Genetic Variation of H-FABP Gene and Association with Intramuscular Fat Content in Laiwu Black and Four Western Pig Breeds*

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ABSTRACT : This study was performed to detect genetic variation of the heart fatty acid-binding protein (H-FABP) gene by PCR-RFLPs approach and its association with intramuscular fat (IMF) content. Data from 223 individuals, including one Chinese native pig breed and four western pig breeds, were analyzed. The results showed that for the H-FABP gene, there was one polymorphic *Hinf* I site in the 5'-upstream region, whereas there were one *Hae*III and one *Hinf*I (marked as *Hinf*I*) polymorphic site in the second intron, respectively. The three PCR-RFLPs were present in all breeds tested. The allele frequencies, however, revealed significant differences between them ($p<0.05$). Furthermore, the allele frequency distribution of *Hinf*I in the Laiwu Black and that of *Hinf*I* in the Hampshire breed were at disequilibrium, which might be the result of selective breeding. Results also indicated that for *Hinf*I, *Hae*III and *Hinf*I* H-FABP RFLP, significant ($p<0.05$) contrasts of 0.78%, -0.69% and 0.72% were detected in the least square means of IMF content between the homozygous genotype HH and hh, DD and dd, BB and bb classes, respectively. It implied that the HHddBB genotype had the highest IMF content in this experimental population and these H-FABP RFLPs could serve, to some extent, as genetic markers for use in improvement of IMF content. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 1 : 13-16)***

Key Words : Pigs, Heart Fatty Acid-binding Protein Gene, Intramuscular Fat, Genetic Markers

INTRODUCTION

Besides the ongoing improvement in quantitative traits for pig breeding, qualitative aspects, in particular meat quality are increasingly attracting more attention. Therefore, pork market is changed from needs of lean meat to that of high quality lean meat. Intramuscular fat (IMF) content is a major determinant of meat quality and is positively correlated with meat tenderness, flavor and juiciness (DeVol et al., 1988; Wood et al., 1988). However, Improving IMF content by selective breeding is difficult because this trait is measured only on the carcass. Marker-assisted selection (MAS) is a promising strategy for genetic improvement of such carcass trait (Meuwissen and Goddard, 1996). Finding genetic markers linked to quantitative trait loci (QTL) is the first step of MAS (Chu et al., 2003a).

Previous researches have studied genetic variation of the porcine heart fatty acid-binding protein (H-FABP) gene (Gerbens et al., 1997; Lin et al., 2002; Zhang et al., 2002) and its effect on IMF content (Gerbens et al., 1999). Functionally, FABP are intracellular proteins that transport fatty acids from the cell membrane to sites of fatty acid oxidation or phospholipid or triacylglycerol synthesis. The H-FABP is expressed predominantly in muscle cells (Veerkamp and Maatman, 1995). Analysis of data from Chinese native pig breed and western pig breed, which differ significantly in IMF content (Zeng et al., 1989), might provide additional insight into H-FABP gene's role in this trait. Therefore, the aim of this study was to detect genetic variants of H-FABP gene in the Laiwu Black and four western pig breeds and to estimate the association of genetic variants of H-FABP gene with IMF deposition.

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MATERIALS AND METHODS

Experimental materials

223 pigs including one Chinese native breed (Laiwu Black-LB) and four western breeds (Landrace-LA, Large Yorkshire-LY, Hampshire-HA, Duroc-DU) were involved in this study. Experimental pigs were housed in groups and fed *ad libitum* at three breeding farms until the slaughter weight of 90-100 kg. At slaughter, ear samples were collected for genomic DNA extraction and muscle samples of the longissimus dorsi at the third lumbar vertebra were obtained for meat quality evaluation.

DNA extraction

The porcine genomic DNA was extracted from the ear samples as described by Sambrook (1989) and Jiang et al. (2002).

