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Association of Three Polymorphisms in Porcine *Ribosomal protein L27a* (*RPL27A*) Gene with Meat-quality Traits

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ABSTRACT

We identified molecular markers associated with meat-quality traits in the porcine *RPL27A* (*ribosomal protein L27a*) gene. Three single nucleotide polymorphisms (SNPs) were discovered in the porcine *RPL27A* gene: *g.920T>C, g.1013T>C, and g.1046T>C*. The g.920 T>C SNP was significantly associated with pH24 ($P < 0.05$) and collagen ($P < 0.05$), while the *g.1013T>C* and *g.1046T>C* SNPs were significantly associated with moisture ($P < 0.05$). Either the TTT or CCC haplotype was significantly associated with moisture, pH24 and collagen ($P < 0.05$, respectively). The genotypes of *RPL27A* associated with meat-quality traits were all located in intron 2. The three SNPs of the *RPL27A* found in this study will provide useful information for genetic characterization or association studies of meat-quality traits in other populations. Additionally, these markers could potentially be applied in pig breeding programs to improve meat-quality traits after validation in other populations.
(**Key words :** Meat-quality, *Ribosomal protein L27a*, Single nucleotide polymorphism)

INTRODUCTION

Meat-quality, which is one of the most important economic traits in farm animals, is controlled by multiple genes as quantitative trait loci. Genetic markers have been applied to improve meat-quality in pigs via marker-assisted selection (Markljung et al. 2008; Li et al. 2010; Fan et al. 2010). Meat-quality is affected by many factors such as genetic effects, muscle characteristics, and production and environmental conditions. Understanding fat formation and the metabolic pathways involved in this process is important to meat production and quality in farm animals. In pork, back-fat thickness and intramuscular fat content (IMF) are known to be major factors affecting the sensory meat-quality that are highly considered as selection criteria in commercial markets (Gerbens et al., 2001; Cho et al., 2011). The *ribosomal protein L27a* (*RPL27A*) gene has recently been identified as a positional and functional candidate gene responsible for marbling in Japanese Black beef cattle. (Yamada et al. 2009; Watanabe et al. 2011). In pigs, the RPL27A gene is located in SSC 9 (http://asia.ensembl.org, ENSSSCT00000015919). Ribosomes, the organelles that catalyze protein synthesis, consist of a small 40S subunit and a large 60S subunit. Together, these subunits are composed of four RNA species and approximately 80 structurally distinct proteins. The *RPL27A* gene encodes a ribosomal protein that is a component of the 60S subunit. However, porcine *RPL27A* has not yet been examined for structural variation or its regulatory functions. Therefore, in this study, genotyping and association analyses of single nucleotide polymorphisms (SNPs) in the *RPL27A* gene were carried out in a Berkshire pig population to identify genetic markers associated with meat-quality traits and explore the possible genetic relationship between these traits.

MATERIALS AND METHODS

The study protocol and standard operating procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science (Suwon, Republic of Korea).

1. Animals and trait measurement

This association study examined 666 Berkshire pigs (325 castrated males and 341 females). These pigs were fed the

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same commercial diet at the same pig farm and slaughtered at an average body weight of 110 kg. Slaughter was conducted according to standard procedures under the supervision of a Korean grading service for animal products. Following slaughter, the hot carcass weight was recorded and the back-fat thickness was measured between the 10^{th} and 11^{th} ribs. Meat-quality traits were then evaluated from the longissimus dorsi muscle. Nine items were measured as meat characteristics, meat pH, water-holding capacity (WHC), drip loss, cooking loss, meat color, muscle shear force, moisture, IMF, and crude protein. Meat pH was measured 24 hours after slaughter. The water-holding capacity of longissimus dorsi immediately sampled after slaughter was determined using the filter-paper method described by Grau and Hamm (1952, 1956). Additionally, drip loss during vacuum storage was determined 1 day postmortem by weighing samples before and after storage. Cooking loss was measured as the difference between sample weights before and after incubation at 75°C for 10 min. Meat color was measured using three coordinates from the Hunter **L, a, b** system, where **L** is a general indication of lightness, **a** represents the degree of green- redness, and **b** represents the degree of blue-yellowness. Shear force was determined using a Warner-Bratzler shear force meter (G-R Electrical, USA). The moisture, fat content, and crude protein were analyzed according to the American Organization of Analytical Chemists methodology (Arlington, 1980). The overall means and standard deviations of the 14 traits are shown in Table 1.

