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QTL fine mapping for intramuscular fat and fatty acid composition using high-density SNP chip array on SSC12 in Korean native pig × Yorkshire F2 population

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Abstract: Intramuscular fat (IMF) and fatty acid composition are characteristics that are used as important indicators of evaluating high quality pork and contribute to the economic benefits of the pig farming industry. In this study, quantitative trait loci (QTL) fine mapping of chromosome 12 was performed in a population of F2 intercross between Yorkshire (YS) and Korean native pigs (KNPs) by adopting combined linkage and linkage disequilibrium method using high-density SNP chips. QTLs for IMF (*H3GA0034813* to *H3GA0034965*) and oleic acid (C18:1) (*ASGA0054380* to *ALGA0066299*) were located at 120 cM (54.112–57.610 kb) and 85 cM (36.097–38.601 kb), respectively, within chromosome 12 (Sscrofa11.1 genomic reference). In addition, 31 candidate genes present within the IMF QTL region and 28 candidate genes existing within C18:1 QTL region were chosen. In order to understand the function of these candidate genes at the molecular level, these candidate genes were functionally categorized by studying gene ontology and analyzing network and pathway. Among the 59 candidate genes within the region of IMF QTL and C18:1 QTL, five (*MYH1*, *MYH2*, *MYH4*, *ACACA*, and *RPS6KB1*) directly interacting candidate genes were found. Furthermore, the *RPS6KB1* gene was assumed to be an important candidate gene that is involved in leptin and insulin signaling pathway and participates in controlling adipogenic differentiation, fat deposition, and fatty acid composition, which is related to obesity of pigs.

Keywords: meat quality; LDLA; mapping; chromosome 12; pig

Livestock breeding has improved tremendously over the past decades owing to the progress of molecular market technology. The characteristics of flesh are vital to the economic feasibility of farms. In addition, fat contents and composition, tenderness, water-holding capacity, colour, oxidation, and stability boost economic profits in pig breeding (Gao et al. 2007). Intramuscular fat (IMF) increases meat flavour as well as tenderness, and serves as a significant criterion for determining the degree of fleshiness in South Korea. Fatty acid composition plays a crucial role in determining pork quality. Fatty acid composition regulates the oxidative stability of pork, which in turn affects muscle colour and meat flavour (Wood et al. 2008). It is positively correlated with contents of unsaturated fatty acid in meats. A previous study reported that the proportion of oleic acid (C18:1) increases as intramuscular fat content rises (Smith et al. 2006). In addition, the fatty acid composi-

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tion of triglycerides in pork sirloin has become an important attribute that is related to human health (Wood et al. 2004).

Quantitative trait loci (QTL) mapping identified the chromosome regions associated with quantitative characters using molecular markers or genes (Gao et al. 2007). Several methods have been developed by using DNA sequencing or genotype data (e.g., single nucleotide polymorphisms (SNPs), microsatellites) in livestock. A correlation between molecular marker and QTL was examined using linkage analysis (LA) and linkage disequilibrium (LD). However, although LA is used in the generation, it does not consider genetic or environmental effects; there are limitations when conducting research on complex traits. LD mapping can detect the level of population, although its application is restrained by marker density (Meuwissen et al. 2002). Meuwissen et al. (2002) proposed a QTL fine mapping method on LDLA that adopted an analysis of maximum likelihood estimation of variance components (Meuwissen et al. 2002; Hernandez-Sanchez et al. 2010). Because this method can obtain both family-level LA and population-level LD, it was reported to determine twining rate (Hernandez-Sanchez et al. 2010). Some studies observed that LDLA performed more accurate QTL mapping and that it is useful for detecting unique and narrower QTL areas as compared with LA (Uleberg and Meuwissen 2007).

