

Effect Analysis of Heart Fatty Acid-Binding Protein Gene Assisted Selection on Intramuscular Fat Content in Chicken

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Abstract: Fatty deposition in organism is an important factor which influences meat quality. Previously, Heart Fatty Acid-Binding Protein (*H-FABP*) gene was studied as a candidate gene which having effect on Intramuscular Fat content (IMF). In current study, we chose four SNPs loci (g.260T>C, g.675G>A, g.783C>T and g.1198T>C) of *H-FABP* which have been proved to have distinct correlation with IMF as molecular markers to scan SNPs in a new breeding Chinese yellow-feather broiler population. Researchers allocated adaptive individuals into high-IMF group and low-IMF group according to the haplotypes which were constructed of genotypes at four SNP sites of each individual in F₁ generation and these haplotypes had been proved to be the preponderant or recessive haplotype. The core group was chose from the F₁ generation based on the traditional breeding method. And then, we detected the genotypes of the four loci by SNPs screening and analyzed the selection effect in F₂ generation which were obtained from mating and crossing within each group. Results indicated that the preponderance alleles were changed at Locus 1, 3 and 4 in the three groups of F₂ and there were no significant differences in IMF among the three groups (p>0.05). However, the IMF of the core group and the low fat group was higher than which of high fat group. The result of variance analysis showed that there were no significant difference in the effect of the different genotypes of the four SNPs on IMF (p>0.05). The four loci on the *H-FABP* gene in the research were not suitable as candidate markers to select IMF in this yellow-feather broiler strain.

Key words: *H-FABP* gene, intramuscular fat content, molecular marker-assisted selection, yellow-feather broiler, breeding, generation

INTRODUCTION

Meat quality is a complex economic characters, researches showed that Intramuscular Fat (IMF) content is the important factor to effect the meat flavor (Gerbens *et al.*, 1999; Mirzaei *et al.*, 2009; Yang *et al.*, 2011). Most of Chinese indigenous chickens have higher IMF and are most welcome for their superior meat quality and delicious taste. However, it is difficult to improve IMF content by traditional selection of phenotypic value because this trait is measured after slaughtering. Now with the development of molecular technology, the candidate gene approach is a powerful method to find the Quantitative Trait Loci (QTL) for improving the rate of genetic progress in meat quality traits (Zhang *et al.*, 2008).

Heart Fatty Acid-Binding Protein (*H-FABP*), a 15 kDa cytosolic protein is a major member of the Fatty Acid-Binding Protein (FABP) family which is the only FABP expressed in various muscle tissues (Zanotti, 1999). The major role of *H-FABP* in cardiac intracellular lipid

utilization and transport, fuel selection and metabolic homeostasis has been demonstrated by *H-FABP*-deficient mice (Binas *et al.*, 1999). As a candidate gene to influence IMF content, researches on *H-FABP* gene have been done and genetic variations of which were shown to be positive association with IMF content in different animals previously (Gerbens *et al.*, 2001; Huang *et al.*, 2006; Wang *et al.*, 2007; You *et al.*, 2009). Therefore, some molecular marks on IMF have been found out. Nevertheless, it was uncommon to see the verification of these markers in their progeny populations.

In this study, researchers chose four SNPs were reported in chicken *H-FABP* gene to identify whether these mutations can be as the molecular markers to select IMF in a new breeding yellow-feather broiler strain. Furthermore, these data allowed us to further clarify the selection effect of *H-FABP* and the reliability of marker-assisted selection which exists in the practical application so as to lay the foundations for MAS in chicken breeding of meat quality traits.

