Detection of Imprinted Quantitative Trait Loci (QTL) for Growth Traits in Pigs

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ABSTRACT : As an experimental reference population, crosses between Korean native pig and Landraces were established and information on growth traits was recorded. Animals were genotyped for 24 microsatellite markers covering chromosomes 2, 6, and 7 for partial-genome scan to identify chromosomal regions that have effects on growth traits. quantitative trait loci (QTL) effects were estimated using interval mapping by the regression method under the line cross models with a test for imprinting effects. For test of presence of QTL, chromosome-wide and single position significance thresholds were estimated by permutation test and normal significance threshold for the imprinting test were derived. For tests against the Mendelian model, additive and dominance coefficients were permuted within individuals. Thresholds (5% chromosome-wide) against the no-QTL model for the analyzed traits ranged from 4.57 to 4.99 for the Mendelian model and from 4.14 to 4.67 for the imprinting model, respectively. Partial-genome scan revealed significant evidence for 4 QTL affecting growth traits, and 2 out of the 4 QTLs were imprinted. This study demonstrated that testing for imprinting should become a standard procedure to unravel the genetic control of multi-factorial traits. The models and tests developed in this study allowed the detection and evaluation of imprinted QTL. *(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 8 : 1087-1092)*

Key Words : Pig, QTL, Imprinting, Microstellite Markers

INTRODUCTION

The use of genetic markers has widely made it possible to dissect quantitative trait variation. Due to the availability of large numbers of polymorphic markers, it is now possible to scan complete genome for genes affecting quantitative traits of interest, so-called quantitative trait loci (QTL) or economic trait loci (ETL). QTL underlying the genetic variance of economic traits of livestock have been mapped in many chromosomal regions using saturated genetic marker maps. On the basis of genetic linkage maps and data from F_2 breed cross resource populations, several recent studies have reported the discovery of a number of QTL affecting economic traits in pigs on a variety of chromosomes (Anderson et al., 1994; Wang et al., 1998). Accordingly, whole genome scans have revealed a number of genomic regions containing QTL and have also provided better insights into the modes of inheritance of such traits.

Recently, several studies showed that the non-Mendelian form of gene expression could be searched for by using genome scanning. There were a number of reports that QTL exhibit non-Mendelian inheritance in pigs. Much attention had been paid to genomic imprinting with respect to identification of gene expression patterns for medical applications as well as animal breeding. In spite of this important implication, there were only few evidences of imprinted genes and their expression modes due to limitations of the approach for experimental populations in humans. In livestock, evidence for non-Mendelian gene expression was reported for one specific chromosomal region in pigs (Nezer et al., 1999; Jeon et al., 1999). De Koning et al. (2000) first presented results of a genome-wide systematic approach for detecting imprinted regions for multi-factorial traits. They detected gametic imprinting for QTL in swine F_2 cross based a on comparison of levels of significance of paternal and maternal imprinting effects against a no-QTL model. But they did not test imprinting against a Mendelian model which is needed to identify deviations from Mendelian inheritance. If imprinting is a more common phenomenon than is previous thought, it is necessary to investigate whether imprinting happened or not for previously published candidate gene for application of marker assisted selection. It is also necessary to include statistical tests for imprinting in humans and animal genetics results of both genome scanning and evaluate on of candidate genes. It is necessary to develop and apply tests that can be done to identify deviations between the Mendelian and the parental competition model which proposes that imprinting acts to maximize the genetic contribution of one of parent at other expense of the other in polygamous species. Results of the partial genome-wide approach are presented in this paper to detect imprinted regions for multi-factorial traits in an F_2 cross between

Table 1. Overall means and phenotypic standard deviations of the traits studied

Description of traits	Abbreviation	No. of Pig	Mean	Standard deviation
Body weight at birth (kg)	Bwt	240	1.30	0.18
Body weight at 5 weeks of age (kg)	Wt5wk	240	7.10	1.61
Body weight at 12 weeks of age (kg)	Startwt	240	23.90	5.65
Average daily gain from 5 to 12 weeks of age (kg/day)	Postwadg	240	0.13	0.04
Carcass weight (kg)	Carcwt	240	70.58	12.77
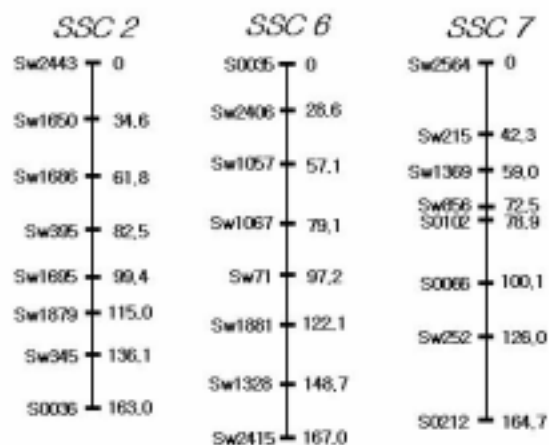
Figure 1. Microsatellite markers and their map distances (Kosambi mapping function; cM) by chromosomes.

outbred lines of pigs.