Table 1. Means, standard deviations (SD), and ranges of the traits measured in 666 pigs

Trait	Mean	SD	Min	Max
CWT (kg)	85.98	5.51	71	105
pH24	5.77	0.19	5.37	6.72
WHC (%)	58.40	3.38	50.13	67.82
Drip loss (%)	4.43	1.91	12.30	14.38
Cooking loss (%)	26.51	4.16	12.30	39.02
MC_L	48.49	2.75	38.00	57.68
MC_a	6.26	1.04	3.40	9.62
MC_b	3.14	1.21	0.33	6.85
BF (mm)	25.10	5.20	12	41
SF (kg/0.5 inch2)	3.08	0.80	1.45	6.14
Moisture (%)	75.17	1.12	69.98	77.57
Fat (%)	2.67	1.18	0.42	10.15
Protein (%)	23.76	0.88	20.95	26.24
Collagen (%)	0.89	0.13	0.53	1.39

SD, standard deviation; CWT, carcass weight; WHC, water-holding capacity; MC_L, CIE_lightness; MC_a, CIE_redness; MC_b, CIE_yellowness; BF, back fat; SF, shear force.

2. SNP detection and genotyping

Genomic DNA was extracted from EDTA-treated blood samples using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The primers used to amplify the porcine *RPL27A* gene were designed from published genomic DNA sequences (http://asia.ensembl.org, ENSSSCT00000015919). The porcine *RPL27A* gene was amplified from 96 genomic DNA samples of Berkshire pigs and then sequenced to detect polymorphic sites. PCR was performed in a 20 μL reaction mixture containing 10 pmol each primer, 0.25 mM each dNTP, 2 μL 10X PCR buffer, 1.25 U DNA polymerase (GenetBio, Chungnam, Korea), and 100 ng genomic DNA. The thermal cycling conditions consisted of initial denaturation for 5 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 62°C, and 1 min at 72°C, with a final 10-min extension at 72°C. Sequencing was conducted using a DNA Engine Tetrad® 2 Thermal Cycler (Bio-Rad, Hercules, CA, USA). To identify differences in the nucleotide sequences, direct sequencing of the PCR products was performed using a Big Dye Terminator Cycle Sequencing Ready Reaction Kit V3.0 (Life Technologies Corp., Carlsbad, CA, USA) and an ABI PRISM® 3730 Genetic Analyzer (Life Technologies Corp.). The sequences were compared to find SNPs using the SeqMan program (DNASTAR Inc., Madison, WI, USA).

Genotypes of *RPL27A* genes produced by direct sequencing of the PCR product from 666 Berkshire pigs were used for the association study. The primers used for direct sequencing were 5'-GCATAGCCAGTCACCAGGTT-3' and 5'-CAGCATTTATCCGTGTCTGC-3' (nucleotide positions relative to the transcription initiation site of the *RPL27A* gene were 788 to 807 and 1167 to 1187, respectively). Haplotype analyses were performed with SNPs identified when genotyping the *RPL27A* gene. Haplotypes were reconstructed from unphased genotypic sequences by the PHASE v2.1.1 algorithm with linkage disequilibrium information (Stephens and Donnelly, 2003; Stephens et al., 2001).

3. Statistical analysis

Association analysis was performed using SAS 9.13 (SAS

Institute Inc., Cary, NC, USA). The following formula was used in a generalized linear model (GLM) analysis: $y_{ijklmn} = \mu + G_i + S_j + P_k + e_{ijkl}$, where y_{ijklmn} is the observed value, μ is the general mean, G*i* is the fixed effect of genotype *i*, S*j* is the fixed effect of sex *j*, P*k* is the fixed effect of the period of slaughter *k*, and e_{ijkl} is the random error. The results were presented as the least squares means for each group and standard errors (SEs) of the least squares means. Genotype, sex, and period of slaughter were included as fixed effects in the statistical model. Differences were considered significant at $p < 0.05$. All data were expressed as the mean ± SE. Haplotypes were computed for each animal using the PHASE v2.1.1 computer program. The association analyses between the copy numbers of certain haplotypes and traits were implemented using a mixed model procedure of SAS as mentioned above.

