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Distribution of *H-FABP* and *ACSL*4 gene polymorphisms and their associations with intramuscular fat content and backfat thickness in different pig populations

J.N. Chen[1]*, Y.Z. Jiang[1]*, W.M. Cen[1], S.H. Xing[1], L. Zhu[2], G.Q. Tang[2], M.Z. Li[2], A.A. Jiang[2], P.E. Lou[3], A.X. Wen[1], Q. Wang[1], T. He[1], G.X. Zhu[1], M. Xie[1] and X.W. Li[2]

[1]College of Life Science, Sichuan Agricultural University, Ya'an, China
[2]College of Animal Science and Technology, Sichuan Agricultural University, Ya'an, China
[3]The Zhejiang Jinhua Pig Farm, Jinhuan, China

*These authors contributed equally to this study
Corresponding authors: YZ Jiang / XW Li
E-mail: jiangyz04@163.com / xuewei.li@sicau.edu.cn

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ABSTRACT. Here, we analyzed the distribution of *H-FABP*/(*Hin*fI, *Msp*I, and *Hae*III) and *ACSL*4/*Rsa*I polymorphisms, and the associations of these 4 polymorphic loci with intramuscular fat (IMF) content and backfat thickness (BFT) in Yanan, Jinhua, Duroc, Landrace, Yorkshire, and Duroc x (Landrace x Yorkshire) (DLY) pigs. *H-FABP*/*Hin*fI polymorphisms were present in all the 6 populations. At the *ACSL*4/*Rsa*I locus, sows had 3 genotypes, whereas boars only had haplotype A or G, in Duroc, Landrace, Yorkshire, and DLY pigs. *H-FABP*/(*Msp*I and *Hae*III) and *ACSL*4/

*Rsa*I polymorphisms were absent in Yanan and Jinhua pigs. Linkage disequilibrium analysis indicated that the 3 loci (*Hin*fI, *Msp*I, and *Hae*III) were separated. Association analysis showed that the *H-FABP*/*Hin*fI locus significantly affected IMF content in DLY ($P < 0.05$) and Yanan ($P < 0.001$) pigs. The highest IMF content was recorded in the adH haplotype of the 3 *H-FABP* polymorphic loci (2.59%, $P < 0.05$) in DLY pigs. At the *ACSL*4/*Rsa*I locus, higher IMF content was recorded for sows with a GG genotype or boars with a G haplotype compared to those with an AA genotype (2.53 *vs* 2.10%, $P < 0.05$) or A haplotype (2.48 *vs* 1.73%, $P < 0.01$) in DLY pigs. Significant differences were not obtained among these 4 polymorphic loci and BFT ($P > 0.05$). The results indicate that *H-FABP* and *ACSL*4 genes might serve as markers to improve IMF content (but not BFT) in the pig breeding system.

Key words: Pig; *H-FABP*; *ACSL*4; Polymorphism; Intramuscular fat content; Backfat thickness

INTRODUCTION

The intramuscular fat (IMF) content of muscle is one of the most important parameters for determining the meat quality (van Wijk et al., 2005). This parameter exhibits a positive correlation with meat tenderness, juiciness, and taste (Fernandez et al., 1999). In contrast, Rincker et al. (2008) observed that marbling has little influence on the eating quality of pork meat. The relationship of IMF content with sensory and eating pork meat quality varies across many studies (Brewer et al., 2001; van Laack et al., 2001; Rincker et al., 2008; Moeller et al., 2010). It is clear that lipid content is not the sole parameter that determines pork sensory quality (Lonergan et al., 2007). Moreover, visible fat content is a major determinant of purchase intent. Today, increasing numbers of consumers in China tend to prefer highly marbled pork, along with some export markets to countries such as Japan and Korea (Sillence, 2008). Hence, pork meat production should reflect varying consumer fat preferences. Therefore, it is necessary to identify ways to control fat deposition through research, taking into consideration both industry needs and consumer demands. However, IMF has been seldom considered as a selection objective in traditional pig breeding systems, since it is difficult to measure this parameter.

One objective of pig breeding programs is the reduction of fat in the carcass to meet consumer demands for lean meat. Generally, fat reduction is perceived as a decrease in backfat thickness (BFT). However, other fat depots, such as IMF content, are reduced as well. Further reduction in IMF would be undesirable, because it is the main fat depot in meat, and is related to the organoleptic characteristics of pig meat (Fernandez et al., 1999; van Wijk et al., 2005). Hovenier et al. (1992) showed that IMF reduction is not completely correlated with BFT reduction; hence, both traits may be treated separately. Lipid deposition and fatty acid composition in pigs are very complex traits that are probably controlled by many genes. The search for IMF content genetic markers was initiated by Gerbens et al. (1997), who suggested that the heart fatty acid binding protein (H-FABP)

might be responsible for this trait. H-FABP is a member of the fatty acid binding protein family, which plays a critical role in intracellular fatty acid transport by binding lipids and regulating metabolic homeostasis (Veerkamp and Maatman, 1995). The porcine *H-FABP* gene is widely distributed, but is primarily expressed in the heart and skeletal muscle (Veerkamp and Maatman, 1995), and is localized on chromosome 6 (Gerbens et al., 1997). Association studies confirmed the effect of this gene on IMF content in pig crossbreeds, including Duroc crosses (Gerbens et al., 1997, 1999; Sieczkowska et al., 2006a); however, these findings were not confirmed in non-Duroc pig populations (Nechtelberger et al., 2001; Sieczkowska et al., 2006b). Pang et al. (2006) also reported that the *H-FABP* gene significantly affects the IMF content of Chinese indigenous breeds.

