Characterization of Quantitative Trait Loci (QTL) for Growth using Genome Scanning in Korean Native Pig

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ABSTRACT

Molecular genetic markers were genotyped used to detect chromosomal regions which contain economically important traits such as growth traits in pigs. Three generation resource population was constructed from a cross between the Korean native boars and Landrace sows. A total of 193 F2 animals from intercross of F1 were produced. Phenotypic data on 7 traits, birth weight, body weight at 3, 5, 12, 30 weeks of age, live empty weight were collected for F2 animals. Animals including grandparents (F0), parents (F1), offspring (F2) were genotyped for 194 microsatellite markers covering from chromosome 1 to 18. Quantitative trait locus analyses were performed using interval mapping by regression under line-cross model. To characterize presence of imprinting, genetic full model in which dominance, additive and imprinting effect were included was fitted in this analysis. Significance thresholds were determined by permutation test. Using imprinting full model, four QTL with expression of imprinted effect were detected at 5% chromosome-wide significance level for growth traits on chromosome 1, 5, 7, 13, 14, and 16.

(Key words : Microsatellite, QTL mapping, Imprinting effect, Korean Native pig, Genome scanning)

INTRODUCTION

Molecular genomic analysis has revolutionized how geneticists examine the genetic difference that exist within the domesticated pig. In the past few years, efforts have been directed toward the development of genomic maps consisting of anonymous genetic markers and known genes.

These QTL linkage analysis involve employing a genomic scan where generally F2 or backcross families are used and genotypes are botained for many markers evenly spaced across the genome. Several such experiments are underway or have recently been completed and are beginning to produce interesting and useful results (De Koning *et al.*, 2000b). Candidate gene and comparative mapping approaches have also been successful in identifying major genes affecting several traits (Maleck *et al.*, 2001). Candidate gene analysis is undertaken when a gene is chosen based on the physiology of the trait. The comparative candidate gene analysis which allows researchers to find "positional candidate genes" in the regions associated with putative QTL. To date, several major genes affecting quantitative traits have been found with the candidate gene approach (Rothchild *et al.*, 1997). Present and future genetic improvements will result from the more detailed genetic maps and our growing understanding of the function and structrue of the individual genes and gene families that are responsible for the economically important trait in pigs.

Accordingly, whole genome scans have derived as number of genomic regions containing quantitative trait loci and also provided a better insight into the mode of inheritance for traits. Recently, several studies show that non-Mendelian form of gene expression can be searched using the genome scans (De Koning *et al.*, 2000b).

And there was the number of report which QTL exhibit no-Mendelian inheritance in pigs. As explicit example of non-Mendelian gene expression, imprinted gene near the IGF-2 locus on chromosome 2, which affects muscle mass and fat deposition in pig, was identified (Nezer *et al.*, 1999: Jeon *et al.*, 1999).

In livestock, evidence for non-Mendelian gene expre-

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ssion was reported for one specific chromosomal region in sheep and pig (Cockett *et al.*, 1996; Nezer *et al.*, 1999; Jeon *et al*, 1999). But De Koning *et al* (2000a) first presented result of a genome-wise systematical approach to detect imprinted regions for multifactorial traits. If imprinting is common phenomena than previous thought, it is need to investigate whether imprinting happen to be or not in previous published candidate gene for application of marker assisted selection. And it is also need to include statistical test for imprinting in human and animal genetic result in both genome scan and evaluating candidate gene. We present results of genome-wise approach to detect imprinted regions for growth traits in an F2 cross between outbred line of pigs.

MATERIAL & METHODS

Experimantal population and performance traits

Three-generation pedigrees have been established on the basis of crosses between genetically divergent breeds. Five Korean native sires have been crossed with nine dams of Landrace breed. A total of 193 F2 individuals has been generated using intercross between 10 boars and 31 sows of F1 animals. This cross design was chosen because of the considerable phenotypic differences between the founder lines (Fig. 1).

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. The performance test was finished at 30 weeks of age. 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 week of age. They were weighed and slaughtered at 210 days of age.

DNA isolation, marker selection and genotyping

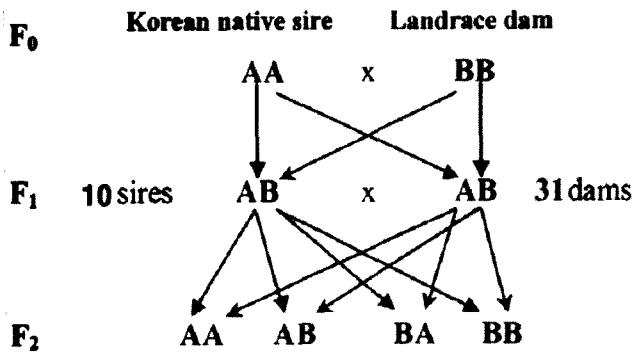

Fig. 1. Design for the resource population. F2 animals were produced by full-sib mating.

