Asian-Aust. J. Anim. Sci.
Vol. 20, No. 12 : 1812 - 1819
December 2007
www.ajas.info

Identification of Single Nucleotide Polymorphism of H-FABP Gene and Its Association with Fatness Traits in Chickens

Yan Wang, Dingming Shu[1], Liang Li, Hao Qu[1], Chunfen Yang[1] and Qing Zhu*
College of Animal Science and Technology, Sichuan Agricultural University, Ya'an, Sichuan, 625014, China

ABSTRACT : Heart fatty acid-binding protein gene (H-FABP) is an important candidate gene for meat quality. One of the objectives of this study was to screen single nucleotide polymorphisms (SNP) of chicken *H-FABP* gene among 252 individuals that included 4 Chinese domestic chicken breeds (Fengkai Xinghua (T04), Huiyang Huxu (H), Qingyuan Ma (Q), Guangxi Xiayan (S1)), 2 breeds developed by the Institute of Animal Science, Guangdong Academy of Agricultural Sciences (Lingnan Huang (DC), dwarf chicken (E4)) and one introduced broiler (Abor Acre (AA)). Another objective of this study was to analyze the associations between polymorphisms of the *H-FABP* gene and fat deposition traits in chickens. PCR-SSCP was used to analyze SNPs in *H-FABP* and 4 SNPs (T260C, G675A, C783T and G2778A) were detected. Associations between polymorphic loci and intramuscular fat (IMF), abdominal fat weight (AFW) and abdominal fat percentage (AFP) were analyzed by ANCOVA method. The results showed that the T260C genotypes were significantly associated with IMF ($p = 0.0233$) and AFP ($p = 0.0001$); the G675A genotypes were significantly associated with AFW, AFP ($p<0.01$) and IMF ($p<0.05$); at the C783T locus, AFW and AFP differed highly between genotypes. However, the G2778A loci did not show any significant effect on fat deposition traits in this study. In addition, we found that there were some differences between AFP and definite haplotypes through a nonparametric statistical method, so the haplotypes based on the SNPs except G2778A loci were also significantly associated with IMF, AFW (g) ($p<0.05$) and AFP (%) ($p<0.001$). Significantly and suggestively dominant effects of *H4H4* haplotype were observed for IMF and the *H2H3* was dominant for AFW (g) and AFP (%). The results also revealed that *H5H7* haplotype had a negative effect on IMF, while the *H5H6* had a positive effect on AFW (g) and AFP (%). (**Key Words :** Chicken, H-FABP Gene, Single Nucleotide Polymorphism (SNP), Fat Deposition Traits, PCR-SSCP)

INTRODUCTION

In the past decades, the poultry breeding has achieved exciting progress. For example, broiler chickens have been improved in many traits such as daily weight gain, feed efficiency and resistance to disease. But now, the high selection intensity for growth rate has caused many problems, especially the increasing trend for abdominal fat deposition. Excessive fat deposition affects the carcass value and flavors of chickens. More and more researchers and producers have been paying attention to this problem and some researchers have found several candidate genes or markers for chicken fat traits (Meng et al., 2005; Choi et al., 2006; Li et al., 2006; Wang et al., 2006).

Fatty acid-binding protein (FABP) genes are important genes that are involved in the development of fatness traits (McArthur et al., 1999) and belong to a superfamily of lipid-binding proteins and occur intercellularly in invertebrates and vertebrates. These proteins are involved in intracellular fatty acid transportation, cell growth and differentiation, cellular signaling, gene transcription, and protection of enzymes from the toxic effects of free fat acid (Glatz and Veerkamp, 1985; Zimmerman and Veerkamp, 2002). In FABPs families, there are many different types of FABPs which can be divided into 2 main groups (Glatz and van der Vusse, 1996): those associated with the plasma membrane ($FABP_{PM}$) and those with the intracellular or cytoplasmic proteins ($FABP_C$). So far, 9 types of tissue-specific cytoplasmic FABPs have been identified, and they are liver (L-) FABP, intestinal (I-) FABP, heart (H-) FABP, adipocyte (A-) FABP, epidermal (E-) FABP, ileal (Il-) FABP, brain (B-) FABP, myelin (M-) FABP, and testicular (T-) FABP (Zimmerman and Veerkamp, 2002; Chmurzynska, 2006). All FABP types are similar in their three-dimensional structures except T-FABP. The common structural feature is

* Corresponding Author: Qing Zhu. Tel: +86-835-2882006, Fax: +86-835-2883153, E-mail: zhuqing5959@163.com
[1] Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, P. R. China.
Received April 17, 2007; Accepted July 22, 2007

a 10-stranded β-barrel, made of two orthogonal antiparallel 5-stranded sheets that form the "clam"-shaped binding cavity (Banaszak, 1994). The overall gene structure is conservative in all family members and consists of 4 exons separated by 3 introns (Hunt et al., 1986; Hayasaka et al., 1993; Treuner et al., 1994; Hertzel and Bernlohr, 2000).

