Tissue Expression and Association with Fatness Traits of Liver Fatty Acid-Binding Protein Gene in Chicken[1]

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ABSTRACT Fatty acid-binding proteins belong to a superfamily of lipid-binding proteins that exhibit a high affinity for long-chain fatty acids and appear to function in metabolism and intracellular transportation of lipids. The current study was designed to investigate expression characterization and association with growth and composition traits of the liver fatty acid-binding protein (L-FABP) gene in the chicken. Northern blot analysis indicated that the gene, similar to the mammal L-FABP gene, was expressed only in liver and intestinal tissues. The mRNA levels of the chicken L-FABP gene in liver and intestine had significant differences between the broilers and Baier layers. The China Agricultural University F_2 population was used in the present study. Body weight and body composition traits were measured in the populations. Primers for the coding region and 5′ upstream region of the L-FABP gene were designed according to chicken genomic and cDNA sequence. Polymorphisms were detected by DNA sequencing, and the PCR single-strand conformation polymorphisms method was developed to genotype the F_2 population. The results indicated that the L-FABP gene polymorphisms were associated with abdominal fat weight and percentage of abdominal fat, and the L-FABP gene could be a candidate locus or linked to a major gene(s) that affects fatness traits in the chicken. The results of the current study provided basic molecular information for studying the role of the L-FABP gene in the regulation of lipid metabolism in avian species.

Key words: liver fatty acid-binding protein gene, tissue expression, polymorphism, fatness trait, chicken

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INTRODUCTION

Modern broilers selected for rapid growth exhibit higher body fat deposition (Mallard and Douaire, 1988; Griffin, 1996). Fat is considered a by-product of meat and has low commercial value. It is not a cost-effective body component from a view of energy utilization, and much fat deposition can depress feed efficiency. Although several strategies of selection for leanness in poultry production have been described, it is still difficult to measure body fat content (Mallard and Douaire, 1988; Jennen et al., 2004). Molecular genetic information can be used to enhance genetic improvement of animal species. Identifying the quantitative trait nucleotide responsible for the economically important traits will facilitate poultry breeding programs.

Fatty acid-binding proteins (**FABP**), which are small molecular weight proteins with high binding affinities for long-chain fatty acids (Ockner et al., 1972), are the members of a superfamily of lipid-binding proteins that occur intracellularly in invertebrates and vertebrates. These proteins are involved in intracellular fatty acid movement (Tipping and Ketterer, 1981), cell growth and differentiation, cellular signaling, gene transcription, and protection of enzymes from the toxic effects of free fatty acids (Glatz and Veerkamp, 1985; Zimmerman and Veerkamp, 2002). They may also play a role in the modulation of enzyme activity and signal transduction (Grinstead et al., 1983).

In mammals, the intracellular or cytoplasmic FABP form a group of at least 9 distinct proteins, which are liver FABP (**L-FABP**), intestinal FABP, heart FABP, adipocyte FABP, epidermal FABP, ileal FABP, brain FABP, myelin FABP, and testicular FABP (Zimmerman and Veerkamp, 2002). Their molecular weights range from 14 to 15 kDa, with 126 to 134 amino acids, and are named according to the tissues from which they are isolated (Ockner et al., 1972). All FABP types show similar structural features, containing 10 antiparallel β strands (βA to βJ) that form a β barrel. The bound ligand is found within the barrel in a central internal water-filled cavity. The overall organization of all members of the FABP family is identical, containing 4 exons and 3 introns. The exon-

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intron positions are similar, but the intron length is variable among the genes (Zimmerman and Veerkamp, 2002).

