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Full Length Research Paper

Polymorphisms of two neuroendocrine–correlated genes associated with body weight and reproductive traits in Jinghai yellow chicken

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In this study, insulin-like growth factor binding protein 2 (IGFBP-2) and signal transducers activators of transcription 5b (STAT5b) gene were studied as candidate gene associated with body weight and reproductive traits of the Jinghai Yellow chicken. Single nucleotide polymorphisms (SNPs) of the IGFBP-2 and STAT5b genes were examined in both Jinghai Yellow chicken and three reference chicken populations by PCR-SSCP. Two SNPs (T3746TG and CC3753TT) were detected in the IGFBP-2 gene. One SNP (C8066T) was observed in the STAT5b gene. For primer 1, the general linear model analysis showed that Jinghai yellow chickens with FF genotypes had significant effect on hatch weight, egg weight at 300 days and body weight at 300 days than those of the EF genotype and had significant effect on body weight at 8 weeks of age than those of the EE genotype ($P < 0.05$). For primer 2, Jinghai yellow chickens with CT genotype had significant effect on hatch weight and age at first egg than CC genotype and TT genotype respectively ($P < 0.05$). SNPs in both IGFBP-2 and STAT5b genes had significant effect on body weight and reproductive traits of the Jinghai yellow chicken than those with either SNP alone. These SNPs may be served as a potential genetic marker for growth and reproduction traits evaluation of the Jinghai yellow chicken.

Key words: Jinghai Yellow chicken, IGFBP-2 gene, STAT5b gene, economic traits, polymorphism.

INTRODUCTION

Traditional breeding technique in chicken has made great improvement in many economically important traits. Based on the pedigree information, traditional breeding is laborious and time-consuming. With the development of genetics and genomics, many new molecular strategies were adopted in chicken breeding. The candidate gene approach is a powerful method for finding the QTL responsible for genetic variation in traits of interest in agricultural animal species (Lamont et al., 1996; Bai et al., 2006). Similar to other economic traits; body weight and reproductive traits are under complex genetic control. Elucidation of genes and their network involved in body weight and reproductive traits determinant is key in chicken genetics and breeding. The IGFBP-2 is an important member of IGFBPs family which has many biological functions. The IGFBP2 is capable of controlling the biological actions of IGFs (Hoeflich et al., 1999) and TGF-ß (Rajaram et al., 1997) *in vivo* via endocrine, autocrine, or paracrine mechanisms and further affects the growth and development of animals. The IGFBP-2 gene has a total length of 32 kb and is composed of four exons; 2.0 kb (rat) and 1.6 kb (human) mRNAs are generated and the mature protein is around 31 kDa in rat and 36 kDa in human (Shimasaki and Ling, 1991). The chicken IGFBP-2 gene spanned approximately 38 kb consisted of four exons and was similarly organized to that of the rat and human (Schoen et al., 1995).

Signal transducers and activators of transcription (STAT) consisting of 7 members are a family of cytoplasmic

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proteins that are involved in the signal transduction pathways of numerous cytokines, growth factors and hormones (Darnell, 1997; Levy and Darnell, 2002). The STAT protein contains five conserved domains. That is DNA-binding domain, SH3-like domain, SH2 domain, ubiquitous tyrosine and C-terminal transactivating domain (Ihle, 2001). STAT5 was firstly identified from sheep mammary gland and initially was known as the mammary gland factor (Wakao et al., 1994). STAT5 exists in two isoforms -A and B which differ from a few amino acids in the carboxylic end of the protein molecule; the STAT5b is an important modulator of the growth hormone, growth hormone receptor, IGF, prolactin and insulin signaling pathways which are involved in growth, reproduction, lactation and metabolism (Bachelot and Binart, 2007; Pilecka et al., 2007; Hennighausen and Robinson, 2008). So, the STAT5b can regulate the growth and lactation performance of animal by these factors. The Jinghai yellow chicken is a national cultivated meat minitype breed which is characterized by the adaptability to poor quality feeds and environment. By contrast, the Bian chicken and the Youxi chicken are local breeds raised for dual-purpose. The Arbor Acre chicken (a commercial broiler) which is well known for rapid growth. The objectives of this study were to identify the SNPs of the IGFBP-2 gene and STAT5b genes in the Jinghai yellow chicken and three reference chicken populations (the Arbor Acre, Youxi and Bian chickens) by the PCR-SSCP technique.

