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Association of T1740C polymorphism of *L-FABP* with meat quality traits in Junmu No. 1 white swine

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ABSTRACT. This study was designed to investigate a single nucleotide polymorphism in intron 1 of the liver fatty acid-binding protein (*L-FABP*) gene in 156 Junmu No. 1 white swine using PCR-single-strand conformational polymorphism. The association between the polymorphism and meat quality traits was also studied. The cloning and sequencing results indicated that the polymorphism in intron 1 was due to a T→C mutation at position 1740 of *L-FABP*, yielding three genotypes (TT, TC, and CC). Association analysis revealed that the polymorphism had a significant effect on marbling ($P < 0.05$): genotype CC had more marbling than TC, and TC had more marbling than TT. The polymorphism also had a highly significant effect on intramuscular fat content ($P < 0.01$). Genotypes CC and TC had higher intramuscular

fat content than TT; there was no significant difference between CC and TC ($P > 0.05$). However, no significant conclusions concerning other traits could be drawn. We tentatively conclude that *L-FABP* is a candidate gene or a quantitative trait locus-linked gene associated with meat quality traits.

Key words: Swine; *L-FABP*; Intron 1; Single nucleotide polymorphism; Marbling; Intramuscular fat

INTRODUCTION

Geneticists are working to improve meat quality traits in pigs using genetic manipulation to control important genes *in vivo*. This study is of particular importance for a healthy diet in China, where large amounts of pork are consumed. Some genes play important roles in metabolic pathways, and many genes that are involved in the growth and development of animals require more detailed study (Curi et al., 2009). Liver fatty acid-binding protein (L-FABP) is a member of the FABP multigene family; FAPBs are small intracellular polypeptides found in many tissues involved in fatty acid transfer and metabolism, and are encoded by various genes (Gomez et al., 2007). L-FABP is present in hepatocytes and small intestine mucosa cell cytoplasm. In mammals, L-FABP is expressed in both the liver and small intestine (Di Pietro and Santomé, 1996) and to a much lesser extent in the kidney (Rolf et al., 1995). It can bind not only fatty acids but also a wide range of hydrophobic ligands, such as acyl-CoAs, bilirubin, lysophosphatidylcholine, retinoic acid, bile salts, prostaglandins, heme, and peroxisome proliferators (Glatz and van der Vusse, 1996). To date, the primary structures of L-FABPs from rat, human, cow, and pig have been reported, and these proteins display 79-90% amino acid identity (Di Pietro et al., 1999). The *L-FABP* gene, which influences the uptake, transport, mitochondrial oxidation, and esterification of fatty acids (Atshaves et al., 2004; Jiang et al., 2006), has been suggested as a candidate gene for meat quality traits. Therefore, studying the genetic variance of the *L-FABP* gene could lead to improvements in the quality of pork.

The pig is an important genetic, genomic, and biochemical model in scientific studies (Liu et al., 2010). Junmu No. 1 white swine are fast growing, and with high forage availability and adaptability; these traits make the pigs suitable for raising in north China. As far as we are aware, no molecular genetic research has been carried out on the association of *L-FABP* with production performance in Junmu No. 1 white swine. Consequently, this study is the first to investigate genetic variation through PCR-single-strand conformation polymorphism (PCR-SSCP) and sequencing, as well as the association of the gene with production performance in this breed of pig. The aim of this research was to identify gene markers for improved meat quality traits in Junmu No. 1 white swine.

MATERIAL AND METHODS

Animals and DNA extraction

Blood samples from 156 Junmu No. 1 white swine were collected from the breeding farm in the Agronomy Ministry of Jilin University; rearing and feeding conditions were standardized. The samples were stored at -20°C after treatment with ethylenediamine tetraacetic

acid. Genomic DNA was extracted according to the protocol of Sambrook et al. (1989) and detected with 1% agarose gel electrophoresis.

Primer design

The primers were designed according to the GenBank *L-FABP* sequence (accession No. DQ182323). The upstream primer was 5'-CCCCTCAGCCTCCAATGCCT-3' and the downstream primer was 5'-CTTGACCTTCTCCCCAGTCA-3'. The primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (China).