Molecular cloning and structural studies have increased the knowledge of the evolutionary and cellular diversity of FABP, and, recently, studies on knockout mice have provided direct evidence for their physiological role in the uptake and transport of long-chain fatty acids and their interaction with other transport systems and enzymes (Zimmerman and Veerkamp, 2002). The L-FABP gene ablation dramatically enhanced many of the effects of dietary cholesterol to greatly induce hepatic cholesterol (primarily cholesterol ester) and triacylglycerol accumulation as well as to potentiate BW gain (primarily as fat tissue mass; Martin et al., 2006). The liver weight of L-FABP gene knockout mice increased significantly, and these mice exhibited macroscopic steatosis (Seneshaw et al., 2005). The L-FABP gene knockout mice fed a diet with and without 1% phytol (a metabolic precursor to phytanic acid) exhibited a gender-dependent lipid phenotype. Livers of phytol-fed L-FABP mice enhanced the accumulation of lipid droplets and exhibited more necrosis than their counterparts in female, but not male, mice (Atshaves et al., 2005).

Although the role of L-FABP in the metabolism of fatty acids has been studied in mammals, the physiological functions need to be demonstrated in avian species. To address this issue, the current study was designed to characterize the chicken L-FABP gene tissue expression, to identify polymorphisms of the gene in different populations, to evaluate the associations of the polymorphism of the gene with growth and body composition traits, and to provide the basic molecular information for studying the role of the gene in the regulation of fatty acid metabolism in chickens.

MATERIALS AND METHODS

Bird Material and Phenotypic Traits

Broilers derived from lines at Northeast Agricultural University divergently selected for abdominal fat and Baier layers were used as tissue expression analysis in the current experiment. The Baier layer is a Chinese local egg-laying breed that has yellow feathers, a yellow beak, yellow feet, and white ears. Adult weights are 1,450 g for the cock and 1,190 g for the hen. Six broilers and 6 Baier layers were slaughtered at 2, 6, and 12 wk of age (3 of each sex), respectively, and leg muscle, breast muscle, abdominal fat, liver, heart, intestine (jejunum part), glandular stomach, and brain samples were harvested at 2 wk of age. In addition, kidney and ovaries (female) were taken at 6 and 12 wk of age, and lung, spleen, muscular stomach, and testicles (male) were also taken at 12 wk of age. All the tissues harvested were stored at −80°C until use.

A China Agricultural University (**CAU**) F_2 population was used for association analysis between growth and body composition traits and the L-FABP gene in the current study. The CAU F_2 population was established by crossing broilers with silkies. Unrelated F_1 birds were mated (1 sire to 5 dams) to produce 33 half-sib families with an average of 75 F_2 offspring per family in 6 hatches. The 445 F_2 individuals, including males and females produced from 4 reciprocal F_1 families, were sampled for the current study. Birds had free access to feed and water. Commercial corn-soybean-based diets that met all NRC (1994) requirements were provided in the study. From hatch to 3 wk of age, birds received a starter feed (3,000 kcal of ME/kg and 210 g/kg of CP), and from 3 to 12 wk of age, birds were fed a grower diet (3,100 kcal of ME/kg and 190 g/kg of CP). Body weight was measured at hatch and weekly up to 12 wk of age. Body composition traits were recorded at 12 wk of age. These measurements included pectoralis major weight, leg muscle weight, abdominal fat weight (**AFW**), and liver weight. All the traits were also expressed as percentage of BW at 12 wk of age.

DNA and RNA Extraction and cDNA Synthesis

Genomic DNA was extracted using phenol-chloroform from venous blood collected in EDTA-Na_2-coated tubes and was stored at −20°C. The RNA was extracted with the Trizol reagent (Invitrogen, Rockville, MD) according to the instructions of the manufacturer from each tissue at different weeks of age, then was resuspended in diethyl pyrocarbonate-treated water. The reverse transcription reactions were carried out using the Takara RNA PCR Kit, Version 2.1 (Takara Biotechnology Co. Ltd., Dalian, China). The reverse transcription reaction contained 1 μg of total RNA derived from the liver tissue of 3 broilers and 3 Baier layers in a final volume of 20 μL, respectively.

