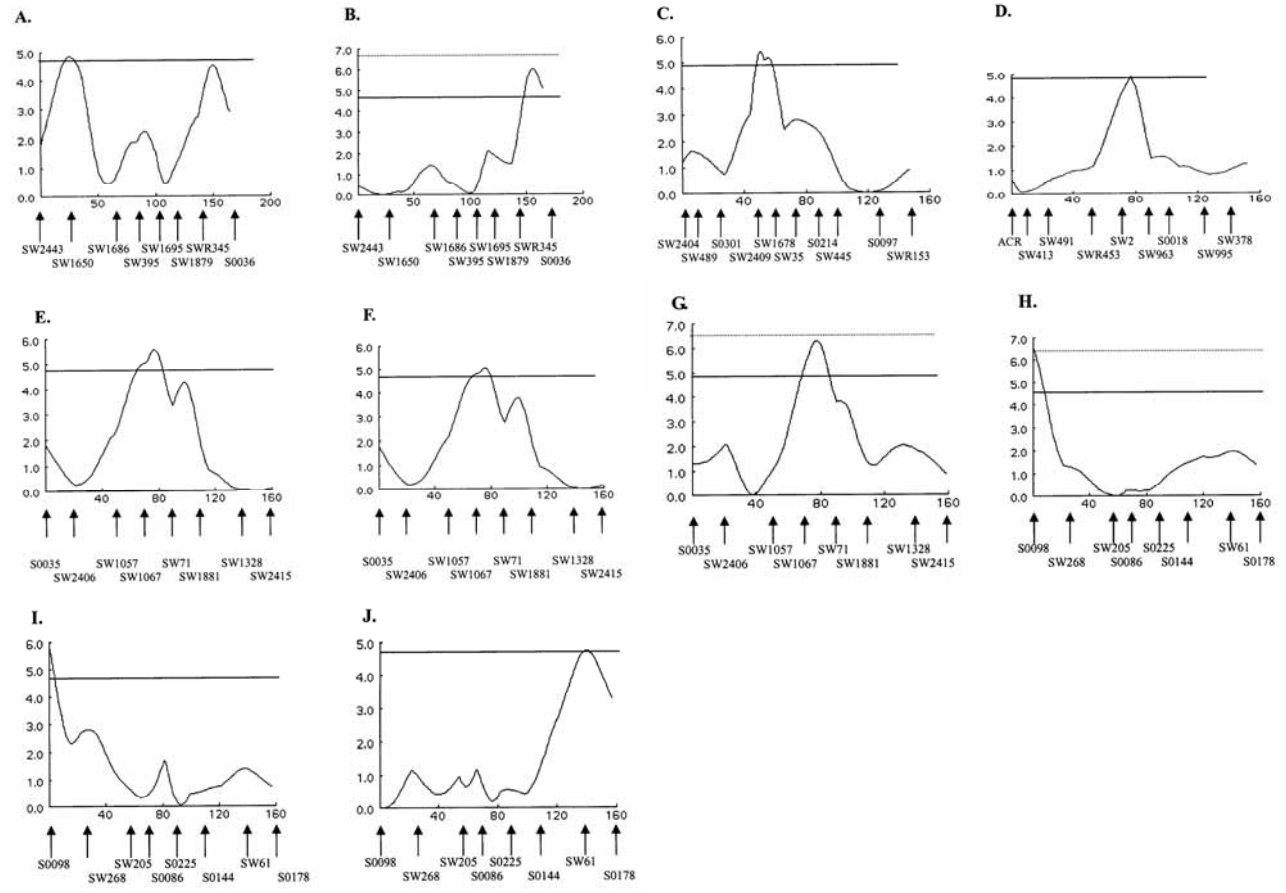

Figure 1. F-ratio curves for birth weight on chromosome 2 (A), average daily gain from 12 to 30 weeks of age on chromosome 2 (B), average daily gain from 12 to 30 weeks of age on chromosome 4 (C), body weight at 12 weeks of age on chromosome 5 (D), body weight at 5 weeks of age on chromosome 6 (E), average daily gain from birth to 5 weeks of age on chromosome 6 (F), body weight at 12 weeks of age on chromosome 6 (G), body weight at 30 weeks of age on chromosome 8 (H), body weight at 12 weeks of age on chromosome 8 (I), and birth weight on chromosome 8 (J). The x-axis indicates the relative position on the linkage map. The y-axis represents the F-ratio. Two lines indicate of 5% (—) and 1% (---) chromosome-wise significance level from the permutation test.

RESULTS

A total of 80 microsatelltes were used for linkage mapping from chromosome 1 to 10. Quantitative trait loci for growth traits were analyzed using a three-generation resource population constructed between Korean native boars and Landrace sows. The results of QTL that were detected at least 5% chromosome-wide level are summerized in Table 2. Ten significant affecting growth traits QTL at the 5% chromosome wide level were identified on chromosome 1 to 10. Seven significant QTL at the 5% chromosome-wide level for body weight were found from SSC1 to SSC10. The other QTL were associated with average daily gain (ADG).

