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Study on the Relationships Between Single Nucleotide Polymorphism of *EX-FABP* Gene and Slaughter and Meat Quality Traits in Chicken

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Abstract: In this study, the single nucleotide polymorphisms of chicken *EX-FABP* gene were detected by PCR-SSCP method with different pairs of primers among 228 chickens from 8 populations. Results indicated that there were 5 mutation sites had been found: 645C→T, 3254C→T, 3266T→C, 4272A→G, 4282A→G, the X^2 test results showed that there was extremity significant difference($p<0.01$) (Fragment 1) in the frequency of genotypes among breeds. Based on the detected polymorphisms, the effects of different genotypes on slaughter and meat quality traits were analyzed. The results indicated that there were significant differences between genotypes and Glu and Ash ($p<0.05$) (fragment 2) and significant differences between genotypes and Val ($p<0.05$) (fragment 8). It was concluded that the single nucleotide polymorphisms of chicken *EX-FABP* gene was not association with slaughter, but it was association between the polymorphisms and partial amino acid which can affect meat quality traits.

Key words: *EX-FABP*, PCR-SSCP, slaughter, meat quality traits

INTRODUCTION

Several studies with molecular genetic methods have identified genomic regions or genes that may be associated with fat deposition. *H-FABP* and *EX-FABP* gene can bind and transport fatty acids to many tissues (Veerkamp and Maatman, 1995; Glatz and Vander Vusse, 1996). Several researches indicated that using of mutations detectable by RFLP in *FABPs* gene as markers to select for improved meat quality traits and possible for growth in pig breeding programs (Gerbens *et al*., 1998, 1999). This experiment was aim to study the relationships between the polymorphisms of *EX-FABP* gene and slaughter and meat quality traits in poultry.

Extra cellular fatty acid binding protein (*Ex-FABP*) is a 21 kDa lipocalin specifically binding fatty acids (Gentili *et al*., 1998), expressed during chicken embryo development in hypertrophic cartilage, in muscle fibers and in blood granulocytes (Descalzi *et al*., 2002). The protein selectively binds with high affinity fatty acids; preferably long chain unsaturated fatty acids. Ex-FABP expression is strongly induced by treatment with inflammatory agents such as the bacterial liposaccharide (LPS) or interleukin-6 (Glatz and Vander Vusse, 1996).

MATERIALS AND METHODS

Experimental populations: In this experiment, 228 chickens (8 populations including 5 pure lines and 3 crossbreds developed by Sichuan Animal Science Academy and Dahen Poultry Breeding Company) were used. 8 populations include S03, S02, S05, S01, D99, S01×S10, S01×S05, S01×D99. The number of each line was 30 and each sex was identical. S03, S02, S05 and S01 belong to pure line and meat type, S01×S10, S01×S05 and S01×D99 were cross line, D99 was pure line and egg-meat type. The samples were randomly selected. The blood samples were collected from vein in wings, stored at -70°C before use.

Management and phenotypic measurements: At the age of 90 day, weight 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

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Table 1: *EX-FHBP* primer sequences corresponding to PCR product sizes and positions

Primer	Sequences	Location	Fragment (bp)
1	TTCCAGGCCAGAAGAATG	489	204
	ATGGCGTAGTGCTCGTAG	692	
2	GGCAGGGCACTACATTG	543	316
	GCTCCTGGCTGTCTTTCT	858	
3	AAGGCCATTGCATCAGC	959	220
	CCAGCAGCAAAGGACGG	1178	
4	GCTGTAGGACCCTCACTCA	1273	291
	TCAGCCTGTGCTCACTTC	1563	
5	CTCCAAGTGCCTACGACC	1581	229
	CCAGCCCTGACAACAAA	1809	
6	TGAGAAGGGCAAGATGAAGA	3110	264
	CCAGAGTAGGACAGGGAGAATA	3373	
7	TCAAGTGCTAGTCCCAAATT	3877	203
	CCATTCATTGATCCACCC	4079	
8	TGAGAAGGGCAAGATGAAGA	4063	290
	CCAGAGTAGGACAGGGAGAATA	4352	