PCR amplification

The PCR mixture contained 50 ng DNA template, 10 pmol/μL upstream or downstream primer, 2.5 mM dNTP mixture, 1.5 mM $MgCl_2$, and 1 U Taq DNA polymerase in a 50-μL reaction volume. Amplification conditions were as follows: denaturation at 94°C for 5 min, 35 cycles of amplification at 94°C for 30 s, 59°C for 45 s, 72°C for 1 min, and an extension step at 72°C for 10 min. The amplification products were detected using 1.5% agarose gel electrophoresis and visualized using a UV transilluminator.

Genetic variation identification and sequencing

The PCR products from 156 pigs were genotyped using 12% native polyacrylamide gel electrophoresis (PAGE; 150 V, 16 h). The PCR product (2 μL) was mixed with 5 μL loading buffer. PCR products were denatured for 10 min at 98°C and the mixture was immediately cooled on ice for 10 min. The denatured samples were loaded on a 12% polyacrylamide gel through a microinjector. After electrophoresis, the gel was shaken gently for 10-15 min in 70% ethanol and for 20 min in $AgNO_3$ staining solution after washing with double-distilled water. Washing was repeated three times after incubation in the staining solution. Coloration solution was added until bands became clearly visible. The reaction was terminated by washing the gel. A refrigerated circulator was used to control the temperature (4°C) of the gels.

PCR products exhibiting homozygous genotypes were cloned and sequenced by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. The DNAMAN software package (USA) was used to confirm the *L-FABP* DNA sequence and detect the polymorphic locus.

Measurement of meat quality traits and statistics

The methods used to assess meat quality are listed in Table 1. Genotypic and allelic frequencies were determined, and a χ^2 test for Junmu No. 1 white swine was performed. Associations between genotypes and meat quality traits were analyzed using the general linear model procedure of SPSS version 13.0. The linear model was:

$$Y_{ijklm} = \mu + A_i + B_j + G_k + P_l + S_m + E_{ijklm},$$

where Y_{ijklm} is the observation of meat quality trait, μ is the overall population mean, A_i is the fixed effect due to the i^{th} age, B_j is the fixed effect of the j^{th} slaughter batch, G_k is the fixed effect associated with the k^{th} genotype (TT, TC, and CC genotypes), P_l is the fixed effect of

sire pedigree l, S_m is the fixed effect associated with sex, and E_{ijklm} is a random error term. The significance of differences was tested using the Duncan multiple comparison.

Table 1. Assessment of meat quality traits.

Traits	Method of assessment
COL (score)	A cross-section of muscle between the reciprocal third and the fourth ribs was assessed by use of a shade guide between 45 and 60 min after butchering. Score 1, gray; score 2, slightly gray; score 3, normal bright red; score 4, normal cardinal red; score 5, modena.
pH	Determined by pH meter after muscle samples between the reciprocal third and the fourth ribs were stored for 24 h at 4°C.
RMR (%)	WT of sample before steaming X100/WT of sample after steaming.
FD	(WT of sample before storage - WT of sample after storing for 24 h) x 100/WT of sample before storage.
WLR (%)	(WT of sample before compression - WT of sample after compression) x 100/WT of sample before compression.
MFA (μm)	After preparation, the free fiber plate was placed in a high-power (8 x 40) microscope and the MFA of 100 randomly selected fibers was measured by eye with the aid of a microruler. The mean was calculated.
MARB (score)	Score 1, fat is insignificantly distributed; score 2, fat is distributed only in small amounts; score 3, fat is distributed q.s.; score 4, fat is distributed copiously; score 5, fat is distributed excessively.
SF (kg)	Psoas major muscle samples were heated in 80°C thermostat-controlled waterbath until the temperature of the center of the flesh was 70°C. Ten pieces of center flesh were determined by C-LM tenderness determinator. The mean was calculated.
IMF (%)	300-500 g of muscle tissue between the reciprocal third and the fourth ribs was determined using Soxhlet petroleum-ether extraction.

