QTL Scan for Meat Quality Traits Using High-density SNP Chip Analysis in Cross between Korean Native Pig and Yorkshire

S. W. Kim¹, X. P. Li¹, 2, Y. M. Lee³, Y. I. Choi¹, B. W. Cho¹, B. H. Choi¹,
T. H. Kim⁵, J. J. Kim³ and K. S. Kim¹*,
¹ Department of Animal Science, Chungbuk National University, Cheongju 361-763, Korea

ABSTRACT: We attempted to generate a linkage map using Illumina Porcine 60K SNP Beadchip genotypes of the F2 offspring from Korean native pig (KNP) crossed with Yorkshire (YS) pig, and to identify quantitative trait loci (QTL) using the line-cross model. Among the genotype information of the 62,136 SNPs obtained from the high-density SNP analysis, 45,308 SNPs were used to select informative markers with allelic frequencies >0.7 between the KNP (n = 16) and YS (n = 8) F0 animals. Of the selected SNP markers, a final set of 500 SNPs with polymorphic information contents (PIC) values of >0.300 in the F2 groups (n = 252) was used for detection of thirty meat quality-related QTL on chromosomes at the 5% significance level and 10 QTL at the 1% significance level. The QTL for crude protein were detected on SSC2, SSC3, SSC6, SSC9 and SSC12; for intramuscular fat and marbling on SSC2, SSC8, SSC12, SSC14 and SSC18; meat color measurements on SSC1, SSC3, SSC4, SSC5, SSC6, SSC10, SSC11, SSC12, SSC16 and SSC18; water content related measurements in pork were detected on SSC4, SSC6, SSC7, SSC10, SSC12 and SSC14. Additional QTL of pork quality traits such as texture, tenderness and pH were detected on SSC6, SSC12, SSC13 and SSC16. The most important chromosomal region of superior pork quality in KNP compared to YS was identified on SSC12. Our results demonstrated that a QTL linkage map of the F2 design in the pig breed can be generated with a selected data set of high density SNP genotypes. The QTL regions detected in this study will provide useful information for identifying genetic factors related to better pork quality in KNP. (Key Words: Illumina Porcine 60K SNP Beadchip, QTL, Meat Quality, Korean Native Pig, Yorkshire Pig)

INTRODUCTION

More than 260 studies on the porcine genome have resulted in the identification of 4,143 meat quality-related quantitative trait loci (QTL) (http://www.animalgenome.org/QTLdb). However, without availability of whole genome sequences, the specific genes or mechanisms involved in the detection of QTL have not been sufficiently well characterized because of the traditional low resolution of a positional cloning approach using microsatellite markers (Sellner et al., 2007). The development of “next-generation” sequencing technologies is helping to make up for this weakness, and make it possible to use stored pig genetic information and high-density SNP analysis, which enable pig QTL to be discovered within a 1 or 2 Mb confidence interval (Fan et al., 2009; Gorbach et al; 2009; Onteru et al., 2009; Naomi et al., 2010).

Because the majority of commercial pig breeds has large differences in genetic composition for selected phenotypes, QTL analysis is performed to identify genetic difference using the line-cross model in which the experimental herd resulting from crossbreeding 2 pig breeds with different phenotypes to generate the first and second generations (Haley et al., 1994b; Alfonso et al., 1998). However, if QTL alleles are not fixed in each of the breeds, a combined line-cross and half-sib model is developed and applied (Kim et al., 2005). Most experimental populations used to detect QTL are generated by crossing 2 breeds with different characteristics, with respect to reproduction, growth or carcass traits, in order to exploit the benefits of

* Corresponding Author : Kwan Suk Kim. Tel: +82-43-261-2547, Fax: +82-43-273-2240, E-mail: kwanskim@chungbuk.ac.kr
heterosis and breed complementarily (Bidanel and Rothschild, 2002). Meat quality traits have been previously studied using crosses between Wild Boar and Large White (Andersson et al., 1998; Nii et al., 2005), Meishan and Yorkshire (Paszek et al., 2001), Meishan and Large White/Landrace (De et al., 2001), Duroc and Landrace/Yorkshire (Grindflek et al., 2001), Berkshire and Yorkshire (Malek et al., 2000), Iberian and Landrace (Ovilo et al., 2002), Pietrain and Meishan and Wild Boar (Geldermann et al., 2003), and Duroc and Berlin Miniature pig (Wimmers et al., 2006).

