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Association of GHRH, H-FABP and MYOG Polymorphisms with Economic Traits in Pigs

Eun Seok Cho, Da Hye Park[a], Byeong-Woo Kim[1], Won Youg Jung, Eun Jung Kwon and Chul Wook Kim*

Department of Animal Resources Technology, Jinju National University

Chilamdong 150, Jinju, Gyeongnam 660-758, Korea

ABSTRACT : The study of candidate genes is an important tool to identify genes associated with economic traits. Three genes were selected to study the association between polymorphism and economic traits and breed of pigs. Growth hormone releasing hormone (GHRH) takes part in growth metabolism and is one of the candidate genes known to be highly associated with economic traits in pigs. Heart fatty acid-binding protein (H-FABP) is related to back fat thickness and intramuscular fat (IMF) content, and myogenin (MYOG) is associated with the amount of growth rate and lean yield in pigs. By PCR-RFLP analysis, the association between the genotypes of the three genes and the average daily gain, back fat thickness, feed conversion, body length and meat percent in 352 pigs (112 Duroc pigs, 132 Landrace pigs and 108 Yorkshire pigs) were analyzed. GHRH polymorphisms showed differences depending on breed ($p<0.01$) and were associated with meat percent. H-FABP polymorphisms also showed significant differences among breeds and sex ($p<0.01$), and were highly associated with average daily gain, feed conversion and back fat thickness ($p<0.01$) and even showed an association with meat percent ($p<0.05$). However, the MYOG gene showed no significant effect in this study. These results reconfirmed that GHRH and H-FABP are potential major genes or markers for economic traits. (**Key Words :** GHRH, H-FABP, MYOG, Genotype)

INTRODUCTION

Studies on the association of porcine candidate genes with economic traits are very important. Until now, a considerable number of gene mapping studies have been performed and constant efforts have been made to clarify the association between the genes and economic traits (Alfonso et al., 2004, 2005). These genes are potential candidate markers because of their important physiological effects associated with economic traits (Franco et al., 2005). Such a marker with a known association between a DNA polymorphism and the important traits can be included in marker-based selection.

The GHRH (growth hormone releasing hormone) gene takes part in growth metabolism according to interaction with various interdependent genes, such as GH (growth hormone), IGF1 (insulin-like growth factor 1), PIT1 (pituitary-specific transcription factor 1), GHRHR (growth hormone releasing hormone receptor) and GHR (growth hormone receptor) (Cogan and Phillips, 1998; Xia et al., 2007). This gene is also known to regulate the release of GH. It is located in SSC 17 (Baskin and Pomp, 1997), and is known to be associated with back fat thickness and average daily gain due to *Alu*I RFLP polymorphism (Franco et al., 2005).

The H-FABP gene is a member of the fatty acid-binding protein (FABP) family that comprises a group of small cytosolic proteins that specifically bind and intracellularly transport fatty acids and other hydrophobic ligands (Veerkamp and Maatman, 1995). In addition, FABP may regulate lipid metabolism and other cellular processes such as gene transcription, cellular signaling, growth and differentiation. For the H-FABP gene, *Msp*I, *Hae*III and *Hin*fI RFLP polymorphisms are known and of these, MspI polymorphism is known to have high associations with IMF (intramuscular fat) content in pigs (Gerbens et al., 1999; Nechtelberger et al., 2001).

The MYOG gene is a member of the MyoD gene family (myogenin, MyoD1, myf-5, and myf-6) which acts on myogenesis (Olson, 1990; Weintraub et al., 1991), synthesizes myofibrillar proteins in the skeletal muscles and

* Corresponding Author: C. W. Kim. Tel: +82-55-751-3289, Fax: +82-55-759-1893, E-mail: cwkim@jinju.ac.kr

[1] Division of Applied Life Science, Institute of Agriculture and Life Sciences, Gyeongsang National University, Gazwa-dong 900, Jinju-city, Gyeongnam 660-701, Korea.