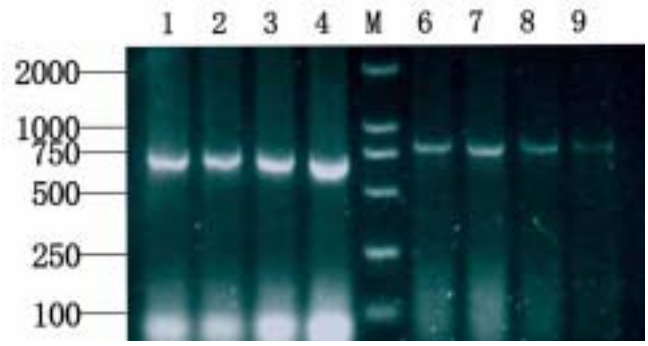

Figure 1. PCR products of 5'-upstream region and the second intron of H-FABP gene. M: DL2000 marker; Lanes 1-4: PCR products of 5'-upstream region; Lanes 6-9: PCR products of the second intron.

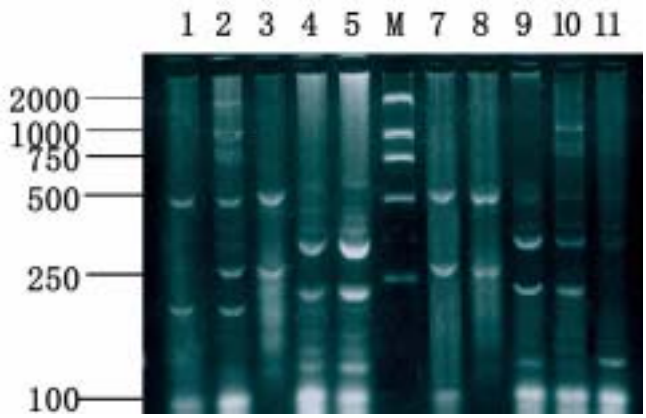

Figure 2. Agarose gel image of digested PCR product with *Hinf*I and *Hinf*I*. M: DL2000 marker; Lane 1: BB; Lane 2: Bb; Lane 3, 7, 8: bb; Lane 4, 5, 9: Hh; Lane 10: hh; Lane 11: HH.

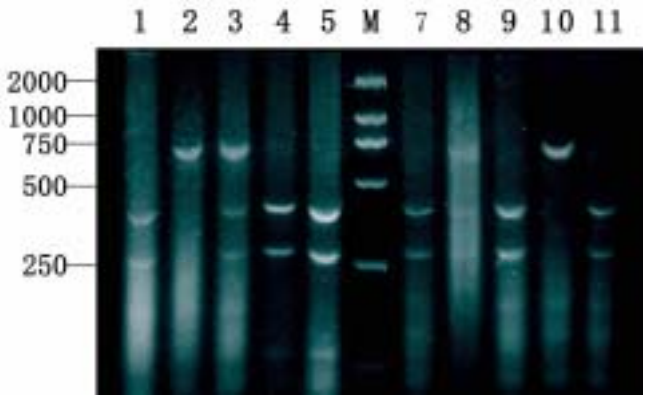

Figure 3. Agarose gel image of digested PCR product with *Hae*III. M: DL2000 marker; Lane 1, 4, 5, 7, 9, 11: dd; Lane 2, 10: DD; Lane 3, 8: Dd.

PCR-RFLP detection

The H-FABP gene was screened for genetic variation in the 5'-upstream and intron 2 by PCR-RFLP. According to the nucleotide sequence of H-FABP gene (Genebank Acc. No. X98558 and Y16180), two pairs of primers were designed as follows: 5'-GGACCCA AGATGCCTACGCC G-3', 5'-CTGCATCTTTGACCA AGAGG-3' and 5'-ATTG CTTCGGTGTGTTTGAG-3', 5'-TCAGGAATGGGAGTTA TTGG-3'. PCR was performed in 25 μl volume of reaction system (Chu et al., 2003b). The conditions of PCR were that after 3 min of denaturation at 94°C, 33 cycles of amplification were carried out: 94°C for 1 min, 57°C for 1 min, 72°C for 50 sec, and 72°C for 7 min to lengthen the expected fragments. 10 μl of the PCR product was used for restriction digestion with *Hinf*I and *Hae*III (TaKaRa Co Ltd., Dalian) in a total volume of 20 μl, then loaded on a 3% agarose gel for electrophoresis and analysis.