The Korean native pig (KNP) has a black coat color and a capacity to produce tender meat which is high in muscular fat, but low in cholesterol; and has more unsaturated fat than saturated fat. However, it also has a low birth weight, slow growth, small litter size and a small adult body size. On the other hand, the Yorkshire (YS) pig has a white coat color, general quality level of pork, but a high rate of growth, large adult body size and a large litter size, therefore, the YS pig is used as a maternal line. The genetic factors for better marbling and pork quality of KNP is largely unknown and the amount of molecular genetics information is relatively insufficient.

Therefore, we determined a high-density linkage map of all porcine autosomes by using genotypes of the Illumina Porcine SNP60K Bead chip, and used line-cross model analysis to identify the QTL that may determine meat quality difference between KNP and YS.

MATERIALS AND METHODS

Animals and phenotypes

The KNP×YS population was produced at Chungbuk National University in order to identify the QTL and functional genes for economically important phenotypes (Kim et al., 2010; Li et al., 2010). Meat quality traits included crude ash (C-ash), crude protein (C-pro), intramuscular fat (IMF), drip loss (DL), water-holding capacity (WHC), moisture, cooking loss (CL), shear force, pH at 24 h (pH), color score (Color), marbling score (MAR), tenderness, juiciness, texture, flavor, total cholesterol, lightness (CIEL), redness (CIE-a) and yellowness (CIE-b) (Table 1). These traits were assessed according to standard methods (Oh et al., 2008).

Genotyping

DNA samples (200 ng adjusted to 50 ng/μl) from 335 pigs (F0, 24; F1, 59; and F2, 252) were prepared from tissue according to standard protocols. The genotyping was done by GeneSeek Inc. (Lincoln, NE, USA) using the Porcine

Table 1. Phenotypic records of meat quality traits in Korean native pig and Yorkshire crossed F2 pigs