RESULTS AND DISCUSSION

1. SNP genotype and haplotype frequencies

The porcine *RPL27A* was amplified by PCR and directly sequenced to identify genetic variation in the Berkshire pig population. In our preliminary sequence analysis, which detected polymorphic sites on the genomic region of porcine *RPL27A* gene from 96 samples, three SNPs were found at *g.920T>C, g.1013T>C* and *g.1046A>T* in intron 2 of *RPL27A* (Fig. 1). To estimate the genotypic and allelic frequencies of the three SNPs and haplotypes in the porcine *RPL27A*, 666 Berkshire pigs were genotyped. The allele and genotype frequencies for individual SNPs and haplotypes in the Berkshire pigs are listed in Table 2 and 3. The genotype distribution of each of the three SNPs in the Berkshire pigs analyzed in this study conformed to Hardy-Weinberg equilibrium. At the *g.920T>C* SNP there was a higher frequency of allele T than allele C, while the TT genotype was found in the highest frequency in Berkshire pigs. The *g.1013T>C* and *g.1046T>C* SNPs showed a slightly higher frequency of T allele than C allele, and the frequency of TC genotype was highest in Berkshire pigs. Overall, the estimated frequencies of haplotypes TTT, TCC, and CCC were 0.531807, 0.171646, and 0.293816, respectively. No structural variation in the porcine *RPL27A* has been reported, and no association study of this region was conducted.

2. Association study

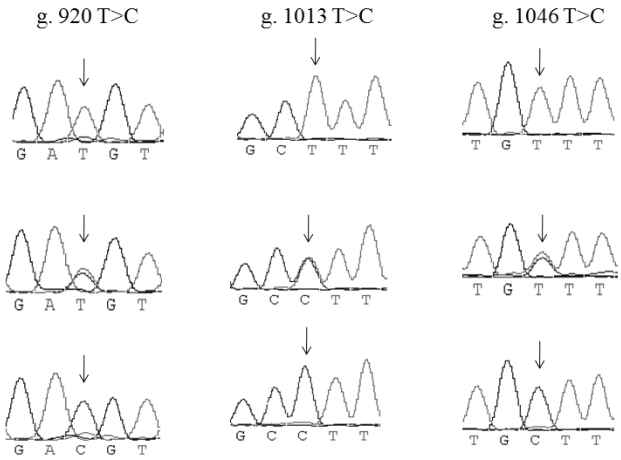

Fig. 1. Sequencing results and polymorphic sites in the 2 intron region of the porcine *RPL27A* gene in Berkshire pig. Adenine of the start codon ATG is counted as +1 (GenBank accession No. XM_003129379).

An association study of the porcine *RPL27A* SNPs with meat-quality traits was performed in 666 Berkshire pigs (Table 1). As shown in Table 2, all three SNPs were significantly associated with meat-quality traits. The g.920 T>C SNP was significantly associated with pH24 and collagen ($p = 0.0318$ and 0.017, respectively), while the *g.1013T>C* and *g.1046T>C* SNP was significantly associated with moisture ($p = 0.0391$) (Table 2). As shown in Table 3, the TTT haplotype was significantly associated with moisture ($p = 0.0348$), while the CCC haplotype was associated with pH24 and collagen ($p = 0.0319$ and 0.0190, respectively Table 3). Consumer assessment of meat-quality is defined by the characteristics of sensory experience such as tenderness, juiciness, flavor, and texture, which are assessed by pH, color, shear force, IMF, moisture content, protein content, and sensory analysis (Reardon et al., 2010). In particular, the pH of pork is correlated with quality traits such ascolor, drip loss, and water-holding capacity. A higher level of acidity within the muscle (lower pH) causes muscle protein to denature and lose its ability to hold water (Yang et al. 2010). The pH of pork is also correlated with glycogen content and glycolysis in postmortem muscle. Fast-twitch fibers mainly carry out the glycolytic pathway, and their metabolism contributes to a rapid metabolic rate in the early postmortem period (Ryu and Kim 2006). Thus, the percentage of type IIB fiber is negatively related to muscle pH and positively related to adenosine/inosine ratio (R-value). These findings support that pigs of the TT genotype in *g.920T>C* may