As a complex trait, IMF is probably shaped by other polymorphic genes involved in lipid synthesis and fatty acid degradation. A gene coding for long-chain acyl-CoA synthetase 4 (ACSL4) seems to be a promising candidate for this role. This is because the *ACSL*4 gene plays an essential role in both lipid biosynthesis and fatty acid degradation (Mercade et al., 2006). The porcine *ACSL*4 gene belongs to a family with 5 *ACSL* isoforms that differ in fatty acid substrate, tissue distribution, location, and regulation. The pig *ACSL*4 mRNA codes a protein of 670 amino acids, with 97% identity to human, mouse, and rat polypeptide sequences. The pig *ACSL*4 gene is located on chromosome X (SSCX), between the SW2456 and SW1943 markers close to a quantitative trait locus (QTL) for IMF (Pérez-Enciso et al., 2002; Čepica et al., 2007). Mercade et al. (2006) reported that the *ACSL*4 gene is expressed in many different tissues. The authors identified 10 polymorphisms within the 3′-UTR region and 2 haplotypes, by the comparative sequencing of 12 pigs from 6 different pig breeds. Association analysis showed that the *ACSL*4/*Rsa*I polymorphism (G2645A) affects the IMF content and composition of fat acid. Ruść et al. (2011) also verified that *ACSL*4/*Rsa*I polymorphism is associated with IMF content in the cross of (Landrace x Yorkshire) x Duroc pigs, while pigs with the GG genotype had the highest IMF content (2.47%).

At present, 3 types of *H-FABP* restriction fragment length polymorphisms (RFLPs), which are defined as *Hin*fI, *Msp*I, and *Hae*III loci, have been described for many pig populations; however, the genetic associations of different genotypes with IMF content and other traits differ in different pig populations, and remain poorly established. Meanwhile, the *ACSL*4/*Rsa*I polymorphism, which is defined by the *Rsa*I locus, and its genetic association with interesting traits has been seldom studied. Therefore, to provide basic data about the 2 genes for marker-assisted selection, along with a theoretical baseline for the improvement of IMF content, we estimated the frequencies of the *H-FABP/*(*Hin*fI, *Msp*I, and *Hae*III) and *ACSL*4/*Rsa*I gene mutations in 6 porcine populations, including 2 native Chinese breeds (Yanan and Jinhua pigs), 3 foreign breeds (Duroc, Landrace, and Yorkshire pigs), and 1 foreign cross-breed pig population of Duroc x (Landrace x Yorkshire) (DLY). We sought possible associations among the different genotypes of the 2 genes with IMF content and BFT in Yanan, Jinhua, and DLY pigs.

MATERIAL AND METHODS

This study was conducted in compliance with the requirements of the Animal Ethics Committee of Sichuan Agricultural University, China.

Animal and phenotype data

In this study, 6 pig populations, including 2 native Chinese breeds (Yanan and Jinhua pigs), 3 foreign breeds (Duroc, Landrace, and Yorkshire pigs), and 1 foreign cross-breed pig population Duroc x (Landrace x Yorkshire) (DLY), were used to detect the genotype distribution of these 4 polymorphic loci. The composition, sample size, and source of the pig populations are showen in Table 1. Blood samples were collected from candidate breeding pigs from Duroc, Landrace, and Yorkshire breeds, while muscle samples were collected from Yanan, Jinhua, and DLY pigs, for genomic DNA extraction.

Table 1. Composition, sample size, and source of pig population.

Populations	Number			Source
	Boars	Sows	Total	
Yanan	43	50	93	Farm of Sichuan Agricultural University, Ya'an city, Sichuan Province
Jinhua	23	62	85	Jinhua Pig Farm, Jinhuan city, Zhejiang Province
Duroc	57	62	119	Farm of Shangqing Company, Chengdu city, Sichuan Province
Landrance	33	53	86	Farm of Shangqing Company, Chengdu city, Sichuan Province
Yorkshire	72	145	217	Farm of Shangqing Company, Chengdu city, Sichuan Province
DLY	50	62	112	Farm of Sichuan Agricultural University, Ya'an city, Sichuan Province

DLY = Duroc x (Landrance x Yorkshire). Yanan pigs were from three herds. Jinhua pigs were from two herds. DLY pigs were from three herds. Duroc, Landrance, and Yorkshire were candidate breeding pigs from the same farm.

For the association study, a total of 93 Yanan, 85 Jinhua, and 112 DLY pigs were randomly selected at 60 days of age. Yanan and DLY pigs were reared in the Farm of Sichuan Agricultural University, Ya'an city, Sichuan Province, China, and Jinhua pigs were reared in the Jinhua Pig Farm, Jinhuan city, Zhejiang Province, China. The treatment conditions were similar for all animals before and after slaughter, and all treatment conditions and experimental procedures were conducted in compliance with the requirements of the Animal Ethics Committee of Sichuan Agricultural University, China. At their predesignated slaughter age, all pigs were slaughtered. The average of 3 BFT measurements was taken with a sliding caliper along the midline of the first rib, last rib, and last lumbar. The longissimus dorsi of the left side of the carcass at the final third/fourth rib was sampled, and used to extract genomic DNA, and measure IMF content. IMF content was analyzed according to the Association of Official Analytical Chemists (AOAC, 1990) procedures.

