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Association of *FABP3* Genotypes and Carcass Characteristics in Pigs

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ABSTRACT

This study was conducted to analyze the genotypes and genes of *FABP3* (Fatty-acid Binding Protein 3) in pigs using *MSPI* restriction enzyme and electrophoresis. Analysis of data collected from a total of 210 crossbred pigs (LYD or YLD) in Chungcheongnam-do, Korea, revealed the following. The AA genotypes of *FABP3* were detected in the 750 bp and 100 bp bands, while the Aa heterotype appeared in the 850, 750 and 100 bp bands and the aa recessive homotype was detected in a single band of 850 bp. The genotype frequency of AA, Aa and aa was 46.67%, 51.43% and 1.90%, respectively. The genetic equilibrium of this population showed a significant difference ($p<0.001$) based on a χ^2-test. The carcass weight, backfat thickness, marbling score, pH, drip loss, cooking loss, and meat color based on the CIE L*, and b* values according to genotypes of *FABP3* did not differ significantly ($p>0.05$); however, the CIE a* values did ($p<0.05$).
(**Key words :** *FABP3, MSPI*, Genotypes, Gene, Carcass characteristics)

INTRODUCTION

Pork is the most frequently consumed meat in South Korea, and its consumption per person has gradually increased from 17.8 kg in 2005 to 19.3 kg in 2010 (Ministry of Food, Agriculture, Forestry and Fisheries, 2011). Recently, meat consumption in South Korea has shown a clear trend towards quality preference rather than quantity; accordingly, production of superior quality pork is important to satisfying consumer demand (Kim, 2012).

Pork was the most preferred meat (62.6%) in a study of meat preference in South Korea (Kim and Kim, 2009), and the most preferred cuts were the belly (61.2%), shoulder loin (23.6%), tender loin (8.4%), ribs (5.1%), front leg (1.1%), and rear leg (0.6%) (Kim, 2005). This consumption pattern arises owing to the preference of consumers in South Korea for meat with high intramuscular fat content for barbecue. Intramuscular fat is an important element that affects taste and meat quality because it contains many unsaturated fatty acids (oleic acid, linoleic acid, etc.) that enhance the flavor of the meat and that are not found in the backfat (Cameron, 1990). In addition, intramuscular fat is stored around the perimysium, which enhances tenderness and juiciness. Furthermore, the low melting point of this fat causes it to rapidly melt and create a layer on the surface of the meat that reduces the formation and evaporation of moisture created by protein denaturation during cooking (Choi, et al., 2003).

The fatty acid-binding protein (*FABP*) gene family, which is involved in regulation of intramuscular fat, comprises *H-FABP* (*heart fatty acid-binding protein FABP3*) and *A-FABP* (*adipocyte fatty acid-binding protein FABP4*) in addition to other forms (Veelamp and Maatman, 1995; Boord et al., 2002; Choi et al., 2003). *FABP3*, which is located between SW316-S0003 on swine chromosome 6, encodes a 15-kDa protein expressed in muscle and heart and is known to be involved in the regulation of lipid metabolism and transfer of fatty acid between cells (Gerbens et al., 1999, 2001). *FABP3* has been reported to be related to marbling score, backfat thickness, and growth rate (Gerbens et al., 1998, 1999; Nechtelberger et al., 2001; Choi et al., 2003; Sato et al., 2003). Correlation analysis showed that polymorphisms in the promoter (*FABP3-Hinf I*) and intron 2 (*FABP3-Hae III, Msp I*) of *FABP3* were significantly associated with intramuscular fat content (Gerbens et al., 1997, 2001; Ovilo et al., 2002b; Urban et al., 2002). Kim et al. (2005) also demonstrated that another nucleotide

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polymorphism in the *FABP3* promoter is significantly associated with intramuscular fat content in Korean native cattle and a Landrace F_2 population.

A high marbling score is essential for producing good quality pork. Therefore, in addition to using advances in feeding and management, pork producers must rear high-quality pig breeds that are obtained via selection of superior quantitative trait loci. Accordingly, this study was conducted to identify any relationships between analysis of *FABP3* genotype and carcass characteristics in pigs based on 210 pigs bred in the Chungcheongnam-do area of Korea.

MATERIALS AND METHODS

1. Animals

The animals in this study were selected at random from a population of crossbred pigs (Landrace × Yorkshire × Duroc, LYD or YLD) in the Chungcheongnam-do area. A total of 210 pigs were used.