MATERIALS AND METHODS

Experimental population and performance traits

Five grand sires of Korean native pig and eleven grand dams of Landrace were used to produce nine F_1 litters. From the F_1 litters, 11 boars and 36 gilts were chosen to produce F_2 animals. The resource population with 240 F_2 progeny were produced by three generations. The body weights of piglets were measured at birth, 3 weeks and 5 weeks of age. The pigs were weighed and transferred to performance testing pens at 12 weeks of age. They were weighed and slaughtered at 210 days of age. The average daily gain was calculated from 5 to 12 weeks of age and from 12 to 30 weeks of age (Table 1).

DNA extraction, PCR and genotyping

Blood samples were collected from F_2 animal and their parents and grandparents. DNA was isolated using the Wizard Genomic DNA purification Kit (Promega, USA). A total of 240 F_2 pigs were genotyped for 24 polymorphic microsatellite markers on chromosome 2 (8 markers), 6 (8 markers) and 7 (8 markers) (Figure 1). The microsatellite markers were amplified for by PCR using 10 ng of genomic DNA with the GeneAmp PCR System 9600 (Perkin-Elmer Co., USA) thermocycler. For microsatellite marker genotyping, one primer of each primer-pair was fluorescence labeled (Tet, Fam or Hex) for the automated DNA sequencer (ABI 310, 370, Perkin-Elmer Co., USA). The fragment length of the PCR products was determined with the Genescan software version 2.1 (Perkin-Elmer Co., USA) and marker genotypes were scored using the Genotyper software version 2.5 (Perkin-Elmer Co., USA).

Statistical analysis

Maximum likelihood analysis was used for ordering marker loci relative to one another with the CRI-MAP program (version 2.4) (Green et al., 1990). For QTL analysis, the public web-based software, QTL express (http://www.qtl.cap.ed.ac.uk) (Seaton et al., 2002) was used. The statistical model was fitted with sex and slaughtering date as fixed effect and live weight as a covariate. A single QTL was fitted in all cases by regressing on additive and dominance coefficients for the QTL at each putative position of the QTL (every 1cM). Additive and dominance coefficients at a given position of the QTL were derived based on marker data following the procedure of Haley et al. (1994). In order to estimate paternal and maternal contribution coefficients, we followed an extension of the traditional Mendelian expression model suggested by Knott et al. (1998) and de Koning (2000). To test for the presence of QTL, we conducted some different tests; Mendelian versus no QTL model, full imprinting versus no-QTL model.

For providing evidence of imprinting and for determining whether QTL could be paternally or maternally expressed in the genome, the model for imprinting was reparameterized to enable direct testing for the contributions of paternally and maternally inherited effects by de Korning et al. (2000). For every F_2 offspring, the probabilities of inheriting two alleles of the first founder breed (P_{AA}), two alleles of the alternative breed (P_{BB}) or one from each founder breed (P_{AB} and P_{BA}) at 1cM intervals across the genome were inferred. P_{AB} is the probability that the F_2 progeny can have the paternal allele originating from breed A and the maternal allele from B. P_{BA} is the probability that the F_2 progeny can also have the paternal allele originating from breed B and the maternal allele from A. Also, breed origin probabilities of paternal and maternal alleles received by an F_2 progeny were determined for each

Table 2. Chromosome-wide and single position significance thresholds by permutation under Mendelian genetic model and full imprinting model

Chr.	Trait	Mendelian model[a]				Imprinting model[b]			
		Chromasome-wide		Single position		Chromasome-wide		Single position	
		5%	1%	5%	1%	5%	1%	5%	1%
SSC2	Bwt	4.80	6.30	2.70	4.29	4.18	5.88	3.07	3.79
	Postwadg	4.64	5.82	2.70	4.65	4.25	5.79	2.67	3.99
SSC6	Carcwt	4.99	6.98	2.90	4.81	4.31	5.88	2.85	4.17
	Startwt	4.78	7.30	3.21	5.04	4.67	6.09	2.98	3.84
	Postwadg	4.57	6.70	3.21	5.27	4.14	5.68	3.05	4.10
	Wt5kw	4.90	7.10	2.85	4.88	4.30	5.79	3.11	3.96
SSC7	Bwt	4.62	6.02	2.59	3.49	4.27	6.10	2.88	4.14
	Postwadg	4.71	6.17	2.60	3.27	4.25	5.90	3.07	3.99

[a] QTL is the test statistics for the presence of a QTL under a genetic model with additive and dominance effect (2 d.f.).