<table>
<thead>
<tr>
<th>Trait</th>
<th>Abbreviation</th>
<th>N</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Max</th>
<th>Med</th>
<th>Mini</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat quality characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjective Intramuscular fat (%)</td>
<td>IMF</td>
<td>252</td>
<td>2.496</td>
<td>1.458</td>
<td>10.443</td>
<td>2.066</td>
<td>0.492</td>
</tr>
<tr>
<td>Subjective crude protein (%)</td>
<td>C-protein</td>
<td>252</td>
<td>22.188</td>
<td>1.596</td>
<td>28.038</td>
<td>22.368</td>
<td>16.878</td>
</tr>
<tr>
<td>Subjective crude ash (%)</td>
<td>C-ash</td>
<td>252</td>
<td>1.059</td>
<td>0.138</td>
<td>1.628</td>
<td>1.093</td>
<td>0.472</td>
</tr>
<tr>
<td>Subjective moisture (%)</td>
<td>Moisture</td>
<td>252</td>
<td>73.963</td>
<td>1.712</td>
<td>77.890</td>
<td>74.313</td>
<td>65.163</td>
</tr>
<tr>
<td>Subjective water holding capacity (g)</td>
<td>WHC</td>
<td>252</td>
<td>58.037</td>
<td>6.347</td>
<td>85.442</td>
<td>57.100</td>
<td>45.178</td>
</tr>
<tr>
<td>Subjective color (loin) (1-5 score)</td>
<td>Color</td>
<td>252</td>
<td>3.066</td>
<td>0.490</td>
<td>4.500</td>
<td>3.000</td>
<td>1.167</td>
</tr>
<tr>
<td>Subjective marbling (loin) (1-5 score)</td>
<td>MAR</td>
<td>252</td>
<td>2.399</td>
<td>1.016</td>
<td>5.000</td>
<td>2.167</td>
<td>1.000</td>
</tr>
<tr>
<td>24-h loin pH</td>
<td>pH</td>
<td>252</td>
<td>5.640</td>
<td>0.257</td>
<td>6.650</td>
<td>5.597</td>
<td>5.167</td>
</tr>
<tr>
<td>Drip loss (loin), %</td>
<td>DL</td>
<td>252</td>
<td>5.112</td>
<td>1.815</td>
<td>12.056</td>
<td>4.803</td>
<td>1.232</td>
</tr>
<tr>
<td>Cooking loss (loin), %</td>
<td>CL</td>
<td>252</td>
<td>32.261</td>
<td>3.538</td>
<td>43.043</td>
<td>32.223</td>
<td>18.734</td>
</tr>
<tr>
<td>Lightness (CIE L)</td>
<td>CIE-L</td>
<td>252</td>
<td>52.694</td>
<td>5.503</td>
<td>70.960</td>
<td>53.053</td>
<td>33.805</td>
</tr>
<tr>
<td>Redness(CIE a)</td>
<td>CIE-A</td>
<td>252</td>
<td>5.721</td>
<td>2.034</td>
<td>12.147</td>
<td>5.575</td>
<td>1.448</td>
</tr>
<tr>
<td>Yellowness (CIE b)</td>
<td>CIE-B</td>
<td>252</td>
<td>7.432</td>
<td>1.810</td>
<td>14.747</td>
<td>7.310</td>
<td>3.273</td>
</tr>
<tr>
<td>Biochemical measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/100 g)</td>
<td>Chol</td>
<td>252</td>
<td>142.026</td>
<td>84.385</td>
<td>398.900</td>
<td>115.481</td>
<td>26.769</td>
</tr>
<tr>
<td>Textural and sensory characteristics</td>
<td>Texture</td>
<td>252</td>
<td>2.866</td>
<td>0.422</td>
<td>4.000</td>
<td>2.833</td>
<td>1.333</td>
</tr>
<tr>
<td>Shear force</td>
<td>Shearforce</td>
<td>252</td>
<td>1,732.460</td>
<td>434.474</td>
<td>3,452.500</td>
<td>1,677.500</td>
<td>730.000</td>
</tr>
<tr>
<td>Sensory tenderness (1-5 score)</td>
<td>Tenderness</td>
<td>252</td>
<td>3.069</td>
<td>0.723</td>
<td>4.833</td>
<td>3.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Sensory juiciness (1-5 score)</td>
<td>Juiciness</td>
<td>252</td>
<td>3.065</td>
<td>1.344</td>
<td>4.167</td>
<td>3.000</td>
<td>1.500</td>
</tr>
<tr>
<td>Sensory flavor (1-5 score)</td>
<td>Flavor</td>
<td>252</td>
<td>2.944</td>
<td>0.394</td>
<td>4.167</td>
<td>3.000</td>
<td>1.333</td>
</tr>
</tbody>
</table>

Marbling, 1: extremely low in intramuscular fat, 5: very abundant in intramuscular fat.
1: very tough, very dry, very mild. 5: very tender, very juicy, very intense.
60K BeadChip (Illumina, San Deigo, CA, USA) and
approved standard techniques outlined by the manufacturer.
Quality control (QC) was performed after we received
the original SNP genotyping data. SNP clusters for assigning
genotypes were determined for all SNPs.

**Markers selection and linkage mapping**

To select SNPs for linkage mapping, we used
genotyping results based on 46,865 SNPs distributed over
all 18 *Sus scrofa* chromosomes (SCCs) of the total 62,136
SNPs on the chip. The selected SNPs were further excluded
for SNPs that were located on the sex chromosome and
those with call rates of <0.95. We selected SNP markers that
satisfied the following two conditions: i) the SNP marker
allele frequency difference between the F₀ KNP (n = 16)
and the Yorkshire (n = 8) population is >0.700; and ii) the
PIC value of the individual SNP marker in the F₂ (n = 252)
population is >0.300. The flow map of the SNP marker
selection process for linkage mapping is illustrated in
Figure 1.

