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FABP3 and FABP4 Genes Are the Potential Candidates for Body Weights in Korean Native Chicken

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ABSTRACT FABPs, 15 kDa organic substances, are small intracellular proteins which have a primary role to regulate fatty acid uptake and intracellular transport. This study was conducted to identify SNPs in the two FABP family genes and their associations with the body weight traits in Korean native chicken (KNC). Two SNPs, namely g.508C>T of FABP3 gene and g.285C>T of FABP4 gene, have been genotyped by using PCR-RFLP method. The results showed that FABP3 was significantly associated with body weight at birth, body weights at 12 to 20 weeks, and also slaughter weight. Moreover, the g.285C>T SNP of FABP4 gene was not associated with any body weight traits. These results suggested that the g.508C>T SNP of FABP3 genes can be used as molecular markers to select KNC having desirable body weights.

(Key words : body weight, FABP3, FABP4, Korea native chicken)

INTRODUCTION

Recently, there are increasing reputations for the native livestock breeds to the consumers including chicken. People thought that native chicken meat has better taste and meat quality than that of commercial broilers. In Korea, native chicken is preferred by Korean consumers because Korean native chicken (KNC) has less fat and better protein content, even though its price is 2 to 3 times higher than that of commercial broilers (Sang et al., 2006; Hoque et al., 2011; Seo et al., 2013). Therefore, improving both meat quality and quantity in Korean native chicken will be great challenge for poultry industry.

Few decades ago, selection of chicken was mainly based on the phenotypes. This phenotype-based selection method requires relatively long time for selection. In contrast, recent advances in molecular genetics can give some guidelines for decreasing generation intervals, which can ultimately increasing the selection intensity. Therefore, the identification and utilization of QTLs and functional candidate genes have been becoming powerful tools for faster genetic improvement in selection program by marker assisted selection (MAS) (Dekkers and Hospital, 2002).

Fatty acid-binding proteins (FABPs), members of the intracellular lipid-binding protein (iLBP) family, are small intracellular proteins that acts in fatty acid uptake regulation, metabolism, and intracellular transport by binding intracellular hydrophobic ligands and trafficking them throughout cellular compartments, including the peroxisomes, nucleus, mitochondria, and endoplasmic reticulum (Smathers and Petersen, 2011). FABP genes are known to be expressed in various types of tissue, such as muscle, adipocyte, liver, heart, brain, intestinal, testis, myelin and epidermal (Chmurzynska, 2006). Until now, at least 11 types of FABP genes have been investigated, and one of them, namely FABP11, is specifically expressed in fish (Agulleiro et al., 2007). The FABP3 gene was mainly expressed in muscle and heart. On the other hand, FABP4 gene has been particularly expressed in adipose tissues. In pigs, both FABP3 and FABP4 genes were higher expressed in subcutaneous adipocyte tissue than

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intramuscular adipocyte tissue during growing time (Gardan et al., 2007). In addition, expression of both FABP3 and FABP4 genes was also increased in *longissimus dorsi* (LD) tissue during growing. Particularly, level of FABP4 mRNA expression was positively correlated with intramuscular fat (IMF) content and body weight in pigs (Cho et al., 2011; Chen et al., 2013). In the case of mice, level of FABP3 protein can significantly increase body weight and other metabolic traits which was suggested that expression of FABP3 was correlated with obesity and insulin resistance (Kusudo et al., 2011). Also, polymorphisms in both FABP3 and FABP4 genes were tightly linked with fat-related traits in chicken (Wang et al., 2006; Li et al., 2008; Wang et al., 2009; Ye et al., 2010). Furthermore, variations within FABP4 gene were associated with slaughter and meat quality traits, especially living weight, carcass weight, breast muscle weight, abdominal fat weight, and abdominal fat percentage (You et al., 2009). Therefore, in this study, we investigated variations in FABP3 and FABP4 genes and their relationships with body weight traits in Korean native chicken.

MATERIALS AND METHODS

1. Chicken Population and DNA Isolation

Two populations used in this experiment were consisted by 15 sires and 73 dams (F_0), and 590 F_1 birds comprised by 110, 90, 125, 131, and 134 offspring from gray, black, white, yellow-brown, and red-brown lines of KNC, respectively. The five lines of KNC were distinguished on the basis of feather colors (Hoque et al., 2012). These animals were reared under the standard breeding procedures in National Institute of Animal Sciences (NIAS), Korea. The data including body weight at birth (BBW), body weight at 2 to 20 weeks of age, and slaughter weight (SW) were recorded every two weeks. In addition, their blood samples were collected from wing veins by using vacutainer tubes containing EDTA. These blood samples were used to extract DNA based on Miller et al. (1988) methods. The isolated DNA was maintained in refrigerator at −20℃ for further analysis.

