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Association of adipocytokine *IL-1A* and *IL-6* genes with intramuscular fat content and fatty acid composition in pigs

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ABSTRACT

Several adipocytokines are involved in inflammatory and immune responses as well as regulated fat deposition and lipid metabolism in mammals. This study aimed to verify the polymorphisms of the porcine interleukin 1A (*IL-1A*) and interleukin 6 (*IL-6*) genes and to assess their association with intramuscular fat (IMF) content and fatty acid (FA) composition in commercial crossbred pigs. Two single nucleotide polymorphisms (SNPs) of the porcine *IL-1A* g.43722547A>G and *IL-6* g.91508173C>T loci were found to be segregating in these crossbred pigs. Furthermore, the porcine *IL-1A* g.43722547A>G polymorphism was found to be significantly associated with myristic, palmitic, palmitoleic, and eicosadienoic acid levels. Moreover, the porcine *IL-6* g.91508173C>T polymorphism was significantly associated with IMF content and homolinolenic acid levels. These results suggest that the polymorphisms of the porcine *IL-1A* and *IL-6* genes correlated with lipid content and FA composition and confirmed the importance of the adipocytokine *IL-1A* and *IL-6* genes as candidate genes for fatty acid composition in the muscles of pigs.

1. Introduction

Intramuscular fat (IMF) content and fatty acid (FA) composition are important traits for the determination of meat quality and are strongly related to the eating quality of pork (Ros-Freixedes, Reixach, Bosch, Tor, & Estany, 2014; Wood et al., 2004). IMF content and FA composition traits are influenced by polygenic and environmental factors (Wood et al., 2008; Zappaterra et al., 2020). Many genomic approaches of quantitative trait loci (QTL) mapping, genome-wide association study (GWAS), and transcriptomes have been carried out to identify QTLs and candidate genes for IMF and FA content in the muscle tissues of pigs (Cánovas, Quintanilla, Amills, & Pena, 2010; Muñoz et al., 2007; Zhang et al., 2016). Moreover, a number of previous studies have demonstrated that many adipocytokine genes play an important role in fat deposition and lipid metabolism in mammalian species (Choi et al., 2005; Matsuki, Horai, Sudo, & Iwakura, 2003; Um, Rim, Kim, Kim, & Hong, 2011). Several associations of adipocytokine genes with fatness traits have been identified in humans, mice, and pigs (Manica-Cattani et al., 2010; Ponsuksili, Murani, Brand, Schwerin, & Wimmers, 2011; Um et al., 2011).

Interleukin 1 (IL-1) is one of the main pro-inflammatory cytokines and is known to be secreted from adipose tissues (Ballak, Stienstra, Tack, Dinarello, & van Diepen, 2015; Matsuki et al., 2003). The IL-1 family consists of four major molecules, namely IL-1A, IL-1B, IL-1 receptor antagonist (IL-1RA), and IL-18 (Manica-Cattani et al., 2010). The IL-1A regulates adipocyte differentiation and insulin signaling in adipocytes

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(Ballak et al., 2015). The *IL-1A* gene is located on pig chromosome 3 (SSC3 q1.2-q1.3) at position 43.7 Mb (Mellink, Lahbib-Mansais, Yerle, & Gellin, 1994). The coding sequence of the porcine *IL-1A* gene is 1735 bp in length (ENSSSCT00000008863.3; http://asia.ensembl.org/index.html). It is composed of eight exons and seven introns and encodes a peptide of 270 amino acids. The porcine *IL-1A* gene is located close to the QTL regions for IMF content (47.5–130.1 Mb), linoleic (48.5 Mb), eicosadienoic (48.5 Mb), arachidonic (48.5 Mb), monounsaturated (48.5 Mb), polyunsaturated (48.5 Mb), and palmitoleic (55.5 Mb) FAs (Revilla et al., 2017; Won, Jung, Park, & Kim, 2018; Yang et al., 2013). A total of 318 SNPs of the porcine *IL-1A* gene have been characterized (Ensembl database, online access 16.09.2020). Moreover, an association of *IL-1A* polymorphisms with the fatness trait has been reported in humans (Carter et al., 2008; Song et al., 2008; Um et al., 2011).