Linkage maps were constructed using CRI-MAP
version 2.4 (Green et al., 1996) with flips and all options to
obtain the optimum order.

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**Statistical analysis**

We analyzed the QTL data using Haley and Knotts’
(1994a) regression method. The following equation
describes a linear mode:

\[ Y = \text{sex} + \text{sire} + \text{slaughter date} + \text{cov} + c_a + c_d + e \]

Where \( Y \) is the phenotype and sex (2 levels), sire and
slaughter date are the fixed effects. Slaughter age served as
a covariate (cov) from the different traits. The value \( c_a \)
corresponds to the probability \([P(QQ) P(qq)]\) and \( c_d \) is the
probability \([P(Qq)]\), with \( Q \) and \( q \) representing the
KNP×YS alleles, respectively. The additive effect and
dominance deviation are represented by \( a \) and \( d \),
respectively. Genotypes of putative QTL were calculated on
the basis of the marker genotypes at each cM. Residual
error is represented by \( e \). The model was tested to obtain the
regression of F-statistics.

Permutation tests were performed with 10,000 replicates
to determine p values empirically at the chromosomal (CW)
significance level. For QTL detected at 5% CW significance,
the F-value for a genome-wise significance level was then
obtained based on chromosome size, relative to that of the

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![Flow map of SNP marker selection](image-url)
complete chromosome. We used the QTL express program for regression analysis of F2 populations (http://latte.cap.ed.ac.uk/documentation/AnalysisBCF2.html).

RESULTS AND DISCUSSION

High-density SNP chip analysis

In this study, we prepared a high-density linkage map and QTL map of 18 autosomes of the KNP×YS resource population using the Illumina Porcine 60K SNP Chip. Among 46,865 SNPs on the 18 SSCs in the Illumina Porcine SNP60K Bead chip, 500 SNPs were selected for use with the line-cross model to identify QTL in this study (Figure 1). The average distance between the SNP markers was 106.7 Kb and the entire length of the 18 SSCs was 2,424,100,086 bp.

Marker linkage maps

Five hundred SNP markers (average of 27.7 SNPs per chromosome) were used for linkage mapping. The average frequency difference of individual SNPs and the average PIC value between the KNP (n = 16) and YS (n = 8) populations were 0.790 and 0.368, respectively. The average SSC linkage map length was 109.4 cM (107,491 kb). The entire length on the Illumina porcine SNP Chip represented 80.5% of the linkage map. Minimum linkage map intervals were 0.2 cM for the following intervals: DIAS0000655 (44,836 kb) to ASGA0002880 (45,564 kb), SSC1; H3GA0012850 (62,956 kb) to MARC0082820 (64,292 kb), SSC4; and ASGA0032902 (39,610 kb) to ALGA0040677 (39,921 kb), SSC7. In contrast, the maximum linkage map interval between markers was 41.4 cM for the SCC7 SNPs, MARC0033066 (111,822 kb) to M1GA0011405 (131,384 kb). The average distance between all markers was 4 cM (3,887 kb).