2. Sampling

Blood specimens were collected from the animals' veins into evacuated blood-collection tubes containing the anti-coagulants heparin (sodium heparin; BD Vacutainer®, USA) or ethylenediaminetetraacetic acid (EDTA; BD Vacutainer®, USA). The samples were then stored at 4℃ and transferred to the laboratory, where they immediately underwent centrifugation to separate the plasma from the blood cells. The cells were subsequently combined with Dulbecco's phosphate buffered saline (DPBS) and then centrifuged with Ficoll-Paque™ PLUS (Amersham Biosciences, Sweden) to isolate the white blood cells, which were stored at −25℃ until the experiment.

For muscle sampling, 60 of the animals were slaughtered at a local slaughterhouse, and samples of approximately 1,000 g each were taken from the area corresponding to the loin cut. Collected samples were then stored at 4℃ until transfer to the laboratory, where they were stored at −25℃ until needed.

3. Gene Analysis

Genomic DNA extraction from frozen white blood cells and tissues were conducted using standard experimental methods with a QIAamp Mini kit (QIAGEN®, Valencia, CA, USA). Briefly, purified white blood cells were suspended in DPBS at 5×10^6 cells/200 μL, cell lysis buffer and proteinase K were added, and the solution was then filtered to isolate the genomic DNA. Next, the genomic DNA was quantified using a Nanodrop spectrophotometer (ND-2000, Nanodrop® Technologies, Wilmington, DE, USA) and stored at −25℃ until the time of experiment.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was conducted using the genomic DNA extracted from the animals as a template and primers specific for the *FABP3* gene described by Gerbens, et al. (1997; GenBankaccession number HM591296; Table 1). Primer synthesis was conducted by Bioneer (Bioneer Co., Korea), and a final concentration of 10 pmol was used.

Qiagen HotStarTaq polymerase (2.5 units; Qiagen®, Valencia, CA, USA) was used for amplification with 1 × buffer (0.01 M Tris-HCl, 0.05 M KCl, 0.08% Nonidet), 1.5 mM $MgCl_2$, 1 mM dNTPs, and 10 pM of each primer in a reaction mixture with a final volume that was adjusted to 10 μL. PCR was performed using the GeneAmp PCR system 9700 (Applied Biosystems, USA) to subject the samples to the following conditions: initial denaturation for 15 minutes at 95℃ followed by 35 cycles of 30 seconds of denaturation at 94℃, 30 seconds of annealing at 52℃, and 40 seconds of elongation at 72℃ with a final elongation step for 10 minutes at 72℃.

Gerbens et al. (1997) reported a SNP located approximately 300 bp away from the second intron that could be identified by RFLP analysis using the restriction enzyme *Msp* I (New

Table 1. Primer sequence and restriction for PCR-RFLP analysis in *FABP3* genes in pigs

Gene name	Restriction enzyme	PCR primer sequence (5'-3')	Product size	Annealing temp. (℃)	SNP genotype	Fragment size (bp)
FABP3	*Msp* I	ATTGCTTCGGTGTGTTTGAG TCAGGAATGGGAGTTATTGG	816 bp	52℃	AA	727, 89
					Aa	816, 727, 89
					aa	816

England BioLabs Inc., England.). The reaction mixture contained 2 μL of the PCR product, 2 μL of 10 × buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol, pH 7.0) and 10 units of *Msp* I diluted to a final volume of 20 μL with water. The digest was then incubated in a 37℃ constant-temperature water bath for 6 hours to ensure complete digestion, after which it was run on agarose gel to determine the genotypes.

TAE (40 mM Tris-acetate, 1.0 mM EDTA) buffer was used for electrophoresis. Briefly, a 1.5% agarose gel was used to confirm the initial PCR product, and a 2.03.0% gel was used determine the genotypes following restriction digestion. To visualize the DNA, ethidium bromide was added at 0.1 mg/mL to both the gel and the running buffer. Electrophoresis was performed for approximately 90 minutes under 8 volts/cm. The size marker used during electrophoresis was 100 bp DNA plus Ladder (MBI Fermentas Inc., USA). After completion of electrophoresis, the gels were photographed using an Image Analyzer (Seoulin Bioscience, Korea). The genotypes were then classified according to the restriction digest results and analyzed statistically.

