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Association of the *ADRB3*, *FABP3*, *LIPE*, and *LPL* gene polymorphisms with pig intramuscular fat content and fatty acid composition

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ABSTRACT: The aim of the present study was to investigate the associations of single nucleotide polymorphisms (SNPs) in candidate genes with fatness traits in the *Longissimus dorsi* muscle of pigs. The polymorphisms of genes were investigated, which included beta-3-adrenergic receptor gene (*ADRB3*), heart fatty acid-binding protein gene (*FABP3*), and hormone-sensitive lipase gene (*LIPE*) as well as lipoprotein lipase gene (*LPL*). The intramuscular fat (IMF) content and fatty acid composition contents in *Longissimus dorsi* muscle samples were measured. Results showed that *ADRB3*, *LIPE,* and *LPL* SNPs were associated with IMF content ($P < 0.05$). *ADRB3 AG* heterozygotes exhibited higher IMF content. *LIPE A* allele was associated with greater IMF content. *LPL CT* heterozygotes exhibited the lowest IMF content. *ADRB3* c.1192G>A had highly significant association with the total monounsaturated fatty acid (MUFA) ($P < 0.01$) and the total polyunsaturated fatty acid (PUFA) ($P < 0.01$). *LIPE* c.442G>A was significantly associated with the contents of C12:0 and C14:0 ($P < 0.05$). *LPL* c.624C>T was significantly associated with the percentage of C16:1 ($P < 0.05$) and the percentage of total saturated fatty acid (SFA) ($P < 0.05$). The pigs with *ADRB3 G* allele had more MUFA, and the pigs with *LPL T* allele had less SFA, implying that the *ADRB3 G* and *LPL T* in pigs may be beneficial to human health. In conclusion, the results suggest that these genetic markers are important sources of the variations for the pork selection to obtain favourable meat with higher IMF levels and appropriate fatty acid composition.

Keywords: fatty acid composition; IMF; *Longissimus dorsi* muscle; Duroc; Shanzhu

INTRODUCTION

The intramuscular fat (IMF) content has a positive influence on porcine meat quality characteristics such as colour, juiciness, flavour, tenderness, and texture level (Fortin et al. 2005; Reardon et al. 2010). The porcine fatty acid composition has been concerned because of its effects on meat quality and its association with human health (Zhang et al. 2009). Recent improvements in porcine carcass lean content are the result of selection pressure on traits associated with production economics (Tyra et al. 2011). The breeding program direction brought about a high carcass lean content and a low level of fat, accompanied by a considerable decrease of IMF content. In order to satisfy the demand for porcine economic value, the genetic improvement for IMF content is an urgent issue.

IMF content is higher in the Chinese local pigs than in the other commercial ones (Zhao et al.

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2009). The Shanzhu pigs, one of the indigenous pigs in Jiangsu Province of China, are fatty pigs characterized by their good meat quality and a high level of IMF content. By contrast, the Duroc pig is an international breed mainly concentrated on the lean growth efficiency (Schwab et al. 2007). Therefore, Shanzhu × Duroc crossbred pigs might possess good meat and fatness traits determined by IMF content and fatty acid composition.

Beta-3-adrenergic receptor gene (*ADRB3*), which encodes a major mediator of lipolytic and thermogenic effects in adipose tissue, can be used as a genetic marker in selection. *ADRB3* gene maps to chromosome 15 within quantitative trait loci (QTL) regions for fatness traits (Nowacka-Woszuk et al. 2008). Heart fatty acid-binding protein gene (*FABP3*) maps to chromosome 6 (Gerbens et al. 1997) and is responsible for the transport and cellular metabolism of fatty acids. *FABP3* gene has been shown to be associated with IMF content (Lee et al. 2010). Hormone-sensitive lipase gene (*LIPE*), which maps to chromosome 6 (Mellink et al. 1993), has been involved in the free fatty acids mobilization (Miyoshi et al. 2008; Zechner et al. 2009). Lipoprotein lipase gene (*LPL*) maps to chromosome 14 (Gu et al. 1992) and is responsible for lipid deposition and mobilization (Luo et al. 2009).

The use of *ADRB3*, *FABP3*, *LIPE,* and *LPL* genes in porcine selection might block the disadvantageous trend related to the decrease of IMF levels and also conduce to improve this parameter. Therefore, the objective of this study was to analyze the polymorphisms of *ADRB3*, *FABP3*, *LIPE,* and *LPL* genes and investigate the association of these polymorphisms with fatness parameters including IMF content and fatty acid composition contents in the *Longissimus dorsi* muscle of Shanzhu × Duroc crossbred pigs.