Carcass weight (CW), evisceration weight (EW), semi-evisceration weight (SEW), breast muscle weight (BMW), leg muscle weight (LMW), abdominal fat weight (AW) and subcutaneous fat thickness (SFT). The ratios of these traits to *CW* were calculated as evisceration percentage (EP), semi-evisceration percentage (SEP), breast muscle percentage (BMP), leg muscle percentage (LMP) and abdominal fat percentage (AP). The meat quality traits were measured too, including sixteen types of amino acids (Asp, Glu, Ser, His, Gly, Thr, Ala, Arg, Tyr, Val, Met, Phe, Ile, Leu, Lys, Pro), Moisture Content, Dry Matter, Protein, Fat, Ash, Inosinic Acid (IMP), Muscle Fiber Number (MFN), muscle fiber diameter (MFD) and Power of Hydrogen (PH).

Development of PCR-SSCP assays and screening the population: Genomic DNA was prepared from blood samples by using proteinase k digested followed by phenol/chloroform extraction and precipitation with ethanol. Base on the nucleotide sequences of *Gallus gallus* extra cellular fatty acid binding protein (*EX-FABP*) gene (Genebank Accession No: AY_545055), 8 different pairs of primers were designed using software Primer 5.0 and oligo 6.0 (Table 1). Amplification was carried out for 35 cycles of 95°C for 45 sec, 56-61°C for 45 sec, 72°C for 45 sec and followed by a final extension of 10 min at 72°C.

A mixture was prepared for each sample by mixing 2 μL PCR product, 4 μL SSCP loading buffer (95% formamide, 20 mmol L^{-1} Na2EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF). The mixture was denatured at 99°C for 10 min and cooled immediately in ice water before being loaded into the polyacrylamide gels. Electrophoresis was carried out at 165 V for 8 h 30 min. The bands were visualized by silver staining. After being rinsed in 3 changes of water, the gel was dried at normal temperature for permanent record. SSCP profiles were interpreted visually. Two bands, representing the two strands, are seen for each allele on an SSCP gel. For each band, two separate panel samples containing only that allele were PCR amplified and then sequenced directly by Shanghai Ying Jun Biology Technique Corporation.

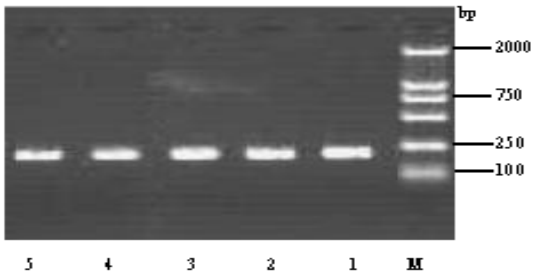

Fig. 1: Analysis of PCR amplification using P1~5: Individual PCR products; M: DNA marker

Data analysis: The genotype distribution of different breeds was computed and the frequency of genotypes was analyzed by x^2 test. The association between the genotypes of *EX-FABP* gene and the traits measured in the birds was analyzed using an animal mixed model in the SAS 8.1 program package.

RESULTS

Fragments were amplified by primers and then PCR products were detected by 1% agerose gel electrophoresis (Fig. 1). In *EX-FABP* gene, Fragment 1-5 (F1-F5) were amplified from intron 1, Fragment 6 (F6) was amplified from some parts of exon 2 and some parts of intron 3, Fragment 7 (F7) was amplified from some parts of intron 4, Fragment 8 (F8) was amplified of exon 4. Only F2, F6, F8 have detected mutations by PCR-SSCP (Fig. 2), then sequencing found there were five mutations, a C/T mutation at position 645nt, a C/T mutation at position 3254nt, a T/C mutation at position 3266nt, a A/G mutation at position 4272nt, a A/G mutation at position 4282nt in DNA sequence of chicken (Accession No: AY_545055) (Fig. 3).

