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EFFECTS OF *LEP*, *GYS1, MYOD1*, AND *MYF5* POLYMORPHISMS ON PIG ECONOMIC TRAITS*

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Abstract

In the present study, we examined the effect of single nucleotide polymorphisms (SNPs) of leptin (*LEP*), skeletal muscle glycogen synthase (*GYS1*), myogenic differentiation 1 (*MYOD1*), and myogenic factor 5 (*MYF5*) genes on economic trait association in pigs. *LEP/Hin*dIII, *MYOD1/Dde*I, *MYF5/Fok*I, and *GYS1/Fok*I genotypes were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) from 466 pigs comprised of Duroc, Landrace and Yorkshire breeds. The *LEP/Hin*dIII polymorphism differed significantly with respect to average daily gain (ADG) in Duroc pigs (P<0.05). However, the *GYS1/Fok*I polymorphism was not significantly associated with any trait. The *MYOD1/Dde*I polymorphism was significantly associated with both ADG and meat percentage (MP) in Duroc pigs, and ADG, backfat thickness (BFT) and feed efficiency (FE) in Landrace pigs, whereas the *MYOD1/Dde*I polymorphism was not significantly associated with any trait in Yorkshire pigs. In addition, the *MYF5/Fok*I polymorphism revealed a close relationship with ADG in Duroc pigs. In conclusion, we believe that the SNPs within *LEP*, *MYOD1* and *MYF5* in certain pig breeds play important roles as potential genetic markers for economic traits of pigs.

Key words: *LEP, GYS1, MYOD1, MYFS,* economic traits

The pig (*Sus scrofa*) is economically important livestock for meat production. Meat quality is associated with various economic traits, such as average daily gain (ADG), meat percentage (MP), backfat thickness (BFT), and feed efficiency (FE). Since single nucleotide polymorphisms (SNPs) are the most abundant type of DNA variation in the vertebrate genome and suitable for genetic markers in the laboratory

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and data analysis, SNPs are widely used for genetic selection to improve meat quality in modern pig breeding (Matsumoto et al., 2012).

Leptin, a product encoded by the LEP gene, is one of the most important adipose tissue-derived hormones that regulates the body's fat storage system and mediates a signal for gastric fullness in the hypothalamus to regulate body weight and energy expenditure (Garcia-Galiano et al., 2014; Park and Ahima, 2014). The level of LEP mRNA in adipose tissue from obese pigs was determined to be higher than that from lean pigs (Piórkowska et al., 2011). In addition, the LEP gene has been reported as a candidate marker for important economic traits used to improve pork production, including growth, obesity and saturated fatty acid content in fat by increasing feed intake (Perez-Montarelo et al., 2012). The porcine LEP gene reportedly contains various polymorphisms within the 3'-untranslated region (UTR), such as c.846C>T, which is weakly associated with abdominal fat weight, and c.2863G>A, which is associated with backfat thickness (Liu et al., 2011; Mankowska et al., 2015).

The glycogen synthase (*GYS1*) gene, which encodes an enzyme that mediates glycogen biosynthesis in skeletal muscles, has been reported as a candidate gene affecting skeletal muscle in pig (Wang et al., 2012 a; Zuo et al., 2005, 2007). GYS1 mRNA is expressed in various organs, especially skeletal muscle (Zuo et al., 2005). Furthermore, the stringent regulatory mechanism of glycogen metabolism in muscles makes it a prime biological candidate gene for production traits (Wang et al., 2012 b).

The *MyoD* gene is a myogenic regulatory factor (MYF) known as a critical regulator of muscle differentiation. The MRF family is composed of four components: *MYOD1* (*MYOD1*; chromosome 2), *MYOG* (*MYF4*, myogenin; chromosome 9), *MYF5* (chromosome 5), and *MYF6* (*MRF4/herculin*; chromosome 5). These highly conserved genes encode basic helix-loop-helix proteins that regulate embryonic muscular development (Acharjee et al., 2014; Daou et al., 2013). *MYOD1* and *MYF5* are predominantly expressed during proliferation of myoblasts and muscle precursor cells and play roles as master transcription factors in myogenesis (Acharjee et al., 2014; Daou et al., 2013). Polymorphisms within the *MYOD1* promoter region influence transcriptional activity. In addition, variation within the *MYOD1* gene affects muscle fiber characteristics and meat quality traits (Bongiorni et al., 2014; Lee et al., 2012). Moreover, SNPs within *MYOD1* show significant effects on mRNA expression (Lee et al., 2012).