DNA extraction and genotyping

Total genomic DNA was isolated from blood or muscle samples using a MasterPure DNA Purification Kit (Epicenter Biotechnologies, Madison, WI, USA) and stored at -20°C.

The *H-FABP* and *ACSL4* fragments were amplified from a genomic template by PCR, using primer sequences reported by Gerbens et al. (1997) and Ruść et al. (2011), respectively. The primer sequences, PCR product sizes, and locations are shown in Table 2. PCR was carried out in a 25-μL mixture containing: 20X buffer, dNTP mix (2 mM each), 100 pM of the primer pair, 25 mM $MgCl_2$, 10X enhancer, 0.7 U Taq DNA Polymerase

(Epicenter), 200 ng DNA, and H_2O to make a final solution of 25.0 µL. The amplification conditions were: 94°C for 5 min, 35 cycles at 94°C for 30 s, 57°C (primers 1 and 2) or 60°C (primer 3) for 30 s, 72°C for 1 min (primers 1 and 2) or 30 s (primer 3), and a final extension at 72°C for 5 min.

Table 2. Primer sequences, corresponding PCR product sizes, and positions for each PCR-RFLP.

Gene	PCR-RFLP	Primer sequences (5'-3')	PCR product size (bp)	PCR product location
H-FABP	*Hin*fI	P 1: (forward) GGACCCAAGATGCCTACGCCG (reverse) CTGCATCTTTGACCAAGAGG	693	5' Upstream 1125-1817*
	*Msp*I/*Hae*III	P 2: (forward) ATTGCTTCGGTGTGTTTGAG (reverse) TCAGGAATGGGAGTTATTGG	816	Intron 2 1401-2216**
ACSL4	*Rsa*I	P 3: (forward) CAGAAGATGCTTAAATATTAAGCATGACA (reverse) TGTCTAACCTACACAACAATTATGAATCC	181	3'-UTR region*** 2539-2719

*Product position corresponds to the sequence accession No. X98558 in GenBank. **Product position corresponds to the sequence accession No. Y16180 in GenBank. ***Product position corresponds to the sequence accession No. DQ144454 in GenBank.

RFLP was used to genotype the porcine. PCR products of 693 bp and 816 bp, and 181 bp were generated using the primers described by Gerbens et al. (1997) or Ruść et al. (2011), respectively. The PCR reaction mixture was used for restriction digestion with 2.5 U of *Hin*fI (693-bp fragments of *H-FABP*), *Msp*I, and *Hae*III (816-bp fragments of *H-FABP*), or *Rsa*I (181-bp fragments of *ACSL4*) in a total volume of 10 µL, respectively. Digestion reactions were carried out at 37°C for 2 h (*Hin*fI and *Rsa*I) or 1 h (*Msp*I and *Hae*III), and DNA fragments were separated on 2.5% agarose gel in Tris-acetate-EDTA buffer.

Statistical analyses

The expectation-maximization (EM) algorithm was used to construct the haplotype. The linkage disequilibrium of the 3 polymorphic loci (*Hin*fI, *Msp*I, and *Hae*III) was analyzed by the Haploview 4.2 software, where the blocks were defined by 95% confidence bounds of D'.

The general linear model procedure was used to determine the association between genotypes of a single locus, or haplotypes and traits, using the statistical software SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). The model was: $Y_{ijkl} = \mu + B_i + S_j + G1_k + G2_l + b_{ijkl}X_{ijkl} + e_{ijkl}$, where Y_{ijkl} was the observation, μ was the general mean, B_i was the effect of herd i, S_j was the effect of sex j, $G1_k$ was the effect of *Hin*fI, *Msp*I, or *Hae*III locus genotype k, $G2_l$ was the effect of *Rsa*I locus genotype l, b_{ijkl} was the regression coefficient of the body weight, X_{ijkl} was the body weight, and e_{ijkl} was the random error. $G1_k$ and $G2_l$ were replaced as the effect of the *H-FABP* haplotype k and allele G of *ACSL4*, respectively, when analyzing the genetic haplotype effect.

RESULTS

RFLP patterns, genotype, and allele frequency

The genotypes of the 4 polymorphic loci were detected by using PCR-RFLP of the

6 pig populations. The 693 bp of the PCR product of *H-FABP* was used for *Hin*fI RFLP. *Hin*fI RFLP genotype classes were HH, Hh, and hh. The H allele was cleaved into 5 fragments of 339, 172, 98, 59, and 25 bp, and the h allele was cleaved into 4 fragments of 339, 231, 98, and 25 bp using *Hin*fI (Figure 1). The 816 bp of the PCR product of *H-FABP* was used for *Msp*I and *Hae*III RFLP. *Msp*I RFLP genotype classes were AA, Aa, and aa. The A allele was identified when the 816 bp PCR products were divided into 750 bp and 66 bp fragments by *Msp*I, and the intact fragment (816 bp) was the a allele (Figure 2). The *Hae*III RFLP genotype classes were DD, Dd, and dd. The d allele was digested into 405-, 278-, 117-, and 100-bp fragments, and the D allele was digested into 683, 117, and 16 bp by *Hae*III (Figure 3). The 181 bp of the PCR product of *ACSL*4 was used for *Rsa*I RFLP. *Rsa*I RFLP genotype classes were AA, AG, and GG. Allele A was cleaved into 2 fragments of 134 and 47 bp, and the G allele was cleaved into 3 fragments of 108, 47, and 26 bp using *Rsa*I (Figure 4).