4. Carcass Traits According to Genotype

The following carcass traits were used for comparison with the genotype analysis obtained from the PCR-RFLP method described above: dressed weight (kg), thickness of backfat (mm), marbling score, pH, drip loss, cooking loss, and mechanical meat color (CIE L*, a*, b*).

The rating method used by the grade judge of the N slaughterhouse was used to evaluate the dressed weight, backfat thickness, and marbling score. To measure the pH of the pork, 5 g of the sample were combined with 20 mL of distilled water and homogenized for 1 minute at 8,000 rpm using an Ultra-Turrax disperser (Utra-Turrax® T25, Janke & Kunkel, Staufen, Germany) and the pH was then determined using a pH meter. To determine drip loss, samples were cut into 2 cm thick pieces and weighed before and after being stored in polyethylene bags at 4℃ for 48 hours. Drip loss was then determined using the following equation:

$$\text{Drip loss (\%)} = \frac{\text{initial sample weight} - \text{sample weight after 48 hours}}{\text{initial sample weight}} \times 100$$

To evaluate cooking loss, samples were cut into 2 cm thick pieces, weighed, placed in polyethylene bags and heated for 40 minutes in a constant-temperature water bath set to 80℃. The samples were then stored on ice for 30 minutes, at which time they were weighed again. Cooking loss was found using the following equation:

$$\text{Cooking loss (\%)} = \frac{\text{weight before heating} - \text{weight after heating}}{\text{weight before heating}} \times 100$$

Meat color was evaluated using a color meter (Model NF333, Nippon Denshoku Co., Japan) to analyze a portion cut from the loin sample and standardized using a standard white board. To determine the CIE value, measurements of L* (lightness), a* (redness), and b* (yellowness) values were repeated three times and the average values were reported. A white tile of Y = 92.40, X = 0.3136, y = 0.3196 was used as the standard board.

5. Statistical Analysis

FABP3 genotype and allelic frequencies from restriction digests with *Msp* I were calculated and compared with those expected according to the Hardy-Weinberg law using the χ^2-test.

For each genotype, the average and standard deviation of the dressed weight, thickness of backfat, marbling score, pH, drip loss, cooking loss, meat color, and other carcass characteristics were found, and significance at the 5% level was verified and analyzed using a *t*-test.

RESULTS AND DISCUSSION

1. *FABP3* Gene Analysis

As shown in Fig. 1, PCR produced a single 816 bp band.

Analysis using *Msp* I to determine polymorphisms in the second intron region gene is shown in Fig. 2. The homozygous AA genotype has one band at 727 bp and another at 89 bp. The heterozygous genotype Aa has one band at 816 bp, one at 727 bp, and one at 89 bp. The homozygous genotype aa has a single band at 816 bp.

Genotype classification and frequency are described in Table 2. The AA genotype, which showed relatively high prevalence, was found in 99 pigs (46.67%). The heterozygous Aa genotype showed the highest prevalence, being present in

Table 2. Distribution of *FABP3* gene intron 2 region and alleles determined by *Msp* I restriction enzyme digestion and χ^2-square test for goodness of fitness with the Hardy-Weinberg equilibrium

Genotypes	*Msp* I site		Gene frequencies	
	No. of pigs	Percentage	A	a
AA	98 (130.14)	46.67		
Aa	108 (73.94)	51.43	0.724	0.276
aa	4 (5.12)	1.90		
Total	210	100.00	1.000	
χ^2-test	χ^2-value: 17.213***	df: 1	p: 0.000	

( ) : No. of animals expected, *** : $p<0.001$.

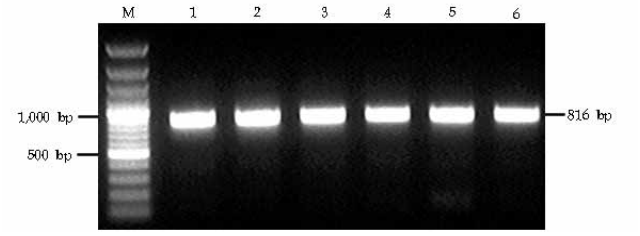

Fig. 1. PCR products of *FABP3* gene (816 bp) of the second intron region in pig samples. M: Molecular size standard (GeneRuler™ 100 bp DNA ladder plus, MBI Fermentas Inc. USA), lanes 1-6: PCR products.