In *EX-FABP* gene, the X^2 test results showed that there was extremity significant difference in the frequency of genotype among breeds ($p<0.01$) (Fragment 2). There were no significant differences in the frequency of genotype among breeds ($p>0.05$) (Fragment 6, 8) (Table 2).

In *EX-FABP* gene, the least square analysis showed that there was significant difference between genotypes and meat quality traits such as Glu, Ash ($p<0.05$) (fragment 2), Glu, Ash of *A2A2* genotype birds were significantly higher than *A1A2*, *A1A1* genotype chickens. There was another significant difference between genotypes and Val of fragment 3 ($p<0.05$). Val of *C1C1* genotype birds were significantly higher than other kinds of genotype chickens (Table 3).

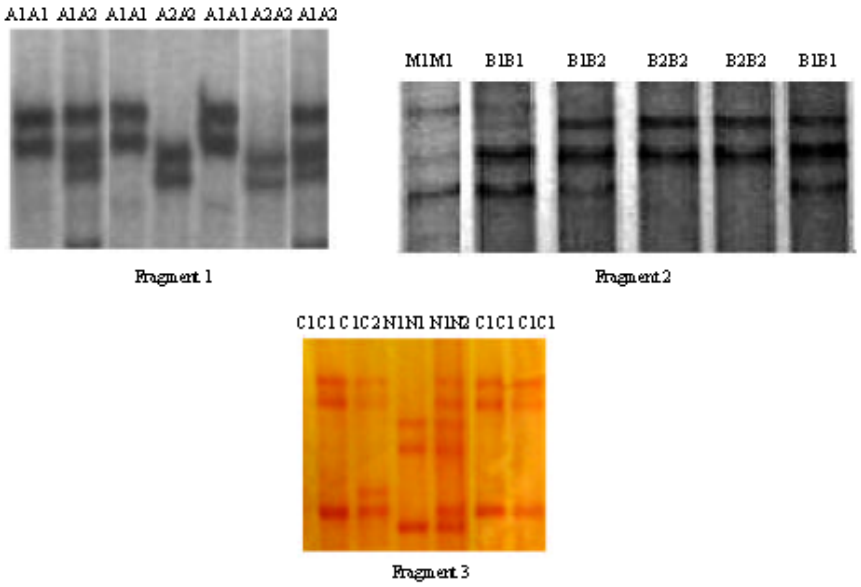

Fig. 2: PCR-SSCP analysis of *EX-FABP* gene, showing the individual genotypes

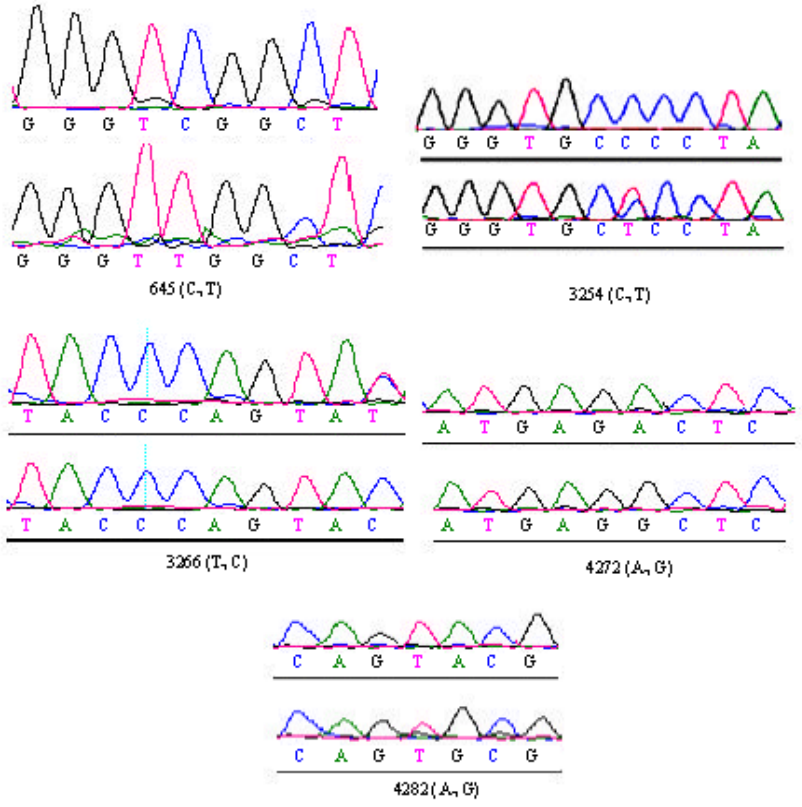

Fig. 3: The sequence map of EX-FABP gene including a C/T mutation at position 645nt, a C/T mutation at position 3254nt, a T/C mutation at position 3266nt, a A/G mutation at position 4272nt, a A/G mutation at position 4282nt in DNA sequence of chicken (Accession No: AY_545055)

Table 2: The comparisons of frequencies of genotype and gene among different breeds in *EX-FABP* gene

Primer	Genotype	S03	S02	S05	S01	S01XS05	S01XS10	S01XD99	D99	X2
F2	A1A1	0.259	0.759	0.379	0.034	0.036	0.172	0.483	0.714	$X^2 = 77.01$
	A1A2	0.556	0.207	0.345	0.621	0.679	0.690	0.310	0.107	$p<0.01$
	A2A2	0.185	0.034	0.276	0.345	0.285	0.138	0.207	0.179	
F6	B1B1	0.371	0.483	0.448	0.414	0.429	0.552	0.310	0.464	$X^2 = 28.23$
	B1B2	0.259	0.345	0.310	0.276	0.357	0.207	0.172	0.143	$p>0.05$
	B2B2	0.111	0.034	0.035	0.138	0.035	0.000	0.173	0.071	
