DETECTION OF QTL ASSOCIATED WITH POST-MORTEM MUSCLE PH USING A THREE-GENERATION POPULATION PRODUCED BY CROSSES BETWEEN LANDRACE AND KOREAN NATIVE PIGS

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***Abstract*— Post-mortem muscle pH is an important factor in determining meat quality due to its close relationships with water-holding capacity, meat color, and the proliferation of microorganisms. It has also been known to be a determinant of PSE, or acid meat. It is affected by muscle fiber type, storage temperature, and types of catalytic enzymes. However, very little has been reported in the literature regarding genetic factors for muscle pH. Therefore, this study aimed to develop QTL (quantitative trait loci) related to muscle pH and pH changes after slaughter in order to find a QTL peak for the chromosome of interest and to suggest positional candidate genes using an F2 population produced by Landrace x Korean native pigs. On SSC3 (Sus scrofa chromosome 3), a 1% chromosome-wide (P<0.01) QTL for muscle pH change between 1 hour and 24 hours (△pH1-24h) after slaughter was detected. On SSC4, another 1% chromosome-wide QTL for muscle pH at 3 hours (pH3h) after slaughter was identified. The physical positions of the pH3h QTL peaks were inferred to be located at 126 ~ 128 Mb on SSC4. Within the physical regions, ten positional candidate genes for pH3h were estimated.**

***Index Terms*—Korean native pig, muscle pH, positional candidate gene, quantitative traits loci**

I. INTRODUCTION

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 locations that affect major economic traits. In particular, the location of genes that affect quantitative traits that are economically important have been identified and labeled "QTL" (Quantitative traits loci). QTL detection is generally accomplished through linkage mapping, QTL mapping. and candidate gene analysis (Rothschild, 1997). During the last 10 years, 5,732 QTL for 558 traits have been reported in pigs (pigQTLdb, http://www.animalgenome.org/cgi-bin/QTLdb/SS/index). Reported QTL can be classified into five catalogues: exterior feature, health, meat quality, production, and reproduction. Among the five groups, meat quality has the largest number of reported QTL, which is about 70% of all QTL reported. QTL related to muscle pH and pH decline after slaughter were found on all porcine chromosomes except for the Y chromosome.

Muscle pH and pH change after slaughter are closely related to water-holding capacity, meat color, texture, and the proliferation of microbes. They also act as a determinant for meat freshness, quality, and the extent of muscle proteins' denaturation. The speed of glycolysis of meat-producing animals is different among species and individuals due to different compositions of muscle fiber types. As a consequence, alternative patterns of pH decline and body temperature after slaughter were shown. That is, metabolism in muscle and pH changes are linked, but their relationship is complicated, as there are several enzymes and control factors involved. Muscle pH after slaughter has been reported as a multi-factorial trait which shows 0.07 ~ 0.41 of heritability (Sellier, 1994).

The two genes identified so far which appear to be related to muscle pH are *RYR*1 and *PRAKG*3. The R615C mutation on *RYR*1 leads to PSE meat, which shows extremely low post-mortem pH at 45 minutes (Fujii et al., 1991), and the R200Q mutation *PRAKG*3 results in acid meat in the Hampshire breed (Milan et al., 2000). However, it has been reported that either no association or an opposite relationship between the mutations and PSE/acid meat exists, thus indicating the presence of an environmental effect or another genetic factor. Consequently, genetic factors and evidence related to muscle pH have been less frequently identified. Thus, this study was conducted with the aim of suggesting positional/functional candidate genes that affect the levels and changes of post-mortem muscle pH through QTL mapping in a pedigreed pig population.

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 slaughter 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 post-mortem pH

To measure muscle pH at 1, 3, 6 and 24 hours after slaughter, longissimus dorsi m. was biopsied every hour. Taken sample were frozen into liquid nitrogen, moved to the lab. About three grams of sample were homogenized with 27 ㎖

of de-ionized water using a homogenizer (IKA T25basic, MALAYSIA) at 13,500rpm for 10 seconds. Then pH were measure using a pH-meter (MP230, METTLER-TOLEDO, SWITZERLAND). Statistics for muscle pH measured at 1, 3, 6 and 24 hours were listed in Table 1.

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 on the USDA-MARC swine linkage map (http://www.marc.usda.gov/genome/swine/swine.html). Allele distribution and informativeness were checked and estimated by genotyping them for 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, 1,000 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 (Table 2).