H-FABP is a 15-kDa cytosolic protein found in heart, muscle, and lactating mammary gland. This protein is the only FABP expressed in various muscle tissues, in both vertebrates and invertebrate species (Zanotti, 1999; Haunerland, 1994). The protein is highly conserved, even in insects and mammals, and is found in all muscles that metabolize fatty acid (Norbert and Friedrich, 2004). In muscle cells, *H-FABP* is believed to be involved in fatty acid uptake and subsequent transport toward mitochondrial β-oxidation systems; thus increased fatty acid exposure *in vitro* and *in vivo* has been shown to result in elevated *H-FABP* expression, both at mRNA and protein levels (Carey et al., 1994; Chang et al., 2001; Clavel et al., 2002). Binas (1999) regarded that mice with *H-FABP* knocked-out could confirm the importance of *H-FABP* for fatty acid transportation and metabolism. The absence of *H-FABP* did not result in any phenotypical difference, and the tissues of skeletal and cardiac muscle appeared normal. However, fatty acid uptake was reduced markedly in cardiac tissue (-80%) and isolated from cardiomyocytes (-45%) (Norbert and Friedrich, 2004). So *H-FABP* may play an integral role in energetic metabolism of skeletal muscle, delivering long-chain fatty acid (LCFAs) to mitochondria and peroxisomes for oxidation or directing LCFAs towards esterification and hence fat storage within muscle fibers (Brandstetter et al., 2002). Polymorphisms in the *H-FABP* gene were shown to be related with genetic variation of intramuscular fat content and growth performance traits in pigs (Gerbens et al., 1997; 1999; 2000). There were adequate investigations of *H-FABP* gene in pigs, but few in chickens. So the objectives of the present study were to identify the SNPs of the chicken *H-FABP* gene, develop the PCR-SSCP methods to genotype the polymorphisms of the individuals in the seven populations and analyze whether there are associations between polymorphisms of the *H-FABP* gene and chicken fat deposition.

MATERIALS AND METHODS

Chicken populations

Seven populations used in this study consisted of 4 Chinese domestic chicken breeds (Fengkai Xinghua chicken (T04), Huiyang Huxu chicken (H), Qingyuan Ma chicken (Q), Guangxi Xiayan chicken (S1)), 2 breeds developed by Institute of Animal Science, Guangdong Academy of Agricultural Sciences (Lingnan Huang chicken (DC), dwarf chicken (E4)) and one introduced broiler (Abor Acre chicken (AA)), most of these chicken have favorable meat quality. The total of 252 birds had free access to feed and water. Commercial corn-soybean diets that met all NRC requirements were provided in the study. From hatch to 3 wk of age, birds received a starter feed (2.90 Mcal of ME/kg and 20.5 g/kg of CP) and from 4 to 6 wk of age, birds were fed a grower diet (3.00 Mcal of ME/kg and 18.5 g/kg of CP). Before slaughter, blood was collected and the genomic DNA was isolated by phenolic extraction, and was used to genotype the *H-FABP* gene.

Phenotypic measurements

At the age of 90 d, body weight (BW) was measured on live birds after 12 h with no access to feed. After slaughter at the same day of age, the carcass traits were measured, including carcass weight (CW), eviscerated weight (EW), semi-eviscerated weight (SEW), breast muscle weight (BMW), leg muscle weight (LMW), abdominal fat weight (AFW) and subcutaneous fat thickness (SFT). CW was measured on the chilled carcass after removal of the feather and SEW on CW after removal of trachea, esophagus, gastrointestinal tract, spleen, pancreas and gonad, as well as EW on SEW after removal head, claws, heart, liver, gizzard, glandular stomach and abdominal fat. The ratios of these traits to CW were calculated as eviscerated percentage (EP), semi-eviscerated percentage (SEP), breast muscle percentage (BMP), leg muscle percentage (LMP), abdominal fat percentage (AFP). Fat thickness under the skin was measured in the back near the tail, while fat width was measured between the leg and breast muscles, and both were performed with a vernier caliper after dressing.

Amplification and population genotyping

Four PCR primers were designed according to the sequence of *Gallus gallus H-FABP* (GenBank accession no: AY648562) using the primer design procedure, Oligo 6.0 and Primer 5.0. All primer sequences were shown in Table 1.

The PCR was performed on a final volume of 10 μl containing 0.6 μl of genomic DNA (2.5 ng/μl), 0.3 μl of each primer (10 pmol/μl), 5 μl of 2×Master (including Mg^{2+}, dNTP, Tag DNA Polymerase). Amplification was carried out on the following procedure: initial denaturation at 94°C for 5 min; 34 cycles of 94°C for 45 s, 56°C-61.5°C for 45 s, and 72°C for 60 s; final elongation at 72°C for 8 min.