MATERIALS AND METHODS

Animals and data collection: In this research, researchers chose four SNP loci of H-FABP (g.260T>C, g.675G>A, g.783C>T and g.1198T>C) which have been proved to have distinct correlation with IMF by Wang as molecular markers to screen the SNPs and determine genotype of *H-FABP* gene in F₁ generation from a new breeding Chinese yellow-feather broiler strain. Subsequently, we got the clear genotypes in the four SNPs sites and constructed the haplotype of every detected individual. Then, we selected adaptive individuals into effect-increasing group (high-IMF group) and effect-decreasing group (low-IMF group) according to the haplotypes which have been proved to be the preponderant or recessive haplotype in Wang's thesis. And we formed a control group (Core group) from the F₁ generation population based on the traditional breeding methods. The total number of three groups was 717. Then, we screened the SNPs of *H-FABP* gene by the same four primers and identified the genotypes in 667 individuals in the three groups of F₂ generation which were obtained from mating freely within the different three groups of F₁ generation. The next, we analyzed the effect of MAS on IMF between the two generations. All 717 birds of F₁ generation and 667 birds of F₂ generation were raised in the same management and nutrition level and had *ad libitum* access to feed and water until a slaughter age of 91 days old. Blood was collected from their vein which was below the wing and the genomic DNA was isolated by the standard phenol/chloroform method.

After slaughter, a slice of right breast muscle was isolated to assess intramuscular fat content. A muscle sample was taken from this slice, carefully avoiding inter-muscular fat depots surrounding the muscle. The IMF content was measured using Soxhlet petroleum-ether extraction.

Amplification and population genotyping: Four pairs of primers which came from Wang's thesis were used to detect the H-FABP polymorphism (Table 1).

The PCR amplification was performed in a volume of 10 µL reaction which includes 0.6 µL DNA template (2.5 ng µL⁻¹), 5 µL 2× Taq PCR MasterMix (Beijing TIAN WEI Biology Technique Corporation, Beijing, China), 3.8 µL ddH₂O, 0.3 µL of each primer (10 pmol µL⁻¹). The PCR reaction was executed with the following condition: one denaturation cycle at 94°C for 5 min followed by 35 cycles at 94°C for 45 sec, 56°C or 61.5°C for 45 sec, 72°C for 60 sec and ended with an extension cycle at 72°C for 10 min. PCR products were separated on 1% agarose gel and were visualized on gel imaging system (Gel DocTMEQ170-8060) and photographed. PCR products were resolved by SSCP analysis. Each PCR product was denatured at 99°C for 10 min and then quickly chilled on ice for 5 min. The 2 µL product was resolved on a 12% polyacrylamide/bis-acrylamide 29:1 gel electrophoresis for 12-16 h approximately at room temperature.

Gels were stained using the silver staining. Individual single strand conformation polymorphism banding pattern was determined under visible light. Samples showing different bands in the gel were further amplified and purified and were sequenced by a commercial sequencing company (Shanghai Yingjun Biology Technique Corporation, Shanghai, China).

Statistical analysis: The association between the polymorphism and the IMF traits was analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC). The following model was used:

$$Y_{ijk} = \mu + S_j + G_k + e_{ijk}$$

Where:

- Y_{ijk} = The dependent variable
- μ = The population mean
- S_j = Fixed effects of sex
- G_k = Genotype value
- e_{ijk} = Random error

Significant differences (p<0.05) were found among different genotypes in the light of least square means using Duncan's multiple-range test.

Table 1: Detailed information of the primers used for the SNPs of chicken H-FABP

Primers	Primer locations ^a	Sequences (5'-3')	Fragments (bp)	Annealing temperatures (°C)
P1	110-280	TGAGTACATGAAGGCGTTGG CGCGTTTCCTATTCCTA	188	61.5
P2	430-661	GTCCTGAATCCTCCATCC AGAGCCCCCTCGGTATT	232	56.0
P3	528-792	AGGTGCAGCATCTGAGTG TCACCGTCGCCTTGT	265	56.0
P4	1106-1283	TGTGAGCAAGGCGGTAGT CAGGCTGGTTATAGTCAAAGC	178	56.0

^aThe corresponding location of primer pairs to the genomic reference sequences of chicken *H-FABP* gene (GenBank Acc. No.: AY648562.1)

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

The data of fat traits were verified for normal distribution by Shapiro-Wilks test in SAS. None of them were normally distributed. The IMF was analyzed as the linear model with parameters estimated on the Square Root scale. The IMF traits was shifted and rescaled to give approximate normality and equality of variance. Genotype effect was considered significant at $p < 0.05$ for F-test of type III sum of squares. Values were presented as Least Square Means (LSM). Standard Errors (SE) after LSM and SE were converted to their original status.