III. RESULTS AND DISCUSSION

Based on the results of the QTL analyses, which considered the additive effect (a) and the additive + dominant effect (a + d), 5% chromosome-wide significant QTLs for post-mortem muscle pH were identified on SSC3, 4, 5, and 6 (Table 3). Among them, the QTL on SSC4 was also significant for muscle pH at 3 hours (pH3h) after slaughter at a 5% genome-wide level. The 5% chromosome-wide QTL for the decline of muscle pH was found on SSC3 and SSC6. Between them, the QTL on SSC3 was significant for both the pH change between 1 hour and 24 hours after slaughter (△pH1-24h) at a 1% chromosome-wide level (Fig. 1A). The highly significant QTL on SSC3 for △pH1-24h and on SSC4 for pH3h were estimated to be located between SW1327 ~ SW2532 (q-arm) and SW445 ~ MP77 (q-arm), respectively. On SSC3, 15 QTL associated with muscle pH have been reported so far; their locations were between SW833 ~ SW1525 (p-arm). Our QTL on SSC3 for △pH1-24h was somewhat far from the other QTL reported on SSC3; therefore, we consider our QTL to be a novel one for muscle pH and have determined that the Q allele is from the Korean native pigs, and the q allele is from the Landrace pigs. Twelve QTLs for muscle pH that were scattered all over SSC4 have been reported so far. A QTL-related PSE (pale, soft, exudative) and a few QTLs for lightness have been reported near our QTL for pH3h on SSC4. This indicates that our QTL would be the same as or have some interaction with the previously-reported QTL.

A linkage map for the QTL analysis uses a unit of centimorgan (cM) that is estimated from the recombination rates between markers; however, an exact estimation of the physical location (base pair, bp) of marker/QTL is not possible with the linkage map. Therefore, we combined the SNPs for which physical locations were known with the MS markers. 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 the QTL peak, the F-ratio (test statistics) was increased, indicating that the selected SNP marker is close to a trait gene for the QTL or is on the same haploblock containing the trait gene.

IV. CONCLUSION

QTL mapping for post-mortem muscle pH was performed through MS genotyping, linkage, and association analysis. We found a QTL on SSC4 for pH3h at a 5% genome-wide level. An additional QTL analysis, which combined SNP

markers, revealed that the QTL on SSC4 were located at 126 ~ 128Mb. Ten positional candidate genes were searched near the QTL on SSC4 (Table 4 and Fig. 1B).

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Table 1. Sample size, mean, minimum, maximum, standard error and standard deviation values for the measured phenotypes in the F_2 animals from an intercross of a Korean native pig and Landrace

Traits	N	Mean	Min	Max	Standard error	Standard deviation
pH1h	421	5.890	5.36	6.71	0.012	0.251
pH3h	421	5.884	5.27	6.96	0.014	0.292
pH6h	421	5.809	5.27	6.97	0.015	0.311
pH24h	421	5.641	5.20	6.84	0.012	0.242

Table 2. SNPs were selected from the Genebank database to estimate the physical location of the QTL peak on SSC4

SNP	Accession Number	Position (Mb)	Symbol
H3GA0012096	NW_001886167.1	14.367	4-SNP1
ASGA0019976	NW_001886219.1	67.935	4-SNP2
ASGA0021815	NW_001886259.1	111.458	4-SNP3
MARC0027882	NW_001886276.1	124.051	4-SNP4
M1GA0006616	NW_001886280.1	127.334	4-SNP5

Table 3. Summary of genome-wide significant QTL, F-value and effects for pH traits

SSC	Trait	Model[1)]	Position	*F*-value[2)]	a ± SE[3)]	d ± SE[4)]
3	pH1h	a	97 cM	11.04 *,¤	-0.060 ± 0.018	
		a+d	92 cM	7.05 *	-0.070 ± 0.019	-0.060 ± 0.033
	△pH1-6h	a+d	97 cM	5.50 *	-0.048 ± 0.020	-0.081 ± 0.314
	△pH1-24h	a	148 cM	12.93 **	-0.071 ± 0.020	
		a+d	98 cM	9.45 **	-0.068 ± 0.018	-0.085 ± 0.030
	△pH3-6h	a+d	107 cM	6.44 *	-0.046 ± 0.015	-0.057 ± 0.026
	△pH3-24h	a+d	109 cM	6.07 *	-0.066 ± 0.021	-0.069 ± 0.038
4	pH3h	a	128 cM	15.75 **,¤	-0.085 ± 0.021	
		a+d	128 cM	8.06 **	-0.084 ± 0.021	0.020 ± 0.034
	pH6h	a	128 cM	10.07 *,¤	-0.072 ± 0.023	
5	pH3h	a	119 cM	10.26 *, ¤	-0.066 ± 0.021	
6	pH3h	a+d	91 cM	7.34 *	0.059 ± 0.020	0.690 ± 0.029
	△pH3-24h	a	89 cM	10.12 *, ¤	0.059 ± 0.019	
		a+d	88 cM	7.16 *	0.058 ± 0.018	0.057 ± 0.028

[1)] The QTL model with additive (a) and additive and dominant (a+d) effects. [2)] ¤ significant at the 5% genome-wide level; ¤¤ significant at the 1% genome-wide level. [3)] Additive effect and standard error. A positive value means that the Jeju native pig allele has a positive additive effect, and a negative value indicates that the Landrace allele has a positive effect. [4)] Dominant effect and standard error.