The *MYF5* gene is expressed during embryonic myogenesis as a core transcriptional factor involved in muscle development (Zhao et al., 2011). Myoblasts proliferate under MYF5 regulation (Verner et al., 2007). Genetic lineage tracing indicates that classical brown adipocytes and skeletal muscles are derived from MYF5-expressing progenitors, while white and beige adipocytes predominantly originated from non-MYF5 lineage progenitors (Seale et al., 2008). Statistically significant associations have been observed between *MYF5* gene polymorphisms and intramuscular fat (IMF)/lean meat content (LMC) in *longissimus dorsi* pH (Liu et al., 2008; Verner et al., 2007).

Polymorphisms within *LEP*, *GYS1*, *MYOD1*, and *MYF5* genes have been reported as candidate molecular markers that may exhibit various associations with

economic traits of pig, as described above. Thus, studies focusing on polymorphisms within these genes have continued to improve meat production via analysis of the association between SNP genotypes and meat quality traits. In our previous study, we reported that SNPs within *GHRH*, *H-FABP* and *MYOG*, which induce productivity, showed significant associations with certain pig economic traits (Cho et al., 2009). Similarly, it is assumed that polymorphisms within *LEP*, *GYS1*, *MYOD1*, and *MYF5* identified in our previous studies maintain significant associations with economic traits in Duroc, Landrace and Yorkshire pigs.

In the study described herein, polymorphisms within *LEP*, *GYS1*, *MYOD1*, and *MYF5* genes were characterized. We also analyzed associations between these polymorphisms and economic traits, including BL, ADG, FC, BFT, and MP, from three pig breeds, Duroc, Landrace and Yorkshire. Moreover, the SNPs investigated in this study will likely contribute to improved economic production in the pig industry.

Material and methods

Animals and data collection

A total of 466 pigs, including 180 Duroc (male, 178; female, 2), 132 Landrace (male, 50; female, 82) and 154 Large Yorkshire (male, 78; female, 76), were used in this study, which were released from the 2nd Porcine Performance Testing Station of the Korea Swine Testing Association. Economic trait data, which were obtained from their pigs, included body length (BL), average daily gain (ADG) until a body weight from 30 to 90 kg was reached, backfat thickness (BFT), meat percentage (MP, PIGLOG 105, SFK-technology, Denmark), and feed conversion ratio (FC). BL, ADG, FC, BFT, and MP traits were determined in the same manner as in previous studies (Cho et al., 2009). Briefly, animals were allowed free access to unlimited fresh water during the experimental periods. The experimental pigs were provided with unlimited feed every day, and the following day, residual feed was analyzed for feed efficiency. Initial and finished body weights (IBW and FBW) were measured for ADG. FC was calculated as food intake amount divided by body weight gain.

For BFT measurements, each animal was scanned using a Piglog105TM ultrasound machine in mode A. Three scanned points, P1 (shoulder), P2 (mid-back) and P3 (loin), were employed (Iowa State University). The average of these three measurements served as the BFT value. The amount of MP was calculated from BFT measurements at the last and 10th rib and 10 cm before the last lumbar vertebrae, and the depth of loin muscle area (LMA) at the 10th rib. MP was automatically calculated by Piglog 105TM.