RESULTS AND DISCUSSION

Gene frequency and genotype frequency of *H-FABP* gene:

A PCR-SSCP method was successfully developed for screening all individuals in two generations. Four target fragments were amplified, denatured and then subject to polyacrylamide gel electrophoresis to find SNPs. PCR products were the same as expected. Three kinds of genotypes were determined and the proportions of different genotypes were counted at the each SNP of *H-FABP* gene in the F_1 and F_2 generation.

Table 2 shows that the gene frequency of four loci changed after selective breeding. In Locus 1, the frequency of allele A declined from 0.5732 in F_1 to 0.3037 in F_2 and the frequency of allele B increased from 0.4268-0.6964. In Locus 2, the gene frequency of A from 0.5397 in F_1 rose to 0.5870 in F_2 and the gene frequency of B went down from 0.4603-0.4131. Meanwhile, the gene

frequency of A decreased in both Locus 3 and 4 whereas the gene frequency of B in these two loci showed a rising trend. Except Locus 2, the preponderant alleles at other three loci have changed negatively.

The allele frequency of each site showed similar change trends between core group and whole group from F_1 to F_2 . In the high-IMF group, the frequency of preponderant alleles at four loci have decreased somewhat. In the low-IMF group, there were positive changes in Locus 1 and 3 and came increases in preponderant allele frequency. However, the opposite relationship occurred in the locus 2 and 4 (Table 3).

Comparisons of IMF between three experimental groups of F_2 population:

The IMF of core group, high-fat group and low-fat group in F_2 were compared by way of Duncan, the conclusion was shown in Table 4. There were no difference for the IMF among each group ($p > 0.05$). The IMF content in high-fat group was the lowest among the three groups and the core-group was the highest one.

Relationship between different genotypes of Four SNPs and IMF in F_2 population:

The results of variance analysis on different genotypes of four SNPs on IMF by GLM were shown in Table 5. Different genotypes at the four SNP loci had no significant influence on the IMF content ($p > 0.05$).

During the past few decades, advances in molecular genetics have led to the identification of multiple genes or genetic markers associated with genes that affect traits of interest in livestock and poultry including genes for single-gene traits and QTL or genomic regions that affect quantitative traits (Andersson, 2001). The main application and potential for use of markers to enhance genetic improvement in farm animal is through within-breed selection (Dekkers and Hospital, 2002). One of the first examples of use a molecular marker for a quantitative trait was the test for the Estrogen Receptor Gene (*ESR*) which has been used in several commercial lines to enhance selection for litter size in pigs (Rothschild *et al.*, 1996).

However, it was unfortunately that the four reported loci failed to enhance genetic and phenotype improvement programs in the research. This suggested that the application of using molecular markers of H-FBAP to select the fat trait of IMF has not achieved the anticipated result which was to improve the IMF in high-IMF group and reduce it in low-IMF group.

