DETECTION OF QTL AFFECTING MEAT COLOR USING AN F2 POPULATION CONSRUCTED BY INTERCROSS BETWEEN A KOREAN NATIVE PIG AND A LANDRACE PIG

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Abstract— **Meat color provides a strong visual impact to consumers in their decision to purchase meat in the market; consequently, it is one of the most important factors in consumers' evaluation of meat quality. However, detailed genetic factors affecting meat color have not been studied and identified in the literature thus far. Therefore, this study was performed to detect quantitative trait loci (QTL) affecting meat color in pigs using an F2 population constructed by crosses between a Korean native pig (KNP) and a Landrace pig. The results show that a 5% chromosome-wide QTL for meatc_a (redness) and meatc_L (lightness) were detected on SSC 2, 3, and 10. A QTL for lightness was found on SSC14 (Sus scrofa chromosome 14) with 1% chromosome-wide significance. In the QTL, the additive effect was -1.087±0.325, indicating that meat from an F2 pig possessing the Landrace allele showed higher lightness. It was inferred that this difference between the KNP and the Landrace pig is affected by the genes that are located in the region of 9Mb ~ 10Mb on SSC14.**

Index Terms—**meat color, meatc_a(redness), meatc_L(lightness), QTL(quantitative trait loci).**

I. INTRODUCTION

Because meat color is an initial factor in distributors' and consumers' evaluation of meat quality, it has been identified as one of the most important components of the sale of meat. The determinants for meat color are the content and chemical status of pigments, which either reflect or absorb waves of light. In fresh meat, myoglobin plays a major role in controlling meat color. The content of myoglobin differs by species, breed, sex, age, and tissue type. Park et al. (2001) reported that meat color is affected by environmental factors such as stress, age, pH level, nutrient level, temperature, and muscle conditions, including the rate of post-mortem glycolysis, the content of intramuscular fat, the content of myoglobin, and the oxidative state of myoglobin. Therefore, it can be inferred that meat color is a quantitative trait which is affected by several environmental and genetic factors.

Since the 1990's, techniques in the field of molecular biology and genetic engineering have been rapidly developing and have been applied to the field of genome analyses of livestock animals to elucidate genes/genetic loci that affect major economic traits (Andersson et al., 1994; Malek et al., 2001; Rohrer et al., 2005; Stearns et al., 2005; Liu et al., 2008; Huang et al., 2009). This study was performed to detect quantitative triat loci (QTL) affecting meat color in pigs using an F2 population constructed by crosses between a Korean native pig (KNP) and a Landrace pig.

II. MATERIALS AND METHODS

1. Animals and DNA extraction

Totally 421 F_2 pigs were produced by an intercross between Landrace and Korean native pigs. Pigs were slaughtered after 24 hours mooring and whole blood were collected. Isolation of genomic DNA was performed using the Sucrose-Proteinase K method (Birren et al., 1997).

2. Measurement of meat color

After blooming of longissimus dorsi m. for 30 minutes, meat color was measured using a Minolta Chromameter (CR-300, Minolta Co., Japan). Measured items were Lightness (meatc_L), Redness (meatc_a), Yellowness (meatc_b), Chroma (meatc_C), Hue (maetc_H). Chromameter was standarized using a white standared color panel (Y=93.5, X=0.3132, y=0.3198). Measurement was repeated five times for each sample.

3. Selection of MS (microsatellite) marker for linkage mapping

Initially, MS markers being spaced 10 ~ 15 cM on each porcine chromosome were selected using the information from the USDA-MARC swine linkage map (http://www.marc.usda.gov/genome/swine/swine.html). Allele distribution and informativeness of pre-selected MS markers were checked by genotyping F_0 and F_1 pigs using a conventional polyacrylamide gel electrophoresis. Finally selected MS markers were labeled with fluorescent dye (FAM or HEX or NED) at the 5'-end of forward primer and used for PCR (polymerase chain reaction) amplification of whole F_0, F_1 and F_2. Each PCR product was resolved on an ABI-3130*xl* (PE Applied Biosystems, USA), and was genotyped by its size.