PCR- RFLP analysis

Genomic DNA was isolated from blood using a Wizard Genomic DNA purification kit according to the manufacturer's instructions (Promega, USA). Each primer pair used to detect polymorphisms within *LEP*, *GYS1*, *MYOD1*, and *MYF5* genes is presented in Table 1. The PCR reaction solution was composed of 1X PCR buffer,

100 μM dNTPs, 0.5 μM primer pair, 50-ng genomic DNA and ddH_2O. PCR amplification was performed by initial denaturation at 95°C for 5 min, followed by 35 cycles consisting of annealing at 95°C for 1 min, 55°C for 1 min (except for LEP, which was performed at 66°C) and 72°C for 1 min, and an elongation step at 72°C for 5 min (GeneAmp PCR system 9600/9700, Applied Biosystems, USA). Amplified PCR products were digested by *Hin*dIII (New England BioLabs) for *LEP*, *Fok*I (New England BioLabs) for *GYS1* and *MYF5*, or *Dde*I (New England BioLabs) for *MYOD1*. Their genotypes (designated 'A' and 'B') were identified using 2.5% agarose gel electrophoresis.

Table 1. Chromosomal position, primer sequence, and sizes of PCR products to each gene

Gene	Chr position	Sequence (5'→3')	Product (bp)
LEP	*Chr18: 21,204,710G>A/ HindIII* 3 UTR	F : ttggagagcctgggcggcgt R : GAAACAGCACCTCGGGAGCC	658
GYS1	*Chr6:50,085,657 G>A/ FokI* 6th intron	F : TATGAGTTCTCCAACAAGGG R :GATGAAGAAAGCAACCACTGT	398
MYOD1	*Chr2:44,483,806 C>A/ DdeI* 1st intron	F : CGGATGCGATCCATCCGAT R : CCCTGTATCATCTCCCAGG	479
MYF5	*Chr5:105,703,640 C>T/ FokI* upstream	F : TTGAGAAGGCAGGTCAGCT R : GGTTGGGGCCCTTTATATG	376

Statistical analysis

The general linear model procedure was used to analyze the association between genotypes and traits using Statistical Software Package version 9.1.3 (SAS, 2004).

The linear model was as follows:

$$Y_{ijklmn} = \mu + B_i + G_j + BG_{ij} + S_k + W_l + e_{ijklmn}$$

where:

Y_{ijklmn} represents the phenotypic values of the target trait (BL, ADG, FC, BFT, and MP),

μ represents the general mean,

B_i represents the fixed effect of breed *i* (Landrace, Duroc, Yorkshire),

G_j represents the *LEP*, *GYS1*, *MYOD1*, and *MYF5* (j = AA, AB, BB) genotypes,

BG_{ij} represents the interaction effect in breed *i*,

S_k represents the fixed effect of sex *k*,

W_l represents the fixed effect of measurement weight *l*,

e_{ijklmn} represents random error.

When significant differences were detected, the mean values were separated by the probability difference option. The results are presented as least square means with standard error (SE). Duncan's multiple range tests (MRT) were employed to verify significant differences ($P<0.05$) between genotype and economic traits.

Results

PCR-RFLP analysis of *LEP*, *GYS1*, *MYOD1*, and *MYF5* polymorphisms

To analyze *LEP*, *GYS1*, *MYOD1*, and *MYF5* genotypes, PCR-RFLP was performed with 466 pigs comprised of Landrace, Duroc and Yorkshire breeds (Figure 1). DNA segments including a transition mutation c*hr18: 21,204,710G>A/ Hin*dIII in the 3'-UTR of the *LEP* gene was identified (658 bp in length) via PCR amplification (Table 1). PCR products were digested by *Hin*dIII, and digested DNA fragments were separated into three lengths. The three genotypes are presented in Figure 1A. The *AA* genotype was represented by an uncut PCR product (658 bp), while the *BB* genotype was characterized by two digested fragments (492 and 166 bp), and the *AB* genotype was shown as three digested fragments (658, 492 and 166 bp) (Figure 1A).