[a] This author equally contributed to this work.

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regulates the number of their myofibers. It is also expressed in all the phases of myoblast differentiation to proliferation (Te pas et al., 1999). Accordingly, it is closely associated with the number of muscle fibers at birth which is most important in determination of maximal lean meat growth capacity in pigs (Hadel and Stkckland, 1988). The *Msp*I polymorphic site of this gene is known to affect myoblasts and number of myofibers taking part in muscle development and growth.

In this study, amongst the many known genes associated with economic traits in pigs, the porcine GHRH, H-FABP and MYOG genes known to have a high association with growth or backfat thickness were selected. We investigated the association between RFLP polymorphism in the genes for GHRH, H-FABP, and MYOG and the economically important traits of pigs.

MATERIALS AND METHODS

Animals and data collection

A total of 352 pigs (112 Duroc, 132 Landrace, 108 Yorkshire) from the 2nd Porcine Performance Testing Station of the Korea Swine Testing Association, were used in this study. The trait data measured in these pigs included body length (BL), average daily gain (ADG) until a body weight of 30-90 kg is reached, back fat thickness (BFT, Mode A) meat percent (MP, PIGLOG 105, SFK-technology, Denmark), and feed conversion ratio (FC); all recorded data were adjusted for age (day) and backfat thickness to 90 kg live weight.

Each animal was scanned by Piglog105^{TM} ultrasound machine in mode A. For backfat thickness (BFT), three scanned points were used at P1 (shoulder), P2 (mid-back) and P3 (loin), adapted from Iowa State University. The average of the three measurements were calculated and adjusted to 90 kg. FC was computed as percent (%) from feed intake divided by body weight gain.

To compute meat percent (MP), the backfat at the 10th rib and 10 cm before the last lumbar vertebrae were measured first then the depth of loin muscle area (LMA) measured at the 10th rib point and Piglog 105^{TM} automatically calculated the MP.

PCR-RFLP analysis

Blood was collected, treated with EDTA, and genomic DNA was isolated with a DNA Wizard genomic DNA purification kit (promega, USA).

The GHRH, FABP and MYOG genes were genotyped by PCR-RFLP. The *Alu*I GHRH PCR-RFLP has been described previously and a 455-bp fragment was obtained by PCR (Baskin and Pomp, 1997). A pair of primers designed by Alfonso and Arena (2004) was used to genotype the *Msp*I H-FABP. A pair of primers designed by Te pas et al. (1999) and Soumillion et al. (1997) was also used to genotype the *Msp*I MYOG.

PCR conditions for the three respective genes were identical in this study. The amplification of PCR was carried out in a total volume of 25 μl reaction buffer (Promega, USA) containing 1.5 mM $MgCl_2$; 10 pmol of primer; 1 U Taq polymerase; 0.5 mmol/L dNTPs; 50 ng DNA sample; and ddH_2O. The cycling conditions consisted of denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 s, 62°C for 30 s, 72°C for 60 s, and then finally extension at 72°C for 5 min (GeneAmp PCR system 9600/9700, Applied Biosystems, USA).

The amplified products for GHRH were digested with *Alu*I restriction endonuclease, separated on a 4% agaorose gel, and visualized under UV light following ethidium bromide staining. A 455-bp PCR product and two GHRH alleles were identified (A, 250 bp+100 bp; and B, 230 bp+100 bp) and each animal was classified as AA, AB, or BB with respect to GHRH genotype. The amplified products for H-FABP and MYOG genes were digested with *Msp*I restriction endonuclease, separated on a 2.5% agarose gel. A 412-bp PCR product and two H-FABP alleles were identified (D, 322 bp+90 bp; and R, 412 bp only) and each animal was classified as DD, DR, or RR with respect to the H-FABP genotype. A 353-bp PCR product and two MYOG alleles were identified (A, 353 bp only; B, 219 bp+134 bp) and each animal was classified as AA, AB, or BB with respect to MYOG genotype.