Northern Blot Analysis

Total RNA (15 μg of each sample) isolated from the different tissues of chickens at 2, 6, and 12 wk of age was electrophoresed and transferred to nylon membranes. A 390-bp L-FABP cDNA fragment was used as probe after labeling by random priming with [α^{32}P]dCTP (3000 Ci.mmol^{-1})[2], which was obtained using primers LFF1 (5′-TCA CTG GAA AGT ACG AGC -3′) and LFR1 (5′-GCA TGC AGG GTC TCT AGA TT-3′), according to the chicken L-FABP gene sequence(Genbank accession no. AF380999). The expression of the chicken glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) gene was used as a control. A 491-bp fragment was used as probe after labeling by random priming with [α^{32}P]dCTP (3000 Ci.mmol^{-1})[2], which was obtained using primers GF (5′-TGA CGT GCA GCA GGA ACA C-3′) and GR (5′-CAG TTG GTG GTG CAC GAT G -3′), according to the chicken GAPDH gene sequence(Genbank accession no. K01458). The ribosomal RNA (5-, 18-, and 28S) markers were used as standards for calculation of the transcript size. The membrane was prehybridized at 42°C in ULTRAhyb (Ambion, Austin, TX) for 60 min, and then the denatured probe (95°C for 10 min) was added at a concentration of

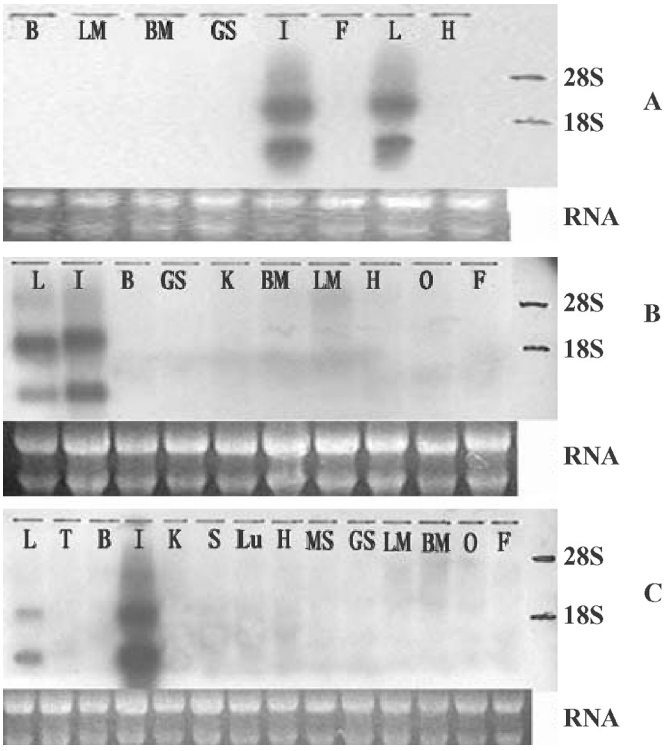

Figure 1. Northern blot of the chicken liver fatty acid-binding protein in different tissues at 2 (panel A), 6 (panel B), and 12 (panel C) wk of age. L = liver; T = testis; B = brain; I = intestine; K = kidney; S = spleen; Lu = lung; H = heart; MS = muscle stomach; GS = gland stomach; LM = leg muscle; BM = breast muscle; O = ovary; and F = fat.