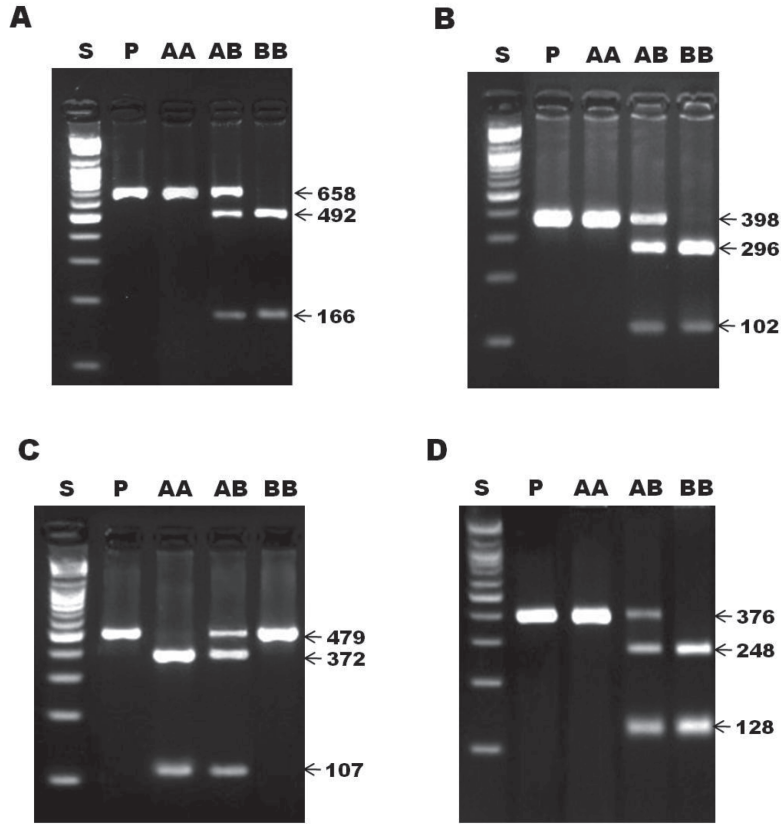

Figure 1. Analyses of PCR-RFLPs to *LEP* (A), *GYS1* (B), *MYOD1* (C) and *MYF5* (D). PCR products amplified from *LEP*, *GYS1*, *MYOD1* and *MYF5* genes were digested by restriction enzymes *Hin*dIII, *Fok*I, *Dde*I and *Fok*I, respectively. The digested DNA fragments were electrophoresed in a 2.5% agarose gel. S; 100 bp ladder DNA size marker, P; uncut PCR products. (A) P and AA; 658 bp, BB; 492+166 bp, AB; 658+492+166 bp in *LEP* gene, (B) P and AA; 398 bp, BB; 296+102 bp, AB; 398+296+102 bp in *GYS1* gene, (C) P and BB; 479 bp, AA; 372+107 bp, AB; 479+372+107 bp in *MYOD1* gene, and (D) P and AA; 376 bp, BB; 248+128 bp, AB; 376+248+128 bp in *MYF5* gene

To identify the *chr6:50,085,657 G>A*/*Fok*I polymorphism within the *GYS1* gene intron 6 (Table 1), PCR products were detected by a 398-bp DNA fragment, and the three genotypes were characterized by digestion of the products with *Fok*I (Figure 1B). The *AA* genotype was represented by an uncut PCR product (398 bp), and the *BB* genotype was represented by two digested fragments (296 and 102 bp). The *AB* genotype was shown as three digested fragments (398, 296 and 102 bp) (Figure 1B).

A transversion mutation within *chr2:44,483,806 C>A/Dde*I, located in intron 1 of the *MYOD1* gene, was established by digestion of the PCR products (479 bp) with *Dde*I (Table 1). The *AA* genotype was represented by an uncut PCR product (479 bp) (Figure 1C). The *BB* genotype was indicated by two fragments (372 and 107 bp), and the *AB* genotype was characterized by three fragments (479, 372 and 102 bp) (Figure 1C).

The transition mutation *chr5:105,703,640 C>T/* contains a *Fok*I site upstream of the *MYF5* gene (Table 1). PCR products were 376 bp in length and three genotypes were identified by digestion with *Fok*I. The *AA* genotype was represented by an uncut PCR product (376 bp), while the *BB* genotype was represented by two fragments (248 and 128 bp). The *AB* genotype was characterized by three fragments (376, 248 and 128 bp) (Figure 1D).