Statistical analysis

To clarify the associations between genotype and economic traits for each of the three genes, statistical analysis was performed with SPSS 12.0 version. A GLM (general linear model) was performed to ascertain the association between genotype and economic traits and subsequently, Bonferroni's multiple range test was carried out to test the statistical significance. The general linear model with fixed effects was assumed to be:

$$Y_{ijkl} = \mu + S_i + Breed_j + Genotype_k + e_{ijkl}$$

Where Y_{ijkl} corresponds to the trait observed (BL, ADG, FC, BFT, and MP) in the k-th genotype group, μ is an overall mean, S_i is fixed effect of i-th sex (male and female), $Breed_j$ is fixed effect of j-th breed (Landrace, Duroc, Yorkshire), $Genotype_k$ represents the GHRH (k = AA, AB, BB), H-FABP (k = DD, DR, RR), and MYOG (k = AA, AB, BB) genotypes, and e_{ijkl} is the random error effect of each individual pig.

The Chi-square test was used to verify the significance of differences between genotypic frequencies in each of the

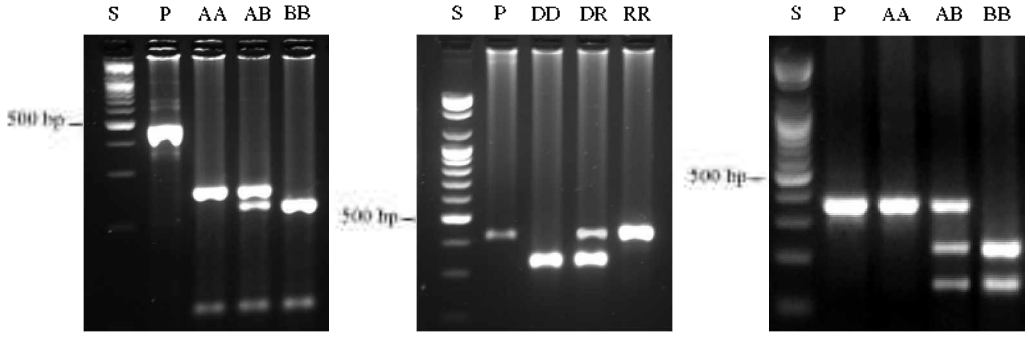

Figure 1. AluI restriction fragment length polymorphism in porcine GHRH PCR products electrophoresed in a 4% agarose gel (A). MspI restriction fragment length polymorphism in porcine H-FABP (B) and MYOG (C) PCR products electrophoresed in a 2.5% agarose gel. S; 100 bp ladder DNA size marker, P; uncut PCR product, AA, AB, BB, DD, DR, and RR; genotypes of genes.

three genes and different breeds and sex.

RESULTS AND DISCUSSION

Results of genotype analysis

The *Alu*I GHRH PCR products were divided into AA genotype where 250 bp and 100 bp bands appeared; BB genotype where 230 bp and 100 bp bands appeared; and AB type where 250 bp, 230 bp and 100 bp bands appeared (Figure 1A). The GHRH gene regulates the release of growth hormones and its structure and functions were known in the 1980s after it came to the fore in research in the early 1950s (Kim, 1990). The human and mouse GHRH cDNAs were cloned in pigs (Moody et al., 1995) and AluI GHRH polymorphism was identified, so that association studies with growth have been conducted. Genotypic and allelic frequencies (52.3% and 71.3%, respectively) at the *Alu*I GHRH polymorphism had higher BB genotype and B allele in a total of 352 pigs. As previous research of Franco et al. (2005) showed, genotype AA and allele A (12.6% and 37.4%) appeared low.

