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Polymorphisms in exons of the *IGFBP-1* gene and their associations with body weight in the Jinghai Yellow chicken*

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Thie study aimed at comparing polymorphisms in exons of the *IGFBP-1* gene encoding insulin-like growth factor binding protein-1 among three chicken breeds and analysing the associations between its genotypes and body weight traits in the Jinghai Yellow chicken. Three mutations (A2276T, T2359C and T5550C) and one insertion/deletion (the inserted/deleted AAT after position 5692bp) were detected in three chicken breeds. The general linear model analysis showed that the Jinghai Yellow chickens with BB and AB genotypes at the position 2276 (A2276T transversion) had significantly higher body weight than those of AA genotype (P<0.05 or P<0.01) at week 4, 8, and 16 of age. Moreover, BB genotype had significantly higher body weight at week 12 of age than those of AB genotype (P<0.01). The B allele had a positive effect on body weight traits.

KEY WORDS: Jinghai Yellow chicken / *IGFBP-1* gene / polymorphism / body weight traits

There are two basic methods of QTL identification: the candidate gene approach and the whole-genome linkage-disequilibrium scanning [Ikeobi *et al*. 2002, Kim *et*

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al. 2005]. At present the candidate gene approach is a powerful method for finding the QTL responsible for genetic variation in traits of interest in livestock [Lamont *et al.* 1996, Bai *et al*. 2006]. Several studies have shown that candidate genes have significant effect on chicken growth traits [Cao *et al*. 2007, Zhang *et al*. 2011].

Insulin-like growth factors IGF-I and IGF-II are growth-promoting polypeptides essential for normal growth and development [Cohick and Clemmons 1993]. The action, however, of IGFs is regulated by many factors; IGFBPs (insulin-like growth factor binding proteins) are ones of them affecting growth and development by regulating IGF transport to tissues and IGF bioavailability to IGF receptors at cell membrane level [Silha and Murphy 2005]. IGFBP-1 is an important member of IGFBPs family, which has many biological functions. IGFBP-1 specifically binds and modulates the mitogenic and metabolic actions of IGF-I and IGF-II [Lee *et al*. 1993]. In humans, low level of IGFBP-1 is associated with impaired glucose tolerance, elevated blood pressure, and obesity [Kajantie *et al*. 2003]. The majority of *in vitro* and *in vivo* results support an inhibitory role of IGFBP-1 on IGF-stimulated growth, differentiation, and metabolic activity. There is evidence for an inverse relationship between IGFBP-1 and bioactive-free IGF-I [Nyomba *et al*. 1997]. The *IGFBP-1* gene is 5.2 kb long and comprises four exons containing coding sequences. A single 1.5 kb mRNA is generated, and the mature non-glycosylated protein is -30 kDa [see reviews by Shimasaki and Ling 1991 and Lee *et al*. 1993].

Many studies focused on the structure and function of IGFBP-1. Nevertheless, few research on associations of the *IGFBP-1* gene with body weight traits were reported in chickens. The objectives of the present research were (1) identification of SNPs in exon regions of the *IGFBP-1* gene by PCR-SSCP technique in the Jinghai Yellow chicken and two reference chicken populations (the Youxi and Bian) and (2) analysis of association between the *IGFBP-1* genotypes and body weight traits of the Jinghai Yellow chicken.

Material and methods

Blood sampling and DNA extraction

Blood samples were withdrawn from 206 female chickens belonging to three populations: Jinghai Yellow (n=146), Youxi (n=30) and Bian (n=30). The Jinghai Yellow chicken is a domestic meat minitype breed, characterized by the adaptability to low quality feeds and poor environment. The remaining two are domestic dual-purpose. breeds. Jinghai Yellow chickens' blood s was collected at the age of 16 weeks (wk) at the Jiangsu Jinghai Poultry Industry Group Co., Ltd. The body weight (g) of each Jinghai Yellow female chicken was measured at hatch, and then at 4, 8, 12, and 16 wk of age. These birds were hatched on the same day, reared in pens and had free access to feed (commercial corn-soybean diets meeting the National Research Council's [NRC] requirements) and water. Bian chickens' blood samples were withdrawn at the age of 18 wk at the Institute of Animal Husbandry and Veterinary of Shanxi Academy of Agricultural Sciences. Youxi chickens' blood samples were

collected at the age of 16 wk at the National Gene Bank for Local Chickens in the 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-1* gene sequences (GenBank accession no. NC_006089), eight pairs of primers were designed using the Primer Premier 5.0 software to amplify the exon regions of the *IGFBP-1* gene. The detailed information of the primers is presented in Table 1.