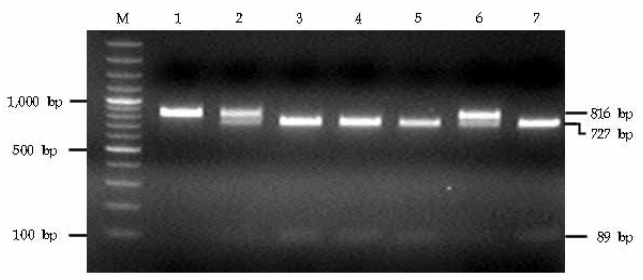

Fig. 2. Agarose gel electrophoresis patterns of different genotypes in *FABP3* of pigs. PCR products digested in *Msp* I. The normal genotype (AA, Lane 3, 4, 5 and 7) produced two digested fragments (727, 89 bp), the heterozygous genotype (Aa, Lane 2 and 6) produced one undigested fragments (816 bp) and two digested fragments (727 and 89 bp), and the mutation genotype (aa, Lane 1) generated one undigested fragment (816 bp). M: Size marker (GeneRuler™ 100 bp DNA ladder plus, MBI Fermentas Inc. USA).

108 pigs (51.43%), while the aa genotype was only seen in 4 pigs (1.9%). A χ^2-test of these allelic frequencies indicated that they were not in Hardy-Weinberg equilibrium ($p<0.001$).

Based on the genotype analysis, the A allele was present at a very high frequency (0.72), but the a allele had a low frequency (0.28). These results contrast with those reported by Nechtelberger, et al. (2001), who found that the AA genotype was very highly prevalent among Landrace and Large White pigs (92.94% and 89.14%, respectively), while the Aa genotype was seen at relatively low prevalence (7.05% and 10.85%, respectively). However, the very low prevalence of the aa genotype was similar in both studies. Analysis of the Berkshire species by Lee et al. (2010) produced different results, with an Aa genotype frequency of 0.29 and an AA genotype of 0.63, but the prevalence of the aa genotype (0.08) was low, which was similar to the results of the present study. Based on these findings, the homozygous genotype aa is assumed to be very low in prevalence or non-existent among various breeds.

2. Carcass Characteristics of each *FABP3* Genotype

Data from the *Msp* I RFLP genotype analysis was correlated with carcass characteristics obtained from muscle samples from 60 pigs selected at random from the 210 pigs used for gene analysis. The aa genotype was excluded from the statistical analysis as its frequency was too low. Table 3 shows the results obtained upon analysis of the AA and Aa genotypes for the primary pig carcass traits (dressed weight, backfat thickness, marbling score, pH, drip loss, cooking loss, meat color (CIE L*, a*, b* value), and others. The average dressed weight for the AA and Aa genotypes was 89.86 kg and 88.83 kg, respectively, which was not a significant difference. The average backfat thickness for the AA genotype was 19.64 mm, while that for the Aa genotype was 19.00 mm, which also did not differ significantly. These

Table 3. Analysis of association between genotypes at the *Msp* I site substitution site of the *FABP3* gene and meat quality characteristics

Traits	Genotypes		
	AA	Aa	Mean ± SD
Carcass weight	89.86±3.84	88.83±4.66	89.55±4.09
Backfat thickness (mm)	19.64±3.21	19.00±0.59	19.45±2.72
Marbling score	2.29±0.60	2.50±0.51	2.35±0.58
pH	5.53±0.09	5.56±0.12	5.54±0.10
Drip loss	2.16±1.30	1.92±0.91	2.09±1.19
Cooking loss	37.12±2.99	36.45±1.80	36.92±2.69
CIE L*	52.30±2.23	52.34±2.61	52.31±2.33
a*	2.89±1.39[a]	1.95±1.13[b]	2.61±1.38
b*	5.58±1.84	5.08±2.17	5.43±1.94

[a, b] Means with different superscripts are significantly different ($p<0.05$).

results were similar to those of a study conducted by Lee et al. (2010) for the Berkshire breed, in which the average backfat thickness for the AA and Aa genotypes was 24.30 mm and 23.90 mm, respectively, which was also not significantly different. These observations indicate that *FABP3* does not affect backfat thickness.

According to the grade ranking (1-5) results, the homozygous AA genotype (2.29) and Aa genotype (2.50) showed similar marbling scores, but the difference between these two groups was not significant. These findings are similar to those in a study conducted by Lee et al. (2010), in which analysis of the National Pork Producers Council marbling scores showed that the scores for the AA genotype (2.36), Aa genotype (2.15), and aa genotype (2.24) were not significantly different.