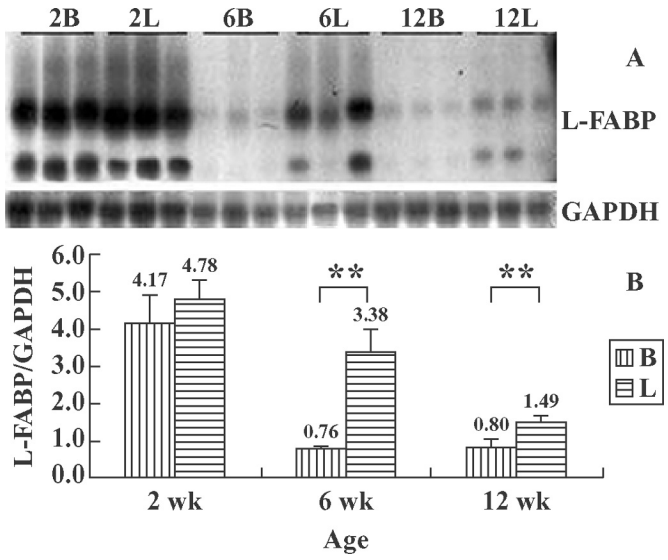

Figure 2. Hepatic expression of liver fatty acid-binding protein (L-FABP) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes evaluated by northern blot. Northern blot of L-FABP in liver tissue of 2-, 6-, and 12-wk-old broiler and Baier layers (panel A). 2B = 2-wk-old broiler; 2L = 2-wk-old Baier layer; 6B = 6-wk-old broiler; 6L = 6-wk-old Baier layer; 12B = 12-wk-old broiler; and 12L = 12-wk-old Baier layer. Quantitative analysis of L-FABP in liver tissue of 2-, 6-, and 12-wk-old broiler and Baier layers (panel B). B = broiler; L = Baier layer. Double asterisks (**) indicate significant difference ($P < 0.01$).

1×10^6 cpm/mL to hybridize with the targeted mRNA transcript overnight at 42°C. Following hybridization, the membrane was washed twice in 2 × SSC (300 m*M* NaCl and 30 m*M* Na citrate, at pH 7.0) containing 0.1% Na dodecyl sulfate for 15 min at 42°C and twice in 0.2 × SSC containing 0.1% Na dodecyl sulfate for 5 min and 15 min, respectively. The membrane was dried in air then radioautography. The bands were analyzed with the Laboratory Imaging and Analysis System (UVP Inc. Upland, CA).

DNA Sequencing of Chicken L-FABP Gene

One fragment of 390 bp including coding sequence and another fragment of 1,086 bp including the 5′ upstream region of chicken L-FABP gene were amplified. Primers for coding region amplification were LFF1 (5′-TCA CTG GAA AGT ACG AGC-3′) and LFR1 (5′-GCA TGC AGG GTC TCT AGA TT-3′), and the primers for 5′ upstream region amplification were LFF2 (5′-AAC TTC GGG AGA TGT CAA CA-3′) and LFR2 (5′-TTC ATG GGA CTG GAG CTC GTA C-3′). The PCR components were 1 × PCR reaction buffer (10 m*M* Tris-HCl, 50 m*M* KCl, and 1.5 m*M* $MgCl_2$, pH 8.3), 200 μ*M* each deoxynucleotide triphosphate, 0.25 μ*M* each primer, 2 μL of cDNA (for the coding sequence) or 50 ng of DNA (for the 5′ upstream region), and 1 U of *Taq* DNA polymerase (TaKaRa Biotechnology Co. Ltd., Dalian, China) in a final volume of 25 μL. The thermal cycling was 94°C for 5 min, followed by 30 cycles of 94°C for 50 s, 54°C for 1 min (for the coding sequence) or 56°C for 1 min (for the 5′ upstream region), and 72°C for 1 min. The PCR products were purified by Gel Extraction Mini Kit (Watson Biotechnologies Inc., Shanghai, China) and then sequenced in forward and reverse directions with an ABI 3730 sequencer (BioAsia Biotechnology Co. Ltd., Shanghai, China). Restriction enzyme sites in these sequences were detected by the DNAMAN package, Version 4.0 (Lynnon BioSoft, Vaudreuil-Dorion, Quebec, Canada).