4. PCR

PCR of 7.5 ㎕ composed of 50 ng of template DNA, 5 pmoles of each forward and reverse primer, 5 nuits of *Taq* DNA polymerase (GENET BIO, Korea), 1x buffer, 0.25mM dNTPs was prepared. Thermal condition was pre-denaturation at 95℃ for 5 minutes and 30 cycles of denaturation at 94℃ for 1 minute, annealing at each annealing temperature (data not shown) for 1 minute and extension at 72℃ for 1 minute. Final extension was at 72℃ for 5 minutes and then it was stored at 8℃.

5. Genotyping of MS marker

Three to six MS markers of which expected PCR product sizes didn't overlap or labeled dye was different were combined in a run set. One ㎕ of combined run set was diluted by adding 15 ㎕ of de-ionized water. Diluted run set : Formamide : Size-standard (GeneScanTM-350 ROX) was mixed as 1 ㎕: 15 ㎕: 0.15 ㎕ and then denatured at 95℃ for 3 minutes. Electrophoresis was performed on an ABI-3130*xl* and each PCR product was resolved by the size and color. GeneMapper version 4.0 (PE Applied Biosystems, USA) was used for genotyping and Microsoft Excel (Microsoft, USA) was used for data processing.

6. Linkage and QTL mapping

Linkage map was constructed using the CRI-MAP software version 2.4 (Green et al., 1990) by estimating recombination rate between MS markers within a chromosome. The build option of the CRI-MAP program was applied for estimating a best order of MS makers. QTL analysis by the least square interval mapping which detect QTL on every cM position was performed using the QTL express software (http://qtl.cap.ed.ac.uk/) under the F_2 analysis platform (Haley et al., 1994). In order to determine 1% chromosome-wide significant level, 5,000 iterations of the permutation test was applied. SNP markers were selected from the Genebank database and genotypes using the PyroMark MD (Biotage, Sweden). The SNP genotypes were combined into linkage and QTL map to estimate physical location of QTL peak and to select positional candidate genes.

III. RESULTS AND DISCUSSION

General statistics for meat color measurements are shown in Table 1. The average value of meatc_a was estimated to be 111.827, while that of meatc-L was 8.606 among 421 heads of F2 pigs. Higher lightness and redness are generally good signs for meat quality. In terms of fat color, higher brightness and lower yellowness is known to be better.

As a result of the QTL analyses for the 5 items related to meat color, significant QTLs at the 5% chromosome-wide level were identified on SSC 2, 3, 10, and 14 under the additive + dominant model. When only the additive model was applied, a QTL for meatc_L on SSC14 was significant at the 1% chromosome-wide level, which is located at the region of SW2038 ~ SW857 (Table 3). QTLs related to meat color in pigs have been found on all chromosomes except for SSC9, 16, 18, and Y. Particularly on SSC14, QTLs for meat color at 0 cM (Malek et al., 2001), at 41 cM, SW295 ~ SW210, (de Koning et al., 2001), at 24.2 cM, SW857 ~ SWC6 (Thomsen et al., 2004), and at 76 cM, SW1081 ~ SW1557, (Rohrer et al., 2005) have been reported. Among the QTLs, the location of the QTL by Thomsen et al. (2004) was similar to that of our QTL on SSC14.

A linkage map for the QTL analysis uses a unit of centimorgan (cM) which is estimated from recombination rates between markers; however, an exact estimation of the physical location (base pair, bp) of the marker/QTL is not possible with the linkage map. Therefore, we combined the SNPs for which physical locations were known with the MS markers to detect the physical location of the QTL peak on SSC 14 for meatc_L, which showed a 1% chromosome-wide significance. A combined linkage and QTL map was re-estimated and used to approximate the physical location of the QTL peak. After the addition of the SNP marker near QTL peak, the F-ratio (test statistics) was increased, which indicated that the selected SNP marker is close to the trait gene for the QTL or is on the same haploblock containing the trait gene. The physical location of the QTL peak elucidated in this study could be useful in developing DNA markers in order to narrow down the candidate region and to select positional/functional candidate genes for meat color. In a future study, positional cloning will be performed to elucidate the major trait gene for meat color.

IV. CONCLUSION

QTL analyses for meat color were performed using an F2 population from crosses between a KNP and a Landrace pig (Fig. 1). Results show that 5% chromosome-wide QTLs (additive + dominat) on SSC2, 3, 10, and 14 were detected. Using the additive model, a 1% chromosome-wide QTL on SSC14 for meatc_L was found. Using the combined analysis of MS and SNP, it was revealed that the QTL peak for meatc_L was located at 9Mb ~ 10Mb, and 14 genes were identified in the estimated physical region (Fig. 2).