Meat pH was also similar between the two groups. Specifically, the pH for the AA genotype was 5.53, while that for the Aa genotype was 5.56, which did not differ significantly. These findings are generally similar to values seen in an investigation of the Berkshire breed conducted by Lee et al. (2010), who found pH values for the AA, Aa, and aa genotypes of 6.12, 6.17, and 6.11, respectively, which were not significantly different.

The drip loss of the two genotypes was not significantly different, although drip loss in the AA genotype (2.16%) was somewhat higher than that for the Aa genotype (1.92%). These findings are similar to those reported by Lee et al. (2010) for the Berkshire breed, in which drip loss for the AA genotype was 2.06% and was not significantly different from that for the Aa genotype (2.43%). Additionally, these results are similar to those reported by Nechtelberger et al. (2001) for the Large White breed, in which drip loss for the AA and Aa genotypes was 2.02% and 2.15%, respectively, while that for the Landrace breed was 1.85% and 1.79%, respectively. The drip loss for the Pietrain breed was 5.69% for AA genotypes and 6.25% for Aa genotypes, there is an overall difference in drip loss; however, the lack of significant difference between genotypes indicates that this difference is due to distinct genetic factors in the breeds (Nechtelberger et al., 2001).

Evaluation of cooking loss revealed that the AA genotype (37.12%) and Aa genotype (36.45%) had similar levels of water loss owing to heat, and that these values did not differ significantly. These findings were similar to those reported by Lee et al. (2010).

In contrast, the CIE a* value (redness) was significantly different between the two genotypes ($p<0.05$). Specifically, the AA genotype had a score of 2.89, which was more red than that of the Aa genotype (1.95). Jung (2010) reported that redness and marbling scores were correlated based on analysis of the intramuscular fat content characteristics of a double cross hybrid of Korean native pigs and Landrace F_2. According to their report, when the marbling score is high, redness decreases. These findings were confirmed by the present study, in which the Aa genotype had a higher marbling score and lower redness. In contrast, the CIE L* (lightness) and b*values (yellowness) between genotypes did not differ significantly. This observation was similar to the results reported by Lee et al. (2010) and Nechtelberger et al. (2001), who also saw no significant differences between the two genotypes for these traits.

Overall, the results of the present study indicate that the prevalence of the recessive homozygous genotype aa is very low among crossbreeds, and that marbling score can be affected by *FABP3* gene polymorphism. Therefore, in addition to future research into the discovery of new genes that affect marbling score as determined by breed or crossbreed genotype classification, more samples from purebred fed pigs should be evaluated to obtain accurate data describing how *FABP3* affects carcass characteristics to analyze breeding plans or meat characteristics of other dressed carcasses. Furthermore, genetic tests should be conducted using genes that are highly correlated with dressed carcass quality to select superior quality individuals for use in breeding to improve farm production.

CONCLUSION

In this study, *FABP3* gene polymorphisms were analyzed to investigate genotype and allelic frequency in 210 pigs (LYD or YLD) bred in the area of Chungcheongnam-do, Korea. The following is a summary of the results based on correlation analysis of carcass characteristics according to genotype.

PCR-RFLP analysis of *FABP3* using *Msp* I revealed that the homozygous AA genotype produced two bands (750 bp and 100 bp), the heterozygous Aa genotype produced three bands (850 bp, 750 bp, and 100 bp) and the recessive homozygous aa genotype generated a single band at 850 bp. The AA genotype frequency (46.67%) and Aa genotype frequency (51.43%) were relatively high, but the aa genotype frequency (1.9%) was very low. The A allele was very highly prevalent (0.72), but the a allele had a low prevalence (0.28). A χ^2-test demonstrated that this allelic frequency was not in Hardy-Weinberg equilibrium.

The pig carcass characteristics of dressed weight, backfat thickness, marbling score, pH, drip loss, cooking loss, and CIE L* value and b* value of meat color were not significantly different between the two genotypes; however, the CIE a* values (redness) were significantly different for the AA genotype (2.89) and Aa genotype (1.95; $p < 0.05$). Porcine *FABP3* should be intensely investigated to enable selection of superior breeding stock by investigating accurate correlations between genotypes and carcass characteristics based on many more samples.

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