LEP, *GYS1*, *MYOD1*, and *MYF5* polymorphisms are significantly associated with pig economic traits

Since selection of pig breeds is one of the most important factors in determining profit, we analyzed the association between pig breed and economic traits. The three pig breeds investigated in this study were analyzed to identify an association with economic traits, as shown in Table 2. Duroc pigs maintained improved values with respect to ADG, FC, BFT, and MP compared with the other breeds. Landrace pigs showed the highest BL value compared with Duroc and Yorkshire breeds, although the value of other economic traits was the poorest among all three breeds. Yorkshire was considered the intermediate breed with respect to all economic traits among the three breeds investigated (Table 2). Next, we investigated the genotypic and allelic frequencies for *LEP*, *GYS1*, *MYOD1*, and *MYF5* in each pig breed. The *B* allele of the *LEP* gene demonstrated the highest frequency among all three breeds, while the *A* allele of *GYS1* and *MYF5* presented the highest frequency. However, the frequency of both the *A* and *B* alleles of *MYOD1* was interspersed (Table 3).

Table 2. Association between each breed and economic traits

	n	BL (cm) M±SE	ADG (g) M±SE	FC M±SE	BFT (cm) M±SE	MP (%) M±SE
Duroc	180	106.07±0.03 a	1120.77±8.55 a	2.24±0.01 c	1.30±0.01 c	58.07±0.19 a
Landrace	132	107.05±0.14 b	984.26±8.63 c	2.38±0.01 a	1.50±0.02 a	55.81±0.28 c
Yorkshire	154	105.89±0.04 a	1010.00±9.03 b	2.35±0.01 b	1.42±0.02 b	57.32±0.26 b
Total	466	106.29±0.05	1045.50±5.80	2.32±0.01	1.40± 0.01	57.18±0.14

Different letters in the same row indicate a significant difference for each trait (*P<0.01).

BL – body length; ADG – average daily gain; FC – feed conversion ratio; BFT – backfat thickness; MP – meat percentage.

Table 3. Frequencies of genotypes and alleles to polymorphisms allocated at specific positions within each gene

Breed		*LEP*					*GYS1*					*MYOD1*					*MYF5*				
		genotype			allele		genotype			allele		genotype			allele		genotype			allele	
		AA	AB	BB	A	B	AA	AB	BB	A	B	AA	AB	BB	A	B	AA	AB	BB	A	B
Duroc	number	10	72	98	46	134	100	72	8	136	44	43	76	61	81	99	145	34	1	162	18
	frequency	5.6	40.0	54.4	25.6	74.4	55.6	40.0	4.4	75.6	24.4	23.9	42.2	33.9	45.0	55.0	80.6	18.9	0.5	90.0	10.0
Landrace	number	2	38	92	21	111	109	22	1	120	12	20	53	59	46.5	85.5	129	3	0	130.5	1.5
	frequency	1.5	28.8	69.7	15.9	84.1	82.6	16.7	0.7	90.9	9.1	15.1	40.2	44.7	35.2	64.8	97.7	2.3	0	98.9	1.1
Yorkshire	number	2	24	128	14	140	150	4	0	152	2	30	71	53	65.5	88.5	103	48	3	127	27
	frequency	1.3	15.6	83.1	9.1	90.9	97.4	2.6	0	98.7	1.3	19.5	46.1	34.4	42.5	57.5	66.9	31.2	1.94	82.5	17.5

Table 4. Analyses of association between economic traits in pigs and polymorphisms of *LEP* gene

Breed		N	BL (cm)	ADG (g/d)	FC	BFT (cm)	MP (%)
Duroc	AA	10	106.0±0.00	1187.4±22.24 a	2.21±0.04	1.23±0.06	58.3±0.62
	AB	72	106.2±0.08	1135.3±10.35 ab	2.23±0.01	1.30±0.02	58.6±0.34
	BB	98	106.0±0.01	1103.3±13.27 b	2.26±0.01	1.30±0.02	57.7±0.25

Different letters in the same row indicate a significant difference for each trait (P<0.05).
BL – body length; ADG – average daily gain; FC – feed conversion ratio; BFT – backfat thickness; MP – meat percentage.