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). In briefly, three milliliters of whole blood were transferred into a 10 ml conical tube containing 9 ml of cell lysis solution and then incubated at room temperature for 10 minutes for lysis red blood cells. White blood cells were collected by centrifugation of 2,000 g for 10 minutes, lysised with 3 ml of nuclei lysis solution, purified with 1 ml of protein precipitation solution, and then recovered DNA with isopropanol. DNAs were resolved in TE buffer (pH 8.0) and measured their quantity by spectrophotometer and agarose gel electrophoresis.

Microsatellite markers were selected from markers distributed from U.S. Pig Genome Coordination Program. Markers were selected based on ease of scoring, informativeness, and location in the genome. Finally, 143 markers from chromosome 1 to 18 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. A list of all the markers used in genetic linkage analysis is listed in Table 2 of supplement.

For microsatellite markers genotyping, one primer of each primer-pair was labeled with a fluorescence (Tet, Fam, or Hex) for using automated DNA sequencer (ABI 310 or 377, Perkin-Elmer Co., USA). Microsatellite markers were amplified by PCR using 10 ng of pig genomic DNA as a template. PCR was performed in 10 ul reactions with 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 uM 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?, followed by 35 cycles of 30 sec at 94?, 30 sec at optimum temperature (Table 2) depends on markers and 1 min at 72?, and a final extension of 10 minutes at 72? in GeneAmp PCR System 9600 (Perkin-Elmer Co., USA), TPC 100 thermal cycler (MJ research), 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 Mapping : Marker linkage maps were computed using Crimap version 2.4 software (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 18 autosomes using the line cross least squares regression interval mapping program developed by Haley and Knott (1994). Marker information was used to calculate the probabilities that an F2 offspring inherited none, one, or two alleles from each breed for a putative QTL at each 1 cM position in the genome. Based on these probabilities, additive and dominance coefficients were derived for the putative QTL, contrasting average QTL alleles from the two breed origins, as represented by the F0 grandparents. The least squares regression model used for QTL analysis included the fixed effects of sex for all traits, along with additive and dominance coefficients for the putative QTL. Slaughter weight size was added as a covariable for birth weight, 3 weeks weight, 5 weeks weight, 12 weeks weight, 30 weeks weight, diet weight and backfat was added as a covariable for growth traits. Significance of each QTL was determined based on an F statistic that was computed from sums of squares explained by the additive and dominance coefficients for the QTL. Significance thresholds of the F statistic were derived at the chromosome wise levels on a single trait basis by the permutation test of Churchill and Doerge (1994). A total of 1,000 random permutations of the data were used.

QTL Mapping : For providing the evidence of imprinting and also identifying whether QTL seems to be paternally or maternally expressed in genome, the model for imprinting was reparameterized to enable a direct test for the contribution of the paternally and maternally inherited effect by De Korning *et al.* (2000a). For every F2 offspring, we inferred the probabilities of inheriting two alleles of first founder breed (PAA), two alleles of alternative breed (PBB) or one from each founder breed (PAB, PBA) at 1cM intervals across the genome. PAB is the probability that F2 progeny can have the paternal allele originated from breed A and maternal allele from B. PBA is the probability that F2 progeny can also have the paternal allele originated from breed B and maternal allele from A. Also, breed origin probabilities of paternal and maternal alleles received by an F2 progeny were determined at each chromosomal position based on marker data (Fig. 1). Breed origin probabilities can be used to derive conditional probability of additive ($Pa = P_{AA} - P_{BB}$), dominance effect ($Pd = P_{AB} + P_{BA}$), and paternal ($Ppat = [P_{AA} + P_{AB}] - [P_{BB} + P_{BA}]$), and maternal ($Pmat = [P_{AA} + P_{BA}] - [P_{BB} + P_{AB}]$) contribution coefficients. Under the traditional line cross approach, the expected performances of offspring that written in term of the additive (a) and dominance (d) contributions are estimated using the regression of the phenotypes on the Pa and Pd.

$$Y_i = m - a\,Pa_i + d\,Pd_i + e_i \text{ ---- Mendelian model (Mend)}$$

Where Y_i is phenotype of *i*th animal, m is overall mean, a is additive effect, d is dominance effect,Pa_i is additive regression coefficient for phenotype of *i*th animal, and Pd_i is dominance regression coefficient for phenotype of *i*th animal.