Intramuscular fat measurement

Intramuscular fat (IMF) content was determined using the Soxleth petroleum-ether extraction method and expressed as the weight percentage of wet muscle tissue according to Hovenier et al. (1992).

Statistical analysis

After electrophoresis the RFLP patterns were scored as described by Zhang (2002) and Lin (2002) and genotype distributions within breeds were tested for Hardy-Weinberg equilibrium as described by Falconer and Mackay (1996).

For data analysis, a fixed effects model was established as follows:

$$Y_{ijkm} = \mu + F_i + B_j + G_k + (B \times G)_{jk} + e_{ijkm}$$

where Y_{ijkm} is the individual IMF observation; μ is the population mean; F_i, B_j and G_k are the estimated effect for farm, breed and genotype, respectively; $(B \times G)_{jk}$ is the interaction effect between breed and genotype; and e_{ijkm} is the random residual effect. Statistical analysis was performed with SAS (1990) by use of the GLM procedure. Duncans Multiple Range Test was performed to separate means of significant difference.

RESULTS

PCR amplification and RFLP

On the basis of the sequence of porcine H-FABP gene, the products of PCR amplification (Figure 1) are fragments including 693 bp of the 5'-upstream region (site 1,125-1,818) and 816 bp of intron 2 region (site 1,401-2,217), respectively.

The fragment of the 5'-upstream had four digested sites for *Hinf*I, whereas that of intron 2 had three for *Hae*III and two for *Hinf*I (marked as *Hinf*I*), respectively. The polymorphic *Hinf*I, *Hae*III and *Hinf*I* site were around at position 1,322, 1,812 and 1,968, respectively. The allele H had five fragments (339+172+59+98+25 bp), and h had

Table 1. Genotype distribution and allele frequency of PCR-RFLPs for the H-FABP gene in various pig breeds

RFLP	Allele	LB	LA	LY	HA	DU
*Hinf*I	HH	28	18	31	7	22
	Hh	17	21	10	12	13
	hh	13	2	2	19	8
	H	0.6293[b]	0.6951[b]	0.8372[a]	0.3421[c]	0.6628[b]
*Hae*III	DD	49	4	14	30	12
	Dd	9	12	19	8	18
	dd	0	25	10	0	13
	D	0.9224[a]	0.2439[c]	0.5465[b]	0.8947[a]	0.4884[b]
*Hinf*I*	BB	41	0	5	19	6
	Bb	13	17	21	10	23
	bb	4	24	17	9	14
	B	0.8190[a]	0.2073[d]	0.3605[c]	0.6316[b]	0.4070[c]
N[e]		58	41	43	38	43

[a, b, c, d] Values with a different letter within a row differ significantly ($p<0.05$). N[e]: The number of pigs tested per breed.

four (339+231+98+25 bp). The allele D had three fragments (683+117+16 bp), and d had four (405+278+117+16 bp). The allele B had four fragments (520+217+47+32 bp), and b had three (520+264+32bp) (Figures 2 and 3).

Genotype and allele frequency

The three RFLPs of H-FABP gene are present in all breeds tested. The allele frequencies, however, revealed significant differences between them (Table 1). For *Hinf*I, the allele H frequency in the Laiwu Black is higher than that in the Hampshire but lower than that in the Large Yorkshire breed ($p<0.05$). For *Hae*III, the allele D frequency in the Laiwu Black is significantly higher than that in the Landrace, Large Yorkshire and Duroc breed. For *Hinf*I*, the allele B frequency in the Laiwu Black is significantly higher than that in the four western breeds tested ($p<0.05$). Moreover, the allele frequency distribution of *Hinf*I in the Laiwu Black and that of *Hinf*I* in the Hampshire breed are at disequilibrium (Table 2).