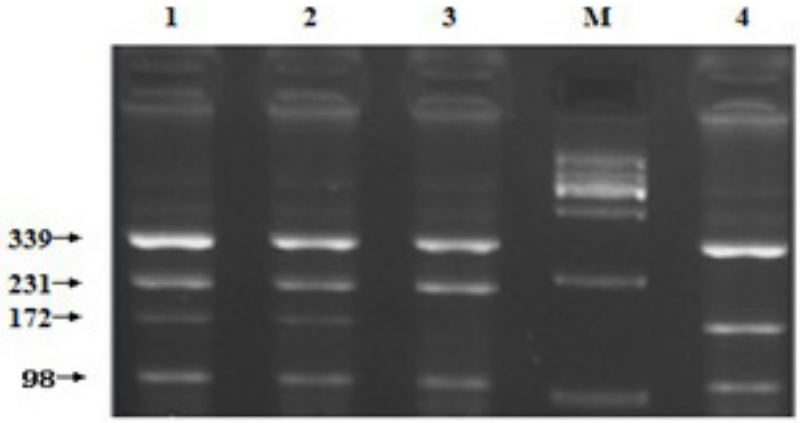

Figure 1. Genotyping of *H-FABP*/*Hin*fI locus by PCR-RFLP. *Lane 3* = hh genotype (339, 231, 98, and 25 bp). *Lane 4* = HH genotype (339, 172, 98, 59, and 25 bp). *Lanes 1* and *2* = Hh genotype (339, 231, 172, 98, 59, and 25 bp). *Lane M* = Marker DL 2000.

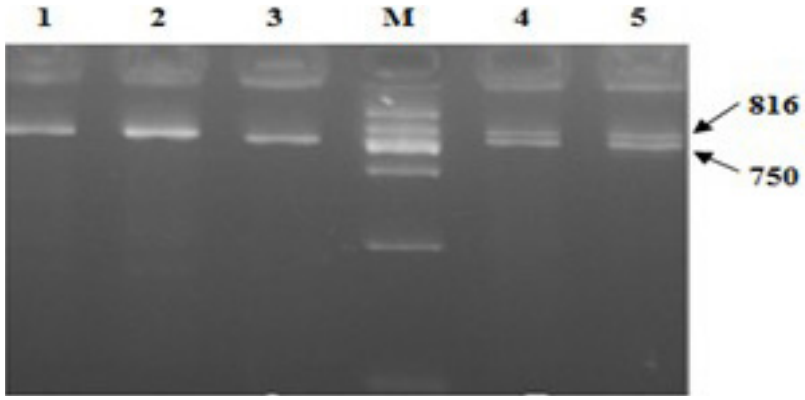

Figure 2. Genotyping of *H-FABP*/*Msp*I locus by PCR-RFLP. *Lanes 1* and *2* = aa genotype (816 bp). *Lane 3* = AA genotype (750 and 66 bp). *Lane 4* and *5* = Aa genotype (816, 750, and 66 bp). *Lane M* = Marker DL 2000.

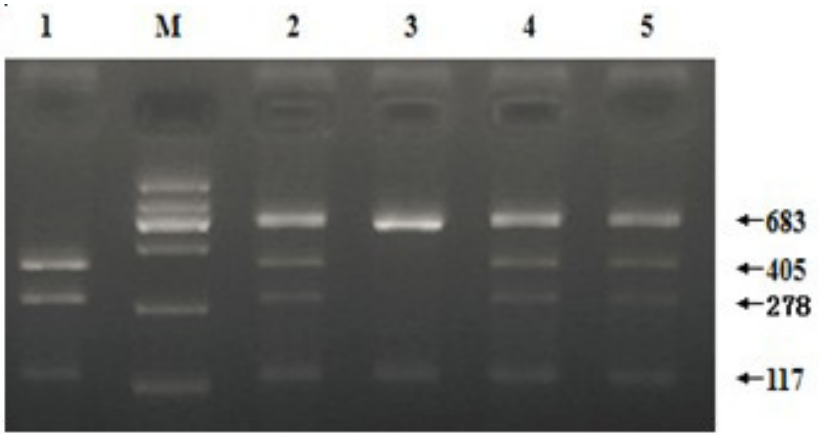

Figure 3. Genotyping of *H-FABP*/*Hae*III locus by PCR-RFLP. *Lane 1* = dd genotype (405, 278, 117, and 16 bp). *Lane 3* = DD genotype (683, 117, and 16 bp). *Lanes 2*, *4* and *5* = Dd genotype (683, 405, 278, 117, and 16 bp). *Lane M* = Marker DL 2000.