However when accounting for the grandparental origin of the alleles by using the multiple marker information, it is available to calculate probabilities of the two alleles in an offspring according to four possible genotypes in the F2 generation. Additional extension model can be fitted with exclusive paternal or maternal expression using the conditional probabilities. To separate contribution of parents, we introduced the probabilities that individual inherited a one founder breed allele from its father (Ppat) or from its mother (Pmat) (De Korning, 2000a). A saturated full model (Full), which include conditional probabilities of paternal (Ppat), maternal (Pmat) and a dominance (Pd), was fitted at 1cM intervals across the genome. The expected performances of offspring that written in term of the paternal (apat), maternal (amat) and dominance (d) contributions also can be estimated using the regression of the phenotypes on the Ppat, Pmat and Pd. On the basis of those concept, we developed another full imprinting model for test of no imprinting QTL (P imp = Ppat − Pmat)

$$Y_i = m + a\,Pa_i + d\,Pd_i + Pimp_i + e_i$$
---------- Full imprinting model (Full)

Where Y_i is phenotype of *i*th animal, m is overall mean, a is additive effect, d is dominance effect,Pa_i is additive regression coefficient for phenotype of *i*th animal, Pd_i is dominance regression coefficient for phenotype of *i*th animal, and $Pimp_i$ is imprinting regression coefficiet for phenotype of *i*th animal.

Due to outbred cross that two type of heterozygotes can be distinguishable, fitting the imprinting genetic model is possible by tracing the four alleles segregation.

Derivation of significance thresholds : 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- wise level empirically by permutation. And to apply to hypotheses test between each alternative genetic model that proposed in this study, permuted data sets were created by randomly shuffling marker data against phenotypic plus fixed effects data for the F2 individuals. 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. For multiple test thresholds, the maximum F statistics were recorded. A total of 1,000 permutated data sets were analyzed, resulting in 1,000 maximum F statistics per trait and every hypothesis test with different alternative

model. Significance thresholds to control type I error rate at a level on the chromosome-wise level were determined by ranking the maximum F statistics and determining the value of the F statistic that marked the $(1-\alpha)\times 100^{th}$ percentile.

RESULT AND DISCUSSION

QTL analysis were performed for nearly whole genome region using a statistical model(Knott *et al.*, 1998) by testing for the presence of an imprinting effect. Genome-scan for putative QTL on the chromosome-wide was conducted.

The presence of four QTL(Table 2) was predicted on the basis of critical value estimated by the permutation test at multiple test significance level(5%).

On the chromosome 1, 7, 13 and 16, four QTL, which might be expressed with imprinted effect were found. So far, QTL affecting birth weight were found on SSC3 (Malek *et al.*, 2001), and 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). A significant QTLs for body weight at 5 and 30 weeks of age were observed on SSC7 (F = 4.53), and SSC16 (F = 4.74), respectively.

As a limitation of the analysis model used in this study, the paternal and maternal expression mode can not be exactly 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. The graphical representations obtained under the full imprinting model are shown in Fig. 2, 3, 4 and 5. For each trait, the peak point indicating the presence of QTL under the imprinting model exceeded the threshold of 5% chromosome-wide level. To derive

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

Trait	Abbreviation	Mean	Standard Deviation	Minimum value	Maximum value
Body weight (kg) at					
- brith	BWT	1.26	0.21	0	1.8
- 3 weeks of ago	21DW	4.66	1.62	0	8
- 5 weeks of ago	35DW	6.92	2.21	0	11
- 12 weeks of ago	STDW	21.33	6.05	0	40.7
- 30 weeks of ago	ETDW	87.70	16.19	45.2	145

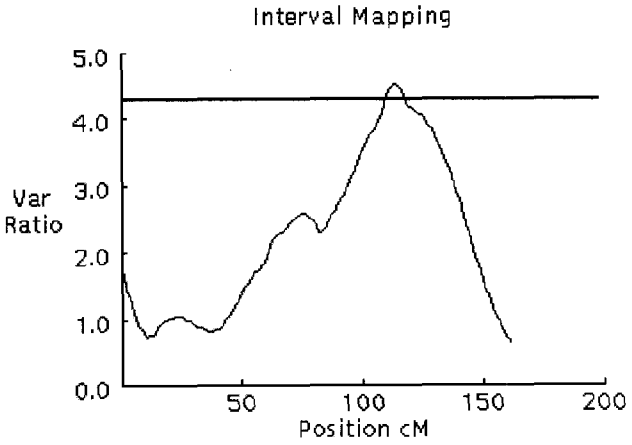

Fig. 2. F-ratio curves for body weight at 5weeks of age on chromosome 7.(chromosome-wide threshold at p = .05)