The *Msp*I H-FABP PCR-RFLP was identified DD (322 and 90 bp), RR (412 bp only) and DR genotype (412, 322, and 90 bp) (Figure 1B). As a candidate gene associated with porcine fatness, this gene is characterized by known polymorphisms at the three variant restriction sites including *Hinf*I in the 5'-upstream region, and *Hae*III and MspI in intron 2. Of these, *Msp*I H-FABP polymorphism is known to be associated with IMF content and backfat thickness thereby affecting the quality of meat (Gerbens et al., 1999; Nechtelberger et al., 2001). Genotypic and allelic frequencies (65.3% and 67.2%, respectively) at the *Msp*I H-FABP polymorphism had higher DD genotype and D allele.

In the case of the MYOG gene, when the 353 bp PCR products were digested with *Msp*I restriction enzyme, AA (353 bp), BB (219 and 134 bp) and AB types (353, 219, and 134 bp) appeared (Figure 1C). In this study, *Msp*I MYOG had higher BB genotypic frequencies (62.5% and 77.7%, respectively). The MYOG gene belongs to the MyoD gene family and is known to have associations with growth and meat deposition performance of pigs. The observed frequencies of the MYOG gene are in disagreement with earlier results (Ernst and Davis, 1995; Te pas et al., 1999; Anton et al., 2002; Verner et al., 2007). This discrepancy is supposed due to difference of breed (Large White) of experimental animals which were used.

Association between breed or sex and genotype

The mean and standard deviation (SD) of the traits for all pigs according to breed and sex are shown in Table 1. Of the 5 traits, ADG, FC, and BFT differed significantly among the breeds. ADG, FC and MP values, out of the 5 traits, appeared highest in Duroc and conversely, BFT appeared highest in Landrace. Four traits, except for MP, differed significantly according to sex.

As known usually, growth rate of males appeared higher

Table 1. Means and standard deviations of traits in studied group of pigs

		Total	BL (cm)	ADG (g)	FC	BFT (cm)	MP (%)
		(352)	M±SD	M±SD	M±SD	M±SD	M±SD
Breed	Duroc	112	106.09±0.37	1,155.88±89.96^{a}	2.20±0.17^{b}	1.35±0.14^{b}	58.14±2.38
	Landrace	132	106.00±.082	982.43±84.63^{b}	2.43±0.13^{a}	1.50±0.24^{a}	56.78±3.09
	Yorkshire	108	105.98±0.33	1,012.47±101.32^{b}	2.38±0.13^{a}	1.38±0.19^{b}	57.99±2.59
Sex	X	152	105.84±0.69	936.91±45.01	2.48±0.09	1.52±0.23	57.14±3.15
	Y	200	106.16±0.42	1,128.11±89.02	2.24±0.15	1.34±0.15	57.92±2.44

Different letters in the same row indicated a significant difference for each traits.

Table 2. Frequencies of GHRH, H-FABP and MYOG genotypes according to each breeds and sex in studied groups of pigs (N, %)

Gene	Genotype	Total	Breeds			X^2	Sex		X^2
			DD	LL	YY	(p)	X	Y	(p)
GHRH	AA	34 (9.66)	7 (6.25)	11 (8.33)	16 (14.81)	31.58**	16 (10.53)	18 (9.00)	1.712
	AB	134 (38.07)	47 (41.96)	31 (23.48)	56 (51.85)		52 (34.21)	82 (41.00)	
	BB	184 (52.27)	58 (51.79)	90 (68.18)	36 (33.33)		84 (55.26)	100 (50.00)	
H-FABP	DD	230 (65.34)	7 (6.25)	124 (93.94)	99 (91.67)	280.23**	133 (87.50)	97 (48.50)	63.550**
	DR	13 (3.69)	3 (2.68)	6 (4.55)	4 (3.70)		6 (3.95)	7 (3.50)	
	RR	109 (30.97)	102 (91.07)	2 (1.52)	5 (4.63)		13 (8.55)	96 (48.00)	
MYOG	AA	25 (7.10)	9 (8.04)	8 (6.06)	8 (7.41)	6.04	9 (5.92)	16 (8.00)	1.380
	AB	107 (30.40)	37 (33.04)	31 (23.48)	39 (36.11)		43 (28.29)	64 (32.00)	
	BB	220 (62.50)	66 (58.93)	93 (70.45)	61 (56.48)		100 (65.79)	120 (60.00)	
Total		352 (100.00)	112 (31.82)	132 (37.50)	108 (30.68)		152 (43.18)	200 (56.82)	