Korean native pigs (KNPs) have good meat qualities such as high glucose content, low fat and cholesterol, and high unsaturated/saturated fatty acid ratio as well as good indigenous adaptability compared to Western breeds. However, the KNPs have unfavourable characteristics for economically important traits, i.e. small birth weight, slow growth, late maturity and small body size. On the other hand, Yorkshire (YS) has good complementary characteristics, i.e. fast growth, high feed efficiency, moderate meat qualities, and excellent reproductive performance and mothering ability. Kim et al. (2011b) reported that multiple QTLs for meat quality traits (IMF content, meat colour, tenderness, among others) were discovered on 45–50 Mb (DIAS0003408–ASGA0055015) regions of chromosome 12 (SSC12) in F2 intercross between YS and KNP populations. In addition, QTLs located in 96–115 cM (S0106-SWR1021) were also found in F2 intercross between Landrace and KNPs (Cho et al. 2015). In previous studies, QTLs for fatty acid composition (C18:1, C20:1, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), among others) in the region S0106-SWR1021 on SSC12 were explored, and several studies revealed identical QTLs in other pig breed populations (Park et al. 2017). The more recent studies aimed to identify the causal genes located in the QTLs on SSC12.

Research on gene networking and biochemical pathway offers very useful information for understanding the biological mechanism of genes at the system level (Kitano 2002). A few earlier studies investigated IMF traits such as fat composition having known pathway, mature fat cell, or gene networks related with candidate genes in QTL areas (Fortes et al. 2011). Recently, an analysis of the gene network responsible for meat fleshiness as an economic trait in pigs and the formation of fatty acids was carried out using these methods (Duarte et al. 2018).

In the present study, QTL fine mapping of SSC12 in F2 intercross between YS and KNPs was implemented using an analysis of LDLA and Porcine 60K SNP chip (Illumina, San Diego, USA). Moreover, gene network and pathway were analyzed to identify the potential functions of the detected candidate genes in metabolism.

MATERIAL AND METHODS

Animals and phenotypic data. Data were collected from the QTL experimental population that was produced by crossing between KNP boars and YS sows at the National Livestock Research Institute (NLRI), Jeju, Republic of Korea. In the reciprocal cross design of the experiment, two F0 grand sires of KNP and five F0 grand dams of YS were mated. Among the F1 offspring, five F1 sires and 14 F1 dams were randomly chosen to produce F2 offspring. Another mating design comprised three F0 grand sires of YS and 14 F0 grand dams of KNP to generate F1 pigs, among which eight F1 sires and 33 F1 dams were randomly chosen. The two reciprocal matings produced 344 F2 individuals. Provision of feed and water was *ad libitum*. Composition and specifications of the basal diets are presented in Supplementary Table S1 in Supplementary Online Material (SOM). The F2 individuals were raised under the general feeding system in Chungbuk province, and slaughtered at approximately 220 ± 23 days of age.

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The IMF content was estimated according to standard method (Oh et al. 2008). The fatty acid profiles were measured based on well-established methods described in a previous study (Lepage and Roy 1986). The composition ratios of the following fatty acids were calculated as described in Maharani et al. (2013): palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), and oleic (C18:1) (Table 1).

All experimental procedures were performed according to national and institutional guidelines and approved by the Ethical Committee of the National Institute of Animal Science.

Genotyping, marker selection, and linkage mapping. DNA samples (200 ng adjusted to 50 ng µl) from 344 F2 pigs were prepared from the sampled tissues according to standard protocols. The genotyping was done by GeneSeek Inc. (Lincoln, USA) using Porcine 60K BeadChip (Illumina Inc., USA) and according to the approved standard techniques outlined by the manufacturer. SNP marker selection for linkage mapping was performed by (*i*) using 1098 SNPs of SSC12 from 62 163 SNPs of all chromosomes, (*ii*) excluding SNPs set Quality Control (QC) with call rates < 0.90, minor allele frequency (MAF) < 0.05 and Hardy–Weinberg equilibrium (HWE) with $P < 0.0001$ condition, (*iii*) estimating and R^2 using the HAPLOVIEW (Version 4.2) program for estimating LD, and detecting 85 haplotype block using the method suggested by Gabriel et al. (2002). The procedure was run with default parameters following the manual for the mentioned program.