Interleukin 6 (IL-6) is a pro-inflammatory adipocytokine and is secreted by adipose tissue, skeletal muscle, and macrophages (Makki, Froguel, & Wolowczuk, 2013). IL-6 is involved in immune response and modulates lipid homeostasis, lipolysis, and fatty acid oxidation in adipocytes, hepatocytes, and skeletal muscle tissues (Hashizume & Mihara, 2011; Makki et al., 2013). The *IL-6* gene is located on pig chromosome 9 (SSC9) (Rettenberger, Bruch, Fries, Archibald, & Hameister, 1996) at position 91.5 Mb. The coding sequence of the porcine *IL-6* gene is 1204 bp in length (ENSSSCT00000023544; http://asia.ensembl.org/index.html). It is composed of five exons and four introns and encodes a peptide of 241 amino acids. The porcine *IL-6* gene is located close to the QTL regions for IMF content (90.6 and 129.4 Mb), polyunsaturated (117.7 Mb), linoleic (121.0–129.2 Mb), and saturated (129.0 Mb) FAs (Iqbal et al., 2015; Park et al., 2017; Uemoto et al., 2012; Won et al., 2018; Zhang et al., 2016). A total of 81 SNPs of the porcine *IL-6* gene have been characterized (Ensembl database, online access 16.09.2020). Moreover, an association of the porcine *IL-6* polymorphism with the fatness trait has been reported in pigs (Szydlowski, Buszka, Mackowski, Lechniak, & Switonski, 2011).

All of this evidence indicates that *IL-1A* and *IL-6* functions are critical for fat deposition in muscle tissues. Therefore, the *IL-1A* and *IL-6* genes can be regarded as positional and functional candidate genes for fat deposition (IMF content and FA composition) traits in pigs. However, information on the association of *IL-1A* and *IL-6* genes with IMF content and FA composition traits in pigs is limited. In the present study, we have verified the polymorphisms in porcine *IL-1A* and *IL-6* genes, while their association with IMF and FA composition traits has also been assessed in commercial crossbred pigs.

2. Materials and methods

2.1. Animals and DNA isolation

A total of 497 pigs were obtained from a stock of crossbred Duroc and Large White × Landrace pigs (224 barrows and 273 gilts) that were reared under commercial conditions at the Betagro Hybrid International Company, Thailand. These pigs were fed a corn-soybean based diet containing 16% crude protein and 3200 kcal/kg digestible energy. All the animals were slaughtered according to standard commercial procedures when they reached a bodyweight of about 90 kg. Therefore, ethical approval was not required for this study. The *longissimus thoracis* (LT) muscle tissues (150–200 g) from the left side of each carcass at the 10-11th rib were taken to determine IMF content and fatty acid composition. DNA samples were extracted from LT muscle tissues using the standard phenol-chloroform method and were kept at 4 °C until analysis.

2.2. Determination of intramuscular fat content and fatty acid composition

IMF content was determined from LT muscle tissues using the ether extraction method as has been established by the Association of Official Analytical Chemists regulations (AOAC, 2000). IMF was expressed as g of lipid in 100 g of muscle tissue. The extracted lipid was converted to fatty acid methyl esters (FAMEs) using the method described in a previous study (Zhang et al., 2019). The fatty acid profiles were analyzed using a gas chromatography-flame ionization detector (SCION 456-GC, Bruker Daltonics Inc., Fremont, USA) with an RT-2560 capillary column (100 m length, 0.25 mm internal diameter, 0.20 μm film thickness, RESTEK, Bellefonte, PA, USA). Injector and detector temperatures were held at 250 °C. The column oven temperature program was increased from 50 to 220 °C at a rate of 10 °C/min, held for 35 min, increased from 200 to 230 °C at a rate of 5 °C/min and then held at 230 °C for 20 min. A sample volume of 1 μl was then injected. A 37-component standard FAME mix (Food Industry Fame Mix, RESTEK, Bellefonte, PA, USA) was used for the identification of FAMEs. Individual fatty acids were expressed as g per 100 g of the total fatty acids (Zappaterra et al., 2019). The proportions of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA), as well as the ratios of MUFA/SFA, PUFA/SFA, and MUFA/PUFA were calculated.