** $p<0.01$.

than females, while females appeared high in BFT. These results confirmed that breed and sex basically influence economic traits.

To clarify the association between breed/sex and genetic polymorphism, statistical analysis was performed with the studied group of pigs comprised of 3 different breeds (112 Duroc, 132 Landrace, and 108 Yorkshire) or sex (152 female and 200 male) (Table 2).

Examination of the association between breed and the GHRH gene showed a higher frequency of AB genotype (51.8%) in Yorkshire, whereas Duroc and Landrace had higher BB genotypic frequency (51.8% and 68.2%, respectively), which suggests that there is significantly association between breed and the *Alu*I GHRH polymorphism ($x^2 = 31.58$, $p = 0.000$).

It was also found that the H-FABP gene was significantly associated with breed ($x^2 = 280.23$, $p = 0.000$). While for Landrace and Yorkshire, the frequency of H-FABP DD genotype was 93.9% and 91.7%, respectively, for Duroc the percent of RR was 91.1%.

Examination of the association between breed and the MYOG gene, showed higher frequency of BB genotype for all of the three breeds, Yorkshire, Landrace, and Duroc, (56.48%, 70.45% and 58.93%, respectively), which suggests that there is no significant association between breed and *Msp*I MYOG polymorphism ($x^2 = 6.04$, $p = 0.197$).

The polymorphisms of the GHRH, H-FABP, and MYOG genes employed in this study were highly associated with breed. Although this finding cannot be compared with any other study, it is surely very important that genetic variation is closely relevant to breed. In frequency of the 3 genes with Sex, only H-FABP showed a difference ($x^2 = 63.55$, $p = 0.001$). The frequency of the BB genotype of the GHRH gene appeared higher than AA independently of sex. In the case of the H-FABP gene, difference of genotype frequency by sex can influence measured economic traits. Therefore, this result may influence association analysis of traits by genotype.

Association between economic traits and genotype

The association between genotype and economic traits

Table 3. Least square means and standard deviations of the RFLP genotypes at GHRH, H-FABP and MYOG loci in economic traits of pigs

Gene	Genotype	N	LS means±SE				
			BL (cm)	ADG (g)	FC	BFT (cm)	MP (%)
GHRH	AA	34	106.03±0.10	1,026.41±20.32	2.37±0.03	1.39±0.04	$58.46±0.48^{A}$
	AB	134	105.99±0.05	1,059.34±10.24	2.32±0.12	1.40±0.02	$57.83±0.24^{AB}$
	BB	184	106.05±0.04	1,041.51±8.74	2.35±0.01	1.44±0.02	$57.24±0.20^{B}$
	F-value		0.48	1.44	1.76	1.83	3.63*
H-FABP	DD	230	106.01±0.04	$1,002.44±6.63^{B}$	$2.40±0.01^{A}$	$1.44±0.01^{A}$	$57.39±0.18^{B}$
	DR	13	105.77±0.16	$1,043.54±27.87^{B}$	$2.35±0.04^{A}$	$1.48±0.06^{AB}$	$56.61±0.77^{AB}$
	RR	109	106.08±0.06	$1,140.93±9.62^{A}$	$2.22±0.02^{B}$	$1.36±0.02^{B}$	$58.11±0.27^{A}$
	F-value		1.94	70.26**	50.74 **	5.50 **	3.33*
MYOG	AA	25	106.00±0.12	1,043.00±23.77	2.34±0.04	1.38±0.04	56.97±0.56
	AB	107	106.02±0.06	1,057.58±11.48	2.33±0.02	1.40±0.02	57.84±0.27
	BB	220	106.03±0.04	1,042.05±8.01	2.35±0.01	1.43±0.01	57.53±0.19
	F-value		0.03	0.63	0.48	1.24	1.09
Total		352	106.01±0.58	1,045.32±118.24	2.35±0.18	1.41±0.21	57.59±2.80