MATERIAL AND METHODS

Animals and sample collection. We analyzed 440 barrows (Shanzhu × Duroc commercial crossbreds) in this study, and all these pigs were reared on the same farm in China. The same corn-meal diets were provided *ad libitum*, and the same feeding allowed us to exclude the influence of dietary intake on fatty acids composition in the *Longissimus dorsi* muscle of the studied crossbreds. Animals were kept under the same feeding and housing conditions. When the pigs reached about 90 kg body weight, they were transported to the same slaughterhouse and slaughtered after electrical stunning. After slaughter, the *Longissimus dorsi* muscle samples from the 13th rib of the left half of the carcasses were collected from each pig for the later analysis of genomic DNA, IMF content, and fatty acid composition. Samples were kept frozen at –20°C until analysis.

IMF content and fatty acid composition determination. The method for fatness traits measurement was described previously (Wang et al. 2013). Briefly, the IMF content was determined as crude fat on thawed *Longissimus dorsi* muscle homogenates by Soxhlet extraction method. The fatty acid compositions were separated and quantified using GC-14B gas chromatography apparatus (Shimadzu Corporation, Tokyo, Japan) equipped with a capillary column. The saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) were calculated. Fatty acid composition contents were expressed as

Table 1. Mean and standard deviation (SD) of the analyzed intramuscular fat content and fatty acid composition in *Longissimus dorsi* muscle of pigs (n = 440)

	Means	± SD
Intramuscular fat (%)	2.53	0.80
Fatty acid composition (%)[1]		
Lauric acid, C12:0	0.08	0.01
Miristic acid, C14:0	1.29	0.17
Palmitic acid, C16:0	24.70	1.82
Palmitoleic acid, C16:1	2.79	0.62
Margaric acid, C17:0	0.22	0.09
Stearic acid, C18:0	11.88	1.29
Oleic acid, C18:1	46.92	3.37
Linoleic acid, C18:2	7.24	1.55
Linolenic acid, C18:3	0.81	0.30
Arachidic acid, C20:0	0.20	0.01
C20:4	1.02	0.49
C22:4	0.22	0.06
SFA	38.38	2.86
MUFA	49.72	3.63
PUFA	9.29	1.99
MUFA/SFA	1.31	0.17
PUFA/SFA	0.24	0.05

SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid

[1]fatty acid composition values are g/100 g of total fatty acids

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a weight percentage of total fatty acids. The main statistics of analyzed parameters are presented in Table 1.

Genotyping. Genomic DNA was extracted from *Longissimus dorsi* muscle samples using the Genomic DNA Purification Kit (Fermentas; Thermo Fisher Scientific, Waltham, USA) following instructions provided in the attached protocol. *ADRB3*, *FABP3,* and *LIPE* genes polymorphisms (Gerbens et al. 1997; Knoll et al. 1998; Cieslak et al. 2009) were genotyped using the PCR-RFLP method. *LPL* gene polymorphisms (Wang et al. 2009) were genotyped using the PCR-SSCP method described by Gasser et al. (2006). Concretely, the amplified and digested DNA fragments of the *ADRB3*, *FABP3,* and *LIPE* genes were separated on 2% agarose gels, and the amplified fragments of the *LPL* gene were separated on 8% non-denaturing polyacrylamide gel at 4°C. The genotypes of pigs for these polymorphisms were determined by analyzing the size of fragments in RFLP or the visible mobility shifts in SSCP. Detailed information about SNPs of the genes *ADRB3*, *FABP3*, *LIPE,* and *LPL* and their respective genotyping approach are listed in Table 2.

Statistical analysis. All statistical procedures were performed using the SPSS statistical software package (Version 17.0, 2008). The genotype frequencies and allele frequencies of each SNP were calculated. Pearson's model was utilized to analyze the associations between the SNP of each gene and the fatness traits (IMF content and fatty acid composition). The effects of genotypes were analyzed by the analysis of variance (ANOVA) procedure. Means were used to compare differences by One-Way ANOVA program. When significant differences were identified, supplementary Tukey's post-hoc multiple tests were performed to investigate differences. The results were presented as mean values ± standard deviations (SD). A value of $P < 0.05$ was considered to be statistically significant in all analyses.