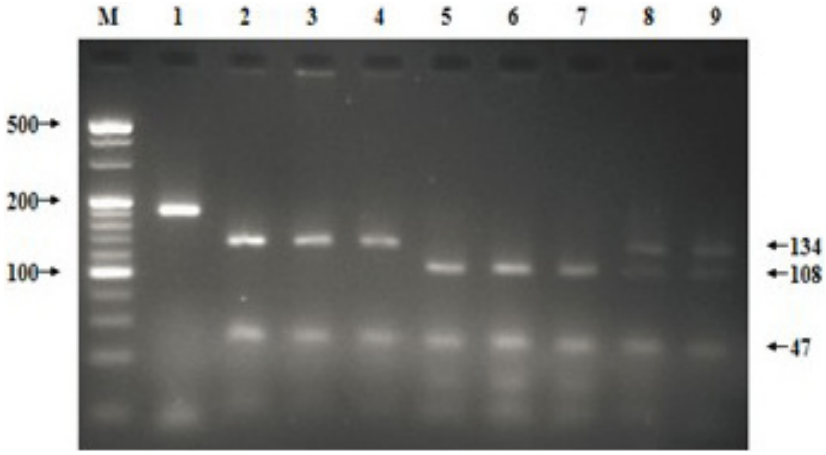

Figure 4. Genotyping of *ACSL4*/*Rsa*I locus by PCR-RFLP. *Lane 1* = 181 bp undigested PCR product. *Lanes 2*, *3*, and *4* = AA genotype (134 and 47 bp). *Lanes 5*, *6*, and *7* = GG genotype (108, 47, and 26 bp). *Lanes 8* and *9* = AG genotype (134, 108, 47, and 26 bp). *Lane M* = Marker DL 500.

H-FABP allelic frequency and genotype distribution from the 6 pig populations were calculated and analyzed (Table 3). The results demonstrated significant differences in the genotype distribution of *H-FABP* among the 6 pig populations ($P < 0.001$); however, sex did not significantly affect the genotype distribution of *H-FABP* ($P > 0.05$, not shown in Table 3). Three genotypes of the *Hin*fI locus were found in these 6 pig populations, with the H allele being dominant in these pig populations. Three genotypes of the *Msp*I or *Hae*III loci were detected in Duroc, Landrace, Yorkshire, and DLY pigs; however, the polymorphisms of the *Msp*I and *Hae*III loci were not detected in Yanan and Jinhua pigs, which only had the AA-DD genotypes.

Table 3. *H-FABP* gene genotype distribution and allele frequency in six pig populations.

Locus	Genotype/Allele	Populations						χ^2 value (P value)
		Yanan	Jinhua	Duroc	Landrance	Yorkshire	DLY	
*Hin*fI-RFLP	HH	34 (0.366)	61 (0.718)	97 (0.815)	72 (0.837)	83 (0.382)	45 (0.402)	152.902 (P < 0.001***)
	Hh	51 (0.548)	20 (0.235)	17 (0.143)	8 (0.093)	95 (0.438)	32 (0.286)	
	hh	8 (0.086)	4 (0.047)	5 (0.042)	6 (0.070)	39 (0.180)	35 (0.312)	
	H	0.640	0.835	0.887	0.884	0.601	0.545	
	h	0.360	0.165	0.113	0.116	0.399	0.455	
*Msp*I-RFLP	AA	93 (1.000)	85 (1.000)	43 (0.361)	42 (0.488)	126 (0.581)	46 (0.411)	234.734 (P < 0.001***)
	Aa	0 (0.000)	0 (0.000)	20 (0.168)	24 (0.279)	72 (0.332)	29 (0.259)	
	aa	0 (0.000)	0 (0.000)	56 (0.471)	20 (0.233)	19 (0.087)	37 (0.330)	
	A	1.000	1.000	0.445	0.628	0.747	0.540	
	a	0.000	0.000	0.555	0.372	0.253	0.460	
*Hae*III-RFLP	DD	93 (1.000)	85 (1.000)	11 (0.092)	10 (0.116)	124 (0.571)	38 (0.339)	415.861 (P < 0.001***)
	Dd	0 (0.000)	0 (0.000)	22 (0.185)	26 (0.302)	74 (0.341)	33 (0.295)	
	dd	0 (0.000)	0 (0.000)	86 (0.723)	50 (0.582)	19 (0.088)	41 (0.366)	
	D	1.000	1.000	0.185	0.267	0.742	0.487	
	d	0.000	0.000	0.815	0.733	0.258	0.513	

DLY = Duroc x (Landrance x Yorkshire). ***Means significant at P < 0.001 level.

ACSL4 allelic frequency and genotype distribution of the 6 pig populations were calculated and analyzed (Table 4). The results showed that both pig population and sex had significant effects on genotype distribution (P < 0.001). Three genotypes were found in sows of Duroc, Landrace, Yorkshire, and DLY pig populations. Because the pig *ACSL4* gene is located on chromosome X (SSCX), boar haplotypes were A or G in these 4 pig populations. These 4 pig populations had high frequencies of the G allele. However, polymorphism was not found in Yanan and Jinhua pigs, in which all sows had the GG genotype and all boars had the G haplotype.

Table 4. *ACSL4* gene genotype distribution and allele frequency in six pig populations.