Different letters in the same row indicated a significant difference for each trait (* $p<0.05$, ** $p<0.01$).

investigated in 352 breeding pigs was analyzed (Table 3). Significant differences were observed among the genes for traits, except for the BL variable.

The GHRH gene was highly associated with genotype concerning MP; AA genotype indicated 1.23% higher MP than the BB genotype ($p<0.05$). However, there was no significance for all the other economic traits in this study, although the GHRH gene was associated with BFT and ADG (Mauricio et al., 2005). The association analysis revealed that pigs with the *Alu*I AA genotype had a higher ADG ($p = 0.0001$), whereas the *Alu*I BB genotype was associated with a higher EPD FT ($p = 0.0004$) (Mauricio et al., 2005). Since MP has high association with ADG, it is considered reasonable to presume the association. Actually, this did not appear significantly, but ADG and BFT value of the BB type appeared high.

For the H-FABP gene, there was high association between *Msp*I RFLP polymorphism and the economic traits. Especially, the polymorphism was highly associated with ADG, FC and BFT ($p<0.01$), and associated even with MP ($p<0.05$). In other words, the RR genotype showed significantly higher ADG than that of the DR and DD ($F = 70.26^{**}$); regarding feed conversion, the DD and DR displayed significantly higher FC than that of RR ($F = 50.74^{**}$); and in respect to BFT, the DR genotype exhibited significantly higher BFT than that of RR ($F = 5.50^{**}$). Although the H-FABP gene is known to have high association with IMF content, it was impossible to ascertain such an association because IMF content was not determined. However, the BFT results obtained in this study were consistent with those in previous studies (Gerbens et al., 1999; Nechtelberger et al., 2001), and H-FABP was associated with ADG, MP and FC as well. Thus, these findings suggest that polymorphism in these genes has a significant effect on the economic traits in pigs.

For the MYOG gene, there was no significant effect of *Msp*I polymorphic sites on the economic traits in this study. Te pas et al. (1999) observed that in Large White pigs the BB genotype was associated with increased birth weight, growth rate and LMC. Cieslak et al. (2000) noted that in Large White the AA genotype was associated with higher half-carcass weight, LMC, ham meat weight, loin meat weight and loin eye area. However, it was revealed that the MYOG BB genotype increased pig birth weight, and increased growth rate and lean weight of the pig without affecting BFT. It was suggested to improve the traits through myogenin-marker-assisted selection due to its very low frequency (Te pas et al., 1999). Nevertheless, association with economic traits and genotype of MYOG was not significant in the current research. Because frequency of the BB type was very high, but AA type was low, this study was not consistent with previous results. Additional experimental animals will be required to repeat these association analyses between economic traits and candidate genes to confirm our results.

There was a significant difference between genotype of the three genes and species. For instance, H-FABP RR genotype was found to be dominant in Duroc, and the DD genotype to be dominant in Landrace and Yorkshire. Such results may cause a serious bias in evaluating genotypic effects. Difference in characters between genotypes may result from difference in species.

This study attempted to investigate the association of the candidate genes GHRH, H-FABP and MYOG with economic traits in pigs. As a result, their high association with economic traits was seen, although some of the results of this study were inconsistent with those reported previously, perhaps because of different experimental animals. Based on the present results, we plan to analyze the effect of genes on traits with a larger number of animals in further studies so that the results will be able to be applied for marker-assisted selection in pigs.

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