[b] QTL is the test statistics for the presence of a QTL under a genetic model with additive, dominance and imprinting effect (3 d.f.).

chromosomal position based on marker data. Breed origin probabilities can be used to derive conditional probability of additive ($P_a=P_{11}-P_{22}$), dominance effect ($P_d=P_{12}+P_{21}$) and paternal ($P_{pat}=[P_{11}+P_{12}]-[P_{22}+P_{21}]$) and maternal ($P_{mat}=[P_{11}+P_{21}]-[P_{22}+P_{12}]$) contribution coefficients. Under the traditional line cross approach, the expected performances of offspring written in terms of the additive (a) and dominance (d) contributions are estimated using the regression of the phenotypes on P_a and P_d.

$$Y_i=m+aP_{ai}+dP_{di}+e_i \qquad \text{Mendelian model}$$

Where, Y_i is phenotype of ith animal, m is overall mean, a is additive effect, d is dominance effect, P_{ai} is additive regression coefficient for phenotype of ith animal, and P_{di} is dominance regression coefficient for phenotype of ith animal.

However when accounting for the grandparental origin of the alleles by using multiple marker information, it is possible to calculate probabilities of the two alleles in an offspring according to four possible genotypes in the F_2 generation. Additional extension models can be fitted with exclusively paternal or maternal expression using the conditional probabilities. On the basis of these concepts, we developed another full imprinting model to test for no imprinting QTL ($P_{imp=}P_{pat}-P_{mat}$).

$$Y_i=m+aP_{ai}+dP_{di}+iP_{impi}+e_i \qquad \text{Full imprinting model}$$

Where, Y_i is phenotype of ith animal, m is overall mean, a is additive effect, d is dominance effect, i is imprinting effect, P_{ai} is additive regression coefficient for phenotype of ith animal, P_{di} is dominance regression coefficient for phenotype of ith animal, and P_{impi} is imprinting regression coefficient for phenotype of ith animal.

Due to outbred cross two type of heterozygotes can be distinguished, so fitting the imprinting genetic model is possible by tracing the four alleles' segregation. The method outlined by Churchill and Doerge (1994) was used to determine significance thresholds for the F statistic to control Type I error rate at the chromosome-wide level empirically by the permutation test. Each permuted data set was then analyzed using the least squares regression interval mapping method and the maximum value of the F statistic was recorded. A total of 1,000 permuted data sets were analyzed, resulting in 1,000 maximum F statistics per trait and every hypothesis tested with different alternative models. Significance thresholds to control type I error rate at a level α on the chromosome-wide level were determined by ranking the maximum F statistics and determining the value of the F statistic that marked the $(1-\alpha)\times100^{th}$ percentile (Lee and Jeon, 2002).

RESULTS

Figure 1 shows the results of linkage analysis using CRI-MAP, including microsatellite marker names and their map positions (cM) in Kosambi mapping function.

Experimental critical values (threshold) accounting for marker-QTL association of five traits on swine chromosome 2, 6 and 7 were calculated. Chromosome-wide and single position significance thresholds (5% and 1%) were derived by 1,000 replicates of permutation test based on each test model for two genetic models (Table 2). Threshold values of 5% and 1% chromosome-wide against the no-QTL model on traits ranged from 4.57 to 4.99, and 5.82 to 7.30, respectively, for Mendelian model. For full imprinting model, threshold of 5% and 1% chromosome-wide ranged from 4.14 to 4.67 and 5.68 to 6.10, respectively. Threshold differences between traits were due to differences in phenotypic distributions and random sampling.

QTL analyses were performed for chromosomes 2, 6 and 7 maps using a statistical model (Knott et al., 1998) by testing for the presence of an imprinting effect. Partial genome-scan for putative QTL linkage chromosome-wide was conducted. For the Mendelian model, the presence of five QTL was predicted on the basis of the critical value estimated by the permutation test at single test significance

Table 3. Test statistics and location of QTL that exceed single test significance (5%) under the regression method

Chr.	Trait	Genetic model			
		Mendelian model		Full Imprinting model	
		Location (cM)	F-value	Location (cM)	F-value
SSC2	Bwt	25	4.88	26	3.29
	Postwadg	155	6.03	155	4.25
SSC6	Carcwt	78	6.55	103	4.76
	Startwt	78	6.31	80	5.87
	Postwadg	0	4.28	0	4.16
	Wt5kw	77	5.58	79	4.25
SSC7	Bwt	126	4.45	126	3.00
	Postwadg	16	4.52	59	4.00

* Significant at the 5% chromosome–wide level, ** Significant at the 1% chromosome–wide level.