The amplified products of *H-FABP* were mixed with single strand conformation polymorphism (SSCP) buffer, 0.1% bromophenol blue and 0.1% xylene cyanol in formamide. Before being loaded into the gel, the samples were denaturized for 8 min at 99.9°C and kept in ice for 5 min. Then 10 μl of this mixture was totally transferred to a

Table 1. PCR forward (F) and reverse (R) primers for the *H-FABP*

Gene	Primer	Sequence	Annealing temperature	Length
H-FABP	P1	F: 5'- TGAGTACATGAAGGCGTTGG -3'	61.5°C	188 bp
		R: 5'- CGCGCTTTCCTATTCCTA -3'		
	P2	F: 5'- AGGTGCAGCATCTGAGTG -3'	56°C	265 bp
		R: 5'- TTCACCGTCGCCTTGT -3'		
	P3	F: 5'- CGACAAGGCGACGGTGAA -3'	56°C	275 bp
		R: 5'- TGGGGCAGGAAGGAGTTT -3'		
	P4	F: 5'- GCTAGTGCGGGAGCTGAA -3'	56°C	160 bp
		R: 5'- TTCTCATAGGTGCGGGTG -3'		

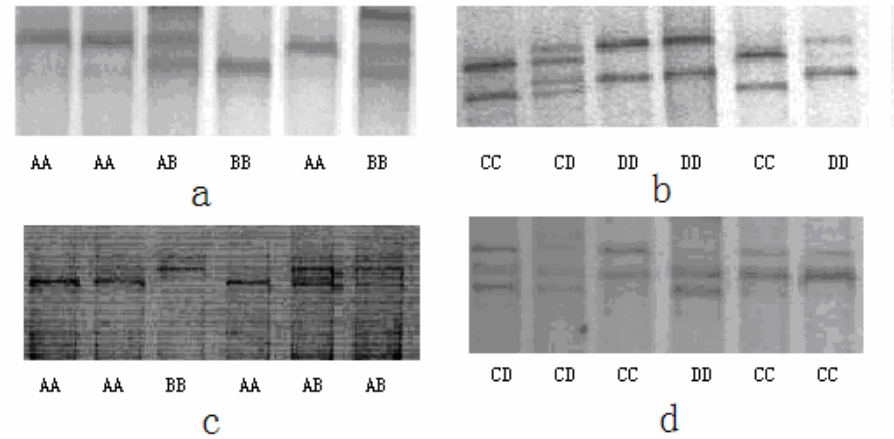

Figure 1. The genotypes of the T+260C, G+675A, C+783T, and G+2778A single nucleotide polymorphisms in the *H-FABP* gene. (a) Genotypes of T+260C, (b) Genotypes of G+675A, (c) Genotypes of C+783T, (d) Genotypes of G+2778A.

12% polyacrylamide gel (29:1) and a 10×TBE buffer. Electrophoresis ran 16h approximately at room temperature. The gel was then stained with silver nitrate in term of a standard protocol, as firstly washed in distilled water for 30 s and then incubated for 20 min in 0.1% silver nitrate. The developing reaction (20-30 min) was carried out in the solution of sodium tetraborate with addition of 4 ml formaldehyde and stopped by 10% acetic acid at last. Genotypes were recorded according to the band patterns.

Statistical analysis

Data were analyzed with GLM procedures of SAS (SAS Inst. Inc., Cary NC). The genetic effects were analyzed by a general linear model procedure in the SAS package, and the following model was used:

$$Y = \mu + B_i + S_j + G_k + e_{ijk}$$

Where Y = the dependent variable, μ = the population mean, B_i = fixed effects of breed, S_j = fixed effects of sex, G_k = genotype value, and e_{ijk} = random error. The interaction G×S was not significant for any trait and therefore was not included in the model. Significant differences ($p<0.05$) were found among different genotypes in the light of least square means using Duncan's multiple-range test.

The data of some fat traits were not normally distributed. The IMF and AFW were analyzed as the linear model with parameters estimated on the Square Root scale. The AFP traits was shifted and rescaled to give approximate normality and equality of variance.

Haplotype construction

Based on 3 SNPs in all of the 252 experimental birds, haplotypes were constructed with PHASE 2.0 programme (Stephens et al., 2001b), the function of which was to reconstruct haplotypes from the population data.

RESULTS

SNP genotypes of the chicken *H-FABP* gene

A PCR-SSCP method was successfully developed for screening the individuals of the population. Four target gene fragments were amplified, denatured, and then subjected to polyacrylamide gel electrophoresis to find SNP. PCR products were the same as expected. Three kinds of genotypes were investigated for each SNP (Figure 1). Both the homozygous and heterozygous individuals of different genotypes were sequenced for further analysis. There were four mutations being found, including a T/C mutation at position 260 nt, a G/A mutation at 675 nt and 2,778 nt respectively, and a C/T mutation at 783 nt in DNA sequence