2.3. Polymorphisms of porcine IL-1A and IL-6 genes and genotyping

The genotyping of the SNPs of the porcine *IL-1A* and *IL-6* genes was carried out using the polymerase chain reaction-fragment length polymorphism (PCR-RFLP) method. Four SNPs of the porcine *IL-1A* and *IL-6* genes were selected based on the restriction enzyme available, as had been ascribed in the Ensembl database (ENSSSCT00000008863.3 and ENSSSCT00000023544; http://asia.ensembl.org/index.html). These four SNPs consisted of *IL-1A* g.43722505C>T (rs707290786), *IL-1A* g.43722547A>G (rs343140378), *IL-6* g.91506415A>G (rs1112378099), and *IL-6* g.91508173C>T (rs1109532035). Specific primers were designed using the porcine *IL-1A* and *IL-6* nucleotide sequences (GenBank accession no. NC_010445.4 and NC_010451.4, respectively) as is shown in Table 1. A mismatched primer was designed to introduce a recognition site of the restriction enzyme for genotyping (Table 1). The PCR amplifications were performed with 50 ng of the DNA template at a total volume of 20 μl containing 0.25 U *Taq* DNA polymerase (Fermentas, USA), 1 × $(NH_4)_2SO_4$ buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 0.4 μM for each primer (Table 1). The PCR conditions were as follows: 94 °C for 3 min at the initial denaturing stage followed by 35 cycles of 94 °C for 30 s, 58–60 °C for 30 s, 72 °C for 30 s, and then 5 min at 72 °C to complete the reaction. The PCR products were digested with restriction enzymes (Table 1). The digested products were separated on 6% polyacrylamide gel electrophoresis and visualized by ethidium bromide staining.

2.4. Statistical analysis

The genotype and allele frequencies of the porcine *IL-1A* and *IL-6* SNPs were calculated. Hardy-Weinberg equilibrium (HWE) was determined using the chi-square test. Associations of the porcine *IL-1A* and *IL-6* polymorphisms with IMF content and FA composition traits in commercial crossbred pigs were analyzed using the general linear model (GLM) as follows: $Y_{ijk} = \mu + S_i + G_j + e_{ijk}$ where Y_{ijk} represents the observed value of IMF content or FA composition traits, μ is a representative of the population mean value of the measurements, S_i represents the fixed effect of the sexes ($i = 1–2$), G_j is representative of the fixed effect of the genotypes ($j = 1–3$), and e_{ijk} represents an error residual. All statistical analyses were considered significant at $P < 0.05$.

3. Results

3.1. Polymorphisms of porcine IL-1A and IL-6 genes

Four SNP loci of the porcine *IL-1A* and *IL-6* genes were verified in the commercial crossbred pigs. Two SNPs (*IL-1A* g.43722547A>G and *IL-6* g.91508173C>T) were found to be segregating among this pig

Table 1
Primer sequences and restriction enzymes used for genotyping of porcine *IL-1A* and *IL-6* genes.

SNP	Primer sequence (5′ to 3′)	Size (bp)	Tm (°C)	Restriction enzyme
IL-1A g.43722505C>T	F: TATGCCTCTGAGTACCTCTAA R: TCGTCATCGGTGATGAACTAA	135	60	*Sat*I
IL-1A g.43722547A>G	F: TATGCCTCTGAGTACCTCTAA R*: TCGTCATCGGTGATGAACTAA	135	60	*Tsp*509I
IL-6 g.91506415A>G	F: TTTCCCTGGTTGTGATTCCT R: GGGATTTCCTTCACTTACTT	295	58	*Hpy*188I
IL-6 g.91508173C>T	F: GCCCATTCTCCACTTGTTTG R: TGCCTGCTTGGTCTACATGT	359	60	*Msp*I

* Mismatched base is underlined to generate a recognition site of the restriction enzyme *Tsp*509I for genotyping.

population. The porcine *IL-1A* g.43722547A>G polymorphism was a non-synonymous SNP leading to a non-conservation amino acid exchange at position N89S of exon 4. The porcine *IL-6* g.91508173C>T was an intronic SNP of intron 3. No polymorphisms of the porcine *IL-1A* g.43722505C>T (A75V) and *IL-6* g.91506415A>G (K7E) loci were observed in this study.

3.2. Genotype and allele frequencies of porcine IL-1A and IL-6 genes

The genotype and allele frequencies of the porcine *IL-1A* and *IL-6* genes are shown in Table 2. Three genotypes of the porcine *IL-1A* g.43722547A>G and *IL-6* g.91508173C>T polymorphisms were found to be segregating among commercial crossbred pigs. The alleles of *IL-1A* g.43722547A and *IL-6* g.91508173C were the major alleles in this pig population. Whereas, the porcine *IL-1A* g.43722505 and *IL-6* g.91506415 loci were fixed as *IL-1A* g.43722505C and *IL-6* g.91506415A among commercial crossbred pigs. The chi-square test indicated that the genotype distributions of the porcine *IL-1A* g.43722547A>G and *IL-6* g.91508173C>T loci deviated from the HWE specifications ($P < 0.01$).

