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Single Nucleotide Polymorphisms in IGFBP-2 Gene and Their Associations with Body Weight Traits on Jinghai Yellow Chicken

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ABSTRACT

Insulin-like growth factor binding protein-2 (IGFBP-2) regulates a broad spectrum of biological activities involved in growth, development, and differentiation. This study aimed at comparing polymorphisms in intron2 of the IGFBP-2 gene among four chicken breeds and at analyzing the associations between its genotypes and body weight in Jinghai Yellow chicken by using PCR-SSCP technique. For primer P2, three genotypes (AA, AB and BB) were observed in the four chicken breeds. Gene sequencing revealed one insertion/deletion (the inserted/deleted TC after position 552bp) in the intron 2 of IGFBP-2 gene. For primer P5, three genotypes were identified in Jinghai Yellow chickens, and named CC, CD and DD. Gene sequencing revealed two SNPs (C1107G, C1130T) and one inserted/deleted GCCAGGT after 1115bp in the intron 2 of IGFBP-2 gene. The results of the linear model analysis showed that Jinghai Yellow chickens with AA genotype had significantly heavier body weight, at hatch and 12 weeks of age, than those of the AB genotype ($p<0.05$). The A allele had a positive effect on body weight. We speculate that mutations in intron 2 could be used as genetic markers for body weight in Jinghai Yellow chicken. This study provides valuable information for the protection of genetic resources and for breeding of Jinghai Yellow chicken.

INTRODUCTION

Traditional genetic selection techniques have resulted in significant improvements in many economically important traits in chickens. Based on pedigree information, traditional selection is laborious and time-consuming. With the development of genetics and genomics, many new molecular strategies were adopted in chicken genetic selection. The candidate gene approach is a powerful method for finding the QTL responsible for genetic variation of the traits of interest in livestock species (Lamont *et al*., 1996; Bai *et al*., 2006). Similar to other economic traits, body weight is under complex genetic control. The elucidation of genes and their networks involved in body weight determination is key in chicken genetics and breeding.

Insulin-like growth factor- I (IGF-I) and IGF- II are growth-promoting polypeptides essential for normal growth and development (Cohick & Clemmons, 1993). The actions of IGFs are regulated by many factors; IGFBPs regulates growth and development by regulating IGF transport to tissues and IGF bioavailability to IGF receptors at cell membrane level (Silha & Murphy, 2005).

IGFBP-2 is an important member of IGFBPs family, which has many biological functions. IGFBP-2 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 it is composed by four exons, 2.0 kb (rat) and 1.6 kb (human) mRNAs are generated, and the mature protein is approximately 31 kDa in rats and 36 kDa humans (Shimasaki & Ling, 1991). The chicken IGFBP-2 gene spans to approximately 38 kb, consists of four exons, and presents similar organizaton compared with rats and humans (Schoen *et al*., 1995).

In the past, the structure and function of the IGFBP-2 gene were analyzed in detail; however, the association of the IGFBP-2 gene with growth traits has not been clarified in chickens yet. The Jinghai Yellow chicken is a Chinese meat-type breed with small size and is characterized by adaptability to poor quality feeds and harsh environments. Bian and Youxi chickens are Chinese breeds raised for both for meat and egg production. Arbor Acre chickens are well known for rapid growth. The objective of this research was to identify SNPs of the IGFBP-2 gene in Jinghai Yellow chickens and three reference chicken populations (Arbor Acre, Youxi, and Bian chickens) using the PCR-SSCP technique. Associations between polymorphisms and body weight of Jinghai Yellow chickens were also evaluated in this study. The results presented in this study may provide molecular tools for the selection for body weight in chickens.

MATERIALS AND METHODS

Chicken populations

Blood samples were collected from 236 chickens belonging to four chicken populations: Jinghai Yellow chickens (146), Arbor Acre chickens (30), Youxi chickens (30) and Bian chickens (30). Blood samples of female Jinghai Yellow chicken and Arbor Acre chicken were collected at the age of 16 weeks (wk) at the Jiangsu Jinghai Poultry Industry Group Co., Ltd. The body weight of each female Jinghai Yellow chicken was measured at hatching, 4, 8, 12, and 16 weeks of age. These birds hatched on the same day, and were reared in floor pens. Birds had access to feed (commercial corn-soybean diets meeting the requirement of National Research Council's [NRC]) and water *ad libitum*. Bian chicken's blood samples were collected at the age of 18 wk at the Institute of Animal Husbandry and Veterinary of Shanxi Academy of Agricultural Sciences. Youxi chicken's blood samples were collected at the age of 16 wk 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°C. The DNA concentrations were quantified spectrophotometrically.

