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ARTICLE

The rs196952262 Polymorphism of the *AGPAT5* Gene is Associated with Meat Quality in Berkshire Pigs

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Abstract

High-quality meat is of great economic importance to the pig industry. The 1-acylglycerol-3-phosphate-O-acyltransferase 5 (AGPAT5) enzyme converts lysophosphatidic acid to phosphatidic acid in the mitochondrial membrane. In this study, we found that the porcine *AGPAT5* gene was highly expressed in muscle tissue, influencing meat characteristics, and we also identified a non-synonymous single-nucleotide polymorphism (nsSNP) (rs196952262, c.673 A>G) in the gene, associated with a change of isoleucine 225 to valine. The presence of this nsSNP was significantly associated with meat color (lightness), lower cooking loss, and lower carcass temperatures 1, 4, and 12 h after slaughter (items T1, T4, and T12 on the recognized quality scale, respectively), and tended to increase backfat thickness and the water-holding capacity. These results suggest that nsSNP (c.673A>G) of the *AGPAT5* gene is a potential genetic marker of high meat quality in pigs.

Keywords *AGPAT5*, gene expression, non-synonymous SNP, meat quality, Berkshire pig

Introduction

For many years, production of high-quality meat has been the prime objective of the pork industry. Meat quality can be assessed from technological, nutritional, and sensory perspectives and may be influenced by multiple interacting factors before and after slaughter (Park *et al.*, 2010). Many studies have focused on genetic factors affecting meat quality (Baby *et al.*, 2014; Casiro *et al.*, 2017; Gonzalez-Prendes *et al.*, 2017; Hwang *et al.*, 2017). These studies found that selective pig breeding and the use of DNA markers played important roles when seeking to enhance pork quality.

The 1-acylglycerol-3-phosphate O-acyltransferases (AGPATs), also known as lysophosphatidic acid acyltransferases, are key enzymes of phospholipid and triacylglyceride biosynthesis. To date, 11 AGPATs have been identified in both mouse and human; however, only the first five (AGPAT1-5) have been proven to catalyze phosphatidic acid synthesis from lysophosphatidic acid; phosphatidic acid is the precursor of all glycerolipids (including triacylglycerides) (Vance and Vance, 2008; Yamashita *et al.*, 2014a). Therefore, AGPATs are important in terms of tria-

cylglyceride biosynthesis because most fatty acids are incorporated into lipids by these enzymes (Coleman and Lee, 2004; Shindou and Shimizu, 2009; Yamashita *et al.*, 2014b). Several studies have shown that fatty acid composition is associated with both meat quality and nutritional value (Choi *et al.*, 2016; Kouba *et al.*, 2003; Yu *et al.*, 2013). However, no study has yet investigated how AGPAT5 affects pig meat quality.

In the present study, we identify a single-nucleotide polymorphism (SNP) in the *AGPAT5* gene and explore the associations between this polymorphism and the meat quality traits of Berkshire pigs.

Materials and Methods

Animals

A total of 430 pigs of a pure Berkshire line (males, 210, females, 220), bred under similar conditions, were randomly selected and slaughtered at body weights of approximately 110 kg. The *longissimus dorsi* muscles were sampled immediately after slaughter and the samples were held at 4°C prior to the assessment of meat quality traits. Animal care and use, and all experimental protocols, conformed to the guidelines of the Animal Care and Use Committee of GNTECH, the Korean Animal Protection Act, and all related laws.

Analysis of *AGPAT5* expression by RT-PCR

Total RNAs from various tissues (liver, stomach, lung, kidney, large and small intestines, spleen, and muscle) of three Berkshire pigs were isolated using the TRI-Reagent (Molecular Research Center, USA) and reverse-transcribed into cDNA with the aid of Superscript II Reverse Transcriptase (Invitrogen, USA), in accordance with the manufacturer's protocol. The cDNAs were then subjected to RT-PCR for evaluation of the relative gene expression level of *AGPAT5* and that of the gene encoding peptidylprolyl isomerase A (*PPIA*) (internal control), using appropriate primer pairs (Table 1). Amplifications proceeded on a Perkin Elmer 9700 system (Applied Biosystems, USA) under the following conditions: 95°C for 5 min; 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s; and final elongation for 7 min at 72°C. The amplification products were separated on 2% (w/v) TAE agarose gels and quantified using a Gel Logic model 200 imaging system (Kodak, USA).

AGPAT5 SNP detection and genotyping

An *AGPAT5* nsSNP was detected in cDNAs synthesized from pooled liver RNAs of three Berkshire pigs using an Illumina GAII analyzer (Illumina, Inc., USA), as described previously (Jung *et al.*, 2012). The nsSNP information was obtained with the aid of the NCBI dbSNP database. To explore *AGPAT5* nsSNP genotypes, genomic DNAs were isolated from whole blood cells of 430 pigs and SNP genotypes were analyzed using an Illumina VeraCode GoldenGate Assay kit (Illumina, Inc.). The relevant oligonucleotide information is shown in Table 1.