QTLs analysis for meat quality traits

Table 2 shows the detected QTL related to meat quality traits. The most important trait that determines the meat quality is IMF; high IMF gives meat a more tender texture and a better flavor (Eilkel enboom et al., 1996; Huff-Lonergan et al., 2002). In this study, the QTL related to the IMF and MAR of the pork loin were found in SSC2 at 54 cM, SSC8 at 0 cM, SSC12 at 74 cM, SSC14 at 79 cM and SSC18 at 11 cM. In particular, the QTL linked with IMF and MAR at a 1% significance level was detected at 74 cM, between the DIAS0003408 (23,353 Kb) and ASGA0055015 (25,707 Kb) markers, within the SSC12 (Table 2). Additionally, the QTL linked with the moisture and meat color of the pork loin were detected in the same region (CIE-a, and CIE-B traits at 74 cM). On SSC12, a texture trait at 62 cM and a tenderness trait at 75 cM also was detected. In the previous study, Edwards et al. (2007) reported that the QTL linked with IMF, moisture and meat color in the Duroc×Pietrain resource population were detected in the region between the S0078 and SW398 (54.2-65.1 cM) markers that contains the same region detected in our study. It was reported that IMF affects the meat moisture and meat color (De Vol et al., 1988; Cameron et al., 1990) but additional studies may be required to determine if the genes regulating the QTL traits are controlled independently or by an interactive biological mechanism.

Other important traits that affect the meat quality of a pig include WHC, meat color, texture, and postmortem pH reduction (Van der Wal et al., 1997). Only one QTL was detected in relation to postmortem pH reduction on SSC6 at 27cM between the ASGA0027741 (3,405 Kb) and ALGA0034911 (5,369 Kb) markers and WHC on SSC14 at 8 cM between the ALGA0075549 (13,584 Kb) and MARC0018903 (13,927 Kb) markers. The pH QTL location was between the SWR1130 and SW1059 (40.2-66.2 cM) markers, where a pH QTL linked was also reported in a study by Malek et al. (2000), indicating that the same region is also the QTL linked with pH in the Berkshire×Yorkshire resource population. It was reported that the overall pH level was increased as the genotypes of YS are converted to those of KNP by the additive gene effect.

In this study, many QTL linked with the meat color traits were detected: 8 QTL (SSC1 at 41 cM; SSC3 at 87 cM; SSC4 at 49 cM; SSC5 at 120 cM; SSC6 84 cM; SSC11 at 29 cM; SSC16 at 50 and 79 cM; SSC18 at 11 cM) at a 5% significance level and 5 QTL (SSC10 at 5 and 23 cM; SSC12 at 71-74 cM) at a 1% significance level. It was shown that the overall meat color traits are improved as the genotypes of YS pigs are converted to those of KNPs by the additive gene effect (Table 2). In general, among the meat color traits, a higher CIE-L value indicates that the meat color is closer to white, and a higher CIE-a value is considered to be an indicator of freshness. Scarlet meat color is preferred by consumers with a lower CIE-b value, which represents the fat color and indicates better quality. Meat color traits are the complex results of many genetic traits other than meat quality traits, but only a few studies have been conducted in this regard (Mancini and Hunt, 2005). It is known that KNP is well matched with the meat color traits than other meat quality traits, but only a few studies have been conducted in this regard (Mancini and Hunt, 2005). It is known that KNP is well matched with the consumer preferences since the meat color is redder than that of other breeds and the fat is solid and white, but nothing is known about the genetic factors. The information regarding the QTL linkage with the meat color traits detected in this study may provide the fundamental research data required to find the genetic factors regulating the color of pig meat (Figure 2).

It was reported that among the meat color traits, CIE-a is positively correlated with C-pro and meat color is proportional to the content of myoglobin, the intramuscular red pigment (Kranen et al., 1999; Choi et al., 2004). It can
Table 2. Quantitative trait loci for meat quality traits that were detected with at least 5% and 1% chromosome-wide evidence for linkage.

<table>
<thead>
<tr>
<th>SSC</th>
<th>Trait</th>
<th>Locus (cM)</th>
<th>Marker (CI, Kb)</th>
<th>SW interval</th>
<th>F-value</th>
<th>LOD</th>
<th>Threshold</th>
<th>Additive</th>
<th>Dominance</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Cie-b</td>
<td>93M</td>
<td>ASGA0037344</td>
<td>SW1213 - SW1240</td>
<td>4.79*</td>
<td>5.97</td>
<td>4.625</td>
<td>1.26</td>
<td>0.43</td>
</tr>
<tr>
<td>5</td>
<td>Cie-a</td>
<td>67M</td>
<td>ASGA0029475</td>
<td>SW1193 - SW1241</td>
<td>3.1*</td>
<td>4.78</td>
<td>2.15</td>
<td>1.09</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>Cie-a</td>
<td>67M</td>
<td>ASGA0029475</td>
<td>SW1193 - SW1241</td>
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<td>1.26</td>
<td>0.43</td>
</tr>
</tbody>
</table>