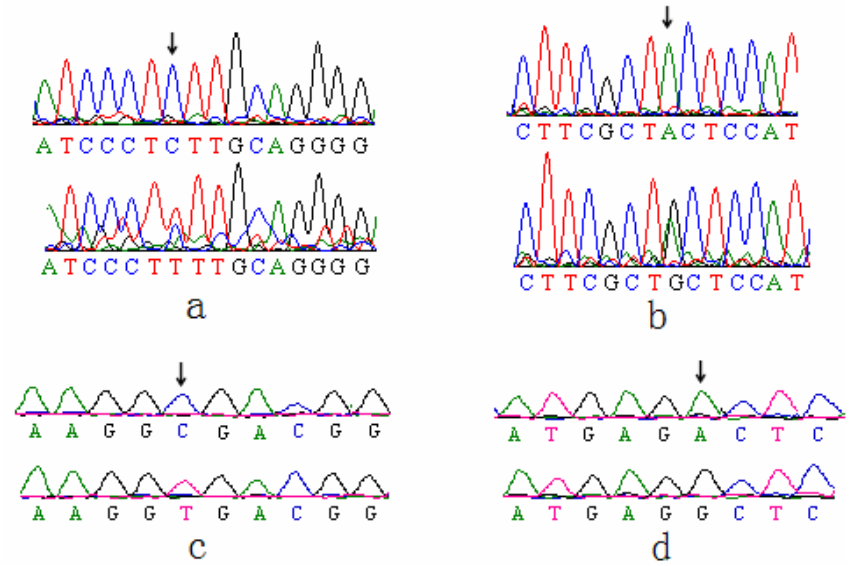

Figure 2. Sequencing analysis of H-FABP gene. (a) The sequencing result of C/C homozygote and T/C heterozygote in the 260 site, the arrow indicates the 260 site; (b) The sequencing result of A/A homozygote and G/A heterozygote in the 675 site, the arrow indicates the 675 site; (c) The sequencing result of C/C homozygote and T/T homozygote in the 783 site, the arrow indicates the 783 site; (d) The sequencing result of A/A homozygote and G/G homozygote in the 2,778 site, the arrow indicates the 2,778 site.

Table 2. Genotype and allele frequencies of *H-FABP* gene in different populations

Primer	Genotype frequency and Gene frequency	DC (36)	T04 (36)	E4 (36)	AA (36)	Q (36)	H (36)	S1 (36)
Primer1	AA	0.0625	0.2222	0.2353	0.1176	0.1667	0.2778	0.3333
T260C	BB	0.0625	0.2778	0.0588	0.1176	0.1667	0.0000	0.0556
	AB	0.8750	0.5000	0.7059	0.7648	0.6666	0.7222	0.6111
	A	0.5000	0.4722	0.5882	0.5000	0.5000	0.6389	0.6388
	B	0.5000	0.5278	0.4118	0.5000	0.5000	0.3611	0.3612
Primer2	CC	0.3750	0.1875	0.5882	0.4706	0.2778	0.2778	0.2222
G675A	DD	0.3125	0.3750	0.4118	0.1765	0.4444	0.3889	0.4445
	CD	0.3125	0.4375	0.0000	0.3529	0.2778	0.3333	0.3333
	C	0.5312	0.4062	0.5882	0.6470	0.4167	0.4444	0.3888
	D	0.4688	0.5938	0.4118	0.3530	0.5833	0.5556	0.6112
Primer3	AA	0.5000	0.3750	0.6471	0.6250	0.6667	0.1176	0.4118
C783T	BB	0.5000	0.5000	0.2353	0.3750	0.2778	0.8824	0.5294
	AB	0.0000	0.125	0.1176	0.0000	0.0555	0.0000	0.0588
	A	0.5000	0.4375	0.7059	0.6250	0.6945	0.1176	0.4412
	B	0.5000	0.5625	0.2941	0.3750	0.3055	0.8824	0.5588
Primer4	CC	0.3125	0.1667	0.2941	0.5294	0.6667	0.3333	0.4444
G2778A	DD	0.3750	0.4444	0.6471	0.2941	0.3333	0.5000	0.4444
	CD	0.3125	0.3889	0.0588	0.1765	0.0000	0.1667	0.1112
	C	0.4687	0.3611	0.3235	0.6176	0.6667	0.4166	0.5000
	D	0.5313	0.6389	0.6765	0.3824	0.3333	0.5834	0.5000

DC: Lingnan Huang chicken; T04: Fengkai Xinghua chicken; E4: dwarf chicken; AA: Abor Acre chicken; Q: Qingyuan Ma chicken; H: Huiyang Huxu chicken; S1: Guangxi Xiayan chicken.

of chicken (Accession No: AY648562) (Figure 2).

Frequencies of genotypes and alleles

The frequencies of the genotypes and alleles are shown in Table 2. In locus T260C, the frequency of *BB* homozygous genotype was the lowest among all populations, even zero in Huiyang Huxu chicken populations, while the *AB* genotype accounted for the highest. In locus G675A, the allele *D* and *C* were respectively identified as the dominant alleles among four native breeds and two breeds developed by Guangdong Animal Science Academy, due to their highest allele frequencies (average = 58.59% and average = 55.97%). In locus C783T, the allele frequency of *A* was higher than that of *B* in Abor Acre chicken, Qingyuan Ma chicken and dwarf chicken, while in Huxu chicken and Guangxi Xiayan

Table 3. The GLM analysis of fat traits in different genotypes (Least square mean, standard error)