Genotyping of Chicken L-FABP Gene

Polymorphisms of the L-FABP gene 5′ upstream regions were genotyped by the PCR single-strand conformation polymorphisms (**SSCP**) method. A genomic fragment of 196 bp responsible for the polymorphism sites of the L-FABP gene was amplified by using primers LFF3 (5′-CAT TTG TTT ATG GAT GCC ACG-3′) and LFR3 (5′-TAA GGA CCT TTT GCC CTA A-3′). The PCR components included 1 × PCR reaction buffer, 200 μ*M* each deoxynucleotide triphosphate, 0.25 μ*M* each primer, 1 μL of genomic DNA (50 ng/μL), and 0.5 U of *Taq* DNA polymerase in a final volume of 10 μL. The thermal profiles were 94°C for 5 min, followed by 32 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s. One microliter of the PCR products was mixed with 5 μL of loading buffer (98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol, 10 m*M* EDTA, and 10% glycerol) after PCR according to the above, then the mixture was denatured in 98°C for 10 min, placed on ice for 5 min, and a silver stain method was developed to display the

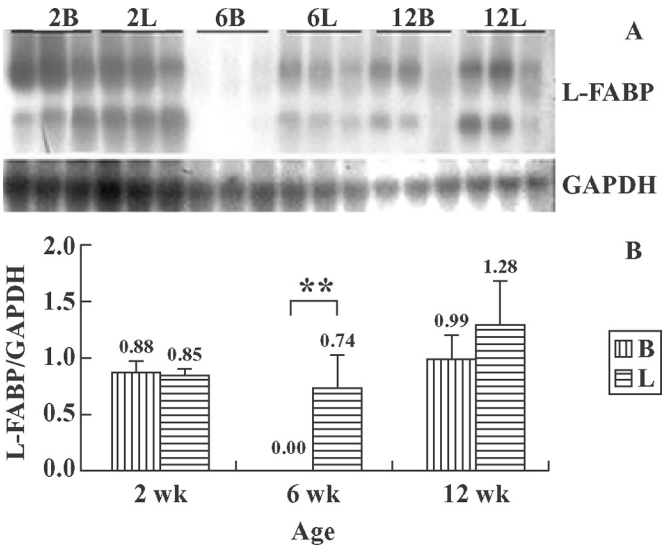

Figure 3. Intestinal expression of liver fatty acid-binding protein (L-FABP) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes evaluated by northern blot. Northern Blot of L-FABP in intestinal tissue of 2-, 6-, and 12-wk-old broiler and Baier layers (panel A). 2B = 2-wk-old broiler; 2L = 2-wk-old Baier layer; 6B = 6-wk-old broiler; 6L = 6-wk-old Baier layer; 12B = 12-wk-old broiler; and 12L = 12-wk-old Baier layer. Quantitative analysis of L-FABP in intestinal tissue of 2-, 6-, and 12-wk-old broiler and Baier layers (panel B). B = broiler; L = Baier layer. Double asterisks (**) indicate significant difference ($P < 0.01$).

bands. Individual PCR-SSCP band patterns were determined by a 14% polyacrylamide gel with 5% glycerol electrophoresis for 8 to 9 h at 10 V/cm and silver nitrate staining under visible light.

Statistical Analysis

Data of differential expression of the chicken L-FABP gene between 2 breeds were analyzed by the *t*-test of SAS 6.12 (SAS Institute Inc., Cary, NC). Data of individual genotypes were subjected to the GLM procedures of JMP (SAS Institute Inc.) with the genotype and sex as fixed effects and hatch, family, and dam as random effects, according to the model

$$Y = \mu + G + S + h + f + d + e$$

where Y = the dependent variable; μ = population mean; G = genotype; S = sex; h = hatch; f = family; d = dam; and e = the random error. The interaction G by S was not significant for any traits and therefore was not included in the model. Statistical significance threshold was $P < 0.05$, unless otherwise specified.

RESULTS

Tissue Expression Pattern of Chicken L-FABP Gene

Northern blot analysis with pool RNA from 3 males and 3 females indicated that 2 transcripts of approximately 2.5 and 0.8 kb for the L-FABP gene were clearly visible. The chicken L-FABP gene was expressed only in liver and intestine among all the tissues tested from chickens at 2, 6, and 12 wk of age (Figure 1).