* p<0.05 for F-statistic threshold. ** p<0.01 for F-statistic threshold.

be assumed that the content of myoglobin is proportional to that of C-pro, resulting in better meat color and quality. A total of 6 QTL linked with C-pro traits were found: 5 QTL (SSC2 at 5 cM; SSC3 at 45 cM; SSC6 at 10 cM; SSC8 at 89 cM; SSC12 at 55 cM) at a 5% significance level and 1 QTL (SSC14 at 96cM) at a 1% significance level (Table 2). The QTL detected within SSC2 and SSC9 were identical to the QTL reported in the previous studies, while the QTL detected within SSC3, SSC6, and SSC12, and SSC14 were different from those in the previous studies (Jiang et al., 2002; Edwards et al., 2008).

The QTL linked to DL and CL was detected in SSC4 at 0 cM, SSC7 at 10 cM, SSC10 at 90 cM and SSC14 at 50 cM. It has been reported that the detected location of the QTL linked to these DL and CL traits differs depending on the breed groups, implying that they may be distributed all over the chromosomes (http://www.animalgenome.org/ QTLdb). This result suggested that the QTL influencing the same trait are difficult to detect in the same region. Such a difference might have been caused by various factors, including the genetic variety of the reference group used in the experiment, statistical analysis models, critical level applied for the statistical analysis and error in the genotype analysis (Dekker et al., 2004).

The QTL related to C-ash was detected on SSC1 at 33 cM and SSC8 at 14 cM, respectively. Tenderness QTL on
Figure 2. F-value curve of meat quality related traits across 18 autosomes (SSC). The horizontal solid and the dashed lines indicates the 5% chromosome-wise and the 5% genome-wise thresholds, respectively.
SSC13 at 103 cM and texture QTL on SSC16 at 8 cM were detected, but no meat-quality-related QTL were detected on SSC15 and 17 where significant QTL were detected in Berkshire and Yorkshire cross (Malek et al., 2000).

General conclusions

Pork quality is a trait with a high economic value for the domestic pig industry. It has been frequently reported that the KNP has excellent meat quality (Choi et al., 2005; Jin et al., 2005; Jin et al., 2006; Cho et al., 2007). Hence, in order to improve KNP as an international, competitive breed with high-quality pork, like the Iberian and Spanish native breed, the genetic properties of the KNP should be investigated for producing differentiated, high-quality pork. In this study, we prepared a high-density linkage map and QTL map of 18 autosomes of the KNP×YS resource population using the Illumina Porcine 60K SNP Chip. We demonstrated that QTL regions can be searched out in small populations using this methodology (Figure 1). Quantitative trait loci can be analyzed using hundreds of SNPs in only the F0 group using Illumina 60K SNP Chip analysis without the F1 and F2 groups. The drawback of using Illumina 60K SNP Chip analysis on many populations is the high cost, but QTL analysis can be carried out at a very low cost if the methods used in this study are implemented. Additionally, the QTL regions related to meat quality traits were detected using the line-cross model and 30 QTL regions at the 5% significance level and 10 QTL regions at the 1% significance level were detected as a result. The results of this study provide fundamental data for the breeding application of KNPs with better meat quality.

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REFERENCES


Green, P., K. Fallis and S. Crooks. 1996. Documentation for CRIMAP version 2.4, Washington University School of Medicine, St. Louis, MO.


with residual feed intake and related traits utilizing the PorcineSNP60 BeadChip. Pig Genome III Conference. November 2-4, Hinxton, UK. Abstract No. 11.


