

Association of Insulin-Like Growth Factor-I Gene with Body Composition Traits in Iranian Commercial Broiler Lines

¹Ali Javanrouh Aliabad, ¹Hamidreza Seyedabadi and ²Bahareh Taheri Dezfali

¹Department of Animal Biotechnology, Animal Science Research Institute of Iran, Karaj, Iran

²Department of Animal Science,
Agriculture and Natural Resources Research Center of Khuzestan, Ahwaz, Iran

Abstract: Insulin-Like Growth Factor-I (IGF-1) may play important roles in growth of multiple tissues and it is encoded by the IGF1 gene. The current study was designed to investigate the associations of IGF1 gene polymorphism on chicken growth and body composition traits. Genomic DNAs were extracted from 400 chickens belonged to four Iranian commercial broiler lines. Genotyping for the IGF1 gene by using PCR-RFLP method and *HinfI* restriction endonuclease showed a mutation in the 5'UTR of the IGF1 gene, near a putative TATA box. In addition, the IGF-1 genotypes were verified by DNA sequencing. Polymorphism in IGF1 gene was significantly ($P<0.05$) associated with abdominal fat weight (AFW), drumstick weight (DW), wing weight (WINW), percentage of carcass weight (%CW), percentage of drumstick weight (%DW), percentage of breast muscle weight (%BMW) and percentage of wing weight (%WINW). This research not only suggests that IGF1 gene could be a candidate gene that affects body composition traits in the chicken, but also suggest that the use of molecular genetic markers associated with the IGF1 gene can be used in a selection program for low abdominal fat.

Key words: IGF1 gene • Body composition • Polymorphism • Iranian broiler lines

INTRODUCTION

For the last 30 to 40 years, one of the business objectives of primary breeders was to select birds that deliver the best commercial performance (growth rate, feed conversion and meat yield) and economic return for their customers. Although traditional selection for phenotypic values of broiler chickens has made significant improvements in growth rates and meat yields during the past half century, but now, the high selection intensity for growth rate has caused many physiological disorders such as obesity, ascites and leg problems, as well as a reduction in overall immunocompetence [1]. Molecular marker-assisted selection may be required. The combination of traditional genetic selection and modern molecular methods may be preferred for breeding chickens in the future [2]. To simultaneously improve production and fitness traits, molecular markers associated with one or both sets of traits may be useful. Understanding the genetic control of growth in chickens will provide an

opportunity for genetic enhancement of production performance and physiology [3].

Insulin-like growth factors (IGF) consist of a family of polypeptide hormones structurally associated with insulin with multiple metabolic and anabolic functions [4]. The IGF are important regulators in stimulating growth, protein synthesis and cell proliferation and differentiation in a variety of cell types [5]. A majority of the functions of the growth hormone are mediated by the insulin-like growth factors (IGF) in chickens. The metabolic effects of avian IGF include an increased amino acid and glucose uptake and the upregulation of DNA and protein synthesis [6]. Plasma levels of IGF1 decrease with fasting and increase with age [7]. Moreover, there is ample evidence suggesting that IGF might influence growth rate, body composition and lipid metabolism in poultry. The molecular characterization of the chicken IGF1 gene has provided valuable clues for understanding how it is regulated and expressed. The chicken IGF1 gene maps to chromosome 1 and encompasses 50 kb.

Corresponding Author: Hamidreza Seyedabadi, Department of Biotechnology, Animal Science Research Institute of Iran (ASRI), First Dehghan Villa, Shahid Beheshti St., Karaj, Iran, P.O. Box: 1483-31585.
Tel: +98-261-4430010 (473), Fax: +98-261-4413258.

Multiple alternative promoters and two different variants generated by alternative splicing have been reported [8]. The profile of IGF1 mRNA expression is remarkably ubiquitous and includes liver, muscle, kidney, testes, heart, ovary, brain, intestine and other tissues [9].