F8	M1M1	0.259	0.138	0.207	0.172	0.179	0.241	0.345	0.322	$X^2 = 29.46$
	C1C2	0.111	0.172	0.035	0.138	0.143	0.000	0.069	0.143	$p>0.05$
	C2C2	0.407	0.586	0.517	0.552	0.643	0.414	0.517	0.393	
	N1N2	0.197	0.207	0.414	0.172	0.143	0.448	0.414	0.321	
	N1N1	0.185	0.035	0.034	0.138	0.071	0.138	0.000	0.143	

Table 3: Effect of different genotype in *EX-FABP* gene on meat quality traits[1]

----------Primer genotype----------		Glu	Val	Ash
Primer2	A1A1	5.9717±0.2142	4.3148±0.1003	4.4859±0.0585
	A1A2	5.5896±0.1629	4.4144±0.0763	4.3275±0.0445
	A2A2	6.2730±0.2275	4.4928±0.1065	4.5055±0.0621
	F Value	3.30*	0.66	3.75*
Primer8	C1C1	5.7148±0.3562	4.8195±0.1579	4.4733±0.0973
	C1C2	5.7872±0.1572	4.3737±0.0697	4.4506±0.0429
	N1N2	6.0283±0.1932	4.3663±0.0856	4.3778±0.0528
	N1N1	5.8795±0.3341	4.261±0.1481	4.3520±0.0913
	F Value	0.37	2.74*	0.64

[1]:*is significant difference ($p<0.05$); ** is extremity difference

DISCUSSION

The palatability is a main factor of meat quality traits, which composed with mineral salt, frees amino acid, peptide, and the productions of nucleic acid metabolism (Kuninaka, 1960; Ugawa *et al*., 1992). Some researchers considered that Glu, Asn, Arg, Ala, Gly, Met, Val, Pro, Ser… are impotent amino acids for palatability (Misako *et al*., 2002), in this experiment, we examined 16 kinds of amino acids. In *EX-FABP* gene, there were significant difference between genotypes and amino acids such as Glu and Val ($p<0.05$). From the results, the conclusion was drawn putatively that A2 and C1allele maybe affect meat quality traits especially palatability in these strains, so we should regard locus A and locus C as potential molecular markers in the process of breeding.

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