Table 4. Summary of genome-wide significant QTL of analysis by combining SNP markers, F-values and effects of pH3h on SSC4

SSC	Trait	Model[1)]	Position	*F*-value[2)]	a ± SE[3)]	d ± SE[4)]
4	pH3h	a	155 cM	18.97 ▫▫	-0.101 ± 0.023	
		a+d	155 cM	9.47 ▫	-0.101 ± 0.023	0.005 ± 0.040

[1)] The QTL model with additive (a) and additive and dominant (a+d) effects. [2)] ▫ significant at the 5% genome-wide level; ▫▫ significant at the 1% genome-wide level. [3)] Additive effect and standard error. A positive value means that the Jeju native pig allele has a positive additive effect, and a negative value indicates that the Landrace allele has a positive effect. [4)] Dominant effect and standard error.

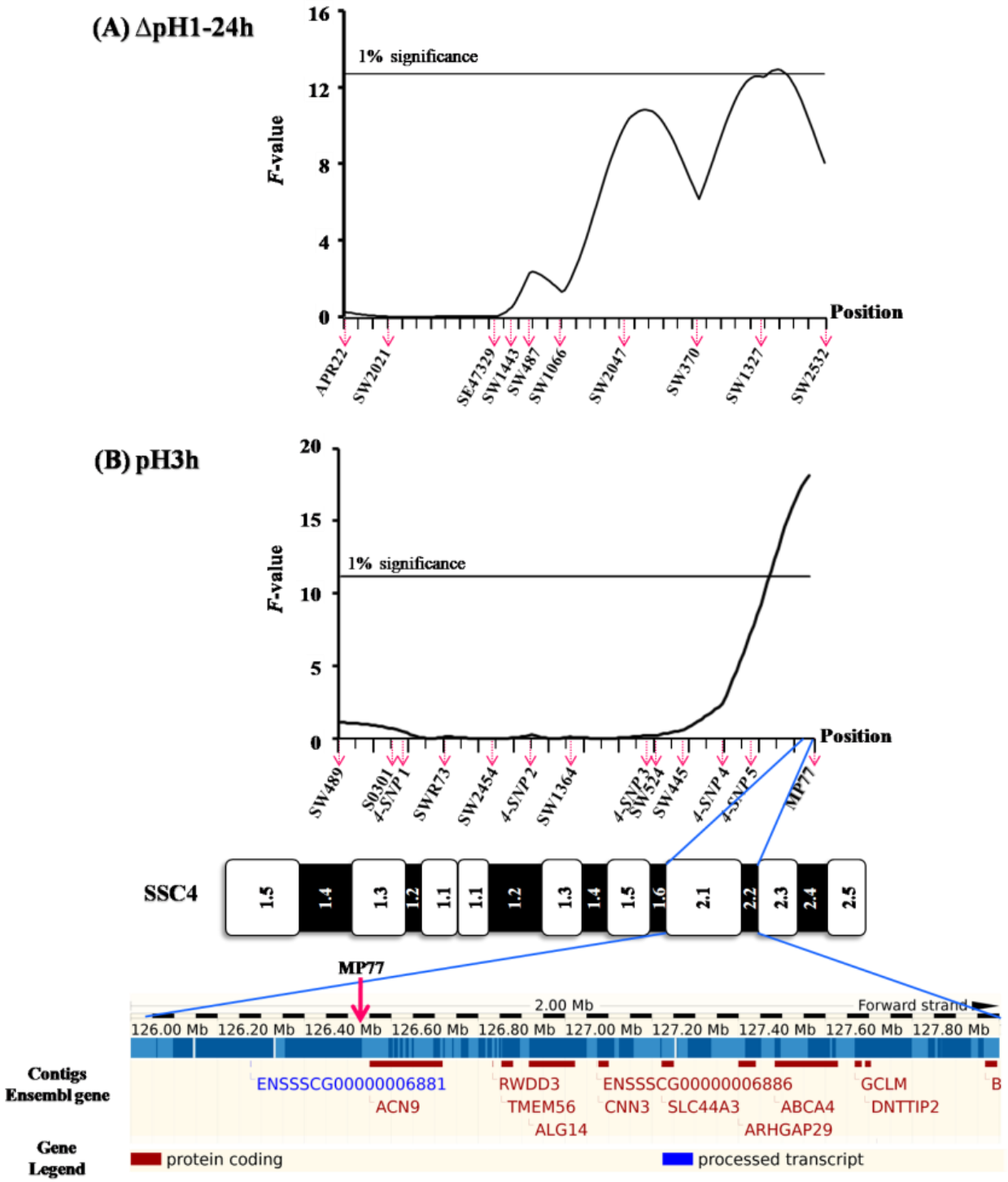

Fig. 1. F-ratio test statistics for (A)△ph3-24h on SSC3 and (B) ph3h on SSC4. The statistical model is A. The horizontal line indicates the 1% chromosome-wide significant thresholds. Positions of MS markers in the linkage map are indicated on the x-axis. Below the QTL map (B), the genomic location of the QTL is marked, and genes located near the QTL peak are depicted.