Associations between the polymorphisms and body weight and reproductive traits of the Jinghai yellow chicken were also evaluated in this study. Results presented in this study will provide molecular tools for chicken's body weight and reproductive traits selection.

MATERIALS AND METHODS

Chicken populations

Blood samples were collected from 290 chickens belonging to 4 chicken populations: Jinghai yellow chicken (JY) (200), the Arbor Acre chicken (AA) (30), Youxi chicken (YX) (30) and Bian chicken (B) (30). Female Jinghai yellow chicken's blood samples and Arbor Acre chicken's blood samples were collected at the age of 16 weeks at the Jiangsu Jinghai Poultry Industry Group Co., Ltd. The body weight of each female Jinghai yellow chickens in grams hatch at 4, 8, 12, 16 weeks of age and seven reproductive traits (egg weight at first egg, BW at first egg, age at first egg, number of eggs at 300 days, egg weight at 300 days and BW at 300 days) were measured. These birds were hatched on the same day and reared in the pens. Birds had access to feed [commercial corn-soybean diets meeting the National Research Council's (NRC) requirements] and water *ad libitum*. Bian chicken's blood samples were collected at the age of 18 weeks at the Institute of Animal Husbandry and Veterinary of Shanxi Academy of Agricultural Sciences. Youxi chicken's blood samples were sampled at the age of 16 weeks at the National Gene Bank for Local Chickens in Poultry Institute, Chinese Academy of Agricultural Sciences.

Genomic DNA was extracted from the whole blood using phenol-chloroform method and stored at -20℃ (Sambrook et al., 1989). The DNA concentrations were quantified spectrophotometrically.

Primers design and PCR amplification

Based on chicken IGFBP-2 gene sequences (GenBank accession no. NC_006094) and STAT5b gene sequences (GenBank accession no. NC_ 006114), two pairs of primers were designed using the Primer Premier 5.0 software to amplify the IGFBP-2 gene and STAT5b genes. The primer 1 (P1: F: 5′- GTGAGGGTGAT CAAATCCCTA-3′; R: 5′- AAGCAGGACCACATCCATCT -3′) was designed to amplify 250 bp fragment of the IGFBP-2 gene parts of intron 3 and exon 4; the primer 2 (P2: F: 5′-GGAGATCATCTG GCAGAACC-3′; R: 5′- AGTCAGGCAGGCAAAGGAG -3′) was designed to amplify 197 bp fragment of the STAT5b gene intron 7. PCR reactions were carried out in 20 µL mixture containing 1 µL chicken genomic DNA (50 ng/µL); 1 µL of each forward and reverse primer (10 µmol/L); 2 µL 10 × buffer (500 mm/L KCL, 100 mmol/L Tris-Cl); 2.2 µL Mg^{2+} (25 mmol/L); 1 U Taq DNA polymerase (Sangon Biological Engineering Technology Company, Shanghai, China); 2 µL dNTPs (2 mmol/L); 11.8 µL sterilized water. The amplification conditions were: initial denaturation at 94℃ for 6 min followed by 30 cycles of denaturation at 94℃ for 30 s, 30 s at annealing temperature of 60℃ and extension at 72℃ for 30 s, and at last 10 min at 72℃. PCR products were verified by electrophoresis on 1% and gels were stained with 'gold view'.

Single-strand conformation polymorphism (SSCP) and sequencing

For SSCP analysis, 2 µL of each amplification product was mixed with 7 µL denaturing buffer (98% formamide, 0.025% xylene cyanole FF, 0.025% bromophenol blue, 10 mmol/L EDTA (pH 8.0) and 10% glycerol) heated for 10 min at 98℃ and then cooled on ice for 5 min. Denatured PCR products were subjected to 10% non-denaturing polyacrylamide gels (29:1) at 150 V for 11 to 13 h at 16℃. SSCP patterns on the gels were visualized by silver staining (Xu et al., 2002). PCR products of homozygous/heterozygous individuals of different genotypes were purified with DNA fragment quick purification/recover kit. The purified PCR products were sequenced in both directions.