2. PCR Condition and Genotyping

Two pairs of primers, one for FABP3 gene and another for FABP4 gene, were designed based on Maharani et al. (2011). The primer and restriction enzyme information are shown in Table 1. Polymerase chain reaction was carried out in 20 μL volume containing 50 ng per μL DNA genome, primers, 10 mM dNTP, 10× reaction buffer, HS Taq Polymerase (GenetBio, Korea), and distilled water. The PCR conditions were as follows: 94℃ for 10 minutes for pre-denaturation, 35 cycles of 94℃ for 30 seconds, 58℃ for 30 seconds, and 72℃ for 30 seconds, and followed by 10 minutes of final extension at 72℃. Reaction was performed using either GeneAmp PCR system 2700 (Applied Biosystems, USA) or C1000^{TM} Thermal Cycler (BioRad, USA). The PCR products were visualized by 2% standard agarose gels stained with ethidium bromide (GenetBio, Korea). For genotyping, PCR restriction fragment length polymorphism (RFLP) was applied. Approximately, 15 μL of PCR product was digested with 2 units of each restriction enzyme (Table 1) based on the protocol recommended by company (Biolabs® Inc., New England). After that, the digested PCR product was separated on 3% agarose gels to identify genotype variations.

3. Statistical Analysis

Genotype and allele frequencies were measured, and Pearson's Chi-square test was applied to verify the Hardy-Wein-

Table 1. Primers for PCR amplification and SNP identification in FABP3 and FABP4 genes

Gene/SNP	GenBank accession No.	Sequence (5' to 3')	PCR product size (bp)	Annealing temperature (℃)	Restriction enzyme
FABP3/ g.508C>T	NC_006110	F: ggtgatgcatgaggacattg R: actaccgccttgctcacact	460	58	*Nla*III
FABP4/ g.285C>T	NC_006089	F: tgtgacctactggcaaagga R: ttcctcccagtcaagctttc	477	58	*Taq*I

berg equilibrium status. The effects of FABP genes variations on body weight traits were analyzed by using general linear model (GLM) procedure in the MINITAB version 14.0 software (Minitab Inc., USA). The following model was used for association analysis between the genotype and body weight:

$$Y_{ijklmno} = \mu + G_i + S_j + B_k + L_l + F_{m(l)} + M_{n(lm)} + \varepsilon_{ijklmno}$$

Where, $Y_{ijklmno}$ is the phenotype of the o^{th} animal, μ is population mean, G_i is the fixed effect of genotype, S_j is the fixed effect of sex, B_k is the fixed effect of batch, L_l is the fixed effect of line, $F_{m(l)}$ is the random effect of the m^{th} sire nested within the l^{th} line, $M_{n(lm)}$ is the random effect of the n^{th} dam nested within the l^{th} line and m^{th} sire, and $\varepsilon_{ijklmno}$ is the residual error associated with the o^{th} animal. In order to test the pairwise differences between the genotypes, Tukey's test was also performed.

RESULTS

1. Genotype and Allele Frequencies

Three genotypes were identified in both FABP3 and FABP4 genes. In case of g.508C>T SNP in FABP3 gene, four DNA fragments, namely 201 bp, 176 bp, 72 bp and 11 bp, were found in animals having CC genotypes, whereas five DNA fragments, 201 bp, 140 bp, 72 bp, 36 bp and 11 bp, were found in animals having TT genotypes (Fig. 1A). On the other hand, the g.285C>T SNP in FABP4 gene was clearly distinguished using 3% agarose gels. The 477 bp of PCR product was separated into 242 bp and 235 bp fragments for animals having CC genotypes (Fig. 1B). It should be noted that DNA fragments under 50 bp could not be identified in agarose gel with bare eyes.