Measurements of meat quality traits

The meat quality parameters examined included carcass weight (kg); backfat thickness (mm); meat colors (L* [lightness], a* [redness], and b* [yellowness]); cooking loss (%); water-holding capacity (%); carcass temperatures at 1, 4, and 12 h after slaughter (T1, T4, and T12, respectively); and the 24-h postmortem pH (pH_{24}). Backfat thickness was measured at the 10^{th} rib at a point 75% along the *longissimus dorsi* (toward the belly). Meat color was recorded by a Minolta Chromameter (CR-400; Minolta, Japan) after 30 min of blooming at 1°C. Cooking loss was the weight difference between before and after cooking. A slice 3 cm in thickness (weight 100±5 g) from the *longissimus dorsi* muscle was placed into a polypropylene bag (Dongbang Co., Korea), cooked for 40 min at 70°C in a water bath, and then cooled to room temperature. The pH_{24} was that at 24 h postmortem and was mea-

Table 1. Oligonucleotides used for genotyping and RT-PCR

Application	Gene name		Sequence (5′ → 3′)
Genotyping	AGPAT5	Allele-specific Oligo1	ACTTCGTCAGTAACGGACGTCGAAAGCCACTGTAACATCGTAAAT
		Allele-specific Oligo2	GAGTCGAGGTCATATCGTGTCGAAAGCCACTGTAACATCGTAAAC
		Locus-specific Oligo	GCATCTAAATAACTCTTCATAGAATCCATGAGCGGGTTCGTACCAG
			TCGTCTGCCTATAGTGAGTC
RT-PCR	AGPAT5	Forward	TTTTCTCAGCATGGAGGGAT
		Reverse	GGCCTTTTTGAGCAGCAAAT
	PPIA	Forward	CACAAACGGTTCCCAGTTTT
		Reverse	TGTCCACAGTCAGCAATGGT

sured with the aid of a portable pH meter (Istek Inc., Korea) equipped with a glass electrode that could be inserted into muscle tissue. The water-holding capacity at 3 d postmortem was measured using a centrifugation method (Fan *et al.*, 2010). Duplicate 10 g minced samples taken from one chop from each loin were placed into centrifuge tubes and spun for 10 min at 40,000 g. After centrifugation, the liquid was removed and the meat re-weighed. The percentage of water loss was measured and used to estimate the water-holding capacity.

Statistical analysis

The frequencies of the various *AGPAT5* genotypes were calculated. To analyze associations between nsSNP genotypes and meat quality traits, we ran a general linear model using SAS software version 9.1.3 (SAS Institute Inc., USA). SNPs subjected to statistical analysis were characterized by a call rate < 0.90, a minor allele frequency > 0.01, and a Hardy-Weinberg equilibrium probability (the *p* value) > 0.05. The linear model employed was: $y_{ij} = \mu + G_i + S_j + e_{ij}$, where y_{ij} is the phenotypic contribution of the target trait, μ the general mean, G_i the fixed effect of genotype i, S_j the fixed effect of sex j, and e_{ij} the random error. Significant differences ($p<0.05$) between the genotypic frequencies associated with various traits were sought with the aid of analysis of variance (featuring the Bonferroni correction) and the Kruskal-Wallis test.

Results and Discussion

AGPAT5 expression in various tissues of the Berkshire pig

We used RT-PCR to evaluate *AGPAT5* expression in various tissues of the Berkshire pig (Fig. 1). *AGPAT5* was ubiquitously expressed in all tissues examined, but its expression was highest in muscle, as is the case for human *AGPAT5* (Agarwal *et al.*, 2006). Murine *AGPAT5* is primarily expressed in skeletal muscle, brain, and heart; and is expressed at high levels in testis and prostate (Biao *et al.*, 2005). Meat quality depends on physiological processes in muscle tissue, potentially involving many genes associated with muscle structure and metabolism. We assumed that AGPAT5 status would be a determinant of meat quality.

Association of the *AGPAT5* nsSNP with meat quality traits

As variations in DNA sequence such as SNPs can enhance phenotypic diversity such as meat quality, we identified a new nsSNP (rs196952262, c.673A>G) in the *AGPAT5* gene and investigated the contribution thereof to meat quality in Berkshire pigs. The nsSNP c.673A>G in *AGPAT5* identified by RNA sequencing of liver tissue samples changes isoleucine 225 to valine in Berkshire pigs. To analyze the association between this nsSNP and meat quality, we genotyped 430 Berkshire pigs using the GoldenGate assay. The genotypic and allelic frequencies of the nsSNP are shown in Table 2. The GG genotype was much more common than the AG and AA genotypes. The frequencies of the G and A alleles were 0.792 and 0.206, respectively. The genotype frequencies were in Hardy-Weinberg equilibrium ($p>0.05$) (Falconer, 1996).