Table 5. Analyses of association between economic traits in pigs and individual polymorphisms of *MYOD1* gene

Breed		N	BL (cm)	ADG (g/d)	FC	BFT (cm)	MP (%)
Duroc	AA	43	106.0±0.02	1093.5±19.62 b	2.25±0.02	1.31±0.02	57.5±0.39 b
	AB	76	106.1±0.06	1138.9±12.48 a	2.23±0.01	1.31±0.02	57.9±0.30 ab
	BB	61	106.7±0.51	1117.4±13.98 ab	2.26±0.02	1.26±0.02	58.7±0.33 a
Landrace	AA	20	106.7±0.51	935.6±16.93 b	2.42±0.02a	1.60±0.04 a	56.6±0.66
	AB	53	107.0±0.21	987.2±13.90 a	2.39±0.02 ab	1.45±0.03 ab	55.6±0.43
	BB	59	107.2±0.19	998.1±13.12 a	2.36±0.01 b	1.52±0.04 b	55.7±0.43

Different letters in the same row indicate a significant difference for each trait (P<0.05).
BL – body length; ADG – average daily gain; FC – feed conversion ratio; BFT – backfat thickness; MP – meat percentage.

Table 6. Analyses of association between economic traits in pigs and individual polymorphisms of *MYF5* gene

Breed		N	BL (cm)	ADG (g/d)	FC	BFT (cm)	MP (%)
Duroc	AA	145	106.1±0.04	1112.7±9.83 a	2.25±0.01	1.29±0.01	58.1±0.22
	AB	34	106.0±0.03	1161.9±14.08 a	2.21±0.02	1.32±0.03	58.1±0.40
	BB	1	106.0±0.00	896.0±0.00 b	2.40±0.00	1.19±0.00	55.9±0.00

Different letters in the same row indicate a significant difference for each trait (P<0.05).
BL – body length; ADG – average daily gain; FC – feed conversion ratio; BFT – backfat thickness; MP – meat percentage.

Table S1. Analyses of association between economic traits in pigs and polymorphisms of *LEP* gene

Breed		N	BL (cm)	ADG (g/d)	FC	BFT (cm)	MP (%)
Landrace	AA	2	106.5±1.50	998.4±98.40	2.44±0.04	1.68±0.02	54.1±4.80
	AB	38	106.7±0.24	978.7±13.95	2.38±0.01	1.53±0.04	55.4±0.56
	BB	92	107.2±0.17	986.2±10.90	2.38±0.01	1.49±0.03	56.0±0.31
Yorkshire	AA	2	106.0±0.00	1106.0±42.00	2.31±0.03	1.61±0.44	58.6±0.65
	AB	24	106.0±0.16	1059.0±25.72	2.30±0.03	1.40±0.04	57.4±0.41
	BB	128	105.9±0.03	999.3±9.50	2.36±0.01	1.42±0.02	57.3±0.30

Different letters in the same row indicate a significant difference for each trait (P<0.05).
BL – body length; ADG – average daily gain; FC – feed conversion ratio; BFT – backfat thickness; MP – meat percentage.

We then examined the effect of these polymorphisms on economic traits in Duroc, Landrace and Yorkshire breeds. The SNP within the *LEP* gene was statistically significantly associated with ADG in Duroc (P<0.05), but not in Landrace or Yorkshire pigs (Table 4 and Table S1). The *Chr6:50,085,657 G>A/Fok*I variant of the *GYS1* gene did not show statistically significant associations with any economic traits in

any breed (Table S2). The SNP within the *MYOD1* gene was significantly associated with ADG and MP in Duroc pigs, as well as ADG, FC and BFT in Landrace pigs ($P<0.05$) (Table 5). On the other hand, no statistically significant association between the *MYOD1* genotype and economic traits was observed in Yorkshire pigs (Table S3). The SNP within the *MYF5* gene was statistically significantly associated with ADG in Duroc pigs ($P<0.05$) (Table 6). However, there was no significant difference between *MYF5* genotype and economic traits in either the Landrace or Yorkshire breed (Table S4).