Statistical analysis

Hardy-Weinberg equilibrium was tested for each SNP using a chi-square test. The general linear model (GLM) was established to analyze the genotype effects of the IGFBP-2 gene and STAT5b gene on body weight and reproductive traits. The following fixed model was used:

$$y_{ij} = \mu + G_1 + G_2 + G_1G_2 + e$$

Where, y_{ij} represented the body weight traits or reproductive traits; μ is the overall mean; G_1 is the fixed effect for IGFBP-2 genotypes; G_2 is the fixed effect for STAT5b genotypes; G_1G_2 are the fixed interaction effect for IGFBP-2 and STAT5b combined genotypes and e is the residual error. These statistical analyses were carried out using the SPSS 11.0 software.

RESULTS

Detection of the mutation of the IGFBP-2 gene and STAT5b gene

Amplicons with expected size were obtained from chicken DNA using different primers. For the IGFBP-2 gene, three

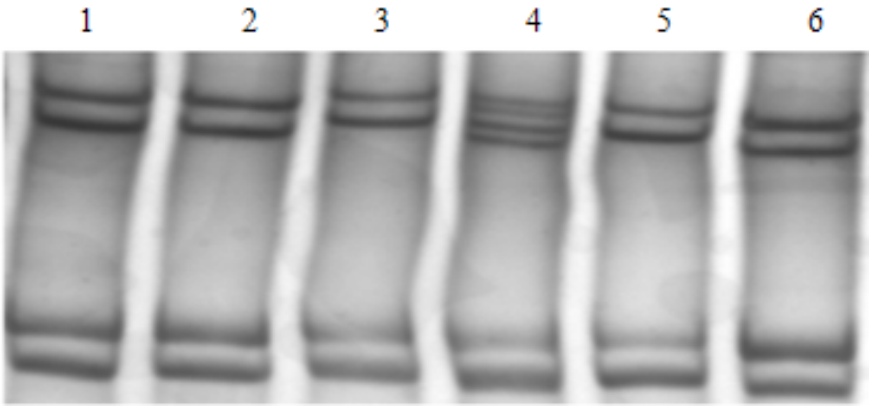

Figure 1. SSCP analysis of PCR amplification of IGFBP-2 gene in chicken. Lanes 1, 2, 6: EE genotype; lanes 3, 5: FF genotype; lane 4: EF genotype.

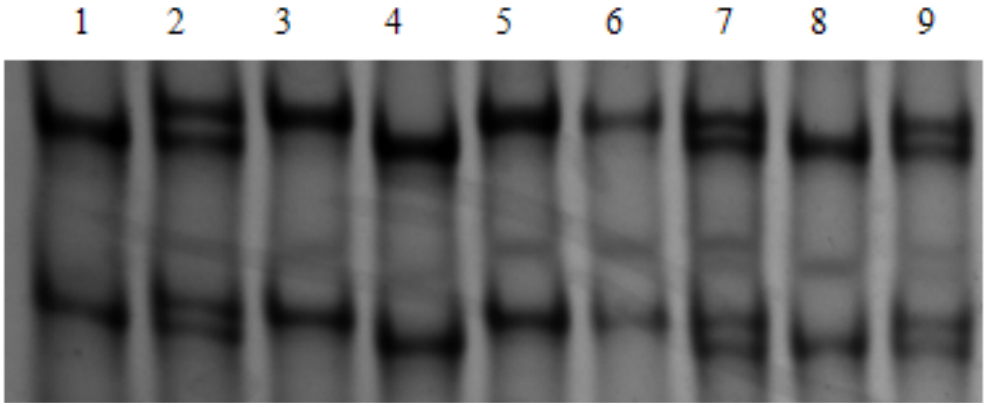

Figure 2. SSCP analysis of PCR amplification of STAT5b gene in chicken, Lanes 1, 4, 8: CC genotype; lanes 2, 7, 9: TT genotype; lanes 3, 5, 6: CT genotype.