Sex	Genotype/Allele	Populations					
		Yanan	Jinhua	Duroc	Landrance	Yorkshire	DLY
Boars[a]	A	0 (0.000)	0 (0.000)	22 (0.386)	6 (0.182)	3 (0.042)	13 (0.260)
	G	43 (1.000)	23 (1.000)	35 (0.614)	27 (0.818)	69 (0.958)	37 (0.740)
Sows	AA	0 (0.000)	0 (0.000)	12 (0.194)	8 (0.151)	9 (0.062)	7 (0.113)
	AG	0 (0.000)	0 (0.000)	28 (0.452)	37 (0.698)	30 (0.207)	19 (0.306)
	GG	50 (1.000)	62 (1.000)	22 (0.355)	8 (0.151)	106 (0.731)	36 (0.581)
	A	0.000	0.000	0.429	0.366	0.143	0.266
	G	1.000	1.000	0.571	0.657	0.857	0.734
	χ^2 value (P value)[b]	-	-	33.756 (P < 0.001***)	45.405 (P < 0.001***)	18.341 (P < 0.001***)	19.755 (P < 0.001***)
Total	A	0.000	0.000	0.409	0.424	0.141	0.246
	G	1.000	1.000	0.591	0.576	0.859	0.754
	χ^2 value (P value)[c]	180.191 (P < 0.001***)					

[a]The genotypes of boars had been represented by alleles. [b]χ^2 test had been done between boars and sows in different pig populations. [c]χ^2 test had been done among different pig populations. (-) = There was no χ^2 value because of no variation in the locus. DLY = Duroc x (Landrance x Yorkshire). ***Means significant at P < 0.001 level.

Haplotype and linkage disequilibrium analysis

According to the 3 *H-FABP* polymorphic loci (*Hin*fI, *Msp*I, and *Hae*III), we con-

structed 8 haplotypes using the EM algorithm in Duroc, Landrace, Yorkshire, and DLY pigs. The type and frequency of haplotypes are showed in Table 5. Haplotype adH had the highest frequency (0.168). Although the 3 polymorphic loci (*Hin*fI, *Msp*I, and *Hae*III) are located on the same gene, the linkage disequilibrium analyses showed that there was on block, and that all D' values were less than 0.95 (Figure 5). The results indicate that the 3 loci are separated.

Table 5. Association between *H-FABP* Haplotypes and IMF content and BFT in DLY pig population.

Haplotypes	Frequency	IMF (%)		BFT (cm)	
		(means ± SE)	P	(means ± SE)	P
ADH	0.148	2.56 ± 0.15	0.827	1.51 ± 0.10	0.084
AdH	0.142	2.38 ± 0.17	0.210	1.45 ± 0.12	0.316
aDH	0.087	2.35 ± 0.19	0.222	1.49 ± 0.13	0.231
adH[a]	0.168	2.59 ± 0.29	0.000	1.33 ± 0.20	0.000
ADh	0.132	2.13 ± 0.15	0.003**	1.26 ± 0.11	0.516
Adh	0.118	2.25 ± 0.16	0.038*	1.67 ± 0.11	0.073
aDh	0.119	2.36 ± 0.16	0.149	1.43 ± 0.11	0.363
adh	0.086	1.87 ± 0.20	<0.001***	1.23 ± 0.13	0.438

[a]Haplotype adH was regarded as the base haplotype and compared with other haplotypes. DLY = Duroc x (Landrance x Yorkshire). IMF = intramuscular fat content of the last third/fourth rib. BFT = Average backfat thickness of the first rib, last rib, and last lumbar. *Means significant at $P < 0.05$ level. **Means significant at $P < 0.01$ level. ***Means significant at $P < 0.001$ level.

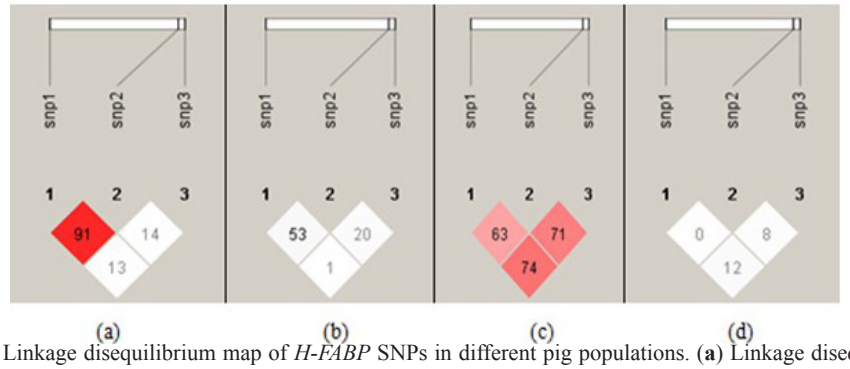

Figure 5. Linkage disequilibrium map of *H-FABP* SNPs in different pig populations. (**a**) Linkage disequilibrium map for Duroc pigs. (**b**) Linkage disequilibrium map for Landrace pigs. (**c**) Linkage disequilibrium map for Yorkshire pigs. (**d**) Linkage disequilibrium map for cross-breed pigs [Duroc x (Landrace x Yorkshire)]. snp1 = *Hin*fI locus. snp2 = *Msp*I locus. snp3 = *Hae*III locus.

Association analyses

Associations of the single *H-FABP* polymorphic locus (*Hin*fI, *Msp*I, and *Hae*III) with IMF content and BFT in DLY, Yanan, and Jinhua pigs are shown in Table 6. The *Hin*fI polymorphic locus was associated with IMF content in both Yanan ($P < 0.001$) and DLY pigs ($P < 0.05$), but not in Jinhua pigs. Furthermore, pigs carrying the HH genotype had the highest IMF content. There was no significant difference for *Msp*I or *Hae*III polymorphic loci versus IMF content in DLY pigs. The 3 polymorphic loci of *H-FABP* did not significantly affect BFT in these 3 pig populations. Haplotype analysis showed that adH had the highest IMF content ($P < 0.001$) in the DLY pig population (Table 5).