Linkage mapping was initially conducted with 85 tag SNPs detected from haplotype blocks using CRI-MAP (Version 2.4). Subsequently, 16 SNPs with extremely small intervals between markers were excluded from linkage mapping; therefore, only 69 SNPs were used for linkage mapping (Supplementary Table S2 in SOM).

LDLA analysis for QTL fine mapping. The QTL on SSC12 was investigated further using an LDLA approach. LDLA analyses implemented historic recombination events in addition to recombination events within genotyped families in order to estimate haplotype effects. The phenotypic data, used to determine the traits record, were fitted to the fixed effects of sire, sex, and slaughter date, and covariate of slaughter age in a linear model to calculate the residuals of the phenotypic data. The analyses were performed using the GRID QTL LDLA program.

QTL fine mapping was performed using LDLA using markers and known pedigree information to estimate identity by descent (IBD) probabilities of haplotypes (Meuwissen et al. 2002). The estimated IBD probabilities were used to construct a genotype relationship matrix at putative QTL region (Gp). The additive genetic matrix (**A**) was constructed based on the knowledge of parent generation of the F1 and F2 genotypes. The metrics Gp and **A** were used to define the covariance structure in a linear mixed model to estimate random QTL and polygenic effects with residual maximum likelihood. The statistical model for single QTL analysis was written as:

$$Y = \mu + \mathbf{Z}_1 h + \mathbf{Z}_2 u + e$$

where:

Y = vector of N observed phenotype traits score

μ = overall mean

h = vector of N random QTL effects

u = vector of N_p random polygenic effects for N_p animals in the pedigree

$\mathbf{Z}_1$, $\mathbf{Z}_2$ = incidence matrices linking observations to QTL (h) and polygenic effect (u)

e = vector with residuals

The random effects are dispersed as $\text{var}(h) = G_p\sigma_h^2$, $\text{var}(u) = A\sigma_u^2$, and $\text{var}(e) = I\sigma_e^2$.

QTL detection was carried out in SSC12 through comparing the "no QTL" versus "one QTL" hypotheses. Chromosome-wide thresholds ($P < 0.00056$: $-\log_{10}P > 3.26$) were calculated according to the Bonferroni correction. A QTL was considered to be significant for a chromosome-wide significance level of 1%.

Functional annotation, gene network, and pathway analysis. Candidate genes were selected inside QTL spanning the position of each SNP marker. To annotate the candidate genes, the gene ontology (GO) terms from the Database for Annotation, Visualization and Integrated Discovery (https://david.ncifcrf.gov/) were used. GO terms are used to categorize candidate genes in terms of their functions. The candidate genes were analyzed and associated with their GO terms for all categories, biological processes, molecular functions, and cellular components.

Gene network and pathway analysis provides important insights into the genetic architecture of complex polygenic traits (Farber 2013). Therefore, we carried out gene network and pathway analysis using the candidate genes by employing

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Table 1. Summary statistics for observations on intramuscular fat (IMF) content and fatty acid composition traits in *m. longissimus* of F2 generation of Yorkshire and Korean native pig

Meat quality traits	Abbreviation	*n*	Average	SD	Min	Max	CV
Intramuscular fat (%)	IMF	344	2.5	1.5	0.5	10.4	60.0
Fatty acid composition (%)							
Palmitic acid	C16:0	344	21.3	1.8	14.8	26.5	8.5
Palmitoleic acid	C16:1	344	3.9	1.1	0.3	8.7	28.2
Stearic acid	C18:0	344	11.0	1.4	7.1	15.6	12.7
Oleic acid	C18:1	344	32.0	4.9	20.3	43.3	15.3

SD = standard deviation, CV = coefficient of variation

Overall, 32 fatty acids (C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, C14:1, C15:0, C15:1, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:2, C20:3, C20:4, C20:5, C22:0, C22:1, C22:2, C22:6, C23:0, C23:1, C24:0 and C24:1) were estimated, but 4 fatty acids (C16:0, C16:1, C18:0 and C18:1) that are known to be related to meat quality were used in this study

GeneMANIA and KEGGParser tool plugin for Cytoscape software 3.6.