Primers design and PCR amplification

Based on chicken IGFBP-2 gene sequences (GenBank accession no. NC_006094), five pairs of primers were designed using the Primer Premier 5.0 software and used to amplify the intron 2 of IGFBP-2 gene. The detailed information of the primers is presented in Table 1.

PCR was performed in a 20-μL mixture containing 1 μL chicken genomic DNA (50ng/μL), 1μL of each of forward and reverse primer (10μmol/L), 2 μL 10×buffer, 2.2 μL Mg^{2+} (25mmol/L), 1U Taq DNA Polymerase (Sangon Biological Engineering Technology Company, Shanghai, China), 2 μL dNTPs (2 mmol/L), and 11.8 μL sterilized water. The amplification conditions were: initial denaturation at 94°C for 6 min, followed by 30 cycles of denaturation at 94°C for 30s, 30s at annealing temperature (58-62°C) and extension at 72°C for 30s, and at last 10min of at 72°C. PCR products were verified by electrophoresis on 1% gel. 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 °C and then cooled on ice for 5 min. Denatured PCR products were subjected to 10% non-denaturing polyacrylamide gels

Table 1 – Primer sequences for amplification of the IGFBP-2 gene

Primer name	Upper Primer sequences (5′→3′)	Down Primer sequences (5′→3′)	Product size
P1	F: CACAACCACGAGGACTCAAA	R: GCTTCAGGCCAAAATAAACG	190bp
P2	F: GCGAGGAGGCGTTATTTTC	R: TAAATCCCTCAGGCCATCC	178bp
P3	F:AAGTAACTCCCCAGGGATGG	R: TTTCCATCCCCATGACATCT	205bp
P4	F: GATGTCATGGGGATGGAAA	R: GGAGATGAGGCCACAAACA	154bp
P5	F:GGCATGATGGTGCTGCTAC	R: TCTTCACGTGGCAAAGAGC	188bp

(29:1) at 150V for 11 to 13 h at 16°C. SSCP patterns on the gels were visualized by silver staining.

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

The Chi-square test was used to verify the significance of differences in genotypic and allelic frequencies between different breeds and the Hardy-Weinberg equilibrium. The following linear model (GLM) was established to analyze the genotype effects of IGFBP-2 gene on body weight traits.

$$y_{ij} = \mu + G_i + e$$

Where, y_{ij} represented the body weight traits, μ was the overall mean, G_i was the genotypic effect of IGFBP-2 gene, e was the residual error.

These statistical analyses were carried out using the SPSS 17.0 software.

RESULTS

Detection of the mutation in IGFBP-2 gene

Amplicons with expected size were obtained from chicken DNA using different primers. Two (P2 and P5) primers showed polymorphisms among the five pairs of primers. For primer P2, three genotypes (AA, AB and BB) were observed in the four chicken breeds (Figure 1).

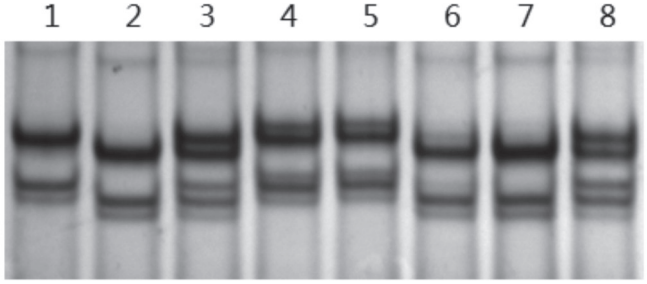

Figure 1 – SSCP analysis of PCR amplification using primers P2 in different chicken breeds 1, 4, 5: BB genotype; 2, 6, 7: AA genotype; 3, 8: AB genotype

Forward and reverse sequencing results revealed that one insertion/deletion (the inserted/deleted TC after position 552bp) in the intron 2 of IGFBP-2 gene. For primer P5, three genotypes were identified in Jinghai Yellow chickens, named CC, CD and DD, respectively (Figure 2).

