Single nucleotide polymorphism analysis of the encoding region of EX-FABP gene and its association with fattiness trait in chicken*

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Abstract Fattiness is an important parameter to estimate meat quality, which has high heritability. In this experiment, F_2 chickens derived from Broilers crossing to Silky were used to study the effect of extracellular fatty acid binding protein (EX-FABP) gene on abdominal fat accumulation. Five exons and partial introns of the gene were amplified by six pairs of primers, and then single nucleotide polymorphisms (SNPs) were detected by single strand conformation polymorphism (SSCP) and subsequently confirmed by sequencing. There were four nucleotides variations found, T-C at 659, C-T at 924, CA-TG at 968 and C-T at 1985 respectively. The result of least square analysis suggests that the chickens with HH genotype defined by the third pair of primers have a lower abdominal fat weight and abdominal fat percentage than the chickens with other genotypes (GG, GI, GH and II). It implies that extracellular fatty acid binding protein gene could be a candidate locus or linked to a major gene that significantly affects abdominal fat traits in chicken.

Keywords: abdominal fat percentage, EX-FABPs, SNPs.

Great progress has been made in poultry breeding in the past 20 years. While with daily gaining of body weight, improved feed conversion and resistance to disease in chicken, the intensive selection in chicken breeding has resulted in some negative effects, such as the degradation of quality and flavor of meat. The problem has drawn the attention from breeders all over the world and some effort has been made, but there is almost no improvement in reducing hypodermal fat and abdominal fat and increasing intramuscular fat (IMF) in breast and legs in broilers. There is a viewpoint that intramuscular fat is closely correlated with meat flavor and succulency[1~3], especially tenderness of meat. A certain amount of fat can keep chicken in a nice appearance of carcass, but too much fatty deposition and abdominal fat is unnecessary. To understand the development and growing mechanism of fatty tissue will be greatly helpful for increasing IMF, controlling fat deposition and improving meat quality.

Gentili et al.[4] extracted extracellular fatty acid binding proteins (EX-FABPs) from chicken embryo. EX-FABPs were found in the myotube in the early stage of embryonic development, which are continuously expressed in muscular fabric, as well as in cardiac muscle and smooth muscle. EX-FABPs specifically integrate with long chain unsaturated fatty acid and stearic acid and convey them to target organs. They are invalid for short chain fatty acid. Giannoni et al. have succeeded in cloning and sequencing chicken EX-FABPs gene, which is 5148 bp in length, including a 5'-end control region, six exons and five introns, and a 157bp untranslated region. The gene encodes 175 amino acids[4~5].

EX-FABPs specifically integrate with fatty acid and also serve as a carrier of fatty acid, so they are important for fatty deposition and metabolism in various tissues; their roles have been proved somehow in mammals[6]. In this study, samples were selected from F_2 chickens derived from Broilers crossing with Silky chicken, and the EX-FABP gene was considered as a candidate gene. The correlation between the EX-FABP genotypes and fattiness traits was studied with single strand conformation polymorphism

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(SSCP) technique and statistic method.

1 Materials and method

1.1 Sample

F_2 chickens derived from Broilers crossing with Silky were used, including six reciprocal families with a total of 510 individuals. The chickens were sacrificed at the age of 12 weeks, and the weight of carcass, breast muscle, leg muscle, abdominal fat, etc. were recorded. DNA of chicken was extracted from blood, and stored at −20℃.

1.2 Primer design and PCR-SSCP

Six pairs of primers were designed based on the known sequence from GenBank, which amplify five exon and partial intron region of chicken EX-FABP gene (Table 1).

PCR amplification was carried out by adding about 25 ng genomic DNA to 25 μL of reaction mixture. The DNA of 29 Broilers and 23 Silky was amplified with six pairs of primers at first, and then SSCP analysis was conducted. After polymorphism was discovered, the DNA from F_2 individuals were amplified and SSCP analysis was performed.

Table 1. Primer pairs designed for amplification of the encoding region of EX-FABP in chicken

Primer pair	Product size (bp)	Forward primer	Reverse primer	Amplified region
1	233	5′-CCAGGTTGCAGGGAAATGGT-3′	5′-TCCCTCCAGGTTTGTGGGTAT-3′	Exon 2
2	231	5′-TGGAGGGAGACTGAATTGGG-3′	5′-TTGTCGCTGCTGGGTTGAC-3′	Intron 2
3	216	5′-TGCGTGACAGGGCATTCTT-3′	5′-CCAGCATGAAGTGGTGTTGA-3′	Exon 3
4	239	5′-AAACGGTGGAGGTGCTGGAC-3′	5′-CAAACTGCTGCTCCCTCCACT-3′	Exon 4
5	183	5′-CCCACAGCCATGGCAATCT-3′	5′-TGCCTACCCACACCACCAA-3′	Exon 5
6	193	5′-AATGGGACTGGCAAGACCAC-3′	5′-TGCTGGTGAAGCGGGAGAT-3′	Exon 6

1.3 Cloning and sequencing of polymorphic fragments

The amplified fragments of different genotypes were recovered from 1.5% agarose gels, and were cloned into pGEM-T vector (Promega). The clones were sequenced by an automated PE377 DNA sequencer.

1.4 Statistic analysis

The least square model was established according to the characteristics of experimental materials, which is $Y = u$ + genotype + sex + reciprocal + family + residual. Y is carcass traits; u the means of carcass traits. Analysis software used was SAS (version 6.12).