genotypes (EE, EF and FF) were observed in the Jinghai yellow and Arbor Acre chicken, the Bian chicken displayed two genotypes (EE and EF), only one genotype (EE) was observed in the Youxi chicken (Figure 1). Forward and reverse sequencing detected two nucleotide mutations (T3746G and CC3753TT) in the intron 3 of the IGFBP-2 gene (Figure 2). For the STAT5b gene, three genotypes were identified in the 4 chicken breeds named CC, CT and TT respectively. A single C/T SNP was found at position 8066 in intron 7 of the STAT5b gene.

Allelic and genotypic frequencies in four chicken breeds

Genotypic and allelic frequencies of the IGFBP-2 and STAT5b gene are presented in Table 1. For the IGFBP-2 gene, allele E was the dominant allele in the four chicken breeds with frequencies of 0.54, 0.80, 1.00 and 0.90 respectively. The result of chi-square test showed that the Jinghai yellow and Arbor Acre chickens were not in Hardy-Weinberg equilibrium ($P < 0.05$). For the STAT5b gene, allele C was the dominant allele in the Jinghai yellow, Arbor Acre and Bian chickens with frequencies of 0.71, 0.82 and 0.77 respectively, while allele T was dominant allele in the Youxi chicken. In the four chicken populations, genotypes frequencies are in accordance with Hardy-Weinberg equilibrium except for the Jinghai yellow chicken ($P>0.05$). Combined genotypic frequencies of the mutations of two genes are presented in Table 2. Combined genotype EECC and FFCC had the highest frequency (0.25), whereas combined genotype EFTT and FFTT had the lowest frequency (0.03) in the Jinghai yellow chicken.

Effects of the IGFBP-2 and STAT5b genes on body weight and reproductive traits

The least squares means and SE for body weight and reproductive traits of different genotypes in the Jinghai yellow chicken are given in Tables 3 to 6. The chickens

Table 1. Allelic and genotypic frequencies of IGFBP-2 and STAT5b gene in chicken breeds.

Breed	Allele frequency		Genotype frequency			χ^2	Allele frequency		Genotype frequency			χ^2
	E	F	EE	EF	FF		C	T	CC	CT	TT	
JY	0.54	0.46	0.48	0.12	0.40	115.06	0.72	0.28	0.54	0.35	0.11	3.99
AA	0.80	0.20	0.73	0.13	0.14	10.21	0.82	0.18	0.67	0.30	0.03	0.00
YX	1.00	0.00	1.00	0.00	0.00	NULL	0.45	0.55	0.20	0.50	0.30	0.00
B	0.90	0.10	0.80	0.20	0.00	0.37	0.77	0.23	0.57	0.40	0.03	0.42

Table 2. Combined genotypic frequencies of the of IGFBP-2 and STAT5b gene in Jinghai yellow chicken.

Parameter	Combined genotype								
	EECC	EECT	EETT	EFCC	EFCT	EFTT	FFCC	FFCT	FFTT
Genotypic frequency	0.25	0.18	0.06	0.05	0.06	0.03	0.25	0.12	0.03

with FF genotypes had significant effect on hatch weight, egg weight at 300 days and body weight at 300 days than those of the EF genotype ($P < 0.05$). However, for body weight at 8 weeks of age, the birds with FF genotypes had significant higher weight than those of the EE genotype ($P < 0.05$). Jinghai yellow chickens with CT genotype had significant effect on hatch weight than CC genotype and had significant effect on age at first egg than TT genotype ($P < 0.05$). There were significant differences between combined genotypes and hatch weight, body weight at 4, 8 weeks of age, egg weight at 300 days, body weight at 300 days, egg number at 300 days and age at first egg ($P < 0.05$).