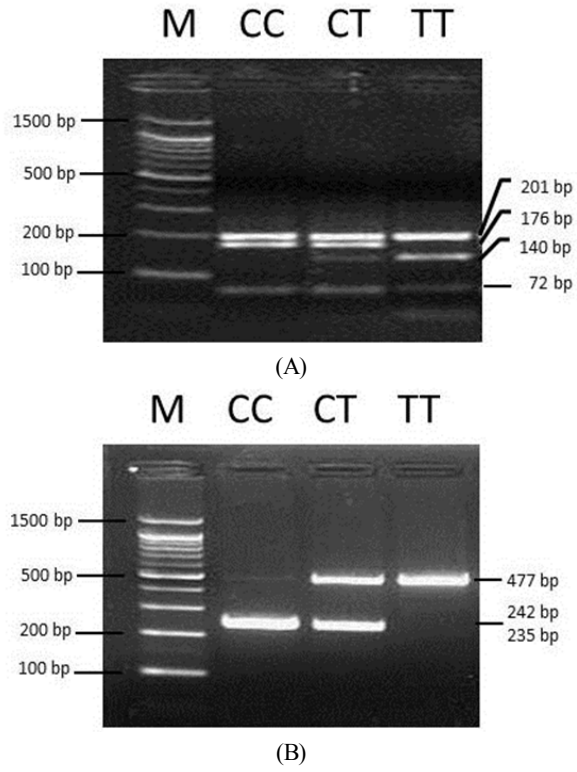

Fig. 1. Genotypes of g.508C>T SNP of FABP3 gene (A) and g.285C>T SNP of FABP4 gene (B) visualized by 3% agarose gel electrophoresis

Table 2. Genotype and allele frequencies of g.508C>T SNP of FABP3 gene and g.285C>T SNP of FABP4 gene in Korean native chicken

Generation	Genotype frequency (Number of animal)			Allele frequency		χ^2 (*P*-value)
	g.508C>T					
	CC	CT	TT	C	T	
Parents	0.49 (43)	0.40 (35)	0.11 (10)	0.69	0.31	0.49 (0.484)
F_1	0.52 (309)	0.41 (242)	0.07 (39)	0.73	0.27	0.84 (0.359)
	g.285C>T					χ^2 (*P*-value)
	CC	CT	TT	C	T	
Parents	0.11 (10)	0.38 (33)	0.51 (45)	0.30	0.70	1.05 (0.306)
F_1	0.10 (61)	0.33 (197)	0.56 (332)	0.27	0.73	13.93 (0.0002)

Either parent or F_1 population was in Hardy-Weinberg equilibrium (HWE) for g.508C>T SNP. Different HWE status was identified for the g.285C>T SNP in FABP4 gene, whereas parent population was in HWE. On the other hand, F_1 population was not in HWE (Table 2). It may be due to selective pressure for desirable phenotypic traits, and inbreeding in Korean native chicken (Falconer and Mackay, 1996; Heo et al., 2011).

2. Association of SNPs in FABP Genes with Body Weight Traits

Twelve body weight-related traits, including body weight at birth, body weights at 2 to 20 weeks of age, and slaughter weight, were used for association analysis. Both sex and line effects were tested in this study. The results showed that either sex or chicken line was affecting body weights in Korean native chicken. Body weights in red-brown and black lines were significantly higher than other lines in all ages. In addition, both dam and sire were strongly affecting body weight in KNC (data not shown).

The allele substitution of g.508C>T in intron 2 region of FABP3 gene was associated with body weights (Table 3). Particularly, this SNP was associated with body weight at birth, body weight at 12 to 20 weeks of age, and slaughter weight. In addition, it was also suggestively correlated with body weights at 4 to 10 weeks of age (P<0.15). Chickens having TT genotypes were higher in body weight at 2 weeks of age. On the other hand, chickens with CC genotypes were higher in body weights at 12 to 20 weeks of age and slaughter weight. In addition, these results revealed that chickens having C alleles were consistently having higher body weights compare to chickens having T alleles in growing to finishing periods. In contrast, g.285C>T SNP in FABP4 gene was not associated with any body weight traits.

