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Association of a Pyruvate Kinase M2 (*PKM2*) Polymorphism with Back Fat Thickness in Berkshire Pigs

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ABSTRACT

Pyruvate kinase M2 (PKM2) is a key regulatory enzyme in the glycolytic pathway. It is one of four pyruvate kinase isoenzymes that widely differ in their occurrence according to tissue type. *PKM2* is expressed in differentiated tissues, such as fat tissues, lung, as well as normal proliferating cells, embryonic cells, and tumor cells. The objective of this study was to investigate the association of single nucleotide polymorphisms (SNPs) in the *PKM2* gene with meat quality traits in Berkshire pigs. We detected a SNP (*g.34341 A>G*) in the 3'UTR region of the *PKM2* gene in 670 Berkshire pigs through DNA sequencing. Three genotypes, AA, AG, and GG, were found for this SNP, but based on an association analysis with meat quality traits, genotype AA was significantly associated with thicker back fat than genotype GG (p=0.027). Therefore, the *g.34341 A>G* polymorphism in the 3'UTR region of the porcine *PKM2* gene could be applied in pig breeding programs to improve back fat thickness.

(**Key words :** Meat quality, Pyruvate Kinase M2, Pig, Single nucleotide polymorphism)

INTRODUCTION

Meat quality is attributed to various factors such as genetics, muscle characteristics, as well as production and environmental conditions. Understanding the metabolic pathways involved in fat formation is particularly important for quality meat production from farm animals. In pork, back fat thickness (BF) and intramuscular fat content (IMF) are major factors that affect the sensory meat quality and highly considered as selection criteria in commercial markets (Gerbens et al., 2001; Cho et al., 2011). Genetic markers have been applied to improve the fatness and meat quality of pigs (Markljung et al. 2008; Li et al. 2010; Fan et al. 2010). In earlier studies, QTLs related to fatness and meat quality traits, such as intramuscular fat content (de Koning et al., 1999; Gerbens et al., 1999, 2000; Ovilo et al., 2000; Grindflek et al., 2001; Uleberg et al., 2005) and backfat thickness (Malek et al., 2001; Ovilo et al., 2002; Szyda et al., 2003; Soma et al., 2011) were identified on chromosome 6 (SSC6).

Pyruvate kinase has four different isoforms: M1, M2, L, and R. M1 and M2 isoforms are in the muscle, heart, and brain, whereas L and R forms are found in the liver and erythrocytes (Takegawa, Shinohara, and Miwa, 1984). M1 and M2 isoforms are encoded from the *PKM* (Pyruvate Kinase Muscle) gene (Noguchi, Inoue, and Tanaka, 1986) located in q12-q23 on porcine chromosome 7 (Davoli et al., 2002; Fontanesi et al., 2004), a region associated with highly reported to contain QTLs related to meat quality, fat deposition, and growth traits (Bidanel et al., 2001; Gilbert et al., 2007; Malek et al., 2001; Nezer et al., 2002; Ovilo et al., 2002; Reiner et al., 2002; Rohrer and Keele, 1998; Yue et al., 2003). *PKM2* is a particularly promising positional candidate gene that plays a crucial role in porcine meat quality (Duan et al, 2009). Therefore, determining the *PKM2* gene polymorphisms associated with meat quality could be useful for marker-assisted selection in pig breeding programs. In this investigation, *PKM2* gene polymorphisms were tested for their association with meat quality traits in a Berkshire pig population. This study contributes to a better understanding of molecular factors associated with meat quality in livestock.

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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 measurements

A total of 670 Berkshire pigs (307 castrated males and 363 females) were used in the association study. The pigs were fed with the 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: meat characteristics, meat pH (measured 24 hours after slaughter), water-holding capacity (WHC), drip loss, cooking loss, meat color, muscle shear force, moisture, IMF, and crude protein. The water-holding capacity of the longissimus dorsi was immediately sampled after slaughter using the filter-paper method described by Grau and Hamm (1952, 1956). In addition, 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. Mean, standard deviation (SD), and ranges for the traits measured in 670 pigs