Although, there were some successful marker-assisted selection was used in the farm animal breeding industry (Bennewitz *et al.*, 2003; Hovenier *et al.*, 1993; Spelman and Garrick, 1997), the presence of restrictions or

Table 2: The analysis of each SNP of *H-FABP* gene in F_1 and F_2 generation

Groups	Population	Locus			
		1	2	3	4
Alleles					
A	F_1	0.5732	0.5397	0.5718	0.5614
	F_2	0.3037	0.5870	0.4145	0.4468
B	F_1	0.4268	0.4603	0.4282	0.4386
	F_2	0.6964	0.4131	0.5855	0.5532
Genotype frequency					
AB	F_1	0.2706 (194)	0.1869 (134)	0.4017 (288)	0.4310 (309)
	F_2	0.4243 (283)	0.0675 (45)	0.5202 (347)	0.4798 (320)
AA	F_1	0.4379 (314)	0.4463 (320)	0.3710 (266)	0.3459 (248)
	F_2	0.0915 (61)	0.5532 (369)	0.1544 (103)	0.2069 (138)
BB	F_1	0.2915 (209)	0.3668 (263)	0.2273 (163)	0.2232 (160)
	F_2	0.4843 (323)	0.3793 (253)	0.3254 (217)	0.3133 (209)

Figures in the parentheses indicate the size of chickens in the different genotypes at the four loci

Table 3: The analysis of each SNP of H-FABP in different experimental groups of F₁ and F₂

Groups	Population	Locus			
		1	2	3	4
Core group (Alleles)					
A	F ₁	0.5977	0.5016	0.557	0.5423
	F ₂	0.3018	0.6246	0.4035	0.4263
B	F ₁	0.4023	0.4984	0.443	0.4577
	F ₂	0.6982	0.3754	0.5965	0.5737
Genotype frequency					
AB	F ₁	0.3094 (95)	0.1889 (58)	0.4951 (152)	0.4658 (143)
	F ₂	0.4772 (136)	0.0561 (16)	0.6316 (180)	0.5789 (165)
AA	F ₁	0.4430 (136)	0.4072 (125)	0.3094 (95)	0.3094 (95)
	F ₂	0.0632 (18)	0.5965 (170)	0.0877 (25)	0.1368 (39)
BB	F ₁	0.2476 (76)	0.4039 (124)	0.1954 (60)	0.2248 (69)
	F ₂	0.4596 (131)	0.3474 (99)	0.2807 (80)	0.2842 (81)
High-fat group (Alleles)					
A	F ₁	0.766	0.835	0.7685	0.7069
	F ₂	0.4441	0.6835	0.5904	0.5691
B	F ₁	0.234	0.165	0.2315	0.2931
	F ₂	0.5559	0.3165	0.4096	0.4309
Genotype frequency					
AB	F ₁	0.2217 (45)	0.1724 (35)	0.2069 (42)	0.3103 (63)
	F ₂	0.5160 (97)	0.1436 (27)	0.4681 (88)	0.4149 (78)
AA	F ₁	0.6552 (133)	0.7488 (152)	0.6650 (135)	0.5517 (112)
	F ₂	0.1862 (35)	0.6117 (115)	0.3564 (67)	0.3617 (68)
BB	F ₁	0.1232 (25)	0.0788 (16)	0.1281 (26)	0.1379 (28)
	F ₂	0.2979 (56)	0.2447 (46)	0.1755 (33)	0.2234 (42)
Low-fat group (Alleles)					
A	F ₁	0.3478	0.3068	0.401	0.4469
	F ₂	0.1701	0.3866	0.2603	0.3582
B	F ₁	0.6522	0.6932	0.599	0.5531
	F ₂	0.8299	0.6134	0.7397	0.6418
Genotype frequency					
AB	F ₁	0.2609 (54)	0.1981 (41)	0.4541 (94)	0.4976 (103)
	F ₂	0.2577 (50)	0.0103 (2)	0.4072 (79)	0.3969 (77)
AA	F ₁	0.2174 (45)	0.2077 (43)	0.1739 (36)	0.1981 (41)
	F ₂	0.0412 (8)	0.3814 (74)	0.0567 (11)	0.1598 (31)
BB	F ₁	0.5217 (108)	0.5942 (123)	0.3720 (77)	0.3043 (63)
	F ₂	0.7010 (136)	0.6082 (118)	0.5361 (104)	0.4433 (86)

Figures in the parentheses indicate the size of chickens in the different genotypes at the four loci

Table 4: Comparisons of IMF and VFR between three experimental groups of F₂

Groups	N	IMF (LSE±SE)
Core group	285	2.0422±0.0328
High-IMF group	188	1.9181±0.0404
Low-IMF group	194	1.9993±0.0397

constrains limited the use of MAS and also were hampered by implementation issues. The genetic variance was revealed by molecular markers covered the whole genome, only by detecting the markers which closely linked to the analyzed traits could apply the molecular marker technology to heredity and breeding of farm animal (Bernardo, 1992).