PCR was performed in 20 μL mixture containing 1 μL chicken genomic DNA (50ng/μL); 1 μL of each of forward and reverse primer (10μmol/L); 2 μL 10×buffer; 2.2 μL Mg^{2+} (25 mmol/L); 1U 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°C for 6 min followed by 30 cycles of denaturation at 94°C for 30s, 30s at annealing temperature (58-62°C), extension at 72°C for 30s, and a final elongation step at 72°C for 10 min.

Single-strand conformation polymorphism (SSCP)

For SSCP analysis, 2 μL of each amplification product were 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 (29:1) at 150V for 11 to 13 h at 16°C. SSCP patterns on the gels were visualized by silver staining.

DNA sequencing analysis

PCR products of homozygous and heterozygous individuals of different genotypes were purified with DNA Fragment Quick Purification/Recover Kit. The purified PCR products were ligated to pGEM-T Easy vector and transformed into DH5-α *Escherichia coli*. The positive recombinant colonies for each genotype were chosen. The sequencing reactions were completed by Shanghai Invitrogen Biotechnology Co., Ltd.

Statistical

The general linear model (GLM) was established to analyse the genotype effects of the *IGFBP-1* gene on body weight traits. The following linear model was used:

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

where:

y_{ij} – the body weight trait;

μ – the overall mean for the trait;

G_i – the genotypic effect of the *IGFBP-1* gene;

e – the residual error.

The statistical analyses were carried out using the SPSS 11.0 software.

Results and discussion

Amplicons of the expected size were obtained using primer pairs shown in Table 1. Those obtained with primer pairs P2, P6 and P7 showed polymorphisms. For primers P2, two alleles (A and B), and three genotypes (AA, AB and BB) were identified in three chicken breeds (Fig. 1). Allele A showed higher frequency in all tested breeds as compared to allele B (Tab. 2). The sequencing analysis of three SSCP variants revealed two SNPs (A2276T, T2359C) in the exon 2 of the *IGFBP-1* gene. Transition

Table 1. Primers for exons of IGFBP-1 gene PCR analysis parameters established for chicken

Primers	Primer sequence (5 3)	Fragment length (bp)	Annealing temperature(°C)	Location
P1	F: CTCACCAGCGACATGAACTG R: AGAGGGCCAGCTTCTCTTGT	154 bp	58	Exon 1
P2	F: CCAGAATCTACCGAGCCTGA R: CCCACTCACCTGTTCTTTCC	192 bp	62	Exon 2
P3	F: TTCTCTGCAGGGACCTTGTC R: GGAGCAGAGGGTTGTGAAAG	219 bp	60	Exon 3
P4	F: GTGCACTGCAGTTCTTGAGC R: AGCGGAATCTCCATCCAGT	158 bp	60	Exon 4
P5	F: TGGGTGCTGGTGTGTCTATC R: GGCTCCTGATGAGTTCAAGC	160 bp	59	Exon 4
P6	F: CCAGGTTCTCTGTGGAGCTT R: GGAGGACCTGATCTTGCAGT	187 bp	60	Exon 4
P7	F: CAAGCAACTGCAAGATCAGG R:GCATCATTTCTCCAACATAACA C	201 bp	61	Exon 4
P8	F: AGCTTTCAGTAGGGAATGTGC R: CTATCCCATCACGAGCTTCAG	151 bp	59	Exon 4

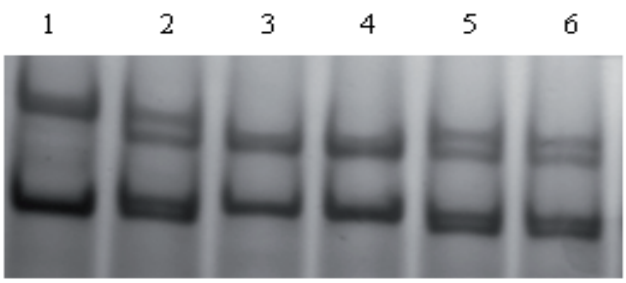

Fig. 1. SSCP analysis of PCR amplification for P2 of IGFBP-1 gene in chicken.