Traits	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 *PKM2* gene were designed from published genomic DNA sequences (Ensembl: ENSSSCG00000001930). The porcine *PKM2* gene was amplified from 96 genomic DNA samples from Berkshire pigs and sequenced to detect polymorphic sites. PCR was performed in a 20 μL volume containing 10 pmol of each primer, 0.25 mM of each dNTP, 2 μL 10X PCR buffer, 1.25 U DNA polymerase (Genet Bio, Chungnam, Korea), and 100 ng genomic DNA. The thermal cycling conditions included an 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 in a DNA Engine Tetrad® 2 Thermal Cycler (Bio-Rad, Hercules, CA, USA). To detect 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). *PKM2* genotypes, determined by direct sequencing of the PCR product from 670 Berkshire pigs, were used for the association study. The primers used for direct sequencing were 5'-TTGGGTGGGGTAGTTCAGAG-3' and 5'-AGACAGTCA-GCAACGGCTTT-3'.

3. Statistical analysis

Association analyses were performed using SAS 9.13 (SAS Institute Inc., Cary, NC, USA). The following formula was used in 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, Gi is the fixed effect of genotype i, Sj is the fixed effect of sex j, Bk is the fixed effect of breed k, Pk 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.

RESULTS AND DISCUSSION

1. Genomic organization of porcine *PKM2* gene

The genomic structure of the porcine *PKM2* gene, a putative exon region, was predicted from the GenBank and Ensembl sequence (Acc. No. XM_003356683, Ensembl: ENSSSCG00000001930) and contained fourteen exons and thirteen introns spanning approximately 46 kb of genomic DNA. The translation initiation codon was located in exon 1 (Table 2). Furthermore, all exon/intron boundary sequences followed the GT-AG rule for splice-donor and acceptor sites reported by Jacob and Gallinaro (1989).

2. SNP identification and genotype frequencies

The porcine *PKM2* wasamplified by PCR and directly sequenced to identify genetic variations in pig samples from the Berkshire population. In our preliminary sequence analysis, one SNP was found at *g.34341 A>G* in the 3' UTR region of the *PKM2* (Fig. 1). To estimate the genotypic and allelic frequencies of this SNP in the porcine *PKM2*, a total of 670 Berkshire pigs were genotyped and the allele and genotype frequencies of *g.34341 A>G* in the Berkshire pigs determined (Table 3). The genotype distribution of *g.34341 A>G* in the Berkshire pigs conformed to Hardy-Weinberg equilibrium in this study. In the Berkshire population, the estimated frequencies of genotypes AA, AG, and GG were 0.17, 0.50, and 0.58, respectively. Hence, the G allele (0.58) was slightly more common than the A allele (0.42).

3. Association study

The statistical analysis results from 670 animals in a commercial Berkshire population are presented in Table 4. The *g.34341 A>G* SNP of the *PKM2* gene was significantly associated with the BF trait and animals with the GG genotype had lower BF values than animals with AA or AG

Table 2. Exon-intron organization of the porcine *PKM2* gene

Exon	Position	Exon size (bp)	Intron	Intron size (bp)
1	1~129	129	1	799
2	929~1,008	80	2	397
3	1,406~1,469	64	3	151
4	1,621~1,705	85	4	19,288
5	20,994~21,175	182	5	512
6	21,688~21,779	92	6	2,856
7	24,636~24,767	132	7	454
8	25,222~25,408	187	8	714
9	26,123~26,393	271	9	1,626
10	28,020~28,170	151	10	240
11	28,411~28,563	153	11	3,335
12	31,899~32,065	167	12	1,803
13	33,869~34,050	182	13	581
14	34,632~35,358	727		

The gene structure was defined by evidence-based gene annotation method using a total of 140 expressed sequence tags and reference genome sequence (accession no. NC_010449) of the porcine *PKM2* gene from NCBI database.

Table 3. Allele and genotype frequencies of *PKM2* polymorphisms in Berkshire pigs

SNP position	Genotype frequency (n = 670)			Allele frequency	
g.34341 A>G	AA (115, 0.17)	AG (338, 0.50)	GG (217, 0.32)	A (0.42)	G (0.58)

The number of genotyped animals and genotype frequency are shown in parentheses. Polymorphisms in 3' UTR region numbered relative to the translation start site; Adenine of the start codon ATG is counted as +1 (Ensembl : ENSSSCG00000001930).