Locus	Genotype	Trait		
		%IMF[1]	AFW (g)	%AFP
T260C	AA (48)	3.141±0.329^{a}	43.290±6.851	2.619±0.431^{AB}
	AB (168)	2.560±0.218^{ab}	40.553±4.522	3.033±0.284^{A}
	BB (36)	2.065±0.507^{b}	41.369±11.410	1.784±0.669^{B}
G675A	CC (88)	2.846±0.278^{a}	47.447±6.046^{A}	2.936±0.367^{A}
	CD (70)	2.228±0.356^{b}	33.111±7.459^{B}	1.945±0.461^{B}
	DD (94)	2.693±0.336^{ab}	44.658±7.198^{A}	2.556±0.441^{A}
C783T	AA (112)	2.413±0.225	48.167±4.709^{A}	3.404±0.293^{A}
	AB (30)	2.207±0.583	32.302±12.330^{B}	1.294±0.769^{B}
	BB (110)	3.146±0.237	44.747±5.390^{AB}	2.738±0.309^{AB}
G2778A	CC (96)	2.627±0.293	39.006±6.249	2.289±0.379
	CD (50)	2.518±0.402	45.168±8.593	2.679±0.531
	DD (106)	2.622±0.285	41.042±6.074	2.467±0.375

[1] IMF = Intramuscular fat, AFW = Abdominal fat weight, AFP = Abdominal fat percentage.
Values with different letter with the same row differ significantly; the lowercase letters show the level of 0.05; the capital letters show the level of 0.01.

Table 4. Haplotypes inferred based on the 3 single nucleotide polymorphisms

Haplotype	T+260C	G+675A	C+783T	Frequency (%)
H1 (ACA)	A	C	A	8.64
H2 (ACB)	A	C	B	13.39
H3 (ADA)	A	D	A	18.65
H4 (ADB)	A	D	B	14.51
H5 (BCA)	B	C	A	12.29
H6 (BCB)	B	C	B	14.33
H7 (BDA)	B	D	A	10.95
H8 (BDB)	B	D	B	7.24

chicken the allele *B* was identified as the dominant one. In locus G2778A, the allele *D* was the dominant allele among Lingnan Huang chicken, Fengkai Xinghua chicken, dwarf chicken and Huxu chicken because of it's highest allele frequency. At the same time, we found that the frequency of allele *A* and *C* was higher than that of *D* and *B* in Abor Acre chicken at all mutation loci.

Associations of SNP in H-FABP gene with fat traits

The associations of *H-FABP* genotypes with fat traits in chickens were analyzed, and the least square means of three genotypes were listed in Table 3. As shown in the Table 3, the T260C genotypes were significantly associated with IMF and AFP ($p = 0.0233$ and $p = 0.0001$). The IMF of *AA* chickens was significantly higher than that of *BB* ($p<0.05$). For AFP trait, *AB* chickens exceeded *BB* chickens by 1.25% ($p<0.01$), whereas no significant difference was detected among other genotypes. In locus G675A, genotypes were extremely significantly associated with AFW and AFP ($p< 0.01$), and also significantly associated with IMP ($p<0.05$). The IMF of *CC* chickens was significantly higher than that of *CD* ($p<0.05$). As regard to both AFW and AFP, *CD* chickens had lower values ($p<0.01$) than *CC* chickens by 16.34 g and 0.99%, than *DD* chickens by 13.55 g and 0.61%. In locus C783T, AFW and AFP were significantly associated with *H-FABP* genotypes. *AA* chickens had higher AFW and AFP than *AB* ($p<0.01$) by 2.11% for the latter. No significant difference was detected for IMF traits. In locus G2778A, there were no significant differences among three genotypes for different carcass traits.

Haplotypes and their frequencies

At the locus G2778A, there was no significant difference among three genotypes for different carcass traits, thus we only analyzed the former 3 SNPs. Haplotypes were constructed with 3 SNPs in all 252 experimental birds, employing the PHASE program (Stephens et al., 2001) of which the main function was reconstructing haplotypes from population data. Table 4 shows that eight haplotypes, with the minor allelic frequencies of above 7%, were identified based on these 3 SNPs. Six main haplotypes, ACB, ADA, ADB, BCA, BCB and BDA accounted for 84.12% of all the observations.

Construction of haplotypes and their associations with chicken fat traits

In this construction of haplotypes, the value of abdominal fat percentage (AFP) as a variance, may lead to incorrect conclusions about association between genomic polymorphism and state of obesity, because this is normalized parameter which generally has not normal statistical distribution. So we must apply the nonparametric statistical method to proof whether there were exist some different between AFP and definite haplotypes. The results indicated that the definite haplotypes were highly significantly associated with AFP ($p = 0.0032<0.01$) through analysis with NPAR1WAY procedures of SAS (SAS Inst. Inc., Cary NC). So we can use the mixed model to analyze the relationship between AFP and haplotypes.