Table 2. Allele and genotype frequencies in exons of IGFBP-1 gene in three breeds of chicken

Primers pair			Jinghai yellow chicken	Youxi chicken	Bian chicken
P2	genotype frequency	AA	0.38 (56)	0.60 (18)	0.23 (7)
		AB	0.35 (51)	0.23 (7)	0.67 (20)
		BB	0.27 (39)	0.07 (5)	0.10 (3)
	allele frequency	A	0.56	0.72	0.57
		B	0.44	0.28	0.43
P6	genotype frequency	CC	1.00 (146)	0.70 (21)	0.93 (28)
		CD	0.00 (0)	0.23 (7)	0.07 (2)
		DD	0.00(0)	0.07 (2)	0.00 (0)
	allele frequency	C	1.00	0.82	0.97
		D	0.00	0.18	0.03
P7	genotype frequency	EE	0.84 (123)	0.23 (7)	0.40 (12)
		EF	0.06 (9)	0.47 (14)	0.23 (7)
		FF	0.10 (14)	0.30 (9)	0.37 (11)
	allele frequency	E	0.87	0.47	0.52
		F	0.13	0.53	0.48

Bracketed are numbers of individual genotypes in the population.

T2359C was a synonymous mutation, whereas transversion A2276T caused the amino acid change (Ile→Leu). Furthermore, these two SNPs (at positions 2276 and 2359) constructed three genotypes: AT/AT, TC/TC and AT/TC named AA, BB and AB, respectively.

For primers P6 only one genotype (CC) was detected in the Jinghai Yellow chicken and two (CC, CD) in the Bian chicken. However, Youxi chicken displayed three genotypes (Fig. 2). Allele C showed higher frequency than allele D in all tested breeds. The sequencing analysis of three SSCP variants revealed one SNP (T5550C) in exon 4 of the *IGFBP-1* gene giving genotypes T/T, C/C and T/C named C, DD and CD, respectively.

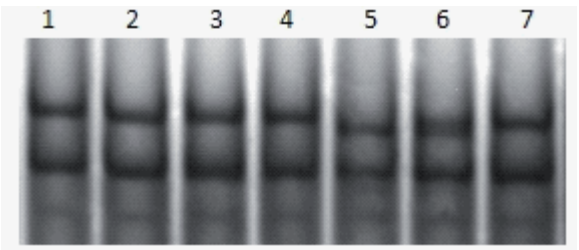

Fig. 2. SSCP analysis of PCR amplification for P6 of IGFBP-1 gene in chicken. 1, 2, 3, 4, 7: CC genotype; 5: DD genotype; 6: CD genotype.

For primers P7 three genotypes detected in the Jinghai Yellow, Youxi and Bian chickens were named EE, EF and FF, respectively (Fig. 3). The frequency of allele E was higher than that of allele F in the Jinghai Yellow and Bian chickens, while in

Youxi chickens both alleles showed a similar frequency. Sequencing revealed one insertion/deletion AAT located in the non-coding region of exon 4 of the *IGFBP-1* gene leading to EE and FF genotypes.

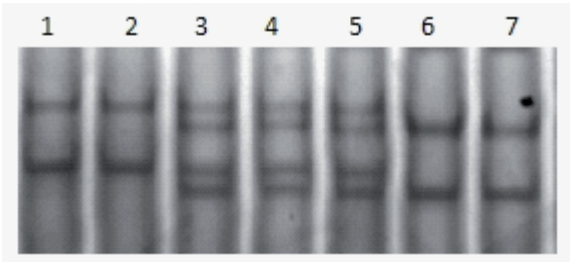

Fig. 3. SSCP analysis of PCR amplification for P7 of IGFBP-1 gene in chicken. 1, 2: EE genotype; 3, 4, 5: DE genotype; 6, 7: EE genotype.