Table S2. Analyses of association between economic traits in pigs and polymorphisms of *GYS1* gene

Breed		N	BL (cm)	ADG (g/d)	FC	BFT (cm)	MP (%)
Duroc	AA	100	106.1±0.06	1126.4±11.72	2.24±0.01	1.29±0.02	58.1±0.26
	AB	72	106.0±0.00	1109.6±13.32	2.26±0.01	1.29±0.02	58.2±0.32
	BB	8	106.0±0.00	1151.4±35.41	2.20±0.05	1.35±0.03	57.2±0.54
Landrace	AA	109	107.2±0.16	992.4±9.80	2.37±0.01	1.48±0.02	55.9±0.30
	AB	22	106.5±0.28	947.3±15.88	2.42±0.01	1.61±0.05	55.2±0.70
	BB	1	106.0±0.00	909.0±0.00	2.50±0.00	1.30±0.00	57.9±0.00
Yorkshire	AA	150	105.9±0.04	1010.6±9.20	2.35±0.01	1.41±0.02	57.3±0.26
	AB	4	105.8±0.25	989.2±51.86	2.37±0.04	1.59±0.09	56.8±1.45
	BB	0	-	-	-	-	-

Different letters in the same row indicate a significant difference for each trait ($P<0.05$).

BL – body length; ADG – average daily gain; FC – feed conversion ratio; BFT – backfat thickness; MP – meat percentage.

Table S3. Analyses of association between economic traits in pigs and individual polymorphisms of *MYOD1* gene

Breed		N	BL (cm)	ADG (g/d)	FC	BFT (cm)	MP (%)
Yorkshire	AA	30	105.8±0.08	971.8±20.60	2.37±0.02	1.45±0.04	57.3±0.47
	AB	71	105.9±0.04	1019.7±13.3	2.35±0.02	1.42±0.03	57.2±0.38
	BB	53	105.9±0.08	1018.6±14.98	2.35±0.02	1.41±0.03	57.5±0.47

Different letters in the same row indicate a significant difference for each trait ($P<0.05$).

BL – body length; ADG – average daily gain; FC – feed conversion ratio; BFT – backfat thickness; MP – meat percentage.

Table S4. Analyses of association between economic traits in pigs and individual polymorphisms of *MYF5* gene

Breed		N	BL	ADG	FC	BFT	MP
Landrace	AA	129	107.1±1.64	983.4±99.68	2.38±0.10	1.51±0.25	55.7±3.17
	AB	3	106.7±1.16	1021.1±81.55	2.40±0.08	1.29±0.27	58.8±2.00
	BB	0	-	-	-	-	-
Yorkshire	AA	103	105.9±0.51	1008.1±116.93	2.36±0.13	1.43±0.23	57.5±3.03
	AB	48	106.0±0.20	1018.0±104.59	2.33±0.11	1.42±0.21	56.9±3.56
	BB	3	106.0±0.00	946.7±21.93	2.46±0.16	1.38±0.08	57.5±0.92

Different letters in the same row indicate a significant difference for each trait ($P<0.05$).

BL – body length; ADG – average daily gain; FC – feed conversion ratio; BFT – backfat thickness; MP, meat percentage.

Discussion

A total of 466 pigs comprising Duroc, Landrace and Yorkshire breeds were employed to analyze associations between SNPs within *LEP*, *GYS1*, *MYOD1*, and *MYF5* genes and economic traits including ADG, BL, BFT, FC, and MP. In this study, we showed that any associations between genotype of the applied genes and economic traits were completely dependent on the breed. The SNPs within *LEP*, *MYOD1* and *MYF5*, but not *GYS1*, demonstrated statistically significant associations with at least one economic trait described above (Tables 4, 5 and 6).