The mixed model analysis indicated that there were significant associations of haplotypes with IMF and other carcass traits (Table 5). Haplotypes were associated with

Table 5. Associations between diplotypes and the chicken carcass traits[1, 2, 3]

Haplotype	Traits		
	IMF%*	AFW (g)*	AFP%**
H1H3	3.85±1.35	9.00±45.88	1.72±2.27
H1H5	3.35±0.41	88.27±13.83	5.38±0.68
H2H2	3.71±0.95	19.00±32.44	2.78±1.60
H2H3	-	148.00±45.88	8.90±2.27
H2H4	2.85±0.61	2.00±20.52	0.25±1.02
H2H5	2.43±0.68	27.00±22.94	3.49±1.14
H2H6	3.06±0.33	61.41±11.13	4.38±0.55
H3H3	2.67±0.45	54.89±15.29	4.16±0.76
H3H4	3.41±0.61	50.00±22.94	3.02±1.02
H3H5	2.43±0.45	46.83±13.24	3.23±0.66
H3H7	2.74±0.35	43.82±11.13	3.83±0.55
H4H4	**4.43±0.61**[1]	47.00±20.52	3.19±1.02
H4H6	2.36±0.38	35.31±12.72	2.36±0.61
H4H7	2.17±1.35	2.00±45.88	0.38±2.27
H4H8	2.99±0.43	20.20±14.51	2.46±0.72
H5H5	1.74±0.78	9.00±26.48	1.17±1.31
H5H6	1.59±1.35	**0.000±45.88**	**0.00±2.27**
H5H7	1.06±0.96[B]	11.50±32.44	2.01±1.61
H6H6	3.45±0.68	52.33±26.49	2.95±1.14
H6H8	2.28±1.35	-	0.00±2.27
H7H7	1.89±1.35	51.00±45.88	4.95±2.27

[1] Represents the advantageous diplotypes.

[2] Least squares means±standard error means.

[3] Underline represents the negative diplotypes. * p≤0.05. ** p≤0.01.
IMF = Intramuscular fat, AFW = Abdominal fat weight, AFP = Abdominal fat percentage.

IMF (%), AFW (g) and AFP (%) ($p<0.05$). Significantly and suggestively dominant effects of *H4H4* haplotype were observed for IMF and the *H2H3* was dominant for AFW (g) and AFP (%). Results also betrayed that *H5H7* haplotype had a negative effect on IMF, while the *H5H6* diplotype had a positive effect on AFW (g) and AFP (%).

DISCUSSION

H-FABP protein is involved in transportation of fatty acid from the cell membrane to the site of fatty acid oxidation and triglyceride and phospholipids synthesis, so there is a big chance that variations within genes have crucial implications for biological traits. Previous studies on the pig genome have elucidated that SNP was associated with intramuscular fat content (IMF), such as those by Gerbens et al. (2000) and Ovilo et al. (2002). The SNP of the pig genome could also have relationships with meat tenderness, juice and taste. Now, more and more studies have focused on chicken *H-FABP*, mostly because of it's important roles in chicken growth regulation, long-chain fatty acid uptake, and metabolic homeostasis. A SNP (C2054T) was found in the second intron of Beijing Oil chicken *H-FABP* gene (Ye et al., 2003). You et al. (2007) reported that genotypes of the 8 SNPs in the Caoke chicken *H-FABP* gene were associated with carcass traits (G332A, G534A, C835T, -1131A, C1294A, C2329T, C2372T and C2636T). In the present study, four SNPs (C260T, A675G, C783T and A2778G) were found in seven populations, and through comparing we found the mutation loci were distinct, though all the SNPs have great effects on body weight, intramuscular fat content (IMF) and abdominal fat weight (AFW) traits. There are some possible reasons for the SNP distinction: (1) different researchers used different species; (2) the variable penetrance of mutations elsewhere in the gene and to cell-type specific differences in gene expression. In addition, several researchers have investigated associations of *H-FABP* polymorphisms with fatness traits or other meat quality traits in different species. A SNP (C1006G) of *H-FABP* gene was found in bovine by PCR-RFLP and the effect of this SNP on tenderness was significant (Li et al., 2004). Li et al. (2006) discovered that the *H-FABP* gene expression levels were significantly correlated with IMF content and carcass weight in chicken. Thus, extensively studying the chicken *H-FABP* gene may lead to a great breakthrough in the understanding of fatness regulation of poultry. In current study of *H-FABP* gene, we found 2 SNPs (C260T, A675G) having associations ($p< 0.05$) with IMF and 2 SNPs (A675G, C783T) related ($p< 0.01$) to AFW and %AFP. The results point to the possible identification of *H-FABP* as a candidate gene of quantitative trait loci useful for regulating abdominal fat.

As a traditional approach to study both trait association (marker vs. trait) and linkage disequilibrium (marker vs. marker), single-marker analysis has taken on many problems, such as noisy, unsatisfied, and obscured important localization information (Daly et al., 2001). Significant associations between the single SNP and phenotypic traits were presented by one factor analysis, but no with the mixed model. Therefore, it was obscure and uncertain whether there was any association between the single SNP and traits. Haplotype or haplotype block provided a practical solution to resolve these problems (Daly et al., 2001). Haplotypes were constructed with the 3 SNPs and were used to analyze the associations of haplotype combinations with IMF and other carcass traits. The *H4H4* and *H2H3* haplotypes were found to be associated with higher IMF content and AFW, AFP than other haplotype combinations respectively, and the frequency of *H3* (ADA) was 18.65% in all experimental chickens. Therefore, *H3* may be the most disadvantageous haplotype for fat trait. Current data showed that associations of haplotypes with IMF and fat traits were more accurate than those of single SNP. This result implied that there was an interaction between different SNPs, and that the haplotypes generally provided more information content (heterozygosity) than one SNP did (Stephens et al., 2001a).