Effect of H-FABP gene on IMF content

Results (Table 3) of the least square means (LSM) of IMF content for different genotypes indicated that genotype classes differed significantly ($p<0.05$). The contrast between the homozygous genotype classes, IMF (XX)-IMF(xx), was estimated, where XX and xx are the homozygous genotype class for each H-FABP RFLP. For *Hinf*Ⅰ H-FABP RFLP, significant ($P<0.05$) contrast of 0.78% (3.56%□2.78%) was observed in the least square means of IMF content between the homozygous genotype HH and hh classes. For *Hae*III and *Hinf*I* H-FABP RFLPs, significant ($p<0.05$) contrasts of -0.69% (2.98-3.67%) and 0.72% (3.55-2.83%) were detected in the least square means of IMF content between the homozygous genotype DD and dd, BB and bb classes, respectively. Obviously, for H-FABP RFLP the HHddBB genotype had the highest IMF content in this experimental population.

Table 2. The Chi-square test for Hardy-Weinberg equilibrium of H-FABP RFLP genotype distribution

RFLP	LB	LA	LY	HA	DU
*Hinf*I	8.0165*	1.7810	0.9270	3.3850	4.5043
*Hae*III	0.4103	1.7475	0.5068	0.5260	1.1332
*Hinf*I*	3.4562	2.8045	0.1509	7.1748*	0.5027

* $p<0.05$, ** $p<0.01$ ($x^2_{0.05}=5.99$, $x^2_{0.01}=9.21$).

Table 3. The least square means (LSM) and standard errors (SE) of intramuscular fat content for various genotypes

RFLP	Genotype	N	LSM, %	SE
*Hinf*I	HH	106	3.56[a]	0.18
	Hh	73	3.19[ab]	0.22
	hh	44	2.78[b]	0.20
*Hae*III	DD	109	2.98[b]	0.17
	Dd	66	3.51[a]	0.24
	dd	48	3.67[a]	0.20
*Hinf*I*	BB	71	3.55[a]	0.19
	Bb	84	3.43[a]	0.21
	bb	68	2.83[b]	0.17

[a, b] Three values with different superscripts within the same column of each RFLP differ significantly ($p<0.05$). N: The number of pigs per actual genotype.

DISCUSSION

Laiwu Black belonging to North China type pig breeds is mainly raised in Shandong province. This breed exhibits excellent germplasm characters such as hardiness, high prolificacy and good meat quality, especially, high in intramuscular fat content (Zeng et al., 1989;1998). As the change of market needs in resent years, consumer and producer have paid close attention to meat quality, Laiwu Black is a precious gene pool for modern high quality pig breeding.

To detect association of the porcine H-FABP gene with IMF content, it is essential to identify polymorphisms within or near this gene. It is feasible to specifically identify polymorphisms in the regulatory or coding regions of this gene. In our study three RFLPs were identified for H-FABP gene. There was one polymorphic *Hinf*I site in the 5'-upstream region, whereas there were one *Hae*III and one *Hinf*I (marked as *Hinf*I*) polymorphic site in the second intron, respectively, of which the polymorphic *Hinf*I* site in the second intron was not found in previous researches (Gerbens et al., 1997; Lin et al., 2002). The three RFLPs of H-FABP gene were present in all breeds tested. The allele frequencies, however, differed significantly between breeds. The Laiwu Black had higher allele D and B frequencies than the four western pig breeds and lower allele H frequency than the three western breeds except the

Hampshire breed. The observed difference in the genotype frequency distribution between breeds may be the result of selective breeding. Compared to the four western pig breeds, the Laiwu Black has a higher IMF content and a lower lean percentage and growth rate. Breeding objectives for different breeds may have changed the allelic distribution of the H-FABP RFLPs.

Previously, Janss et al. (1997), De Koning et al. (1999) and Bo Zuo et al. (2003) found some evidence suggestive of QTL and a major gene affecting IMF content. Findings from this study with Laiwu Black and four western breeds support the association of H-FABP genetic variation with intramuscular fat content (Gerbens et al., 1999). The evidence that the H-FABP gene is responsible for part of the genetic variation in IMF content in pigs reveals the possibility to improve meat quality of pigs by marker-assisted selection according to polymorphisms in the H-FABP gene. However, the role of H-FABP in IMF deposit and associations in other pig breeds that have a polymorphic H-FABP gene need to be investigated further. Moreover, it is of interest to study genetic variants of the H-FABP gene and its association with IMF content on a larger scale of pig population.

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