Table 6. Association between *H-FABP* genotype and IMF content and BFT in three pig populations.

Populations/Traits	*Hinf*-RFLP						*Msp*I-RFLP						*Had*III					
	Genotype (means ± SE)			P			Genotype (means ± SE)			P			Genotype (means ± SE)			P		
	HH	Hh	hh	HH-Hh	HH-hh	Hh-hh	AA	Aa	aa	AA-Aa	AA-aa	Aa-aa	DD	Dd	dd	DD-Dd	DD-dd	Dd-dd
DLY	45	32	35				46	29	37				38	33	41			
IMF (%)	2.67 ± 0.68	2.18 ± 0.82	2.02 ± 0.68	0.004**	0.000***	0.336	2.28 ± 0.80	2.49 ± 0.65	2.27 ± 0.82	0.202	0.983	0.216	2.34 ± 0.79	2.32 ± 0.71	2.33 ± 0.82	0.864	0.914	0.943
BFT (cm)	1.61 ± 0.39	1.55 ± 0.56	1.55 ± 0.56	0.593	0.576	0.992	1.65 ± 0.60	1.54 ± 0.34	1.50 ± 0.45	0.332	0.163	0.750	1.56 ± 0.48	1.60 ± 0.52	1.57 ± 0.50	0.735	0.916	0.808
Yanan	34	51	8				93	0	0				93	0	0			
IMF (%)	5.17 ± 1.75	3.97 ± 0.94	3.81 ± 1.21	0.000***	0.011*	0.741	4.40 ± 1.43	-	-	-	-	-	4.40 ± 1.43	-	-	-	-	-
BFT (cm)	3.49 ± 0.43	3.45 ± 0.37	3.73 ± 0.44	0.665	0.113	0.060	3.49 ± 0.40	-	-	-	-	-	3.49 ± 0.40	-	-	-	-	-
Jinhua	61	20	4				85	0	0				85	0	0			
IMF (%)	3.91 ± 1.91	4.23 ± 1.58	4.26 ± 1.60	0.501	0.715	0.978	4.00 ± 1.82	-	-	-	-	-	4.00 ± 1.82	-	-	-	-	-
BFT (cm)	4.07 ± 0.47	4.12 ± 0.54	4.51 ± 0.66	0.716	0.089	0.151	4.10 ± 0.50	-	-	-	-	-	4.10 ± 0.50	-	-	-	-	-

DLY = Duroc x (Landrance x Yorkshire); IMF = intramuscular fat content of the last third/fourth rib; BFT = average backfat thickness of the first rib, last rib, and last lumbar; (-) = there are no statistical results. *Significant at $P < 0.05$ level. **Significant at $P < 0.01$ level. ***Significant at $P < 0.001$ level.

Associations of the *ACSL4*/*Rsa*I polymorphic locus with IMF content and BFT in DLY pigs are presented in Table 7. The polymorphic locus was associated with IMF content but not BFT. Sows carrying the GG genotype and boars carrying the G haplotype had higher IMF content compared to those carrying the AA genotype ($P < 0.05$) or A haplotype ($P < 0.01$), respectively. Because the 3 loci (*Msp*I, *Hae*III, and *Rsa*I) only showed genotype AA-DD-GG in Yanan and Jinhua pigs, it was not possible to conduct an association analysis for the 3 three loci in the 2 pig populations.

Table 7. Association between *ACSL4* genotype and IMF content and BFT in DLY pig population.

	Sows						Boars		
	Genotype (means ± SE)			P			Haplotype (means ± SE)		P
	AA (7)	AG (19)	GG (36)	AA-AG	AA-GG	AG-GG	A (13)	G (37)	A-G
IMF (%)	2.10 ± 0.44	2.24 ± 0.51	2.53 ± 0.76	0.904	0.041*	0.156	1.73 ± 0.41	2.48 ± 0.91	0.004**
BFT (cm)	1.43 ± 0.23	1.63 ± 0.52	1.68 ± 0.44	0.376	0.242	0.743	1.50 ± 0.55	1.49 ± 0.53	0.951

DLY = Duroc x (Landrance x Yorkshire). IMF = intramuscular fat content of the last third/fourth rib. BFT = average backfat thickness of the first rib, last rib, and last lumbar. *Significant at $P < 0.05$ level. **Significant at $P < 0.01$ level.

DISCUSSION

Three *H-FABP* polymorphisms have been previously detected; 1 in the upstream region (*Hin*fI) and 2 in the second intron (*Hae*III and *Msp*I) (Gerbens et al., 1997). In this study, we identified the polymorphisms of these 3 loci in Duroc, Landrace, Yorkshire, and DLY pigs. Our results indicate that the genotypes of these 3 polymorphic loci are broadly distributed in western pig populations. However, in this study, we only found *Hin*fI polymorphism, and not *Msp*I and *Hae*III polymorphisms, in Chinese native breed Yanan and Jinhua pigs. The genotypes of *H-FABP*/(*Msp*I and *Hae*III) of these 2 populations were AA-DD. Several studies also found that many local Chinese pig breeds do not have *Msp*I or *Hae*III polymorphisms, and that they have AA-DD genotypes (Gerbens et al., 1997; Pang et al., 2006; Liu, 2008; Chao et al., 2012). The genotype results for *Msp*I or *Hae*III in Yanan and Jinhua pigs indicate the Chinese local breeds had similar genetic background for IMF content. Although the 3 polymorphic loci (*Hin*fI, *Msp*I, and *Hae*III) are located on the same gene, the linkage disequilibrium analyses revealed that they were separate. Therefore, exerting selection pressure on 1 locus should not influence the allelic frequencies of the other loci.