RESULTS AND DISCUSSION

Distribution of IMF content and general statistics of SNP arrays. In the present study, we genotyped 344 F2 pigs obtained by crossing YS and KNP with the Porcine 60K BeadChip (Illumina) and phenotyped IMF content. The mean IMF content was 2.5 ± 1.5% ranging from 0.5 to 10.4%. Among the 1098 SNPs, 210 SNPs had MAF less than 0.05, 74 SNPs departed from HWE and 65 SNPs had call rate less than 90% (10% missing). In addition, 85 haplotype blocks with complete LD ($r^2 = 1$) structure were selected by LD analysis of SSC12. Linkage mapping analysis was performed using 85 tag SNPs, and 69 tag SNPs were available to linkage map (Supplementary Table S3 in SOM). A total of 69 SNPs that met the quality control criteria were used for the linkage and QTL mapping analysis.

Linkage maps. The information on genotypes for the 69 SNP markers was used to construct an SSC12 linkage map, which was in agreement with previous evidence on the order and distances between markers. Sixty-nine SNP markers were used for linkage mapping (Supplementary Table S2 in SOM). The average SSC12 linkage map length was 145.3 cM (59 117 kb). Minimum and maximum linkage map intervals were 0.3 and 7.1 cM, respectively, for the following intervals: *CASI0006966* (27 383 kb) to *ASGA0053936* (27 842 kb), *ALGA0065876* (30 603 kb) to *MARC0078793* (31 581 kb) and *ASGA0054380* (36 097 kb) to *MARC0101217* (36 776 kb), and *H3GA0034916* (56 325 kb) to *ASGA0099848* (57 345 kb). The average distance between two markers was 2.1 cM (804 kb).

QTL fine mapping for IMF and fatty acid composition. After the QTL fine mapping using the LDLA method, the QTL region for IMF and oleic acid (C18:1) content in SSC12 was identified in the F2 population (Table 2).

As shown in Figure 1, the QTL for IMF and oleic acid (C18:1) content was higher than 1% chromosome-wide ($-\log_{10}P > 3.26$) significance level. The QTL for IMF showed the highest level of significance ($-\log_{10}P = 4.81$) at 120 cM, lo-

Table 2. Results of QTL mapping for intramuscular fat and oleic acid content on SSC12 using linkage disequilibrium and linkage analysis (LDLA) method

Trait	cM[1]	LRT[2]	$-\log_{10}$P[3]	QTL effect (SE)[4]	Marker interval (kb)	Genes *n*
Intramuscular fat (%)	120	14.1	4.81	0.51 (0.13)	H3GA0034813 (54 112) – H3GA0034965 (57 610)	31
Oleic acid (%)	85	20.3	6.66	2.72 (0.52)	ASGA0054380 (36 097) – ALGA0066299 (38 601)	28

QTL = quantitative trait locus, LRT = likelihood ratio test, SE = standard error

[1]position at which the test-statistic value was maximized for the inferred QTL model, [2]$2(\log LH_1 - \log LH_0)$, [3]negative logarithm of the comparison-wise *P*-value of the test statistic against the null hypothesis of no QTL effect at the QTL position, [4]estimates of additive effects for LDLA QTL