According to the quantitative genetics theories, the phenotype of trait depend mainly on its genotype and environment that is $P = G + E$ and there have mutual effect between genotype and environment ($G \times E$) (Kang and Gauch, 1996). When the living environment of animal changes, the $G \times E$ effect may happen, its are same individuals represent different productive performances in the different environments as a result. The magnitude and direction of $G \times E$ effect direct influence on the animal production and the working out of breeding plan (Jansen *et al.*, 1995). The four markers were applied in the study were screened by Wang in a breeding farm which was different from the experiment farm therefore, different environment may be a factor to affect the IMF content.

Recent advances in genotyping technologies and increases in genetic marker availability have paved the way for association studies on genomic scales (Marchini *et al.*, 2004). Linkage disequilibrium in population was dependant on multiple factors including genetic drift, population stratification and natural selection therefore, some amorphs also can be combine with QTL became linkage disequilibrium and to show the correlation with traits, namely false positive (Lander and Kruglyak, 1995). What's more, population stratification was the most important factor for false positive (Cardon and Palmer, 2003) and the potential problem for every population-based association study was the presence of population stratification that can mimic the signal of association and lead to more false positives or to missed real effects (Freedman *et al.*, 2004). However, researchers ignored this problem in the process of identifying the molecular makers of *F-HABP* gene in the F₁ generation in the study. Maybe, we can investigate genetic relationships among individuals (genetic stratification) using neutral markers like SSRs in further study (Stich *et al.*, 2005).

Molecular marker is not a gene, the variation of which is hardly to fully account for the genetic variation of traits. Most traits of economic value to animal are sophisticated quantitative characters and involve numerous genes expression, regulation and interaction among them (Lande and Thompson, 1990). But the actual MAS methods have been mostly based on ordinary additive models or single locus and double loci models and which cannot analyze the complicated genetic

Table 5: Result of variance analysis on different genotypes of four SNPs on IMF in F₂

Traits	Genotypes in SPNs	df	SS	MS	F-value	p-value
IMF	SNP1	2	0.5526	0.2763	1.11	0.3296
	SNP2	2	0.2483	0.1241	0.50	0.6077
	SNP3	2	0.4499	0.2249	0.90	0.4053
	SNP4	2	0.0032	0.0016	0.01	0.9935

df= Degree of freedom, SS = Sum of Squares and MS = Mean Square

phenomenon of traits expression during different space and time (Xie *et al.*, 1998). A great of experimental investigations in chicken IMF has been reported that EX-FABP (Qiong *et al.*, 2010), A-FABP (Chen *et al.*, 2004), H-FABP (Ye *et al.*, 2010), PPAR γ (Huang *et al.*, 2006), AMPD1 (Chen *et al.*, 2009), LPL (Meng *et al.*, 2009) and ADFP (Zhao *et al.*, 2009) were the important factors to influence it. Thus, the other genes may be also effect the IMF except H-FABP in the study.

CONCLUSION

Success of commercial application of marker-assisted selection was unclear and will depend on the ability to integrated marker information in selection and breeding programs (Dekkers, 2004). Since, the publication of draft sequence of the whole chicken genome in early 2004, the construction of high-density molecular marker linkage map, the development of research on the chicken function genomics and the organic combine of molecular biology technology and quantitative genetics theory, MAS will play more important role in modern chicken breeding.

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