Association studies between mutations at the IGF1 gene and productive traits are very scarce in chicken. Nagaraja *et al.* [10], described one PstI RFLP in the 5' end of the IGF1 gene that was associated to egg and egg shell weight in one White Leghorn chicken population. Moreover, Yan *et al.* [11], reported an association between phenotypic variation at several growth and carcass traits with one polymorphism at exon 2 of the chicken IGF2 gene. The main goal of the current work was to identify single nucleotide polymorphisms (SNP) in the IGF1 gene, develop PCR-RFLP methods to detect those DNA polymorphisms in Iranian commercial broiler line and evaluate associations between IGF1 SNP and growth and fatness traits.

MATERIALS AND METHODS

Chicken Populations: Four different Iranian commercial broiler lines were used in the current study. All birds had free access to feed and water. The individuals were raised in floor pens and fed commercial corn-soybean diets that met NRC requirements. The fifteen generation individuals (n=400) was used in the present study. Live body weight was measured at 6 wk of age. Chickens were slaughtered, carcasses were eviscerated and dissected. Carcass weight (CW), breast muscle weight (BMW), drumstick weight (DW), back weight (BAKW), wing weight (WINW) and abdominal fat weight (AFW) traits were determined. All traits were also expressed as percentage of BW at 6 wk of age.

DNA Extraction: Whole blood samples were collected from 400 chickens at 6 weeks of age. They were obtained from four different commercial broiler lines, which were selected for production and reproduction traits for 15 generations. Genomic DNA were extracted using salting-out method with some modifications [12]. Optimization includes utilization of separate buffer instead of Buffy coat isolation. Chloroform for DNA phase isolation and achievement to purified DNA and sodium acetate for more concentrated DNA. The optimized protocol would be more safe, simple, cheap and rapid.

PCR Amplifications and Genotyping: The IGF1 primers (5' GCT GGG CTA CTT GAG TTA CTA C 3'; 5' TTG CGC AGG CTC TAT CTG CTC 3') were chosen based on the

primers design by Amills *et al.* [13] to amplify a 813bp corresponding to the 5' end of the chicken IGF1 gene. Fifteen µl of each PCR reaction contained; 1X PCR buffer; 2mM MgCl₂; 0.25µM primers; 200 µM dNTPs; 1 unit of Taq polymerase (Takara Biotechnology Dalian Co, Ltd) ; 150 ng/reaction genomic DNA and ddH₂O. The PCR was performed by using a PTC-200 Programmable Thermal Controller (MJ-Research). Thermal cycling included initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 1 min, 65°C for 1 min, 72°C for 1 min and an extension at 72°C for 7 min. A single nucleotide polymorphism (SNP) of the IGF1 region was detected by digesting 10 µl of the PCR product with *HinfI* restriction endonuclease at 37°C overnight. Restriction patterns were visualized by agarose gel electrophoresis and ethidium bromide staining. Restriction patterns were visualized by agarose gel electrophoresis and ethidium bromide staining.

DNA Sequencing: After the gel electrophoresis process, the amplicons of 813 bp was purified using a Qiaamp Mini Kit (QIAGEN, Valencia, CA, U.S.A.). The purified samples were sequenced by a big dye terminator chemistry on an ABI 3130-Avant DNA sequencer (Applied Biosystems, Foster City, CA, U.S.A.). The DNA sequences were analyzed using the Sequencing Analysis Software Version 3.3 (Applied Biosystems, Foster City, CA, U.S.A.).

Statistical Analysis: Data were subjected to the MIXED procedures of SAS (SAS Inst. Inc., CARY, NC) with genotype (G), line (L) and sex (S) as fixed effects according to the models:

- $y_{ijkl} = \mu + \text{Genotype}_i + \text{Sex}_j + \text{Line}_k + \text{Sire}(\text{Line}) + \text{dam}(\text{Line Sire}) + e_{ijkl}$. In the formula, Y was the response variable, μ represents population mean and e stands for the random error. Statistical significance threshold was determined as $P < 0.05$, unless otherwise specified.