Table 2. Associations between SNPs of porcine *RPL27A* and meat-quality traits in Berkshire pigs

SNP position	Genotype frequency (n = 666)			Allele frequency		Traits	Genotype TT (n = 327)	Genotype TC (n = 283)	Genotype CC (n = 56)	P-value
g.920T>C	TT (327, 0.49)	TC (283, 0.42)	CC (56, 0.09)	T (0.70)	C (0.30)	pH24	5.74 ± 0.02[1)]	5.70 ± 0.02	5.72 ± 0.03	0.0318*
						Collagen	0.91 ± 0.01	0.89 ± 0.01	0.86 ± 0.02	0.0170*
g.1013T>C / *g.1046 T>C*	TT (191, 0.29)	TC (330, 0.49)	CC (145, 0.22)	T (0.53)	C (0.47)		TT (n = 191)	TC (n = 330)	CC (n = 145)	
						Moisture	75.73 ± 0.11	75.54 ± 0.10	75.49 ± 0.11	0.0391*

The number of genotyped animals and genotype frequency are shown in parentheses. Polymorphisms in the 2 intron region are numbered relative to the translation start site; Adenine of the start codon ATG is counted as +1 (GenBank accession No. XM_003).
[1)] Values are expressed as the least squares means and standard errors, *; $P < 0.05$

Table 3. Associations of haplotypes of three SNPs of porcine *RPL27A* gene with meat-quality traits in the Berkshire pig

Haplotype	Frequency	Traits	LSM (SE) of copies of 1 -TTT- 0 (n = 145)	1 (n = 333)	2 (n = 188)	P-value
TTT	0.531807	Moisture	75.50 ± 0.11	75.54 ± 0.10	75.74 ± 0.11	0.0348*
			LSM (SE) of copies of 2 TCC			NS
TCC	0.171646		NS			
			LSM (SE) of copies of 3 -CCC-			
			0 (n = 330)	1 (n = 280)	2 (n = 56)	
CCC	0.293816	pH24	5.74 ± 0.02	5.70 ± 0.02	5.72 ± 0.03	0.0319*
		Collagen	0.91 ± 0.01	0.89 ± 0.01	0.86 ± 0.02	0.0190*

Haplotypes with frequencies less than 1% were excluded from analyses.
*, $P < 0.05$.

produce better quality meat than those with other genotypes (Table 2). This study was conducted to investigate the possibility of using SNPs of porcine *RPL27A* gene as molecular markers for the improvement of meat-quality with good pork production. In this study, three novel SNPs (*g.920T>C, g.1013T>C*, and *g.1046T>C*) within intron 2 of the *RPL27A* gene were detected. Three SNPs and two haplotypes were all found to be significantly associated with pH24, collagen content and moisture. The potential gain of marker-assisted selection would be in terms of reduced costs for sib testing after slaughter and reduction in sophisticated meat-quality measurements, as well as additional improvement of meat-quality by early information from genetic markers (Ovilo et al. 2006). Accordingly, the results of this study indicate that SNPs or haplotypes of the porcine *RPL27A* gene are potential markers for assisted selection of Berkshire pigs. However, further studies to confirm the results presented herein are needed prior to their application for the selection of pigs.

CONCLUSION

The purpose of this study was to identify a molecular marker for improvement of meat-quality using candidate gene analysis. The following conclusions can be made based on the results of this study. The SNPs in the intron 2 region of the porcine *RPL27A* gene may affect meat-quality traits (pH24, collagen and moisture). However, the genotypes of the *RPL27A* gene associated with meat-quality traits were all located in intron 2, which suggests that these SNPs are not causal, but linked to genotype associated meat-quality traits. Therefore, the SNPs of the *RPL27A* gene found in this study

will provide useful information for genetic characterization or association studies of meat-quality traits in other populations. Additionally, these markers could potentially be applied in pig breeding programs to improve meat-quality traits after validation in other populations.

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