Two QTL for birth weight were identified between sw2443 and sw1650 on SSC2, and between sw61 and s0178 on SSC8 (Figure 1A, J). The additive effect suggested that Korean native pig alleles were associated with lower birth weight on SSC2, and higher birth weight on SSC8 compared to Landrace pig alleles. The phenotypic variances accounted for by the QTL were 16.26 and 18.68%, respectively. QTL for body weight at 5 weeks of age was located between sw1057 and sw1067 (Figure 1E). Korean native pig alleles were associated with lower body weight. QTL for body weight at 30 weeks of age were located between s0098 and sw268 on chromosome 8 (Figure 1H). The additive effect for the QTL suggested that Korean native pig alleles tended to be associated with lower body weight compared to alleles of Landrace pigs. Moreover, three QTL for body weight at 12 weeks of age were found on SSC5, SSC6, and SSC8. For these QTL, the additive effects suggested that Korean native pig alleles were associated with lower body weight compared to alleles of Landrace.

Three QTL for ADG were detected from SSC1 to SSC10. Average daily gain between 12 to 30 weeks of age was suggestively affected on SSC2 and SSC4, which were located between marker sw1650 and sw1686 on SSC2

(Figure 1B), and between marker sw1678 and sw35 on SSC4 (Figure 1C). The additive effect suggested that Korean native pig alleles were superior to Landrace allele on SSC2, and inferior to Landrace alleles on SSC4. Additionally, a suggestive QTL for ADG between birth to 5 weeks of age was detected between sw1057 and sw1067 on SSC6 (Figure 1F). Korean native pig alleles were associated with higher growth rate compared to Landrace alleles. The QTL accounted for 12.5% of phenotypic variance. Especially, QTL for body weight at 5 and 12 weeks of age, and ADG from birth to 5 weeks of age on chromosome 6 were detected at same region (Figure 1 E, F, G).

DISCUSSION

In the present study, ten QTL affecting growth traits were found on SSC2, SSC4, SSC5, SSC6, and SSC8. All QTL were significant at 5% chromosome-wide level. Two suggestive QTL for birth weight were identified on SSC2 and SSC8. There are no previous reports of QTL for birth weight on SSC2 and SSC8. So far, QTL affecting birth weight were found on SSC3 (Malek et al., 2001a), on SSC4, SSC5, SSC9, and SSC16 (Paszek et al., 1999), on SSC1 (Wada et al., 2000), and on SSC1, SSC12, and SSC13 (Knott et al., 1998). Also, Bidanel et al. (2001) reported that QTL for birth weight were identified on SSC4 and SSC7 using a line-cross regression method, and on SSC11 using a half-/full-sib maximum likelihood methods. Also, Rothschild et al. (1995) reported that TNFα gene, which is located close to the swine major histocompatibility complex on SSC7, was associated with birth weight.

The QTL for ADG from 12 to 30 weeks of age were identified on SSC2 and SSC4 in this study. The QTL for late growth in similar chromosomal region of SSC4 were reported by Andersson et al. (1994), Knott et al. (1998), Milan et al. (1998), Wang et al. (1998), Marklund et al. (1999), Paszek et al. (1999), Walling et al. (2000), Bidanel et al. (2001), and Malek et al. (2001a). Also, QTL affecting growth rate of SSC2 were identified by Knott et al. (1998) and Malek et al. (2001a). These results were almost consistent with results of this study. Rohrer et al. (2000) also found QTL for growth on SSC2 using genomewide scan. QTL on SSC5 was suggestively affected for growth in this study. Only Paszek et al. (1999) reported QTL for growth on SSC5. QTL on SSC6 were associated with body weight at 5 weeks and 12 weeks of age, and ADG between birth to 5 weeks of age. This QTL was same chromosomal region affecting growth traits of SSC6 reported by Rohrer et al. (2000) and Bidanel et al. (2001). Malek et al. (2001a) reported a QTL affecting ADG on test on SSC8. Also, Bidanel et al. (2001) found QTL for early growth on SSC8. QTL for body weight at 12 weeks and 30 weeks of age was found at the similar position of SSC8 in the present study. Cassas-Carrillo et al. (1997) reported a QTL on SSC3 for ADG, which was not confirmed in this study. Rohrer et al. (2000) reported a QTL on SSC1 that significantly affected growth prior to 18 weeks of age, which confirmed the results of Cassas-Carillo et al. (1997), and Paszek et al. (1999). This result also was not identified in this study. Milan et al. (1998), Wang et al. (1998), Rohrer et al. (2000) and Bidanel et al. (2001) also reported a QTL affecting late growth trait on SSC7, which was not observed in this study. Wada et al. (2000), Malek et al. (2001a) reported a QTL for ADG on SSC9, which was not found a QTL affecting growth in the present study.

In this study, the data of F_2 individuals from a cross between the Korean native boars and landrace sows were analyzed using interval mapping procedure of least squares regression method under line cross concept. We detected 10 QTL affecting growth traits at least 5% level of chromosome-wide scans. It was suggested that QTL on *SSC*6 effected early growth, 5 weeks, and 12 weeks of age, whereas QTL on p arm of *SSC*8 controlled late growth. Lee et al. (2003) reported that two putative QTL imprinted for body weight at 12 and 30 weeks of age. A significant QTL, however, was not found for growth traits at 1% level of chromosome-wide. Although a few of the detected QTL may be false positives, the results of QTL at this level of significance will provide further our knowledge on the inheritance of QTL for growth traits. Additional markers should be genotyped for detected regions to obtain more precise estimates of the position of QTL.

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