3.3. Association of porcine IL-1A and IL-6 genes with IMF content and FA composition

The effects of *IL-1A* and *IL-6* genes on IMF content and FA composition are shown in Tables 3 and 4. There was no association of porcine *IL-1A* g.43722547A>G with the IMF trait. However, porcine *IL-1A* g.43722547A>G was significantly associated with myristic, palmitic, palmitoleic, and eicosadienoic acid levels. The pigs with the GG genotype had higher myristic, palmitic, palmitoleic, and eicosadienoic acid levels when compared to those of the pigs with the AG and AA genotypes. Therefore, the porcine *IL-1A* g.43722547G allele was associated with higher FA levels in these crossbred pigs. Moreover, the porcine *IL-1A* g.43722547A>G polymorphism showed a trend toward being associated with lauric ($P = 0.06$), homolinolenic ($P = 0.07$), SFA ($P = 0.07$), and the ratio of MUFA to SFA ($P = 0.10$) levels in the LT muscle tissues of pigs (Table 3). Moreover, porcine *IL-6* g.91508173C>T was significantly

Table 2
Genotype and allele frequencies of porcine *IL-1A* and *IL-6* genes in pigs.

SNPs	n	Genotype frequency			Allele frequency[a]		*P*-value[b] (χ^2)
		AA	AB	BB	A	B	
IL-1A g.43722505C>T	457	1.00	0.00	0.00	1.00	0.00	–
IL-1A g.43722547A>G	482	0.27	0.58	0.15	0.56	0.44	<0.01**
IL-6 g.91506415A>G	467	1.00	0.00	0.00	1.00	0.00	–
IL-6 g.91508173C>T	478	0.21	0.66	0.13	0.54	0.46	<0.01**

[a] Allele A represents major alleles of the porcine *IL-1A* g.43722505C, *IL-1A* g.43722547A, *IL-6* g.91506415A, and *IL-6* g.91508173C loci and allele B represents minor alleles of the porcine *IL-1A* g.43722505T, *IL-1A* g.43722547G, *IL-6* g.91506415G, and *IL-6* g.91508173T loci.
[b] The *P*-value is considered a significant level of the chi-square (χ^2) test for Hardy-Weinberg equilibrium of each locus.
** $P < 0.01$.

Table 3
Association of porcine *IL-1A* g.43722547A>G with IMF content and fatty acid composition traits in *longissimus thoracis* muscles in pigs.

Traits[1]	Genotypes (least squares mean ± SE)			*P*-value
	AA (n = 132)	AG (n = 278)	GG (n = 72)	
IMF	2.025 ± 0.441	2.380 ± 0.313	2.334 ± 1.266	0.6372
Lauric (C12:0)	0.086 ± 0.007	0.099 ± 0.004	0.118 ± 0.012	0.0640
Myristic (C14:0)	1.012 ± 0.073[a]	1.195 ± 0.037[b]	1.450 ± 0.116[c]	0.0037
Palmitic (C16:0)	12.381 ± 0.666[a]	13.337 ± 0.336[a]	15.675 ± 1.055[b]	0.0270
Stearic (C18:0)	12.095 ± 0.600	10.959 ± 0.303	12.223 ± 0.951	0.1463
Arachidic (C20:0)	0.278 ± 0.049	0.259 ± 0.025	0.339 ± 0.078	0.6139
SFA	25.766 ± 1.075	25.750 ± 0.542	29.687 ± 1.704	0.0697
Palmitoleic (C16:1n-7)	3.696 ± 0.255[a]	4.091 ± 0.129[b]	4.878 ± 0.404[b]	0.0392
Oleic (C18:1n-9)	33.847 ± 1.909	34.458 ± 0.963	29.172 ± 3.026	0.2556
Eicosenoic (C20:1n-9)	2.125 ± 0.212	1.792 ± 0.107	1.603 ± 0.335	0.2543
MUFA	39.689 ± 1.949	40.342 ± 0.983	35.653 ± 3.089	0.3555
Linoleic (C18:2n-6)	23.237 ± 1.413	23.933 ± 0.713	24.440 ± 2.239	0.8609
Linolenic (C18:3n-6)	0.165 ± 0.039	0.161 ± 0.020	0.173 ± 0.062	0.3830
Eicosadienoic (C20:2n-6)	1.848 ± 0.255[a]	1.640 ± 0.129[a]	2.821 ± 0.404[b]	0.0240
Homolinolenic (C20:3n-6)	0.321 ± 0.053	0.205 ± 0.027	0.346 ± 0.085	0.0724
Arachidonic (C20:4n-6)	0.203 ± 0.219	0.249 ± 0.111	0.264 ± 0.347	0.6925
PUFA	25.776 ± 1.396	26.189 ± 0.705	27.617 ± 2.214	0.7657
MUFA/SFA	1.528 ± 0.092	1.602 ± 0.046	1.281 ± 0.145	0.1076
PUFA/SFA	1.092 ± 0.082	1.082 ± 0.040	0.931 ± 0.128	0.5077
MUFA/PUFA	1.710 ± 0.162	1.680 ± 0.080	1.449 ± 0.254	0.6509