Table 4. QTL analysis for pig chromosome 2, 6 and 7 for crossbred between Korean native pig and Landrace

Chr.	Trait	QTL[a]		Impritinting[b]		Map position (cM)
		F ratio	LOD	F ratio[c]	LOD	
SSC2	Bwt	3.29	2.04	NS	2.07	26
	Postwadg	4.25	2.49	NS	2.64	155
SSC6	Carcwt	4.76	2.72	1.07	2.95	103
	Startwt	5.87	2.62	4.30	3.61	80
	Postwadg	4.16	-	4.44	2.59	0
	Wt5kw	4.25	2.32	1.03	2.64	79
SSC7	Bwt	4.00	2.42	0.39	2.49	59

[a] QTL is the test statistic for the presence of a QTL under a genetic model with additive, dominance, and imprinting effects (3 d.f.).
[b] Imprinting is the test statistic for the presence of an imprinting effect (1 d.f.).
[c] The test statistic showed evidence of suggestive linkage. NS. Non significance, * $p < 0.05$ (F>3.92).

level (5%). Four QTL were found as putative QTL under the full imprinting model. From the Mendelian and imprinting model, the locations and F-statistics of QTL affecting growth traits that exceeded the single position threshold are shown in Table 3.

Comparing the results between the Mendelian and the full imprinting model, detection of the presence of QTL was slightly different by the fitted model. For comparison of the two genetic models (Mendelian and imprinting), test statistics for the presence of QTL that exceeded the suggestive linkage (LOD>3) under the maximum likelihood method was shown in Table 4. Test statistics that exceeded suggestive linkage threshold of maximum likelihood for the full imprinting model by chromosome and traits are presented in Table 4.

Although significance that exceeded the 5% chromosome-wide level under the full imprinting model including additive, dominance, and imprinting effect was found for Postwadg (SSC 2) and Carcwt (SSC 6), presence of imprinting effect, was not accepted under reduced imprinting model (1 d.f.) (Table 4). According to this result, those traits might be expressed as the Mendelian mode. As a limitation of the analysis model used in this study, the paternal and maternal expression mode cannot be discriminated for test statistics that exceed the linkage significance threshold under the full imprinting model including additive, dominance, and imprinting. For the critical position with peak point at the 5% chromosome-

wide significance level, presence of imprinted QTL could be inferred as the suggestive significance degree. Table 4 presented evidence of QTL linkage inferred by a different genetic mode of Mendelian and imprinting at 5% chromosome-wide level.

Of all the revealed QTL there were two putative QTL imprinted for Startwt and pdstwadg in chromosome 6. To illustrate the presence of QTL for each trait, graphical comparisons of results obtained under the Mendelian and the full imprinting model are shown in Figures 2 and 3. For each trait, the peak point indicating the presence of QTL under the imprinting and the Mendelian model exceeded the threshold of 5% chromosome-wide level.