Polymorphisms of the *IGFBP*-1 gene presented in this study have not been reported earlier in the other chicken breeds and homologous mutations have not been reported in any other species so far. However, many other SNPs have been found in *IGFBP-1* gene of the other species. Inage-Miyake *et al.* [2005] using PCR-PFLP technique found two SNP in the intron 2 of the *IGFBP-1* gene in pigs. In humans, identified were many SNPs in the *IGFBP-1* gene. Stephens *et al*. [2005] genotyped 13 single nucleotide polymorphisms (SNPs) in the *IGFBP-1* gene of human and they were located in the 5′ regulatory region , 3′ regulatory region and introns. Van der Kayy *et al.* [2009] detected one SNP (–575 G/A) in the 5′ regulatory region of *IGFBP-1* gene of human. However, there were few studies reported on the polymorphism of the *IGFBP-1* gene in chickens. Ou *et al*. [2009] detected SNP in the 5' upstream region of the *IGFBP-1* gene using a modified PCR-RFLP method and found one SNP (T-808C) in this region in the Beijing You chicken.

Table 3. Least squares means analysis between P2 of the IGFBP-1 gene and body weight traits

Body weight (g)	Genotype AA (n=56)	Genotype AB (n=51)	Genotype BB (n=39)
Day 1	35.68±0.42	34.98±0.44	36.00±0.51
Week 4	180.50±3.40^{abB}	182.57±3.56^{bBb}	196.28±4.07^{aA}
Week 8	447.93±10.19^{b}	454.29±10.67^{ab}	482.03±12.21^{a}
Week 12	848.13±15.38^{AB}	827.02±16.11^{B}	887.49±18.43^{A}
Week 16	1129.48±13.10^{B}	1154.71±13.72^{AB}	1184.56±15.69^{A}

$^{aA,...}$Means in rows bearing different superscripts differ significantly at:P<0.05 – small letters P<0.01– capitals. Bracketed are numbers of individual genotypes wihtin the Jinghai Yellow chicken.

Polymorphism A2276T identified in this study caused the amino acid change (Ile→ Leu). Thus, we hypothesized that the amino acid change from Ile to Leu may cause a functional difference of the IGFBP-1 protein. The association of this polymorphism with the body weight traits was analysed (Tab. 3). The results showed that chickens of the BB and AB genotypes had significantly higher body weight at the age of 4, 8 and 16 weeks, than those of the AA genotype ($P < 0.05$ or $P < 0.01$). Moreover, BB genotypes had significantly higher body weight at week 12 of age than those of the AB genotype ($P < 0.01$). Thus, chickens of BB genotype showed the highest body weight. Furthermore, there were no significant differences between AB and BB genotypes for body weight traits ($P>0.05$).

Until now, the research focusing on the relationship between the *IGFBP-1* gene variants and economic traits of chicken was rare. A T-808C SNP in the *IGFBP-1* gene had been reported to be associated with body weight at week 10 of age [Ou *et al*. 2009]. Recently, numerous QTL have been found in chickens using different DNA markers. Several QTLs for growth are located on GGA2 or GGA7, which contains chicken *IGFBP-1* gene [Tatsuda and Fujinaka 2001, McElroy *et al*. 2006, Park *et al*. 2006].

The results of the present study indicate that Jinghai Yellow chickens with the mutant allele (T at 2276 nt) had increased body weight compared to the wild type (A at 2276 nt) – primers pair P2. This suggested that the *IGFBP-1* gene is, therefore, a potential marker for use in marker-assisted selection programmes. This needs to be further confirmed by evaluating polymorphism and traits of growth in other breeds and lines of chickens, and conducting further function studies for defining the effects of SNP in the *IGFBP-1* gene at a molecular level.

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