The *H-FABP* polymorphisms have been studied in many pig populations, with significant associations being observed between these polymorphisms and the IMF content (Gerbens et al., 1997, 2000; Arnyasi et al., 2006; Lee et al., 2010; Li et al., 2010; Han et al., 2012). These studies indicated that the ordering of IMF *H-FABP* genotypes is HH > Hh > hh, DD < Dd < dd, and AA < Aa < aa, and that porcine meat quality might be improved by increasing the frequency of genotype aa-dd-HH in pig breeds (Pang et al., 2006). In this study, we found that IMF content was significantly associated with *Hin*fI polymorphism in Yanan and DLY pigs, but not in Jinhua pigs. In addition, we found that the adH haplotype had the highest IMF content in the DLY pig population. However, *Hae*III and *Msp*I polymorphisms were not significantly associated with IMF content in DLY pigs. Previous studies have also found no significant associations between *H-FABP* polymorphisms and IMF content (Nechtelberger et al., 2001; Sieczkowska et

al., 2006b). In addition, several studies have addressed the effect of the *H-FABP* gene on BFT (de Koning et al., 1999; Chmurzyńska et al., 2007). Gerbens et al. (1997) observed significant differences among the homozygous *Hae*III RFLP genotype classes. These authors also stated that the effect of *H-FABP* on BFT accretion might be indirect, as subcutaneous adipocytes do not express *H-FABP*; instead, they express an adipocyte-specific FABP homeostasis protein (Veerkamp and Maatman, 1995). In contrast, Gardan et al. (2007) suggested that both the A- and H-FABP proteins are expressed in subcutaneous and intramuscular porcine adipocytes. In this study, none of the *H-FABP* genotypes had any significant effects on BFT in Yanan, Jinhua, and DLY pigs. Some previous studies also found the *H-FABP* genotypes do not affect BFT (Lee et al., 2010; Chao et al., 2012). These discrepancies might be explained by an effect of specific breeds, sex, growth rate, age, or amount of daily feed intake. Luo et al. (2006) recorded higher *H-FABP* gene expression in the local Chinese breed Yanan pig compared to the foreign cross-breed pig population of Duroc x (Landrace x Yorkshire), both in the heart and skeletal muscle.

In this study, *ACSL*4/*Rsa*I polymorphism was found in Duroc, Landrace, Yorkshire, and DLY pigs, with these populations having high frequencies of the G allele; however, there was no polymorphism in Yanan and Jinhua pigs, which only had the G allele. The results of this study support a previous report (Mercade et al., 2006), in which the frequency of the G allele was highly expressed in Landrace (0.62), Duroc (0.78), and Yorkshire pigs (0.95). In comparison, the Chinese pig breed Menshan pigs had the highest frequencies of the G allele (1.0). Kamiński et al. (2009) and Ruść et al. (2011) also found that crosses of Landrace x Yorkshire or Duroc x (Landrace x Yorkshire) pigs had the high frequencies of the G allele. In the investigations by Liu (2008), the polymorphic site was only found in Yorkshire, Landrace, and Duroc pigs, along with their hybrids, but was not found in the Min pig and wild pig, which all had GG genotypes. In this study, we found that male pigs only had A or G haplotypes, which confirmed that the pig *ACSL*4 gene is located on chromosome X (SSCX).

Ruść et al. (2011) obtained highly significant differences between *ACSL*4/*Rsa*I genotypes and IMF content but not BFT in DLY pigs, with the GG genotype expressing the highest IMF content (2.47%). In this study, significant differences were also observed between *ACSL*4/*Rsa*I genotypes and IMF content, but not BFT, in DLY pigs, with the GG genotype also expressing the highest IMF content (2.49%). The results of this study indicate that the *ACSL*4/*Rsa*I locus represents an effective genetic marker for IMF content in foreign breeds, but not native Chinese pig breeds, because of the absence of polymorphism in these breeds.

CONCLUSIONS

Our results demonstrated that *H-FABP*/(*Hin*fI, *Msp*I, and *Hae*III) polymorphisms occur in Duroc, Landrace, Yorkshire, and DLY pigs; however, there was only a single AA-DD genotype for *H-FABP*/(*Msp*I and *Hae*III) polymorphic loci in Yanan and Jinhua pigs. The *H-FABP*/*Hin*fI polymorphic locus significantly affected IMF content, but not BFT, in Yanan and DLY pigs, with the HH genotype expressing the highest IMF content. At the *ACSL*4/*Rsa*I polymorphic locus, polymorphism was recorded in Duroc, Landrace, Yorkshire, and DLY pigs, but not in Yanan and Jinhua pigs. In addition, the GG genotype in sows or G haplotype in boars significantly improved IMF content, but not BFT, in DLY pigs. The present results indicate that the porcine *H-FABP* and *ACSL*4 genes may be of practical use as markers to improve IMF content, without altering BFT, in pig breeding systems.

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