[a,b,c]Values in each row with different superscript letters are significantly different ($P < 0.05$).
[1] IMF: intramuscular fat content, SFA: saturated fatty acids (C14:0 + C16:0 + C18:0 + C20:0), MUFA: monounsaturated fatty acids (C16:1n-7 + C18:1n-9 + C20:1n-9), PUFA: polyunsaturated fatty acids (C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6). IMF is expressed as g of lipid in 100 g of *longissimus thoracis* muscle tissue, while fatty acids are expressed as g of fatty acid in 100 g of total fatty acids.

Table 4
Association of porcine *IL-6* g.91508173C>T with IMF content and fatty acid composition traits in *longissimus thoracis* muscles in pigs.

Traits[1]	Genotypes (least squares mean ± SE)			*P*-value
	CC (n = 101)	CT (n = 314)	TT (n = 63)	
IMF	3.298 ± 1.101[b]	3.450 ± 0.452[b]	2.140 ± 0.575[a]	0.0049
Lauric (C12:0)	0.083 ± 0.021	0.101 ± 0.004	0.090 ± 0.010	0.4186
Myristic (C14:0)	1.069 ± 0.208	1.221 ± 0.035	1.031 ± 0.100	0.1567
Palmitic (C16:0)	13.009 ± 1.858	13.579 ± 0.319	12.620 ± 0.897	0.5659
Stearic (C18:0)	10.868 ± 1.611	11.197 ± 0.277	12.119 ± 0.778	0.4983
Arachidic (C20:0)	0.223 ± 0.126	0.265 ± 0.022	0.245 ± 0.061	0.9042
SFA	25.170 ± 2.963	26.262 ± 0.509	26.015 ± 1.431	0.9263
Palmitoleic (C16:1n-7)	3.180 ± 0.706	4.213 ± 0.121	3.871 ± 4.213	0.2436
Oleic (C18:1n-9)	31.016 ± 5.149	33.993 ± 0.884	34.869 ± 2.487	0.5877
Eicosenoic (C20:1n-9)	1.497 ± 0.679	1.852 ± 0.117	2.187 ± 0.328	0.5209
MUFA	35.693 ± 5.223	40.059 ± 0.897	42.176 ± 2.52	0.4946
Linoleic (C18:2n-6)	26.821 ± 3.720	23.909 ± 0.639	23.835 ± 1.797	0.2968
Linolenic (C18:3n-6)	0.080 ± 0.104	0.156 ± 0.018	0.096 ± 0.050	0.4125
Eicosadienoic (C20:2n-6)	1.568 ± 0.705	1.756 ± 0.121	1.689 ± 0.341	0.9507
Homolinolenic (C20:3n-6)	0.894 ± 0.126[b]	0.197 ± 0.022[a]	0.275 ± 0.061[a]	0.0001
Arachidonic (C20:4n-6)	0.217 ± 0.140	0.036 ± 0.024	0.149 ± 0.068	0.1396
PUFA	30.580 ± 3.693	26.055 ± 0.634	26.044 ± 1.784	0.2516
MUFA/SFA	1.411 ± 0.250	1.565 ± 0.042	1.599 ± 0.126	0.7983
PUFA/SFA	1.286 ± 0.218	1.048 ± 0.037	1.066 ± 0.109	0.5626
MUFA/PUFA	1.351 ± 0.429	1.699 ± 0.073	1.615 ± 0.216	0.6898

[a,b]Values in each row with different superscript letters are significantly different ($P < 0.05$).

[1] IMF: intramuscular fat content, SFA: saturated fatty acids (C14:0 + C16:0 + C18:0 + C20:0), MUFA: monounsaturated fatty acids (C16:1n-7 + C18:1n-9 + C20:1n-9), PUFA: polyunsaturated fatty acids (C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6). IMF is expressed as g of lipid in 100 g of *longissimus thoracis* muscle tissue, while fatty acids are expressed as g of fatty acid in 100 g of total fatty acids.

associated with IMF content and homolinolenic acid levels. Pigs with the CC and CT genotypes revealed a significantly higher IMF content than the pigs with the TT genotype. Meanwhile, pigs with the CC genotype presented higher homolinolenic acid levels than the pigs with the CT and TT genotypes ($P < 0.05$). Thus, the porcine *IL-6* g.91508173C allele was associated with an increase in IMF content and homolinolenic acid levels in these crossbred pigs.