2 Results and analysis

2.1 PCR amplification and SSCP

The products of PCR amplification were obtained using six sets of primers; they were with correct sizes as we expected and analyzed respectively with SSCP. The result showed that the PCR products from primer pairs 2, 3, 5 displayed polymorphisms among Broilers and Silky. So 510 individuals of F2 generation were analyzed using these three pairs of primers. Primer pair 2 detected two homozygotes, defined as EE and FF and with frequency of 0.28 and 0.18 respectively, and one heterozygote, defined as EF and with frequency of 0.54. Primer pair 3 identified 6 genotypes including 3 homozygotes, defined as GG, HH and II, and 3 heterozygotes, defined as GH, GI and HI respectively. Primer pair 5 found three genotypes of MM, NN and MN, with frequency of 0.60, 0.20 and 0.20 respectively (Fig. 1).

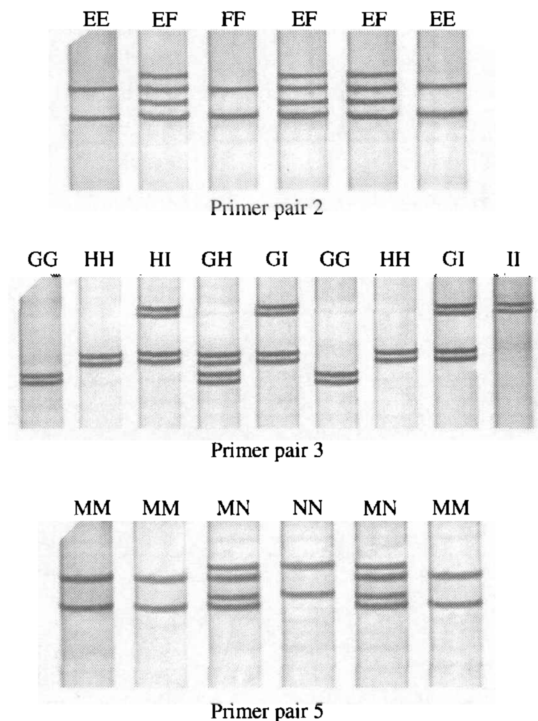

Fig. 1. SSCP analysis of PCR products amplified from different F_2 individuals.

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2.2 Sequencing of polymorphic fragments

The sequencing of EE and FF showed that EE genotype had the same nucleotide sequence as that in GenBank, and FF genotype had a T-C mutation at nt659. The sequence of GG fragment was the same as recorded in GenBank. In the sequencing of GG fragment a C to T mutation at nt924 for HH and a CA-TG mutation at nt968 for II were detected. Also MM fragment had the same sequence as the GenBank's, and NN genotype had a C-T mutation at nt1985 (Fig. 2). The mutations detected by primer pairs 2 and 3 are located in intron 2 and intron 3, and the mutation detected by primer pair 5 is located in the exon 5, which is a silent mutation.

EE TCTGGCACCCATTGCTGCA**T**GAGTGGCAGCTCTCCCCAG
FF TCTGGCACCCATTGCTGCA**C**GAGTGGCAGCTCTCCCCAG
Primer pair 2
GG GGGAACAGAGTCGTGGGC...ACCTGGGA**CA**CCCCCCACCACT
HH GGGAACAGAGT**T**GTGGGC...ACCTGGGA**TG**CCCCCCACCACT
II GGGAACAGAGTCGTGGGC...ACCTGGGA**TG**CCCCCCACCACT
Primer pair 3
MM ACTACACGGATGAGATGGTCGC**C**GTGCTGCCCAGCCAGGGT
NN ACTACACGGATGAGATGGTCGC**T**GTGCTGCCCAGCCAGGGT
Primer pair 5

Fig. 2. Nucleotide sequence comparison of the fragments amplified with three primer pairs. The differences in sequences are indicated in bold letters.

2.3 The least square analysis

There were high positive correlations among carcass, breast muscle, leg muscle and abdominal fat weight. The result of the least square analysis showed that the chickens with HH genotype had a lower abdominal fat weight and abdominal fat percentage than the chickens with other genotypes (GG, II, GI and GH) (Table 2), and the difference was very significant ($P < 0.001$). But carcass, breast muscle, leg muscle weight had no significant difference among different genotypes identified by primer pair 3. No significant difference was found in the carcass, breast muscle, leg muscle and abdominal fat weight of F_2 individuals between different genotypes detected by primer pairs 2 and 5.

Table 2. Association of the different genotypes with abdominal fat weight (AFW) and abdominal fat percentage (AFP)

Genotypes	*n*	Frequency	AFW(g)	AFP(%)
GG	27	0.053	58.82a	0.0384a
HH	108	0.212	41.15c	0.0279c
II	69	0.135	56.80a	0.0366a
GH	68	0.133	49.93ab	0.0336ab
GI	69	0.135	49.40ab	0.0329ab
HI	169	0.332	46.33bc	0.0308bc

The difference is not significant for the means being denoted by the same letter ($P > 0.05$).

In conclusion, our genotyping results imply that extracellular fatty acid binding protein gene could be a candidate locus or linked to a major gene which significantly affects abdominal fat traits in chicken. The DNA markers we identified in this experiment can be used as genetic markers in selection of chicken fat trait.

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