Polymorphisms within the *LEP* gene have been reported to be significantly associated with various traits (Kennes et al., 2001; van der Lende et al., 2005). The *LEP* genotype has been reported as a potential candidate marker for performance traits in native Brazilian pigs, feed efficiency in Duroc pigs, and BFT in Landrace and Yorkshire pigs (Chen et al., 2004; de Oliveira Peixoto et al., 2006). Some of the SNPs within *LEP*, including *c.2728G>A*, *c.798C>T* and *c.3469T>C*, maintain extremely low frequencies of a certain genotype that have no significant association with economic traits (Kennes et al., 2001; de Oliveira Peixoto et al., 2006). Specifically, the frequency of the *AA* genotype in *LEP c.2728G>A* was 0% in Yorkshire, 0.09% in Duroc and 0.15% in Landrace pigs (Kennes et al., 2001). Similarly, in this study, we demonstrated that the frequency of the *AA* genotype in c*hr18: 21,204,710G>A/ Hin*dIII was 1.3% in Yorkshire, 1.5% in Landrace and 5.6% in Duroc pigs (Table 3). Although the frequency of the *AA* genotype of the *LEP* gene was very low, its association with ADG was significantly different in Duroc pigs. In this breed, the *AA* genotype maintained higher ADG than both *BB* (84.1 g/d) and *AB* genotypes (52.1 g/d) (Table 4). Therefore, we hypothesize that the *AA* genotype of *LEP* in Duroc pigs is involved in growth rate by influencing ADG. Since the SNP within *LEP* differed significantly from ADG in Duroc, but not Landrace or Yorkshire pigs, this SNP was linked to QTL disequilibrium in the pigs described herein (Table 4 and Table S1).

GYS1 is one of the candidate genes for production traits due to its involvement in muscle glycogen metabolism regulation. It was previously determined that the genotype digested with *Fok*I by RFLP is associated with pig economic traits (Zuo et al., 2005). Frequencies of the *B* allele and *BB* genotype of *GYS1* in Chinese Meishan pigs were as low as 12.45% and 1.93%, respectively. However, the frequency of the *B* allele and *BB* genotype of *GYS1* in pig breeds including Duroc, Huainan, Tongcheng, Qingpin, and Erhualian, are believed to have an important effect on economic traits (Zuo et al., 2005). In this study, we investigated the effect of the SNP within *GYS1 chr6:50,085,657 G>A* on economic traits, although the results were not significantly associated with any of the economic traits in the pig breeds investigated (Table 3).

SNPs within the *MYOD1* gene are associated with muscle fiber characteristics, loin eye area, lightness, and intramuscular fat content of pig (Lee et al., 2012; Verner et al., 2007). The allelic and genotypic frequencies were evenly interspersed (Table 3). The *MYOD1* gene exhibited the most diverse association with economic traits among all genes examined in this study (Table 5). The *MYOD1* genotypes differed significantly with respect to ADG and MP in Duroc pigs and ADG, FC and BFT in Landrace pigs (Table 5). The *AB* genotype of *MYOD* was associated with higher

ADG in Duroc pigs compared with both the *AA* (45.4 g/d) and *BB* (21.5 g/d) genotypes. The *BB* genotype of *MYOD1* in Duroc pigs exhibited a greater association with MP (1.2% higher than the *AA* genotype). These results suggest that the *B* allele of *MYOD1* is associated with more economic traits, including ADG and MP in Duroc and ADG, FC and BFT in Landrace pigs. Therefore, it is hypothesized that the *B* allele of *MYOD1* promotes pig production through improvement of ADG, FC, BFT, and MP. However, the *MYOD1* SNP in Yorkshire pigs did not show any significant difference among any of the economic traits examined. Therefore, linkage disequilibrium to QTL may exist in these pigs.

SNPs within *MYF5* (*Chr5:105,703,640 C>T*/*Fok*I) exhibited statistically significant differences with respect to ADG in Duroc pigs. Although a statistically significant difference between *AA* or *AB* genotype versus *BB* genotype in ADG was detected in Duroc pigs, the frequencies of the *B* allele and *BB* genotype were as low as 1.1–17.5% and 0–1.94%, respectively (Table 3). All SNPs within *MYF5* in Landrace and Yorkshire pigs showed no significant associations with any of the economic traits investigated in the present study.

In conclusion, we investigated the effects of polymorphisms within *LEP*, *MYOD1*, *MYF5*, and *GYS1* genes on pig economic traits. The key findings were as follows: (1) polymorphisms within *LEP* are associated with ADG in Duroc pigs, (2) *MYOD1* is associated with ADG and MP in Duroc pigs and ADG, FC and BFT in Landrace pigs, and (3) *MYF5* is associated with ADG in Duroc pigs. Therefore, we believe that among the SNPs investigated in this study, the *MYOD1* SNP plays the most important role as a molecular marker associated with pig growth performance traits.

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