4. Discussion

Intramuscular fat deposition is an important trait for the meat quality of pigs and can enhance the eating quality of pork (Fernandez, Monin, Talmant, Mourot, & Lebret, 1999; Wood et al., 2004). IMF deposition is associated with the size and number of adipocytes, and the balance between lipogenesis and the lipolysis rate in muscles (Oliveira et al., 2018). Moreover, the fatty acid composition is considered an indicator of lipid quality and is closely related to the nutritional value of pork (Sanchez et al., 2007; Wood et al., 2004; Wood et al., 2008). Currently, there has been considerable focus on the association of adipocytokines with obesity, lipid accumulation, and lipid metabolism in humans and mice (Matsuki et al., 2003; Um et al., 2011). Moreover, an association of adipocytokine genes (e.g. adiponectin, leptin, and leptin receptor) with fatness and IMF traits has been reported in pigs (Ros-Freixedes et al., 2016; Stachowiak & Flisikowski, 2019; Wang et al., 2020).

In the present study, we have verified the polymorphisms of the porcine *IL-1A* and *IL-6* genes and assessed their effects on IMF content and FA composition in LT muscle tissues. In this regard, two SNPs of the porcine *IL-1A* g.43722547A>G and *IL-6* g.91508173C>T loci were segregating in these commercial crossbred pigs. The chi-square test revealed that the porcine *IL-1A* g.43722547A>G and *IL-6* g.91508173C>T loci were a significant deviation from the HWE specifications. Therefore, it can be expected that there was an excess of the observed heterozygous genotype of the porcine *IL-1A* g.43722547A>G and *IL-6* g.91508173C>T loci due to the outcrossing of the Duroc and Large White × Landrace pigs, and this could have caused these pigs to be deviating from the HWE. In this study, the porcine *IL-1A* g.43722547A>G polymorphism had significantly affected FA composition traits of myristic, palmitic, palmitoleic, and eicosadienoic acid levels in LT muscle tissues. The porcine *IL-1A* g.43722547G allele seems to be a favorable allele for these FA composition traits. However, the porcine *IL-1A* g.43722547A allele displayed a trend toward lower SFA levels as well as a greater ratio of MUFA to SFA values in the LT muscle tissues of pigs (Table 3). Therefore, by decreasing the content of SFA and simultaneously increasing MUFA content in muscles, it could contribute to the enhancement of the nutritional value of pork (Zhang et al., 2019). The porcine *IL-1A* g.43722547A>G polymorphism was a non-synonymous SNP and revealed a leading association with a non-conservative amino acid exchange at position 89 from asparagine to serine (N89S). However, the variant N89S of the porcine *IL-1A* gene has not yet been functionally identified. Porcine *IL-1A* N89S was located in the functional domain (^{80}LKKRRLSLSQS^{91}) of the precursor IL-1A molecule that was observed to bind to chromatin and act as a transcription factor, which has been described in humans and mice (Kim et al., 2013; Stevenson, Turck, Locksley, & Lovett, 1997). Thus, it could be expected that the observed amino acid change of porcine *IL-1A* N89S might contribute to the variations of FA deposition or might be in close linkage of disequilibrium with the causal SNPs that have a positive effect on FA levels in the muscle tissues of pigs.

Although the role of IL-1A in lipid accumulation and fatty composition in adipose and muscle tissues has been scarcely studied, it has displayed a crucial role in inhibiting insulin signaling downstream of the insulin receptor substrate-1 (IRS-1) in adipocytes and has been positively correlated with insulin resistance (He et al., 2006). This may change the lipid buffering capacity of adipocytes, which is conducive to lipid accumulation in muscles (Wang & He, 2018). Moreover, a conducted transcriptome analysis revealed that the *IL-1A* gene was differentially expressed in the porcine intramuscular adipocytes during differentiation and interacted with *IL-6*, *IL-7*, and *IL-8* genes (Mo et al., 2017). This evidence suggests that these adipocytokine genes might play an important role in the early phase of intramuscular preadipocyte differentiation. Recently, *IL-1A* deficient mice displayed a decrease of adiposity, plasma triglyceride, glucose intolerance in diet-induced obese mice, and the expression of stearoyl-CoA desaturase 1 (*SCD1*), fatty acid synthase (*FASN*), the elongation of long-chain fatty acid family number 6 (*ELOVL6*), and acetyl-CoA carboxylase (*ACC*) genes that promote *de novo* lipogenesis (Almog et al., 2019). Moreover, these genes play a major role in FA metabolism and lipid synthesis in pigs (Muñoz et al., 2007; Muñoz et al., 2018; Stachowiak & Flisikowski, 2019; Zappaterra et al., 2019). All of the above information indicates that *IL-1A* seems to play an important role in fatness and lipid metabolism by regulating insulin sensitivity and genes related to lipid deposition and FA oxidation in adipocytes and muscle tissue. The results of this study indicate that the porcine *IL-1A* polymorphism had effectively reduced SFA, especially