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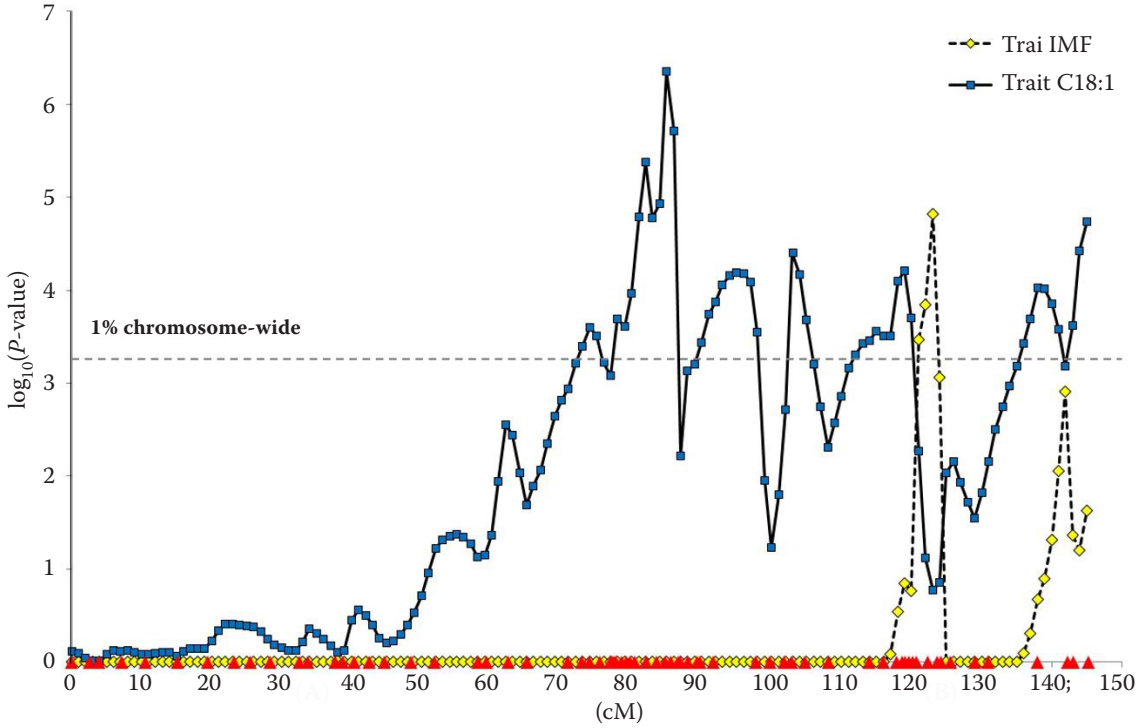

Figure 1. Profiles for quantitative trait loci (QTL) on SSC12 using data from a Yorkshire × Korean Native Pigs cross. Shown is the negative of the logarithm of the comparison-wide significance value for the inferred QTL models against position on the linkage map. Also, shown are profiles for the inferred models; linkage analysis and linkage disequilibrium (LDLA) model for intramuscular fat (IMF) and oleic acid content (C18:1) in *m. longissimus*. The average 1% chromosome-wide (3.26) was obtained by 10 000 permutations for the inferred QTL models on SSC12. Filled red triangles below X-axis indicate marker positions

cated between the *H3GA0034813* (54 112 kb) and *H3GA0034965* (57 610 kb) SNP markers. As compared with the allele of the YS pigs, that of the KNPs had an effect of increasing IMF (0.51 ± 0.13). Previous studies showed that a QTL (*S0106-SWR1021*) related to IMF was present in the same region as that identified in the present study, within SSC12, and that the allele of the KNPs had an effect of increasing IMF (Kim et al. 2011a; Cho et al. 2015). Moreover, previous studies suggested that a family of genes encoding the myosin heavy chain (MYH) in the QTL region associated with the same IMF in SSC12 is associated with the IMF (Lee et al. 2012; Xiong et al. 2015).

In addition, the QTL for oleic acid, which is an unsaturated fatty acid, had the highest level of significance ($-\log_{10}P = 4.81$) at 120 cM, intermediate between that of the *ASGA0054380* (36 097 kb) and *ALGA0066299* (38 601 kb) SNP markers. In addition, it showed that the allele of the KNPs had a greater effect on increasing C18:1 (2.72 ± 0.52) than that of the YS pigs. According to Park et al. (2017), a QTL related to fatty acid composition traits (C10:0, C12:0, C16:0, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, and C20:0) was present in the same region (*S0106-SWR1021*) as that of the QTL for IMF on SSC12 of pigs. However, the results of the present study showed that the QTL was not present on SSC12 for palmitic acid (16:0) and stearic acid (C18:0), which are saturated fatty acids, and for palmitoleic acid (C16:1), which is an unsaturated fatty acid.