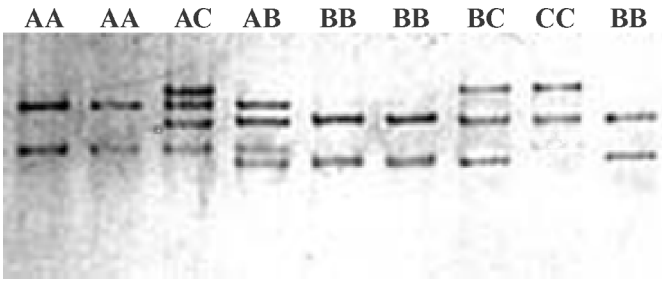

Figure 4. Polymerase chain reaction single-strand conformation polymorphisms pattern for the liver fatty acid-binding protein gene.

Differential Expression of Chicken L-FABP Gene Between 2 Breeds

The quantification of L-FABP mRNA levels expressed relative to GAPDH mRNA in liver and intestinal tissues were investigated with 18 male birds from the 2 breeds. Northern blot analysis indicated that the L-FABP mRNA levels of the Baier layers were higher ($P < 0.05$) than those of broilers in liver at 6 and 12 wk of age (Figure 2) and in intestine at 6 wk of age (Figure 3).

Identification of Polymorphism and PCR-SSCP Analysis

All of the coding sequence and the 5′ upstream region of the chicken L-FABP gene had been amplified successfully by using either cDNA or genomic DNA samples. Sequence alignment of the chicken L-FABP sequences revealed that there was no polymorphism in the coding region. However, 2 novel mutations were found at base 204 and 205 upstream of the ATG initiation codon of the L-FABP gene. There was a G to A substitution at position 204 and a T to C substitution at position 205. The results of SSCP showed that there were 6 genotypes in the F_2 offspring, named AA, BB, CC, AB, AC, and BC, respectively (Figure 4). Three kinds of homozygote individuals (AA, BB, and CC) had been sequenced respectively. The sequence of the AA genotype was TG, BB genotype was CA, and CC genotype was CG in the 2 mutations (Figure 5). The result of the motif search for nucleotides showed that the mutations had not changed protein-binding sites or the transcript start site for the chicken L-FABP gene.

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AA  TCTGCCCCTGCATTGTAAATCGAGGTCTCTACATTGTAGAGCAATTTATC
BB  TCTGCCCCCACATTGTAAATCGAGGTCTCTACATTGTAGAGCAATTTATC
CC  TCTGCCCCCGCATTGTAAATCGAGGTCTCTACATTGTAGAGCAATTTATC
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Figure 5. Sequence comparison of different genotypes in the chicken liver fatty acid-binding protein gene.

Table 1. Effects (*P*-value) of liver fatty acid-binding protein (L-FABP) polymorphisms on chicken growth and body composition traits

Traits[1]	Age (wk)	CAU F_2 population[2]
BW	0	NS[3]
BW	1	NS
BW	2	NS
BW	3	NS
BW	4	NS
BW	5	NS
BW	6	0.132
BW	7	NS
BW	8	NS
BW	9	NS
BW	10	0.081
BW	11	NS
BW	12	NS
CW	12	0.159
PMW	12	NS
LMW	12	NS
LW	12	0.174
AFW	12	0.017
%PMW	12	NS
%LMW	12	NS
%LW	12	NS
%AFW	12	0.032

[1]CW= carcass weight; PMW = pectoralis major weight; LMW= leg muscle weight; AFW = abdominal fat weight; LW = liver weight; and % indicates that traits are expressed as a percentage of BW at 12 wk of age.

[2]CAU = China Agricultural University.

[3]$P > 0.2$.

Association of L-FABP Gene Polymorphisms with Growth and Body Composition

The genotypes based on the 2 mutations of the chicken L-FABP gene were used in the genetic analysis of the population. There was a significant association ($P < 0.05$) between the polymorphisms and fatness traits (AFW and percentage of AFW) in F_2 birds of the CAU F_2 population (Table 1).