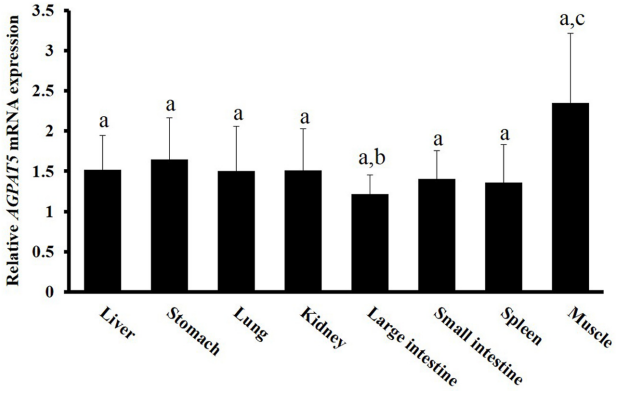

Fig. 1. The *AGPAT5* mRNA expression was determined in various tissues by RT-PCR. RNA was isolated from various tissues, including the liver, stomach, lung, kidney, large and small intestines, spleen and muscle. Letter a, b, and c above each bar indicate statistically significant differences among tissues ($p<0.05$). Values are mean±SD.

We investigated the association between the new nsSNP and meat quality traits (Table 3). All three genotypes (AA, AG, and GG) were detected in the pig population. The *AGPAT5* nsSNP was significantly associated with lightness (the CIE L* value), less cooking loss, and lower carcass temperatures (T1, T4, and T12). The AG genotype was associated with higher meat quality than the AA and GG genotypes.

AGPAT-encoded enzymes convert lysophosphatidic acid to phosphatidic acid, a critical substrate for the synthesis of important lipid signaling molecules including phosphatidyl inositol (a second messenger of insulin signaling) and cardiolipin (a mitochondrial membrane phospholipid) (Yamashita *et al.*, 2014a). Of the various AGPAT isoforms, several exhibit lysophospholipid acyltransferase activity, but only AGPAT4 and AGPAT5 are known to be

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Table 2. Genotype and allele frequencies of non-synonymous SNP in *AGPAT5* gene

SNP	Genotype	Genotype frequency	Allele	Allele frequency
AGPAT5 c.673A>G	GG (n=267)	0.631	G	0.794
	AG (n=149)	0.327	A	0.206
	AA (n=14)	0.042		

χ^2=1.55, 0.10 < p < 0.50

Table 3. Association between *AGPAT5* nsSNP, c.677A>G, and meat quality traits

SNP		*AGPAT5,* c.673A>G		
Genotype		GG (n=267)	AG (n=149)	AA (n=14)
Carcass weight (kg)		85.775±5.567	85.805±5.756	87.214±6.518
Backfat thickness (mm)		24.738±5.337	25.624±5.220	23.429±3.857
Meat color	CIE L*	48.510±2.894*	48.758±2.816*	50.371±0.611*
	CIE a*	6.149±1.058	6.131±0.977	6.228±1.332
	CIE b*	2.887±1.112	2.871±1.090	2.725±1.140
Cooking loss (%)		27.574±3.545*	26.615±4.241*	28.121±3.159*
Water holding capacity (%)		58.213±2.774	58.413±2.704	56.830±1.692
T1 (°C)		37.588±3.569*	36.860±4.613*	39.955±1.949*
T4 (°C)		26.588±4.132*	26.151±5.174*	30.955±3.567*
T12 (°C)		16.978±2.980*	16.729±3.526*	20.491±3.162*
pH_{24}		5.835±0.213	5.793±0.214	5.793±0.167

Data is shown as Means±SD. Superscript indicates statistically significant differences among genotypes (p<0.05).
CIE L*, a* and b* respectively represent the meat color lightness, redness and yellowness.
T represents a postmortem temperature.

located in mitochondria (Prasad *et al.*, 2011). However, unlike AGPAT4, AGPAT5 is active on several lysophospholipid substrates, including lysophosphatidylinositol, lysophosphatidyl ethanolamine, lysophosphatidyl choline, and lysophosphatidyl serine (Prasad *et al.*, 2011). Fats and fatty acids of adipose tissue and muscle are important contributors to various aspects of meat quality. Intramuscular fats are composed primarily of phospholipids located in the cell membranes and neutral lipids consisting of mainly triacylglycerols in the adipocytes (Smet *et al.*, 2004). Fats vary greatly in melting point, and fat composition thus affects meat firmness/softness (Knothe and Dunn, 2009; Wood and Enser, 1997). Negative correlations were evident between various fatty acid profiles and meat quality traits (Razmaitė *et al.*, 2009). Moreover, Kim *et al.* (2016) suggested that fat content affected meat quality by controlling the water-holding capacity and drip loss. Meat from heavy pigs (which were also fatter and faster growing) had lower Warner-Bratzler Shear Force and cooking loss than meat from light weight pigs (Magowan *et al.*, 2011). The decrease in cooking loss with increased ultimate muscle pH is likely to be a reflection of improvements in water-holding capacity which are to be expected as the pH moves away from the average isoelectric point of muscle proteins (Monin *et al.*, 1986). These suggest that *AGPAT5* status may affect meat quality by regulating fatty acid synthesis.

In summary, we found that the porcine *AGPAT5* gene was highly expressed in muscle and we explored the association between an *AGPAT5* polymorphism and meat quality in the Berkshire pig. The *AGPAT5* AG genotype reduced all of meat color, cooking loss, and carcass temperatures. Therefore, this nsSNP may help the breeding industry to select pigs of high meat quality.

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