COL = coloration; pH = pH 24; RMR = ripe muscle rate; FD = fail drip; WLR = water loss rate; MFA = muscle fiber diameter; MARB = marbling; SF = shear force; IMF = intramuscular fat content; WT = weight. Method of assessment, pork quality standards of the People's Republic of China was used for determination of COL and MARB (Evaluation method of pork quality, 1987).

RESULTS

Polymorphism of the *L-FABP* gene and the χ^2 test

Three different genotypes designated TT, TC, and CC were identified in the pig population using PCR-SSCP. The TT and CC genotypes produced two bands and the TC genotype produced three bands (Figure 1A). The genotypic frequency of TC in *L-FABP* was 0.52, and that of TT (0.31) was higher than that of CC (0.17; Table 2). The frequencies of alleles T and C were 0.57 and 0.43, respectively (Table 2). The χ^2 test showed that the population of Junmu No. 1 white swine was in Hardy-Weinberg equilibrium (HWE) for the polymorphism in the *L-FABP* gene ($\chi^2 = 0.004$, $P > 0.05$).

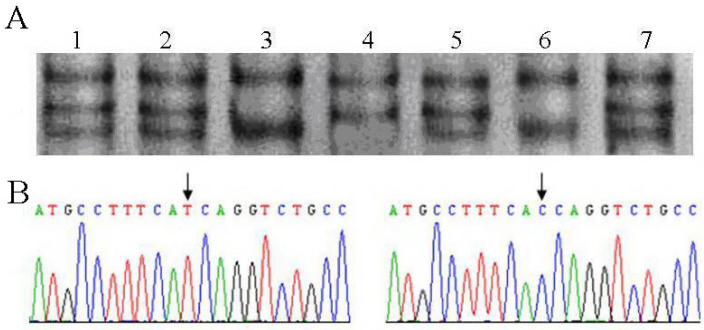

Figure 1. A. Electrophoresis patterns of PCR-SSCP intron 1 of the Junmu No. 1 white swine *L-FABP* gene. *Lanes 1*, *2*, *5*, and *7* = TC genotype; *lanes 3* and *6* = TT genotype; *lane 4* = CC genotype. **B.** Chromatograms showing mutations at intron 1 of the Junmu No. 1 white swine *L-FABP* gene.

Table 2. Gene and genotypic frequencies of the *L-FABP* gene in Junmu No. 1 white swine.

N_1	Gene frequency		Genotypic frequency (N_2)			χ^2 (HWE)
	T	C	TT	TC	CC	
156	0.57	0.43	0.31 (48)	0.52 (82)	0.17 (26)	0.004

N_1 = number of experimental pig population; N_2 = number of observations; χ^2 (HWE) = Hardy-Weinberg equilibrium by the χ^2 test; d.f. = 2; $\chi^2_{0.05} = 5.99$; $P > 0.05$.

Sequencing

The results of PCR fragment sequencing of the representative homozygous genotypes revealed a point mutation at position 1740 of *L-FABP*. The sequencing figures of the homozygous genotypes are shown in Figure 1B.

Association between different *L-FABP* genotypes and meat quality traits

The single nucleotide polymorphism exhibited statistically significant associations with marbling and with intramuscular fat (IMF) content, but not with other traits in Junmu No. 1 white swine (Table 3). The differences between the TT (marbling, 2.051), TC (marbling, 2.319), and CC (marbling, 2.643) genotypes were significant ($P < 0.05$). Genotype CC (2.765) and TC (2.719) had higher IMF measurements than genotype TT (2.216) ($P < 0.01$), but there was no significant difference between the CC and TC genotypes ($P > 0.05$).

Table 3. Least squares analysis of different genotypes with meat quality traits in intron 1 of *L-FABP*.