Thus, it was observed that both haplotype diversity and the method of SNP selection based on maximizing haplotype diversity were preferred to single SNP (Huang et al., 2003; Zhang et al., 2004).

In summary, commercial breeding programs of broiler chickens have became more and more complex, so it would be wise for us to use molecular MAS method to improve growth rate, increase breast muscle yield, decrease abdominal fat, at the same time to maintain good development and overall fitness. The results of current research indicated that 3 SNP markers were associated with fatness traits, so we could draw the conclusion that *H-FABP* gene played an important role in the regulation of fat deposition and IMF in chickens, in other words, the *H-FABP* gene manifested a great potential for use in molecular MAS programs to control fat deposition.

ACKNOWLEDGEMENTS

The work was financially supported by Chinese National Programs for High Technology Research and Development (863 Program) (No. 2005AA001220, NO. 2006AA10Z1D8), Natural Science Research Fund of Guangdong Province (Grant No. 05200576, 04002074) and Academies of Agricultural Sciences Fund of Guangdong Province (No. 04-fund-07B).

REFERENCES

Banaszak, L., N. Winter, Z. H. Xu, D. A. Bernlohr, S. Cowan and T. A. Jones. 1994. Lipid-binding proteins: A family of fatty acid and retinoid transport proteins. J. Adv. Prot. Chem. 45: 89-151.

Binas, B., H. Danneberg, J. McWhir, L. Mullins and A. J. Clark. 1999. Requirement for the heart-type fatty acid binding protein in cardiac fatty acid utilization. J. FASEB. 13:805-812.

Brandstetter, A. M., H. Sauerwein, J. H. Veerkamp, Y. Gaey and J. F. Hocquette. 2002. Effects of muscle type, castration, age and growth rate on H-FABP expression in bovine skeletal muscle. J. Livest. Prod. Sci. 75:199-208.

Choi, C. H., B. W. Cho, G. J. Jeon and H. K. Lee. 2006. Identification of novel SHPs with effect on economic traits in uncoupling protein gene of Korean native chicken. Asian-Aust. J. Anim. Sci. 19(8):1065-1070.

Chmurzynska, A. 2006. The multigene family of fatty acid-binding proteins (FABPs): functions, structure and polymorphism. J. Appl. Genet. 47(1):39-48.

Chang, W., J. Rickers-Haunerland and N. H. Haunerland. 2001. Induction of cardiac FABP gene expression by long chain fatty acids in cultured rat muscle cells. J. Mol. Cell. Biochem. 221: 127-132.

Carey, J. O., P. D. Neufer, R. P. Farrar, J. H. Veerkamp and G. L. Dohm. 1994. Transcriptional regulation of muscle fatty-acid binding protein. J. Biochem. 298:613-617.

Clavel, S., L. Farout, M. Briand, Y. Briand and P. Jouanel. 2002. Effect of endurance training and/or fish oil supplemented diet on cytoplasmic fatty acid binding protein in rat skeletal muscles and heart. Eur. J. Appl. Physiol. 87:193-201.

Daly, M. J., J. D. Rioux and S. F. Schaffner. 2001. High-resolution haplotype structure in the human genome. J. Nat. Genet. 29:229-232.

Glatz, J. F. and J. H. Veerkamp. 1985. Intracellular fatty-acid binding proteins. Int. J. Biochem. 17:13-22.

Glatz, J. F. C. and G. Van der Vusse. 1996. Cellular fatty acid-binding proteins: their function and physiological significance. J. Prog. Lipid. Res. 3535(3):243-282.

Gerbens, F., G. Rettenberqer, J. A. Lenstra, J. H. Veerkamp and M. F. te Pas. 1997. Characterization, chromosomal localization, and genetic variation of the porcine heart fatty acid-binding protein gene. J. Mamm. Genome. 8(5):328-332.

Gerbens, F., A. J. van Erp, F. L. Harders, F. J. Verburg, T. H. Meuwissen, J. H. Veerkamp and M. F. te Pas. 1999. Effect of genetic variants of the heart fatty acid-binding protein gene on intramuscular fat and performance traits in pigs. J. Anim. Sci. 77(4):846-852.

Gerbens, F., D. J. de Koning, F. L. Harders, T. H. Meuwissen, L. L. Janss, M. A. Groenen, J. H. Veerkamp, J. A. Van Arendonk and M. F. Te Pas. 2000. The effect of adipocyte and heart fatty acid-binding protein genes on intramuscular fat and backfat content in Meishan crossbred pigs. J. Anim. Sci. 78:552-559.

Hunt, C. R., J. H. Ro, D. E. Dobson, H. Y. Min and B. M. Spiegelman. 1986. Adipocyte P2 gene: Developmental expression and homology of 5′-flanking sequences among fat cell specific genes. J. Proc. Natl. Acad. Sci. 83:3786-3790.