However, the present study showed that the QTL for C18:1 and the QTL for IMF were not in the same region (the region from 54 112 kb to 57 610 kb), and the QTL for IMF was in the region from 38 601 to 36 097 kb, which is approximately 20 Mb from the QTL for C18:1. In the fatty acid composition, C18:1 is very closely related to marbling and flavour of meat, and many studies have been performed in cattle as well as in pigs (Gallardo et al. 2009; Kim et al. 2011b).

In the present study, QTL fine mapping showed that the IMF is closely related to fatty acid com-

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Table 3. Functional annotation of candidate genes within putative IMF and C18:1 QTL

Traits	QTL region (kb)	Category	Term	Genes	*P*-value
IMF	54 112–57 610	cellular component	GO:0030016 (myofibril)	*LOC100517855, MYH1, MYH2, MYH4*	0.0000002
			GO:0032982 (myosin filament)	*MYH1, MYH2, MYH4*	0.0000023
			GO:0043292 (contractile fibre)	*LOC100517855, MYH1, MYH2, MYH4*	0.0000071
			GO:0016459 (myosin complex)	*MYH1, MYH2, MYH4*	0.0000846
			GO:0015629 (actin cytoskeleton)	*MYH1, MYH2, MYH4*	0.0043334
		molecular function	GO:0003774 (motor activity)	*MYH1, MYH2, MYH4*	0.0002188
			GO:0003779 (actin binding)	*MYH1, MYH2, MYH4*	0.0004318
			GO:0005516 (calmodulin binding)	MYH1, MYH2, MYH4	0.0002792
			GO:0008092 (cytoskeletal protein binding)	*MYH1, MYH2, MYH4*	0.0065417
C18:1	36 097–38 601	cellular component	GO:0005622 (intracellular)	*TBX2, TBX4, ACACA, RPS6KB1, INTS2, MRM1, LHX1, TUBD1, CA4, AATF, APPBP2, BCAS3, GGNBP2*	0.0407537
			GO:0005634 (nucleus)	*TBX2, LHX1, TBX4, TUBD1, ACACA, AATF, INTS2, RPS6KB1, BCAS3, GGNBP2*	0.0046247
			GO:0031974 (membrane-enclosed lumen)	*TUBD1, ACACA, AATF, INTS2, RPS6KB1, BCAS3*	0.0479070
			GO:0031981 (nuclear lumen)	*TUBD1, ACACA, AATF, INTS2, RPS6KB1, BCAS3*	0.0319941
			GO:0043226 (organelle)	*TBX2, TBX4, ACACA, RPS6KB1, INTS2, MRM1, LHX1, TUBD1, CA4, AATF, APPBP2, BCAS3, GGNBP2*	0.0125954
			GO:0043227 (membrane-bound organelle)	*TBX2, TBX4, ACACA, RPS6KB1, INTS2, MRM1, LHX1, TUBD1, CA4, AATF, APPBP2, BCAS3, GGNBP2*	0.0050792
			GO:0043229 (intracellular organelle)	*TBX2, TBX4, ACACA, RPS6KB1, INTS2, MRM1, LHX1, TUBD1, CA4, AATF, APPBP2, BCAS3, GGNBP2*	0.0042431
			GO:0043231 (intracellular membrane-bound organelle)	*TBX2, TBX4, ACACA, RPS6KB1, INTS2, MRM1, LHX1, TUBD1, CA4, AATF, APPBP2, BCAS3, GGNBP2*	0.0013744
			GO:0043233 (organelle lumen)	*TUBD1, ACACA, AATF, INTS2, RPS6KB1, BCAS3*	0.0451957
			GO:0044424 (intracellular part)	*TBX2, TBX4, ACACA, RPS6KB1, INTS2, MRM1, LHX1, TUBD1, CA4, AATF, APPBP2, BCAS3, GGNBP2*	0.0231349
			GO:0070013 (intracellular organelle lumen)	*TUBD1, ACACA, AATF, INTS2, RPS6KB1, BCAS3*	0.0451957
		molecular function	GO:0097159 (organic cyclic compound binding)	*TBX2, LHX1, TBX4, TUBD1, ACACA, AATF, RPS6KB1, MRM1*	0.0296934
			GO:1901363 (heterocyclic compound binding)	*TBX2, LHX1, TBX4, TUBD1, ACACA, AATF, RPS6KB1, MRM1*	0.0274261

IMF = intramuscular fat, QTL = quantitative trait loci

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position. However, in SSC12, the QTL for IMF was 20 Mb from the QTL for C18:1. In addition, identification of additive QTLs for IMF and C18:1 in SSC12 suggested that the KNP sire was commercially useful, because the allele in KNP had a significant effect on increasing IMF and C18:1. The useful alleles found in the KNP breed support the utility of using breeds that have not been selected due to inaccurate information (Kim et al. 2005).