DISCUSSION

Previous reports suggested that family of FABP gene members were tightly linked with fatness traits, especially intra-

Table 3. Association between g.508C>T SNP of FABP3 gene and g.285C>T SNP of FABP4 gene with body weight traits in Korean native chicken

Traits	g.508C>T SNP			*P*-value	g.285C>T SNP			*P*-value
	CC (309)	CT (242)	TT (39)		CC (61)	CT (197)	TT (332)	
BBW (g)	38.45±0.20[a]	38.62±0.22[a]	39.94±0.49[b]	0.019	38.64±0.40	38.79±0.23	38.51±0.20	0.631
BW2 (g)	147.91±1.92	141.92±2.06	139.46±4.63	0.057	140.30±3.73	145.15±2.20	145.53±1.89	0.401
BW4 (g)	277.75±5.44	262.16±5.86	254.65±13.16	0.084	253.14±10.58	268.86±6.24	273.34±5.36	0.237
BW6 (g)	444.35±10.16	420.00±10.93	395.91±24.58	0.105	400.94±19.75	433.43±11.64	435.13±10.00	0.237
BW8 (g)	631.99±15.54	595.77±16.72	567.80±37.59	0.150	583.17±30.21	622.85±17.80	611.98±15.30	0.403
BW10 (g)	787.30±16.95	752.57±18.24	706.09±41.00	0.134	737.32±32.94	784.23±19.41	762.94±16.68	0.293
BW12 (g)	1,028.10±20.11[a]	970.62±21.63[ab]	915.02±48.64[b]	0.042	947.60±39.19	1,002.50±23.09	1,002.32±19.85	0.362
BW14 (g)	1,215.11±19.69[a]	1,154.82±21.18[ab]	1,071.70±47.62[b]	0.010	1,141.61±38.49	1,189.89±22.68	1,182.18±19.49	0.451
BW16 (g)	1,407.95±21.51[a]	1,356.13±23.13[ab]	1,269.92±52.01[b]	0.035	1,334.49±41.94	1,386.13±24.71	1,379.89±21.24	0.465
BW18 (g)	1,602.94±18.63[a]	1,566.15±20.03 [ab]	1,465.46±45.04[b]	0.018	1,541.91±36.34	1,593.05±21.41	1,576.63±18.40	0.342
BW20 (g)	1,796.83±19.22[a]	1,756.62±20.67[ab]	1,673.46±46.48[b]	0.042	1,743.14±37.46	1,788.49±22.07	1,767.32±18.97	0.407
SW (g)	1,707.16±19.09[a]	1,665.54±20.53[ab]	1,574.69±46.15[b]	0.026	1,658.95±37.24	1,696.14±21.95	1,676.16±18.86	0.517

Values in table represent Least Squares Mean (LSM) ± SE.
Abbreviation: BBW: birth body weight; BW2, BW4, BW6, BW8, BW10, BW12, BW14, BW16, BW18, BW20: body weight at 2 to 20 weeks of age; SW: slaughter weight.
[a,ab,b] Subscript in the same row showed significant effects between subject (P<0.05).

muscular fat content in chicken (Wang et al., 2007; Wang et al., 2009; Li et al., 2010; Ye et al., 2010). Among the FABP gene member, adipose fatty acids binding protein (FABP4) and heart-muscle fatty acid binding protein (FABP3) genes were the potential candidate genes for fat-related traits. In human, FABP4 gene has been reported to have the relationships with obesity and metabolic syndrome. Decreasing expression of FABP4 gene has been significantly demoting both body weight status and fat mass index in obese adult and children (Reinehr et al., 2007; Engl et al., 2008; Corripio et al., 2010; Khalyfa et al., 2010). Furthermore, FABP4 genes have also been affecting body weight and IMF in pig, whereas high level of FABP3 expression was linked with obesity in mice and human (Cho et al., 2011; Kusudo et al., 2011; Chen et al., 2013). Therefore, we hypothesized that polymorphisms of both FABP3 and FABP4 genes may be associated with body weight traits in Korean native chicken.

Body weight is one of growth-related traits which have achieved great progress in the broiler breeding program recently (Ikeobi et al., 2004; Bai et al., 2012). These body weight traits directly affect economic values of poultry products (Nassar et al., 2012). In present study, two single polymorphisms (SNPs), an SNP in intron region of FABP3 gene and an SNP in exon 1 region of FABP4 gene, were tested as potential candidate markers for body weight in Korean native chicken. As the results, an SNP in FABP3 gene was significantly associated with body weight at 2 weeks, and at the end stage of age in KNC, while a SNP in FABP4 was not associated with any body weight traits. In conclusion, g.508C>T SNP in FABP3 gene have significant effects with body weight traits, and it can be used as molecular markers to select desired body weights in KNC.

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