DISCUSSION

Until now genomic imprinting is regarded as a rare phenomenon and is consequently ignored in most studies. Based on some of the results that are related to imprinting QTL for multi-factorial traits in pigs, it is suggested that genomic imprinting may be a more common phenomenon than is previously expected. Four QTL, of which two were subjected to imprinting at the suggestive significance level were detected.
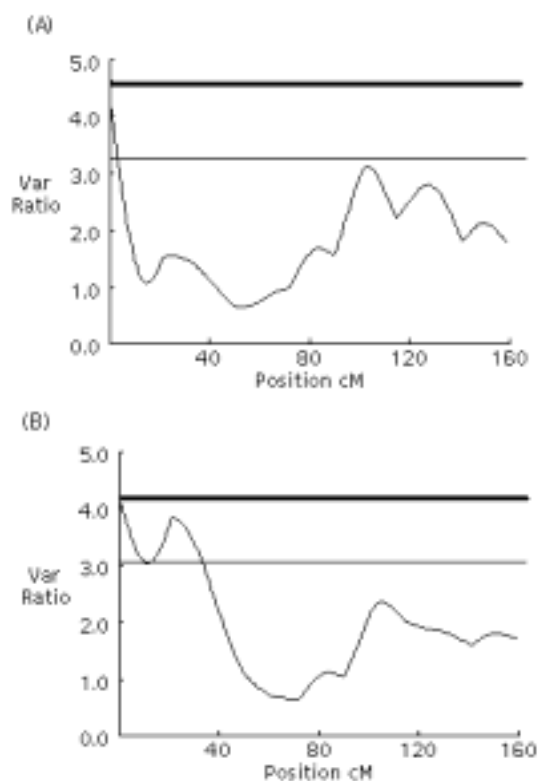
It is necessary to test with massive real data or to pool data sets produced from other resource populations with the mapping results of F_2 families and to construct fine map in the target region that has been found. In this study,
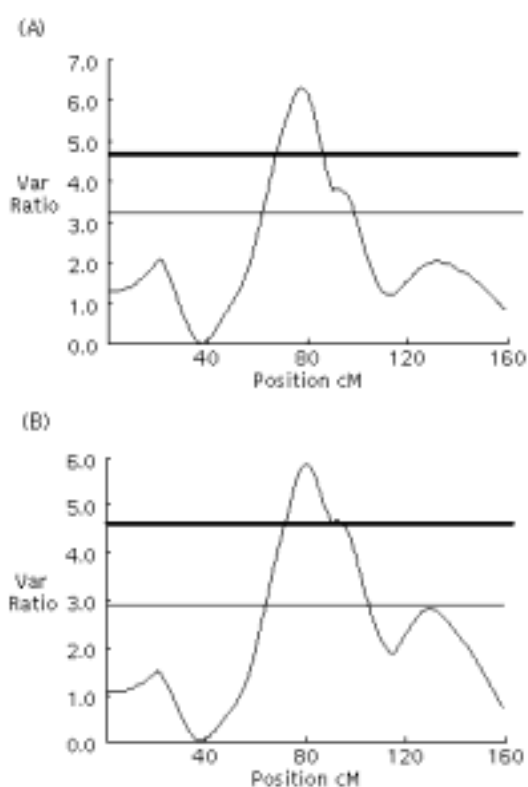
Figure 2. Test statistics profiles for chromosome 6 that exhibit characteristics of QTL expression for Startwt: Mendelian model (A) and imprinting model (B). The two lines are provided for 5% single test significance (——) and 5% chromosome–wide significance (━━)

Figure 3. Test statistics profiles for chromosome 6 that exhibit characteristics of QTL expression for Postwadg: Mendelian model (A) and imprinting model (B). The two lines are provided for 5% single test significance (——) and 5% chromosome–wide significance (━━)

imprinted QTL with evidence of suggestive significance was not found on chromosome 2 and 7 (Table 4). But QTL that expressed the Mendelian mode were found for Postwadg (SSC 2) and Carcwt (SSC 6). It has been reported that many of QTL affecting economic trait of pigs might be located in chromosome 2 (de Koning et al., 2000; Rattink et al., 2000). The distal tip of porcine chromosome 2 has shown to be homologous to human chromosome 11 p, where a large cluster of imprinted genes is located. Among these, IGF-2 has been mapped to chromosome 2 and has been shown to be imprinted in pigs as well (Jeon et al., 1999; Nezer et al., 1999). Our results showing evidences of QTL for various traits were in agreement with the results of the two studies reffered to before.

Using only phenotypic data, de Vries et al. (1994) showed that genomic imprinting may influence the rate and composition of growth in pigs. De Vries et al. (1994) pointed out that it was difficult to discriminate between a maternal imprinting effect and a genetic maternal imprinting using quantitative genetic analyses because these two effects are almost completely confounded. But analysis at the molecular level permits investigation of the imprinting effect because it allows separation of the maternally inherited alleles within litter. Also knowledge of the fact that the QTL is subjected to imprinting will be helpful for identifying the genes. An outbred cross, such as a cross between two pig breeds with different genetic backgrounds each other, is the ideal resource for the detection of imprinted regions.

For the practice of animal breeding, the identification of major imprinted loci affecting economic traits has several implications. If much stronger imprinting genes affecting economic traits are found and confirmed as a real phenomenon in livestock performance, revision of methods for genetic evaluation that currently ignore non-Mendelian expression is required. The identification of imprinted loci opens new perspectives for cross breeding, which is a common practice in pig breeding.

ACKNOWLEDGEMENT

2003. The National Livestock Research Institute (NLRI) directed the whole project and Hankyong National University in cooperation with NLRI, Yonam College of Agriculture and Seoul National University participated in the statistical anayses of QTL.

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