Table 4. Associations between *g.34341 A>G* SNP of porcine *PKM2* and meat-quality traits

Traits	Genotype			*P*-value
	AA (n = 115)	AG (n = 338)	GG (n = 217)	
CWT (kg)	86.09±0.70	86.70±0.54	86.96±0.56	0.403
BF (mm)	25.63±0.59	25.47±0.46	24.43±0.48	0.027*
pH24	5.73±0.02	5.72±0.02	5.71±0.02	0.488
WHC (%)	58.23±0.29	58.03±0.22	57.98±0.23	0.626
Drip loss (%)	4.82±0.22	4.79±0.17	4.96±0.17	0.552
Cooking loss (%)	26.03±0.53	25.71±0.41	26.25±0.43	0.347
MC_L	49.01±0.35	49.09±0.27	49.35±0.28	0.482
MC_a	6.14±0.13	6.01±0.10	6.05±0.10	0.457
MC_b	3.35±0.13	3.14±0.10	3.22±0.11	0.167
SF (kg/0.5inch2)	3.10±0.08	3.18±0.06	3.11±0.06	0.297
Moisture (%)	75.40±0.12	75.55±0.10	75.63±0.10	0.118
IMF (%)	2.61±0.13	2.59±0.10	2.49±0.10	0.514
Protein (%)	24.04±0.01	24.06±0.07	24.07±0.07	0.948
Collagen (%)	0.89±0.02	0.90±0.01	0.89±0.01	0.917

Abbreviations: CWT, carcass weight; BF, back fat thickness; WHC, water-holding capacity; MC_L, CIE_lightness; MC_a, CIE_redness; MC_b, CIE_yellowness; SF, shear force; IMF, intramuscular fat content;
*, $P < 0.05$.

genotypes (P=0.027). However, the *g.34341 A>G* was not a source of variability for the other traits. The *PKM2* gene encodes the muscle isoform of a rate-limiting enzyme that catalyses the conversion of phosphoenolpyruvate to pyruvate in the final step of glycolysis (Cairns et al, 2011) and has also been reported to have a role in muscle glyconeogenesis (Gleeson, 1996). In addition, this gene is on porcine chromosome 7, where several studies have indicated the presence of a QTL that affects meat quality traits. *PKM2* is a strong candidate for meat quality in this region and has also been reported to impact back fat thickness as a powerful candidate gene (Fontanesi et al., 2008).

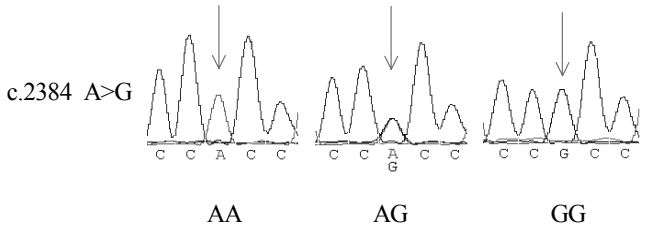

Fig. 1. Sequencing results and polymorphic sites found in the 3' UTR region of the porcine *PKM2* gene in Berkshire pigs. Adenine of the start codon ATG is considered +1 (Ensembl: ENSSSCG-00000001930).

CONCLUSION

The purpose of this study was to find a molecular marker for the improved meat-quality using candidate gene analysis. The *g.34341 A>G* SNP within the 3' UTR of the *PKM2* gene was significantly associated with BF ($p < 0.05$), an economically important trait in pigs. The potential advantage

of marker-assisted selection would be reduced costs for sib testing after slaughter, a reduction in sophisticated meat-quality measurements, as well as additional improvements in meat-quality by early information from genetic markers (Ovilo et al. 2006). However, although the results of this study provide evidence for the potential of SNP marker-assisted selection of Berkshire pigs, the effects of SNP markers need to be compared to the meat-quality traits of pigs carrying different genotypes, as the effects of an allele may vary between pig populations. In addition, since the SNP is located in the 3' UTR, it is difficult to determine the direct effect of the *PKM2* genotypes on meat-quality traits. Whether the association is due to the candidate gene requires further verification and association studies in other regions are also needed.

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