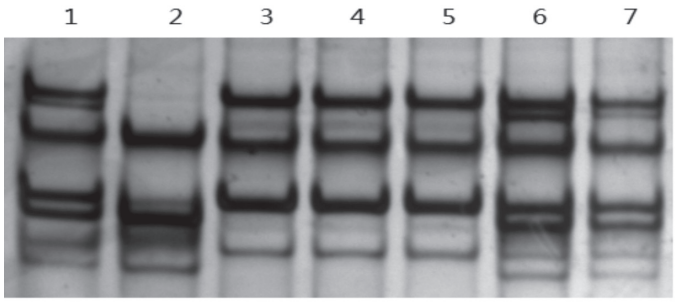

Figure 2 – SSCP analysis of PCR amplification using primers P5 in different chicken breeds 3, 4, 5: CC genotype; 1, 6, 7: CD genotype; 2: DD genotype

The nucleotide sequence obtained from genotype DD was identical to the wild-type CC, except for C1107G, C1130T and one insertion/deletion (the inserted/deleted GCCAGGT after 1115bp) in the intron 2 of IGFBP-2 gene.

Allele variation and genotype distribution of IGFBP-2 gene

Genotype and allele frequencies of IGFBP-2 gene and P-values for the Hardy-Weinberg equilibrium test were presented in Table 2.

For primer P2, allele A was the dominant allele in the Jinghai Yellow and Bian chickens with 0.68 and 0.62 of frequency, respectively. Allele B was the dominant allele in Arbor Acre and Youxi chickens with 0.40 of frequency. The result of Chi-square test showed that the four chicken breeds were in Hardy-Weinberg equilibrium ($p>0.05$). For primer P5, allele C was the dominant allele in Jinghai Yellow, Arbor Acre, Youxi and Bian chickens with frequencies of 0.86, 1.00, 1.00, and 1.00, respectively. The Chi-square test showed that the four chicken breeds were in Hardy-Weinberg equilibrium ($p>0.05$).

Table 2 – Distribution of genotypes and allele frequencies in four chicken breeds

Breeds	No.	P2					χ^2	P5					χ^2
		Allele frequency		Genotype frequency				Allele frequency		Genotype frequency			
		A	B	AA	AB	BB		C	D	CC	CD	DD	
JY	146	0.68	0.32	0.45(66)	0.45(66)	0.10(14)	0.18	0.86	0.14	0.75(109)	0.23(34)	0.02(3)	0.03
AA	30	0.40	0.60	0.23 (7)	0.33(10)	0.44(13)	2.80	1.00	0.00	1.00(30)	0.00(0)	0.00(0)	Null
YX	30	0.40	0.60	0.17(5)	0.47(14)	0.36(11)	0.02	1.00	0.00	1.00 (30)	0.00(0)	0.00(0)	Null
B	30	0.62	0.38	0.30(9)	0.63(19)	0.07(2)	3.46	1.00	0.00	1.00(30)	0.00(0)	0.00(0)	Null

JY= Jinghai Yellow chicken; AA= Arbor Acre chicken; YX= Youxi chicken; B= Bian chicken; df=1, $\chi^2_{0.05\,(1)}$=3.84, $\chi^2_{0.01(1)}$=6.64; Number in the brackets represents number of the genotype in population

Association of SNP with body weight of Jinghai Yellow chicken

The associations between IGFBP-2 gene and body weight in Jinghai Yellow chicken are summarized in Table 3.

Table 3 – Least squares means (LSM) and standard deviation (SD) for body weight traits of different genotypes in P2 locus for IGFBP-2 in Jinghai Yellow chicken [(1)]

Genotype	AA(66)	AB(66)	BB(14)
Hatch weight	36.07±0.84[a]	34.89±0.39[b]	36.03±0.39[ab]
4 week-age-weight	188.35±3.22	183.67±3.22	180.07±6.99
8 week-age-weight	463.65±9.53	456.58±9.53	451.21±20.69
12 week-age-weight	872.06±14.27[a]	832.06±14.27[b]	843.79±30.98[ab]
16 week-age-weight	1168.35±12.24	1138.68±12.24	1148.21±26.58

[(1)]a, b Means within a line with no common superscript differ significantly ($p<0.05$)

For primer P2, chickens of the AA genotype had significantly higher body weight, at hatch and 12 weeks of age, than those of the AB genotype ($p<0.05$). Furthermore, there were no significant differences among different genotypes in body weight at 4, 8, or 16 weeks of age ($p>0.05$). For primer P5, there were no significant differences in body weight among genotypes ($p>0.05$) (Table 4).