Functional annotation and pathway analysis of candidate genes. A total of 59 candidate genes, consisting of 31 candidate genes within the detected IMF QTL region (54 112–57 610 kb) and 28 candidate genes within the detected C18:1 QTL region (36 097–38 601 kb) were detected. Functional annotation was done for those 31 and 28 candidate genes detected by using DAVID web site tool (http://david.abcc.ncifcrf.gov/). For the 31 candidate genes within the IMF QTL region, nine GO terms (five cellular components and four molecular functions) from two categories were found, whereas 13 GO terms (11 cellular components and 2 molecular functions) were found from two categories, as well for the 28 candidate genes within C18:1 QTL region (Table 3).

By using GeneMANIA and KEGGParser analysis tool, pathways for the 31 candidate genes within IMF QTL region and the 28 within C18:1 region were searched and the interactions were investigated through gene network analysis. Among the 59 candidate genes, only three (*MYH1*, *MYH2*, and *MYH4*) within the IMF QTL region and only two

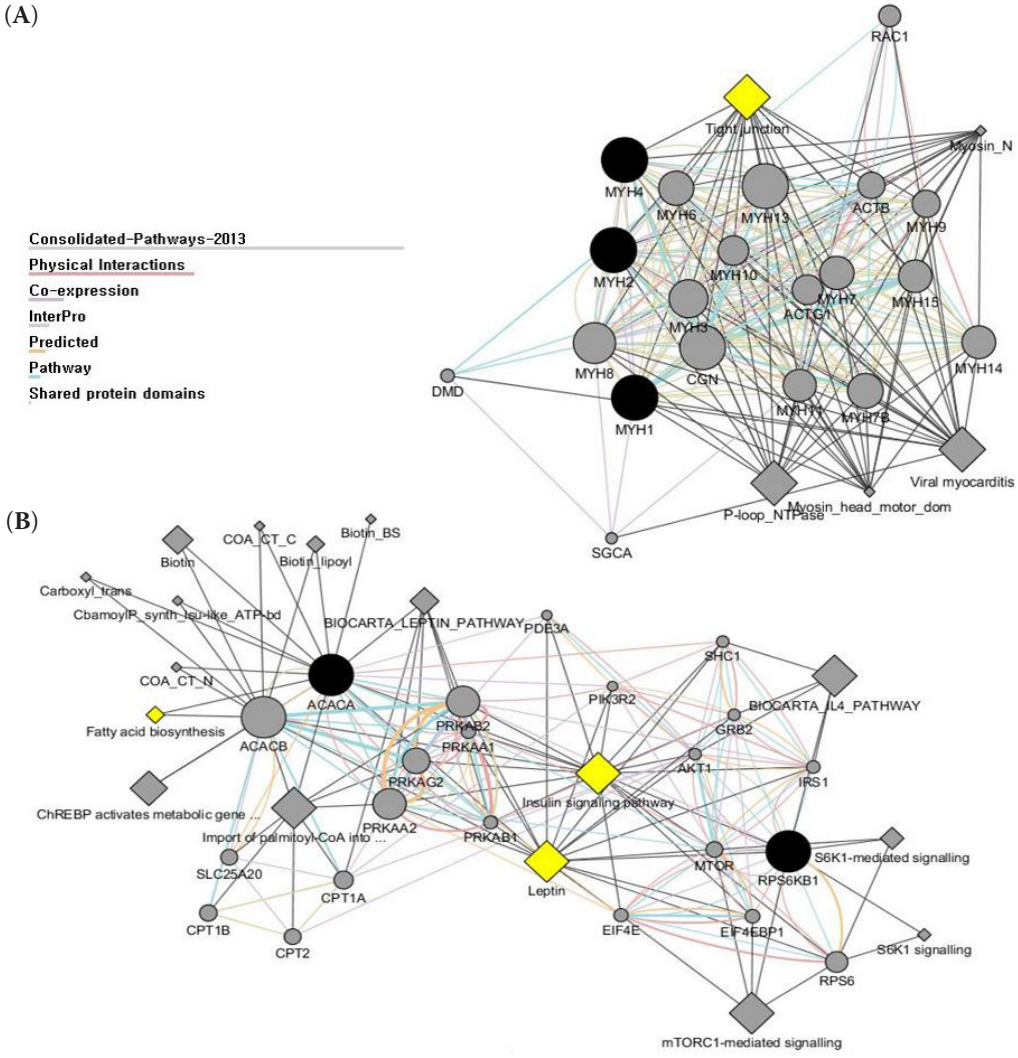

Figure 2. Pathway and network analysis of the candidate genes. Direct interactions between (**A**) intramuscular fat and (**B**) oleic acid content candidate genes and their regulatory relationships. Black and yellow highlight marked genes and pathway indicates candidate genes for QTL and pathway analysis

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candidate genes, including acetyl-CoA carboxylase alpha (*ACACA*), and ribosomal protein S6 kinase B1 (*RPS6KB1*) within C18:1 QTL region had a direct interaction (Figure 2).

Myosin, which occupies approximately 25% of the entire protein pool, is the most abundant protein that is expressed in striated muscle cells and a major protein for contraction that converts chemical energy to mechanical energy through adenosine triphosphate (ATP) hydrolysis. In mammals, 10 kinds of MYH isoforms have been studied, and such isoforms play important roles in the development of skeletal muscles because they are expressed in skeletal muscles during various developmental stages, particularly including the embryonic period (Baldwin and