Hayasaka, K., M. Himoro, G. Takada, E. Takahashi, S. Minoshima and N. Shimizu. 1993. Structure and localization of the gene encoding human peripheral myelin protein 2 (PMP2). J. Genomics. 18(2):244-248.

Hertzel, A. V. and D. A. Bernlohr. 2000. The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. J. Trends. Endocrinol. Metab. 11(5): 175-180.

Haunerland, N. H. 1994. Fatty acid binding protein in locust and mammalian muscle. Comparison of structure, function and regulation. J. Comp. Biochem. Phys. B Biochem. Mol. Biol. 109:199-208.

Huang, Q. Q., Y. X. Fu and E. Boerwinkle. 2003. Comparison of strategies for selecting single nucleotide polymorphisms for case/control association studies. J. Hum. Genet. 113:253-257.

Ovilo, C., A. Oliver, J. L. Noguera, A. Clop, C. Barragan, L. Varona, C. Rodriguez, M. Toro, A. Sanchez, M. Perez-Enciso and L. Silio. 2002. Test for positional candidate genes for body composition on pig chromosome 6. J. Genet. Sel. Evol. 34(4): 465-479.

Stephens, M., N. Smith and P. Donnelly. 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68:978-989.

Stephens, J. C., J. A. Schneider, D. A. Tanguay, J. Choi, T. Acharya, S. E. Stanley, R. Jiang, C. J. Messer, A. Chew, J. H. Han, J. Duan, J. L. Carr, M. S. Lee, B. Koshy, A. M. Kumar, G. Zhang, W. R. Newell, A. Windemuth, C. Xu, T. S. Kalbfleisch, S. L. Shaner, K. Arnold, V. Schulz, C. M. Drysdale, K. Nandabalan, R. S. Judson, G. Ruano and G. F. Vovis. 2001a. Haplotype variations and linkage disequilibrium in 313 human genes. J. Sci. 293:489-493.

Treuner, M., C. A. Kozak, D. Gallahan, R. Grosse and T. Muller. 1994. Cloning and characterization of the mouse gene encoding mammary-derived growth inhibitor/heart-fatty acid-binding protein. J. Gene. 147(2):237-242.

Li, C. Y. and H. Li. 2006. Associaion of MC4R gene polymorphisms with growth and body composition traits in chicken. Asian-Aust. J. Anim. Sci. 19(6):763-768.

Li, W. F., S. Z. Xu, H. H. Chao and H. B. Li. 2004. Genetic Variation in Intron1 of H-FABP Gene in Three Bovine Hybrids and the Relationships with Meat Quality Trait. J. Acta. Veterinaria et Zootechnica. Sinica. 35(3):252-255.

Li, W. J., H. B. Li, J. Wen, J. L. Chen, G. P. Zhao and M. Q. Zheng. 2006. Association of the H-FABP and A-HABP gene expression with intramuscular fat content in chicken. J. Acta. Veterinaria et Zootechnica. Sinica. 37(5):417-423.

Meng, H., J. G. Zhao, Z. H. Li and H. Li. 2005. Single nucleotide polymorphisms on peroxisome proliferators-activated receptor genes associated with fatness traits in chicken. Asian-Aust. J. Anim. Sci. 18(9):1221-1225.

McArthur, M. J., B. P. Atshaves, A. Frolov, W. D. Foxworth, A. B. Kier and F.Schroeder. 1999. Cellular uptake and intracellular trafficking of long chain fatty acids. J. Lipid. Res. 40:1371-1383.

Norbert, H., Haunerlanda and Friedrich Spener. 2004. Properties and physiological significance of fatty acid binding proteins. J. Adv. Mol. Cell. Biol. 33:99-123.

Wang, Y., H. Li, Y. D. Zhang, Z. L. Gu, Z. H. Li and Q. G. Wang. 2006. Analysis on association of a SNP in the chicken OBR gene with growth and body composition traits. Asian-Aust. J. Anim. Sci. 19(12):1706-1710.

Ye, M. H., H. H. Cao, J. Wen, H. B. Li, J. L. Chen and G. P. Zhao. 2003. RFLPs at Heart and Adipocyte Fatty Acid Binding Protein Genes in Beijing Oil Chick and Aijiao Chick. J. Acta. Veterinaria. et Zootechnica. Sinica. 34(5):422-426.

You, X. Y., Y. P. Liu, Q. Zhu and Z. Q. Yang. 2007. Study on SNP of the H-FABP gene and its association with slaughter performance in chicken. J. Hereditas (Beijing). 29(2):230-234.

Zimmerman, A. W. and J. H. Veerkamp. 2002. New insights into the structure and function of fatty acid-binding proteins. J. Cell. Mol. Life Sci. 59:1096-1116.

Zanotti, G. 1999. Muscle fatty acid-binding protein. J. Biochim. Biophys. Acta. 1441:94-105.

Zhang, W. H., A. Collins and N. E. Morton. 2004. Does haplotype diversity predict power for association mapping of disease susceptibility. J. Hum. Genet. 115:157-164.