RESULTS

The genotype and allele frequencies of *ADRB3*, *LIPE,* and *LPL* genes displayed high degree of polymorphisms (Table 3). For *FABP3* polymorphism, identified by the restriction enzyme *Hin*fI, only two out of three genotypes were observed, and allele *T* of *FABP3* c.-314T>C comes near to fixation in Shanzhu × Duroc crossbreds of pigs

Table 2. PCR primers and amplicons of *ADRB3*, *FABP3*, *LIPE*, and *LPL* genes

Gene	Ensembl ID or GenBank Accession No.	SNPs	SNP location	Primer sequences F/R (5'→3')	Amplicon (bp)	T_a (°C)	PCR-RFLP or PCR-SSCP pattern (bp)	Enzyme for RFLP
ADRB3	ENSSSCT00000017229	c.1192G>A (missense substitution)	exon 1	CGTTCAACCCGCTCATCTACTGC/ GGTTCCCTACTCTGTGCCCGTCTT	315	63	*G*: 143+172 *A*: 315	*Taq*I
FABP3	HM591296	c.-314T>C (silent substitution)	5'upstream region	GGACCAAGATGCCTACGCCG/ CTGCATCTTTGACCAAGAGG	706	62	*T*: 25+59+111+172+339 *C*: 25+111+231+339	*Hin*fI
LIPE	ENSSSCG00000003018	c.442G>A (missense substitution)	exon 1	CGCACAATGACACAGTCACTGGT/ AGGCAGCGGCCGTAGAAGCA	497	57	*G*: 67+190+240 *A*: 190+307	*Bsa*HI
LPL	ENSSSCT00000010522	c.624C>T (silent substitution)	exon 5	GGACCTAACTTCGAGTATGC/ CAATCACACGGATGGCTT	202	55	*CC*: four bands *TT*: three bands *CT*: six bands	–

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Table 3. Genotype and allele frequencies in the studied polymorphic sites (n = 440)

Gene	SNP type	Genotype frequencies[1]			Allele frequencies[1]	
		11	*12*	*22*	*1*	*2*
ADRB3	G/A	0.093	0.543	0.364	0.365	0.635
FABP3	T/C	0.918	0.082	0.000	0.959	0.041
LIPE	G/A	0.082	0.582	0.336	0.373	0.627
LPL	C/T	0.077	0.723	0.200	0.438	0.562

[1]the genotypes *11*, *22,* and *12* represent homozygote and heterozygote, respectively (*1* and *2* indicate the allele type for SNPs)

analyzed in our study. As regards the other gene polymorphisms, *ADRB3 A*, *LIPE A,* and *LPL T* alleles had higher frequencies in these pigs.

Comparison of means of the IMF content and fatty acid composition of these animals classified according their genotypes are shown in Table 4. In general, *ADRB3*, *LIPE,* and *LPL* SNPs had significant associations with IMF content ($P < 0.05$), while *FABP3* SNP showed no significant ($P > 0.05$) association with IMF content. *ADRB3* c.1192G>A SNP was significantly associated with the content of C20:4 and MUFA/SFA ratios ($P < 0.05$), and also highly significantly associated with oleic acid (C18:1), linoleic acid (C18:2), total MUFA, total PUFA and PUFA/SFA ratios ($P < 0.01$). *FABP3* c.-314T>C SNP did not show significant ($P > 0.05$) association of its SNP with any kind of fatty acid percentage. *LIPE* c.442G>A SNP was found to be significantly associated with lauric acid (C12:0) and miristic acid (C14:0) ($P < 0.05$). *LPL* c.624C>T SNP showed significant associations with C14:0, palmitoleic acid (C16:1), and stearic acid (C18:0) as well as total SFA ($P < 0.05$).

DISCUSSION

Recent improvement in porcine carcass lean content in pig breeding was related with considerable decrease of IMF content, one of the main determinants of meat quality (Wood et al. 2004).

Table 4. Association of polymorphisms of candidate genes with porcine fatness traits[1]