Haddad 2001; Wang et al. 2013). There are four major fibre types in postnatal pig muscles, characterized by the expression of the slow/I/b, IIa, IIx, and IIb MyHC gene isoforms, encoded by *MYH7*, *MYH2*, *MYH1*, and *MYH4* genes, respectively (Davoli et al. 2003). Additionally, tight junction and adherens junction pathways are known to act in muscle contraction and development of muscular structure (Chen et al. 2014). In the present study, *MYH1*, *MYH2*, and *MYH4* genes were found to network through the tight junction pathway (Figure 2A).

The *ACACA* gene, which encodes the enzyme that catalyzes the rate-limiting step during the synthesis of fatty acid, is one of the most important candidate genes that has been studied in relation to the fatty acid composition of meat in pigs and cattle (Gallardo et al. 2009; Zhang et al. 2010). Eusebi et al. (2017) reported variability in the coding region of the *ACACA* gene in Duroc pig and found significant correlation of the variability with carcass traits, including the deposition of back fat and amount of meat. Moreover, they proposed candidate genes other than the *ACACA* gene; e.g., the ribosomal protein S6 kinase (*RPS6KB1*) gene, which is known to play an important role in controlling adipogenic differentiation, conversion from embryonic stem cells to fat cells, and obesity (Martelli et al. 2014). *ACACA* and *RPS6KB1* genes were found to network in relation to the insulin signaling pathway and leptin pathway. In addition, *ACACA* was found to be involved in fatty acid biosynthesis pathway as well. Previous studies related to the function of genes (*ACACA* and *RPS6KB1*) indicate the same results in KNP.

CONCLUSION

The QTL regions of SSC12 for IMF and C18:1 were identified using LDLA mapping in the F2 generation between YS × KNP. Three genes (*MYH1*, *MYH2*, and *MYH4*) in the QTL region for IMF content, and 2 genes (*ACACA* and *RPS6KB1*) for C18:1 were identified through network and pathway analyses. Further studies are needed to verify the detected QTLs and candidate genes in other pig breed populations for commercial application via marker-assisted selection.

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