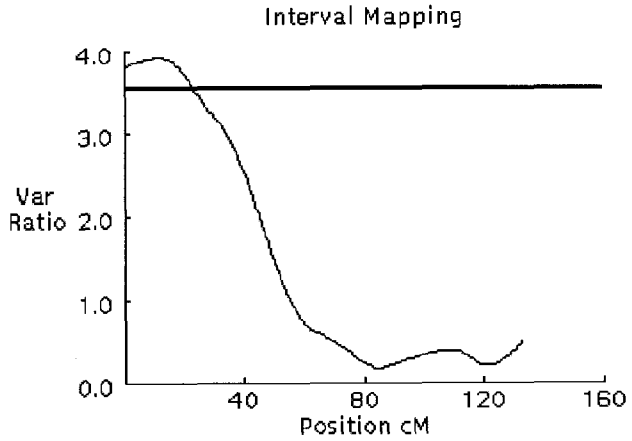

Fig. 3. F-ratio curves for birth weight on chromosome 1. (chromosome-wide threshold at p = .05)

Table 2. Chromosome-wide significant association of chromosomal regions with growth

SSC	Trait	Loci (cM)	Fratio	LOD	Mean	Additive effect	Dominance effect	Imprinting effect	Threshold	
									0.05	0.01
1	BWT	11	3.92	2.475	1.077± 0.089	0.016± 0.027	- 0.115± 0.045	- 0.067± 0.029	3.777	5.146
7	35DW	113	4.53	2.840	2.776± 0.639	- 0.08± 0.166	0.196± 0.263	0.619± 0.178	4.306	5.741
13	BWT	132	4.75*	2.972	0.997± 0.086	- 0.076± 0.022	0.014± 0.035	- 0.035± 0.027	4.343	5.832
16	ETDW	51	4.74*	2.970	9.512± 4.923	- 2.013± 1.111	5.579± 1.679	0.887± 1.123	3.894	4.968

test significance threshold from the imprinting and Mendelian genetic model, specialized permutation test were conducted using real phenotypic data. Significance thresholds estimated by permutation test differed only slightly by traits (Table 2), despite large differences in trait distributions and number of QTL detected in agreement with the results of De Koning *et al.* (1999).

In this study, the data of F2 individuals from a cross between the Korean native pigs and Landrace breed were analyzed using a chromosome-wise scan under outbred cross analyses including a systematic test for imprinting, and thereby some proving evidences for QTL affecting growth trait were obtained.

Until now genomic imprinting is regarded to be rare phenomenon and consequently is ignored in most studies. As some of results that is related to imprinting QTL for multifactorial traits in pig, recently have been reported, it is said that genomic imprinting might be a more common phenomenon than previously expected.

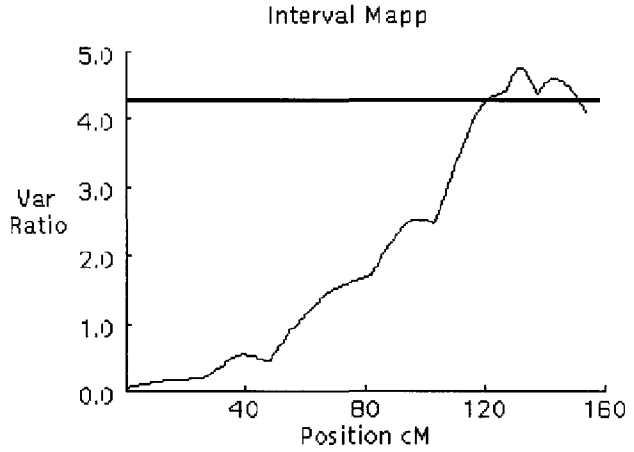

Fig. 4. F-ratio curves for birht weight on chromosome 13. (chromosome-wide threshold at p = .05)

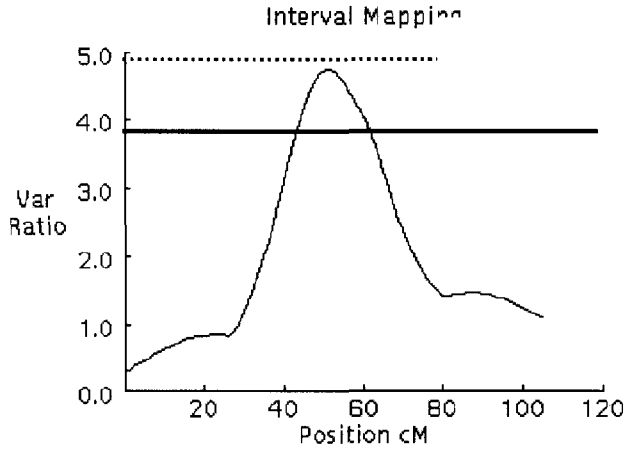

Fig. 5. F-ration curves for body weight at start test day on chromosome 16. (chromosome-wide threshold at p = .05, -------- chromosome-wide threshold at p = .01)

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 implication. 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.

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