myristic and palmitic acid deposition in muscles. This would indicate that decreasing the SFA content of pork could be beneficial to human health due to the fact that an over consumption of myristic and palmitic acids could lead to an increase in the risk of cardiovascular disease and type 2 diabetes in humans (Zhang et al., 2019). Moreover, the porcine *IL-1A* polymorphism was associated with palmitoleic MUFA and eicosadienoic PUFA levels, which are converted from palmitic and linoleic acids. This evidence suggests that *IL-1A* may be correlated with the fatty acid metabolism of MUFA and PUFA, which would then enhance the proportion of MUFA to SFA.

Numerous studies have reported that the human *IL-6* g.-174G>C polymorphism in the promoter region is associated with obesity, plasma triglycerides, very low-density lipoprotein (VLDL), fasting plasma lipid, and postglucose load free fatty acid (FFA) concentrations in Caucasian populations (Fernández-Real, Broch, Vendrell, Richart, & Ricart, 2000; Klipstein-Grobusch et al., 2006). Moreover, a previous study has reported on the associations of the *IL-6* polymorphism with the fatness trait in pigs (Szydlowski et al., 2011). Interestingly, the porcine *IL-6* gene was found to be located within the QTL regions for IMF content, as well as linoleic, SFA, and PUFA (Iqbal et al., 2015; Park et al., 2017; Uemoto et al., 2012; Won et al., 2018; Zhang et al., 2016). Thus, the porcine *IL-6* gene may have a relationship with fat accumulation and FA composition traits. However, to date, there has been no report on the association of the *IL-6* polymorphism with IMF content and FA composition in pigs. In the present study, we found a significant association of the porcine *IL-6* g.91508173C>T polymorphism with IMF content and the levels of homolinolenic acid. The porcine *IL-6* g.91508173C allele was positively associated with IMF content and the homolinolenic acid levels in crossbred pigs. However, the porcine *IL-6* g.91508173C>T polymorphism was an intronic variant and did not exhibit any effect on the coding sequence of the porcine *IL-6* gene. Thus, it might be in a close linkage of disequilibrium with the causal SNPs associated with the positive effect on IMF content and the FA composition traits in pigs.

The molecular mechanism of *IL-6* on lipid accumulation in muscles is still unclear but may involve increased FA uptake and/or decreased FA oxidation (Kim et al., 2004). However, several previous studies have demonstrated a strong relationship between *IL-6* and lipid metabolism with insulin sensitivity in mice, rats, and humans (Bruce & Dyck, 2004; Hashizume & Mihara, 2011; Sharma & Dabur, 2020). It has been hypothesized that *IL-6* may be involved in activating 5′-AMP-activated protein kinase (AMPK) in skeletal muscles, while it has been proposed that AMPK may act as a metabolic master switch that stimulates glucose uptake and FA oxidation in skeletal muscles (Bruce & Dyck, 2004; MacDonald, Wojtaszewski, Pedersen, Kiens, & Richter, 2003; Muñoz-Cánoves, Scheele, Pedersen, & Serrano, 2013). In addition, it has been reported that *IL-6* deficient mice indicate obesity development, increased intramuscular lipid accumulation and SFA composition (Chabowski et al., 2008; Wallenius et al., 2002). Higher serum levels of triglycerides and VLDL were found in *IL-6* deficient mice, while intracerebroventricular IL-6 treatment increased energy expenditure (Wallenius et al., 2002; Wang & He, 2018). It has been suggested that IL-6 may possess anti-obesity effects by regulating the central nervous system (CNS), especially in the hypothalamus (Wallenius et al., 2002). It also promotes lipolysis in adipose tissues, as well as suppresses lipid synthesis and decreases blood lipids (Wang & He, 2018). However, these results are inconsistent with those of previous studies, which have indicated that acute IL-6 treatment can induce insulin resistance in skeletal muscles and increase intramuscular fatty acyl-CoA levels (Kim et al., 2004). Moreover, plasma IL-6 expression levels revealed a relationship with obesity-related insulin resistance (Kern, Ranganathan, Li, Wood, & Ranganathan, 2001). Although the above-mentioned studies have produced conflicting results, it can be suggested that the different pleiotropic effect of IL-6 on fatness and lipid metabolism probably is indicative of an interaction with the specific target cells at various pathway levels. However, the outcomes of this present study indicate a significant association of the porcine *IL-6* gene with IMF content and homolinolenic PUFA levels, as well as a trend toward being associated with arachidonic PUFA levels. Homolinolenic acid is known to be converted from linoleic and γ-linolenic acid. It is an essential substance for the synthesis of arachidonic acid, which is a precursor of certain bioactive molecules, e.g. prostaglandins and leukotrienes. It has been reported that the linoleic and arachidonic acid levels, as well as the ratio of arachidonic to linoleic acid levels in muscles showed negative genetic correlation with the IMF content of pigs (Gol et al., 2019). The results of this study suggest that the porcine *IL-6* polymorphism affected the fatty acid metabolic pathway of linoleic acid (PUFA) and was related to IMF content in pigs.