Traits	Genotypes			F	P
	TT	TC	CC		
	LSM ± SE	LSM ± SE	LSM ± SE		
COL (score)	3.257 ± 0.096	3.208 ± 0.109	3.243 ± 0.116	0.029	0.973
pH	5.564 ± 0.063	5.681 ± 0.085	5.584 ± 0.106	1.712	0.201
RMR (%)	59.964 ± 1.267	60.634 ± 1.378	61.045 ± 1.615	0.676	0.583
FD	2.341 ± 0.279	2.416 ± 0.258	2.613 ± 0.264	1.224	0.318
WLR (%)	36.487 ± 0.756	34.964 ± 0.864	35.613 ± 1.056	0.461	0.633
MFA (μm)	40.125 ± 0.981	41.034 ± 1.086	39.968 ± 0.948	0.341	0.827
MARB (score)	2.051 ± 0.613[a]	2.319 ± 0.515[b]	2.643 ± 0.491[c]	4.458	0.020
SF (kg)	21.368 ± 1.063	20.986 ± 1.207	21.674 ± 1.162	0.276	0.761
IMF (%)	2.216 ± 0.066[a]	2.719 ± 0.061[b]	2.765 ± 0.059[b]	46.298	0.000

COL = coloration; pH = pH 24; RMR = ripe muscle rate; FD = fail drip; WLR = water loss rate; MFA = muscle fiber diameter; MARB = marbling; SF = shear force; IMF = intramuscular fat content. LSM = least square mean; SE = standard error. In the same row, means without a common superscript letter differ ($P < 0.05$).

DISCUSSION

Hereditary features and characteristics of pig breeds are formed through continual selection under both natural and anthropogenic conditions. The χ^2 fitness test indicated that the Junmu No. 1 white swine population was in HWE, probably the result of long-term natural and artificial selection. Generally, the more extensively varied the hereditary basis and the lower the purity of varieties, the more abundant is DNA polymorphism. A single base substi-

tution was found within intron 1 of the Junmu No. 1 white swine gene *L-FABP*. The genetic polymorphism could be due to the relatedness of Junmu No. 1 white swine to the Three River white pig and Shi Ge pig. Herein, we present evidence that the mutation is associated with enhanced IMF and increased marbling.

IMF content refers to the deposits of fat within muscles (Jurie et al., 2007) that affect the sensory properties and nutritional value of meats (Geay et al., 2001). The suggested optimal range of IMF content for achieving eating satisfaction and meeting dietetic requirements is 2.5-3.0% (Li et al., 2010). There was a significant influence of the *L-FABP* gene polymorphism on IMF in Junmu No. 1 white swine. Animals with the C allele in this breed had significantly better performances with regard to IMF.

The effect of the *L-FABP* polymorphism on IMF accretion could be due to the physicochemical properties and function of *L-FABP*. There are considerable data supporting the idea that L-FABP is structurally and functionally different from other FABP types: it binds two fatty acids per molecule (Thompson et al., 1997), whereas the other FABP types have a single-fatty acid-binding site (Richieri et al., 1994); and unlike other FABPs, it undergoes a significant conformational change upon fatty acid binding (Nemecz et al., 1991). *L-FABP* is involved in the intake and intracellular transport of long-chain fatty acids and very long-chain fatty acids (Gertow et al., 2004), influencing the deposition of IMF.

This study confirms that the *L-FABP* locus is involved in the regulation of IMF accretion, which agrees with the results of Jiang et al. (2006). In our pig population, the effects on IMF might be due to linkage disequilibrium between the investigated *L-FABP* loci and one or more other loci.

Marbling plays a key role in establishing the quality of pork (Switonski et al., 2010). Kamalakar et al. (2009) found that marbling is positively related to pork tenderness. Other studies have shown a positive correlation between marbling and juiciness (Heyer and Lebret, 2007). To date, there has been no report about the association between polymorphisms in the *L-FABP* gene and marbling in pigs. Findings from our study of Junmu No. 1 white swine support the association of *L-FABP* genetic variation with marbling. Animals with the C allele in this breed have significantly better performances on marbling. The presently known polymorphism in the *L-FABP* gene can be used in marker-assisted selection to improve the marbling of pork.

The current study led us to hypothesize that *L-FABP* is a candidate gene or a quantitative trait locus-linked gene associated with pork quality. For Junmu No. 1 white swine, it appears that the main task is to protect the genetic resource of high IMF content so that it may provide valuable experimental materials for further research on the *L-FABP* gene.

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