Effects (least square means) of the L-FABP genotypes on fatness traits in F_2 offspring of the CAU F_2 population are shown in Table 2. The AFW was lower ($P < 0.05$) in F_2 birds of the CC genotype than of the AA, AB, AC, BB, and BC genotypes. The percentage of AFW in F_2 birds was lower in the CC genotype than in the AA, AB, and AC genotypes.

Table 2. Effects (least square means) of liver fatty acid-binding protein (L-FABP) genotype on body composition traits in the China Agricultural University (CAU) F_2 population

	Traits[1]	
Genotypes[2] (number)	AFW (g)	%AFW
AA (155)	48.67 ± 6.17^{ab}	3.28 ± 0.36^{a}
AB (72)	54.46 ± 6.55^{a}	3.51 ± 0.38^{a}
AC (117)	46.71 ± 6.29^{b}	3.12 ± 0.36^{a}
BB (28)	49.35 ± 8.57^{ab}	3.14 ± 0.49^{ab}
BC (41)	46.83 ± 7.16^{ab}	3.08 ± 0.41^{ab}
CC (32)	34.53 ± 7.31^{c}	2.50 ± 0.42^{b}

[a–c]Means within a column with no common superscript are different ($P < 0.05$).

[1]AFW = abdominal fat weight; % indicates that trait is expressed as percentage of BW at 12 wk of age.

[2]Numbers shown in parentheses are the individuals selected.

DISCUSSION

The chicken L-FABP gene was expressed only in liver and intestine tissues and existed in 2 transcripts, which was similar to that previously reported in rats or humans (Veerkamp et al., 1991; Schroeder et al., 1998). These results suggest that the function of the chicken L-FABP gene may be similar to that of the mammalian L-FABP gene mammals and play an important role in lipid metabolism.

Microarray analysis of liver genes indicated that L-FABP gene expression was higher (1.66-fold) in the hyperthyroid (triiodothyronine-treated and lean) chickens than in the hypothyroid (propylthiouracil-treated and fat) chickens (Wang et al., 2002; Cogburn et al., 2003). The current results showed that mRNA levels of chicken L-FABP were different between breeds at 6 to 12 wk of age in liver tissue, with the Baier layers having higher levels than broilers. The broilers were derived from a founder meat-type chicken population and had been selected for abdominal fatness at 7 wk of age for 7 generations. The Baier layer is a Chinese local breed chicken. Lipid metabolism is obviously different between these 2 breeds. The AFW and percentage of AFW of the broilers were 17- and 4-fold than those of Baier layers at 12 wk of age, respectively (unpublished data). Liver is an important organ for lipid metabolism in the avian species. The higher expression of the L-FABP gene in the livers of the Baier layers than in the broilers might result in increased fatty acids transportation into mitochondria or peroxisomes and enhanced fatty acids oxidation and reduced depot fat. So the differential expression of the L-FABP gene could have an effect on fat deposition in the chicken.

In addition, the current results showed the polymorphisms of the 5′ upstream region of the L-FABP gene were related ($P < 0.05$) with AFW and percentage of AFW at 12 wk of age in the CAU F_2 population. In an F_2 cross among outbred lines, however, the linkage disequilibrium was substantial, and the associations of the L-FABP polymorphisms with AFW and percentage of AFW in the F_2 population might have been produced by linkage disequilibrium between the L-FABP polymorphisms and another mutation in the L-FABP locus or another linked gene directly involved in the regulation of these phenotypic traits. As it currently stands, there was some hint that the L-FABP gene may be associated with fat traits, but this was tentative and difficult to assess with the current information.

Based on tissue distribution and differential expression of the chicken L-FABP gene, the current results will allow L-FABP gene assessment as an important candidate gene in chicken QTL detection programs focusing on phenotypes related to fat traits.

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