ACKNOWLEDGEMENT

This work was supported by grant no. 20080401034053 and 20070401034029 from the BioGreen 21 Program, Rural Development Administration, Korea. G. J. Lee, C. K. Yoo and E. J. Jung were supported by scholarships supported by a grant from BK 21 Program, the Ministry of Education, Science and Technology.

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Table 1. Sample Sample size, mean, minimum, maximum, standard error, and standard deviation values for the measured phenotypes in the F2 animals from an intercross of a Korean native pig and a Landrace pig

Traits	n	mean	min	max	Standard error	standard deviation
meatc_L	421	8.606	0.86	27.92	0.003	2.50
meatc_a	421	111.827	36.41	64.30	0.005	4.37
meatc_b	421	19.283	2.92	28.34	0.003	2.64
meatc_C	421	9.217	-0.72	55.78	0.005	4.39
meatc_H	421	23.519	2.98	286.14	0.014	13.15

Table 2. . SNPs were selected from the Genebank database to estimate the physical location of the QTL peak on SSC14.

SNP	Accession Number	Position(Mb)	Symbol
ALGA0074155	NW_001885375.1	0.389	SNP-1
ALGA0074714	NW_001885386.1	6.749	SNP-2
ALGA0075053	NW_001885394.1	9.799	SNP-3
H3GA0038865	NW_001885395.1	10.586	SNP-4
ALGA0075247	NW_001885397.1	11.142	SNP-5
MARC0018903	NW_001885401.1	13.898	SNP-6
ALGA0075684	NW_001885407.1	15.035	SNP-7
ASGA0063134	NW_001885453.1	45.021	SNP-8
ALGA0077953	NW_001885469.1	59.355	SNP-9
DRGA0014729	NW_001885548.1	136.491	SNP-10

Table 3. Summary of experiment-wide QTL, F-value, and effects for meat color

SSC	Trait	Model[1)]	Position	*F*-value[2)]	a ± SE[3)]	d ± SE[4)]
2	meatc_C	a+d	26 cM	5.72 *	-0.287±0.208	0.980±0.316
3	meatc_L	a+d	141 cM	5.82 *	-0.315±0.301	1.524±0.465
10	meatc_C	a+d	94 cM	5.87 *	-0.534±0.187	-0.487±0.292
14	meatc_L	a	21 cM	11.18 **	-1.087±0.325	

[1)] The QTL model with additive (a), additive and dominant (a+d) effects. [2)] * significant at the 5% chromosome-wide levels; ** significant at the 1% chromosome-wide levels. [3)] Additive effect and standard error. A positive value mean the Korean native pig allele has a positive additive effect, and a negative value indicate that the Landrace allele has a positive effect. [4)] Dominant effect and standard error.

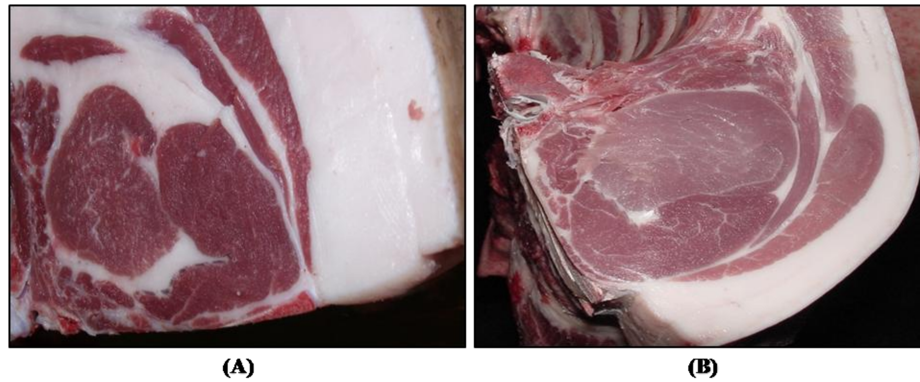

Fig. 1. Meat color of longissimus dorsi m. from KNP (A) and Landrace (B).

Fig. 2. F-ratio test statistics for meatc_L on SSC14. The horizontal line indicates the 1% chromosome-wide significant thresholds. Positions of MS markers in the linkage map are indicated on the x-axis. Below the QTL map, the genomic location of the QTL is marked, and genes located near QTL peak are depicted.