Gene	Fatness trait	Genotype[2]		
		11	*12*	*22*
ADRB3 c.1192G>A	IMF (%)	2.46 ± 0.35^{ab}	3.64 ± 2.14^{b}	1.55 ± 0.45^{a}
	C18:1 (%)	49.14 ± 0.77^{b}	49.66 ± 2.87^{b}	44.66 ± 2.49^{a}
	C18:2 (%)	6.85 ± 0.78^{b}	5.45 ± 0.49^{a}	8.37 ± 1.03^{c}
	C20:4 (%)	1.10 ± 0.24^{b}	0.68 ± 0.19^{a}	1.30 ± 0.33^{b}
	MUFA (%)	52.11 ± 1.34^{b}	52.77 ± 3.17^{b}	47.22 ± 2.43^{a}
	PUFA (%)	6.85 ± 0.64^{a}	9.17 ± 1.02^{b}	10.69 ± 1.23^{c}
	MUFA/SFA	1.42 ± 0.11^{b}	1.40 ± 0.09^{b}	1.22 ± 0.04^{a}
	PUFA/SFA	0.18 ± 0.03^{a}	0.25 ± 0.04^{b}	0.28 ± 0.04^{b}
LIPE c.442G>A	IMF (%)	1.60 ± 0.18^{a}	2.03 ± 0.38^{ab}	2.47 ± 0.64^{b}
	C12:0 (%)	0.07 ± 0.01^{a}	0.08 ± 0.01^{a}	0.10 ± 0.01^{b}
	C14:0 (%)	1.14 ± 0.04^{a}	1.27 ± 0.16^{ab}	1.46 ± 0.14^{b}
LPL c.624C>T	IMF (%)	2.85 ± 0.42^{b}	2.10 ± 0.31^{a}	3.03 ± 0.30^{b}
	C14:0 (%)	1.61 ± 0.02^{b}	1.28 ± 0.15^{ab}	1.10 ± 0.04^{a}
	C16:1 (%)	1.91 ± 0.13^{a}	2.76 ± 0.52^{ab}	3.08 ± 0.47^{b}
	C18:0 (%)	12.43 ± 1.10^{b}	12.00 ± 1.20^{b}	9.50 ± 0.81^{a}
	SFA (%)	38.65 ± 2.92^{b}	37.27 ± 1.00^{b}	35.43 ± 1.05^{a}

[1]values are expressed as mean ± SD and values with different superscripts are significantly different at the 0.05 level ($P < 0.05$)
[2]genotypes *11*, *22,* and *12* represent homozygote and heterozygote, respectively (*1* and *2* indicate the allele type for SNPs)

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Shanzhu pigs are animals with a satisfactory level of carcass fatness and simultaneously are characterized by high IMF level, but they have relatively poor growth characteristics such as daily gain. Duroc pigs are animals with great lean growth efficiency. So the Shanzhu × Duroc crossbreds were developed to obtain pigs with good meat quality and fatness traits. Klensporf-Pawlik et al. (2012), who compared pure breeds of Duroc, Pietrain, Polish Large White, and Polish Landrace, reported that the highest IMF content was observed in *Longissimus dorsi* muscle of Duroc pigs. Tao et al. (2013) also presented the data that the IMF content was the highest in Duroc pigs. In the present study, the mean value of IMF contents (%) of *Longissimus dorsi* muscle in Shanzhu × Duroc crossbreds was 2.53. This value is much higher than 1.56 which was measured in Duroc pigs (Klensporf-Pawlik et al. 2012).

The degree of unsaturation of the fatty acids is shown as the sum of MUFA content and PUFA content. This degree is strongly associated with the meat softness, texture, and possibility of oxidation (Wood et al. 1999). For total MUFA, values in Duroc pigs were much higher (42.14%) than in the other breeds studied by Raj et al. (2010). In our study, the mean value of total MUFA content was even higher (49.72%) in *Longissimus dorsi* muscle of Shanzhu × Duroc crossbreds.

Meanwhile, we analyzed the polymorphisms of *ADRB3*, *FABP3*, *LIPE,* and *LPL* genes and tried to define the association of these SNP genotypes with the IMF contents and fatty acid composition of the *Longissimus dorsi* muscle in these crossbreds.

ADRB3 gene encodes the adrenergic beta-3 receptor which is predominantly found on the surface of adipocytes, and mainly regulates the energy balance under sympathetic neural control (Cieslak et al. 2009). Here, *ADRB3* c.1192G>A was found to be associated with IMF at a significant level ($P < 0.05$). This result is concordant with the findings obtained in gilts (Hirose et al. 2009) and in sheep (Horrell et al. 2009), though it is different from Cieslak's report (Cieslak et al. 2009) wherein *ADRB3* gene polymorphism has no association with porcine fatness traits. It is noteworthy that the *ADRB3 AG* heterozygotes exhibited higher IMF content than did the *AA* and *GG* homozygotes, implying that over-dominance may be partially responsible for the variation in IMF content. Moreover, highly significant association of the *ADRB3* gene polymorphism with the total MUFA and the total PUFA as well as no association with the total SFA will provide us with a revealing insight into how this gene influences the lipid composition in muscle. This finding indicates that the polymorphism of *ADRB3* gene may affect MUFA and PUFA deposition rather than SFA deposition in adipose tissue. The MUFA content in meat has an important role in decreasing the blood LDL-cholesterol concentration which contributes to cardiovascular disease (Zhang et al. 2009). According to our results, the pigs with the *ADRB3 G* allele had greater percentage of MUFA than those with the *ADRB3 A* allele, implying that the *ADRB3 G* in pigs may be conducive to human health. Furthermore, the *ADRB3 A* allele was associated with more PUFA, suggesting that *ADRB3* gene may influence colour traits potentially because the content of PUFA influences the susceptibility of meat to oxidation.