Table 4 – Least squares means (LSM) and standard deviation (SD) for body weight traits of different genotypes in P5 locus for IGFBP-2 in Jinghai Yellow chicken

Genotype	CC(109)	CD(34)	DD(3)
Hatch weight	35.33±0.30	35.85±0.54	38.67±1.82
4 week-age-weight	185.99±2.52	183.91±4.51	182.67±15.18
8 week-age-weight	459.90±7.42	458.56±13.29	444.00±44.74
12 week-age-weight	848.98±11.25	859.82±20.14	837.33±67.80
16 week-age-weight	1151.90±9.50	1147.00±17.01	1261.33±57.27

DISCUSSION

Sequence variation of IGFBP-2 gene in animals was previously reported. Wang *et al*. (2008), using the PCR-SSCP technique, found three SNP (C502T, A603G, and T1218G) in the IGFBP-2 gene in pigs. Leng *et al*. (2009) detected one SNP (C1996A) in the 3'-flanking region of the IGFBP-2 gene of the Northeast Agricultural University F_2 resource population chicken. Based on PCR-RFLP and PCR-SSCP methods, Lei *et al*. (2005) detected five SNPs in the chicken IGFBP-2 gene. Li *et al*. (2006) using PCR-RFLP method detected a C/T SNP in intron 2 of IGFBP-2 gene of chickens.

In this study, the PCR-SSCP approach was used for identification of SNP of the IGFBP-2 gene. The frequency distribution of alleles in four chicken breeds indicated significant differences, which implied that the four chicken breeds demonstrated different polymorphisms at these loci of the IGFBP-2 gene. Results of Chi-square test showed that four chicken breeds were in Hardy-Weinberg equilibrium ($p>0.05$). These results indicated that allele frequency was not changed by selection, mutation, or hybridization factors in the evaluated chicken populations.

The Jinghai Yellow chicken is a new meat minitype breed developed from the Chinese yellow chicken, which is characterized by the adaptability to poor quality feeds and harsh environments. The traditional breeding technique used for Jinghai Yellow chickens has made great improvement in many economically important traits. With the development of molecular genetics, researchers tend to improve the Jinghai Yellow chicken at molecular level. Researchers found that many genes had effects on the body weight of Jinghai Yellow chickens (Tao *et al*., 2008; Wei *et al*., 2009; Yu *et al*., 2010; Hou *et al*., 2010).

Leng *et al*. (2009) reported that one SNP (C1996A) of the IGFBP-2 gene was associated with abdominal fat weight and percentage. Li *et al*. (2006) detected a C/T SNP of the IGFBP-2 gene, which was related to BW, metatarsus length, shank length, femur length, shank weight, femur weight, metatarsus claw weight, and abdominal fat weight of chickens. Lei *et al*. (2005) studied the association between five SNP of IGFBP-2 gene and growth traits. Results showed that the difference induced by the haplotypes derived from five SNP was more significant than that by the single SNP in the genotype-phenotype association analysis. The haplotypes were associated with BW at hatch and at 21, 28, 42, 49, 56, and 90 d of age.

In our research, we analyzed IGFBP-2 gene polymorphisms and found two SNPs (C1107G and C1130T) and two insertions/deletions (the inserted/deleted TC after position 552bp and GCCAGGT after 1115bp) in intron 2. The following GLM analysis results showed that chickens with AA genotypes had a significant effect on hatchling weight and body weight at 12 weeks of age compared with the AB genotype ($p<0.05$). 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 the chicken IGFBP-2 gene (23 to 24 Mb); however, the mechanism by which this gene affect carcass weight still requires further study for confirmation.

CONCLUSION

In this study, two SNPs and two insertions/deletions in intron 2 of IGFBP-2 gene were found in four chicken populations (Jinghai Yellow, Youxi, Arbor Acre, and Bian chickens). The identified SNPs influence the function of chicken IGFBP-2 gene and are related with body weight. The findings of the present study not only provide a basis for maker-assisted selection of Jinghai yellow chicken, but also represent a reference for further studies using other chicken breeds.

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