Several previous studies have demonstrated that genetic factors affect IMF content and fatty acid composition in the muscle tissues of pigs (De Smet, Raes, & Demeyer, 2004; Estany, Ros-Freixedes, Tor, & Pena, 2017; Martin, Fredeen, Weiss, & Carson, 1972). It is obviously clear that the endogenous synthesis of SFA and MUFA increases with IMF content, which leads to a decrease in the proportion of PUFA in muscle tissues (Ros-Freixedes et al., 2016; Wood et al., 2008). Moreover, there is evidence to indicate that backfat thickness and IMF content consistently exhibit positive correlations with oleic and MUFA, as well as palmitic, stearic, and SFA levels. Conversely, both of them have displayed negative correlations with linoleic and PUFA levels (Estany et al., 2017; Wood et al., 1978; Zhang et al., 2019). The present study has revealed a positive correlation between IMF content and SFA levels. Notably, there was no correlation between IMF content with linoleic, MUFA, and PUFA levels. However, a significant negative correlation of MUFA with SFA and PUFA was observed in this pig population (data not shown). The results of this study indicate that the polymorphism of the porcine *IL-1A* gene had a significant association with SFA (myristic and palmitic acids), MUFA (palmitoleic acid), and PUFA (eicosadienoic acid) levels. The porcine *IL-1A* polymorphism seemed to be independent of IMF content, which confirmed that the porcine *IL-1A* variation at this locus affected FA composition but not IMF content. Moreover, the polymorphism of the porcine *IL-6* gene had a significant association with IMF content and PUFA (homolinolenic acid) levels. It has been hypothesized that *IL-6* may regulate fat deposition and may also be related to the processing of enzymes in the metabolizing of fatty acids of PUFA in the muscle tissues of pigs. In consideration of all the above-referenced data, it seems reasonable to assume that *IL-1A* and *IL-6* genes may be involved in fatness and skeletal muscle lipid metabolism. In this study, we have provided evidence to indicate that genetic variants in the *IL-1A* and *IL-6* genes exist that can enhance IMF content and proportions of MUFA and SFA, as well as PUFA levels. Further studies are needed in order to clarify the function of the *IL-1A* and *IL-6* genes in regulating lipid accumulation and FA composition in the muscle tissues of pigs. Moreover, confirmation of the effects of *IL-1A* and *IL-6* genes on IMF content and FA composition traits is required in order to confirm this association in various pig populations.

5. Conclusion

In the present study, we have verified variant SNPs in the porcine *IL-1A* and *IL-6* genes and assessed their effects on IMF content and the fatty acid composition traits in the LT muscles of pigs. The porcine *IL-1A* polymorphism revealed an association with myristic, palmitic, palmitoleic, and eicosadienoic acid levels. Moreover, the porcine *IL-6* polymorphism revealed a further association with IMF content and homolinolenic acid levels. The practical implications of these results were to promote the importance of the porcine *IL-1A* and *IL-6* genes in lipid deposition and fatty acid composition in pigs. Therefore, the porcine *IL-1A* and *IL-6* genes may be used as candidate markers for the genetic improvement of lipid content and fatty acid composition in the muscles of pigs. Moreover, genetic variants of *IL-1* and *IL-6* genes are a relevant issue, not only in terms of lipid and FA depositions in the muscles of pigs, but also in terms of an ability to fully understand the relationship between skeletal muscle lipid metabolism and incidences of

obesity in humans.

Conflict of interest declaration

The authors declare that they have no conflicts of interest.

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