DISCUSSION

Body weight and reproductive traits are under complex genetic control and uncovering the molecular mechanisms of these traits will make a progress in selection of broiler chickens. Studies of the IGFBP-2 biological function showed that reduced growth of mice selected for low body weight was associated with increased hepatic IGFBP-2 mRNA expression and elevated serum IGFBP-2 levels (Hoeflich et al., 1999). Wang et al. (2008) found three SNP (C502T, A603G and T1218G) in the IGFBP-2 gene in pigs, further suggesting that IGFBP-2 was associated with production performance of swine. In chickens, Leng et al. (2009) detected one SNP (C1996A) of the IGFBP-2 gene was associated with abdominal fat weight and percentage of abdominal fat. Li et al. (2006) detected a C/T SNP of the IGFBP-2 gene which had effect on BW, metatarsus length, shank length, femur length, shank weight, femur weight, metatarsus claw weight and abdominal fat weight of chicken. DeKoning et al. (2003) reported that a QTL for carcass weight was mapped between marker brackets MCW0030 and MCW0236 (about 2.3 to 29 Mb) on GGA7, a region that contains chicken IGFBP2 gene (23 to 24 Mb). In our reaearch, two mutations (T3746G and CC3753TT) of the IGFBP-2 gene were observed. The result of chi-square test showed that the Jinghai yellow and Arbor Acre chickens were not in Hardy-Weinberg equilibrium ($P < 0.05$). The plausible reason for this may be that genetic drift, selection and other factors may affect the allele frequency. These SNPs had significant effect on hatch weight, body weight at 8 weeks of age, egg weight at 300 days and body weight at 300 days which have difference with the aforementioned results (Leng et al., 2009; Li et al., 2006). STAT5b is the important modulator of the growth hormone, growth hormone receptor, IGF, prolactin and insulin signaling pathways.

Many studies showed that these genes play an important role in growth and reproductive traits in chicken. Gouda and Essawy, (2010) analyzed the polymorphism of IGF- I gene among Egypt chicken breeds and indicated their effect on the growth traits of chicken was significant. Researchers detected the 24 bp insertion/deletion in 5' flanking region of PRL gene and found this mutation had effect on egg production and broody traits (Jiang et al., 2005; Cui et al., 2006). These results laid foundation for our research, effect of STAT5b gene on body weight and reproductive traits in the Jinghai yellow chicken. In this study, the SNP (C8066T) of the STAT5b gene was novel. We do not find this SNP in other animals. The Jinghai yellow chickens with CT genotype had significant effect on hatch weight and age at first egg than other genotypes ($P < 0.05$). These results have difference with previous studies by domestic and foreign researchers. Ballester et al. (2006) who sequenced one fragment of STAT5b gene from animals of six breeds (Duroc, Iberian, landrace, large white, Piétrain and Meishan) and detected a G/A single nucleotide polymorphism in intron 14 creates a polymorphic PstI restriction site. He et al. (2011) reported a g. 13598C >T SNP in the STAT5b gene of Chinese Holsteins. Based on a modified PCR-RFLP method, Ou et

Table 3. Least squares means and SE for reproductive traits of different IGFBP-2 and STAT5b genotypes in chicken.

Primer	Genotype	Body weight at first egg	Egg weight at the first egg	Age at the first egg	300-day-egg-weight	300-day-egg number	Body weight at 300 day
Primer 1	EE	32.78±0.59	1570.44±17.49	140.43±0.99	50.33±0.41^{a}	106.51±1.25	1789.92±30.10^{ab}
	EF	31.58±1.17	1532.00±34.98	140.84±1.98	48.67±0.83^{b}	111.42±2.50	1714.92±60.19^{b}
	FF	32.60±0.64	1592.84±19.16	140.46±1.09	50.89±0.45^{a}	107.79±1.37	1827.64±32.97^{a}
Primer 2	CC	32.88±0.55	1584.83±16.53	140.28±0.93^{ab}	50.36±0.40	107.43±1.19	1839.13±25.65
	CT	31.73±0.68	1554.90±20.53	139.60±1.15^{b}	50.31±0.49	107.41±1.47	1770.61±31.86
	TT	33.68±1.22	1588.73±36.63	144.36±2.05^{a}	50.46±0.88	109.14±2.63	1752.68±56.83

$^{a, b}$ Means within a row and loci with no common superscript differ significantly ($P < 0.05$).