FABP3 gene codes for an intracellular protein which plays a critical role in trafficking of fatty acids and has been considered as a candidate gene related to lipid metabolism and fat deposition (Gerbens et al. 1997; Chmurzynska 2006). In our study, there was no significant ($P > 0.05$) association between *FABP3* c.-314T>C and IMF content in pigs as it was displayed in Berkshire breed (Lee et al. 2010), in addition, there were no significant ($P > 0.05$) associations between *FABP3* c.-314T>C and any fatty acid composition contents. The possible reason might be various genetic backgrounds of different pigs. In Shanzhu × Duroc crossbreds, allele *T* of *FABP3* c.-314T>C was close to be fixed. Therefore, it may not be suitable to evaluate any association of this *FABP3* gene polymorphism with fatness traits in these crossbreds.

Hormone-sensitive lipase encoded by *LIPE* gene cleaves free fatty acids from the intracellular triacylglycerol (Mersmann 1998; Large et al. 1999), so it contributes to the mobilization of free fatty acids. *LIPE* gene is involved in the antilipolysis and participates in the lipid homeostasis (KEGG PATHWAY database, http://www.genome.jp/kegg/pathway.html). In the case of *LIPE* SNP, the performed analysis of *LIPE* c.442G>A revealed significant association with IMF content, and the *LIPE A* allele was associated with greater IMF ($P < 0.05$). Moreover, *LIPE* c.442G>A was found to be significantly associated with the C12:0 content and the C14:0 content ($P < 0.05$), and had a trend of association with the C18:0 content, indicating *LIPE* gene polymorphism acts to affect the contents

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of some kinds of SFA in pigs. Concretely, the pigs that carried the *AA* genotype of *LIPE* gene showed greater contents of C12:0 and C14:0 than those carrying the *AG* and *GG* genotypes.

Lipoprotein lipase encoded by *LPL* gene is an important enzyme to hydrolyze triacylglycerol from triacylglycerol-rich lipoprotein particles and to liberate the fatty acids to different tissues (Luo et al. 2009). As for *LPL* gene, it participates in the PPAR signalling pathway and mainly relates with the fatty acid transport and lipid metabolism (KEGG PATHWAY database). In the present study, highly significant association was observed between *LPL* c.624C>T and IMF content ($P < 0.01$), suggesting the *LPL* polymorphism has a great influence on the pork fat quality. It is worth mentioning that the *CT* heterozygotes exhibited the lowest IMF content in all three genotypes of *LPL* gene. Furthermore, association was observed between the C14:0 content, the C16:1 content, the C18:0 content, the total SFA, with the polymorphism of *LPL* gene ($P < 0.05$). Actually, the pigs carrying *TT* genotype not only had higher contents of IMF than the pigs carrying either *CC* or *CT* genotypes, but also produced pork with a greater content of C16:1, indicating that *LPL* SNP may have influence on the total fat contents as well as the fatty acid profile in muscle of pigs. In contrast, the contents of C14:0, C18:0, and total SFA were lower in pigs that carried *TT* genotype than in pigs that carried other genotypes, suggesting that *LPL T* allele may help reduce the percentages of total SFA which increase the risk of cardiovascular disease.

In conclusion, this study illustrates that the SNP genotypes of *ADRB3*, *LIPE,* and *LPL* genes were significantly associated with the IMF content in the *Longissimus dorsi* muscle of Shanzhu × Duroc crossbreds. Concretely, *ADRB3 AG* heterozygotes exhibited higher IMF content than *AA* and *GG* homozygotes. *LIPE A* allele was associated with greater IMF content. *LPL CT* heterozygotes exhibited the lowest IMF content in all three genotypes. Moreover, our study demonstrates that the polymorphism of *ADRB3*, *LIPE,* and *LPL* genes showed significant association with the fatty acid composition contents. The pigs with *ADRB3 G* allele had greater MUFA, and the pigs with *LPL T* allele had lower SFA, implying that the *ADRB3 G* and *LPL T* in pigs may be beneficial to human health. However, this study shows no significant association of *FABP3* SNP with IMF or any fatty acid composition content. The results of our study suggest that *ADRB3*, *LIPE,* and *LPL* genes are important sources for the pork selection to obtain favourable meat with higher IMF levels and thus better taste properties improving porcine fat quality. Nevertheless, efforts should be made to achieve better understanding of the control of the fat deposition along with genetic regulation in pigs.

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