Table 4. Least squares means and SE for body weight traits of different IGFBP-2 and STAT5b genotypes in chicken.

Primer	Genotype	Hatch weight	4 week-age-weight	8 week-age-weight	12 week-age-weight	16 week-age-weight
Primer 1	EE	36.20±0.35^{A}	181.91±3.01	448.97±8.81^{b}	846.88±13.56	1147.41±11.58
	EF	33.11±0.72^{B}	187.72±6.14	448.17±17.98^{ab}	846.83±27.67	1163.39±23.64
	FF	35.38±0.42^{A}	189.66±3.58	477.60±10.48^{a}	858.98±16.12	1157.40±13.78
Primer 2	CC	35.03±0.35^{b}	187.32±2.94	460.58±8.72	857.34±13.17	1155.17±11.30
	CT	36.18±0.45^{a}	181.06±3.69	456.16±10.96	836.80±16.55	1148.16±14.20
	TT	35.88±0.76^{ab}	189.59±6.33	462.24±18.79	865.59±28.38	1155.82±24.35

$^{a, b}$ Means within a row and loci with no common superscript differ significantly ($P < 0.05$). $^{A, b}$means within a row and loci with no common superscript differ significantly ($P < 0.01$).

Table 5. Least squares means and SE for reproductive traits of different combined genotypes in chicken.

Trait	Combined genotype								
	EECC	EECT	EETT	EFCC	EFCT	EFTT	FFCC	FFCT	FFTT
Body weight at first egg	1574.92±24.48	1556.83±29.26	1593.36±52.20	1568.56±57.71	1535.46±52.20	1498.00±77.42	1597.94±24.73	1561.00±35.34	1655.83±70.68
Egg weight at the first egg	32.70±0.82	32.57±0.98	33.82±1.74	31.22±1.92	30.82±1.74	34.20±2.58	33.67±0.82	30.92±1.18	33.00±2.36
Age at the first egg	140.32±1.38^{ab}	138.89±1.65^{b}	145.82±2.93^{a}	140.00±3.24^{ab}	141.64±2.93^{ab}	141.40±4.35^{ab}	140.29±1.39^{ab}	139.71±1.99^{ab}	144.17±3.97^{ab}
300-day-egg-weight	50.16±0.58^{ab}	50.31±0.69^{ab}	51.18±1.23^{ab}	50.22±1.36^{ab}	48.46±1.23^{ab}	47.00±1.83^{b}	50.59±0.58^{ab}	51.17±0..83^{a}	52.00±1.67^{a}
300-day-egg number	107.02±1.73^{b}	106.91±2.07^{b}	102.91±3.70^{b}	106.78±4.08^{ab}	111.73±3.70^{ab}	119.20±5.48^{a}	107.96±1.75^{ab}	106.17±2.50^{b}	112.17±5.00^{ab}
Body weight at 300 days	1797.26±37.66^{ab}	1799.03±45.01^{ab}	1727.55±80.28^{ab}	1797.11±88.75^{ab}	1693.18±80.28^{b}	1663.80±119.08^{ab}	1889.57±38.04^{a}	1764.67±54.35^{ab}	1872.83±108.70^{ab}

$^{a, b}$ Means within a line with no common superscript differ significantly ($P < 0.05$).

al. (2009) detected SNPs (C-1591T, G-250A and G-110C) of the 5' upstream region of STAT5b gene in the Beijing You chicken and found SNP of the STAT5b gene were associated with age at first egg and body weight at 8 weeks of age. The effect of combined genotype was not the simple addition of single genotype.

In this study, mutations in both IGFBP-2 and STAT5b genes had significant effect on body weight and reproductive traits of the Jinghai Yellow chicken than those with either mutation alone, so the IGFBP-2 and STAT5b gene had interactions in the signaling pathways and gene networks of growth and reproduction. To confirm the afore-mentioned results, our following research is to study the functions of two genes in the level of RNA or protein.

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