RESULTS

Allele Frequency: The genotype and allele frequencies at IGF1 loci calculated by PopGene.S2 software, are shown in Table 1. The B allele was more frequent than A allele in three broiler lines (B,C,D). The AB genotype was more frequent than other genotypes in three broiler lines (B,A,D). The Chi-square test ($P < 0.05$) indicated that the genotype distributions were not in Hardy-Weinberg equilibrium (Table 1). Agreement of the genotype frequencies with the Hardy-Weinberg equilibrium expectations tested indicated that IGF1 gene frequency was significantly different ($P < 0.01$) among lines.

Table 1: Genotype and gene frequency of IGF1 gene in chicken population

Chi-square test (χ^2) p < 0.05	Gene frequency		Genotype frequency		Population	
	B	A	BB	AB	AA	
0.00	0.50	0.50	0.25	0.50	0.25	Line A
	0.59	0.41	0.36	0.48	0.16	Line B
	0.61	0.39	0.47	0.38	0.16	Line C
	0.58	0.42	0.34	0.48	0.18	Line D

Table 2: Effects of Insulin-Like Growth Factor-I (IGF1) genotype on growth and body composition (least squares means)

Trait	P-value	AA	BB	AB
BW6(g)	0.720	2485.4± 52.28 a	2506.45± 34.07a	2483.86± 19.94 a
CW(g)	0.493	1747.03± 33.01 a	1712.95± 20.95 a	1721.53± 12.66 a
BMW(g)	0.073	550.24± 15.67a	570.81± 9.81 a	557.49± 5.79 a
DW(g)	0.003	516.07± 12.07a	486.80± 7.74 b	506.15± 4.54 a
WINW(g)	0.01	192.33± 4.54 b	202.20± 2.89 a	197.16± 1.70 ab
BAKW(g)	0.801	377.10± 8.98 a	381.34± 5.66 a	383.17± 3.35 a
AFW(g)	0.028	22.03± 1.76 b	26.85± 1.09 a	25.88± 0.70 a
%CW	0.049	0.685± 0.004 b	0.687± 0.003 b	0.694± 0.002 a
%BMW	0.0002	0.217± 0.003 b	0.228± 0.002 a	0.223± 0.001 a
%DW	0.0001	0.203± 0.003 a	0.195± 0.002 b	0.203± 0.001 a
%WINW	0.0005	0.084± 0.001 b	0.089± 0.0007 a	0.087± 0.0004 a
%BAKW	0.736	0.152± 0.002 a	0.153± 0.001 a	0.153± 0.001 a
%AFW	0.303	0.088± 0.0007 a	0.097± 0.0004 a	0.011± 0.0002 a

^{ab} Means with no common superscripts differ significantly (P<0.05)

¹BW6(g)= Body Weight at 6 week; CW= carcass weight; BMW= breast muscle weight;

DW= drumstick weight; WINW= Wing weight; BAKW= back weight; AFW= abdominal fat weight, %CW= carcass weight as percentage of BW at 6 wk of age, %BMW= breast muscle weight as percentage of BW at 6 wk of age, %DW= drumstick weight as percentage of BW at 6 wk of age, %WINW= Wing weight as percentage of BW at 6 wk of age, %BAKW= back weight as percentage of BW at 6 wk of age, %AFW= abdominal fat weight as percentage of BW at 6 wk of age

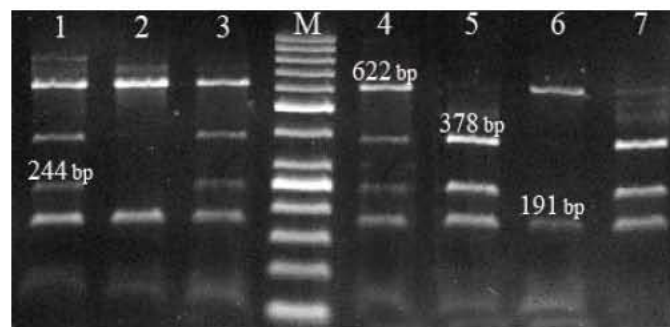


Fig. 1: PCR-RFLP pattern for IGF1 gene with *HinfI* digestion. Lanes 2 and 6, AA genotype; Lane 5 and 7, BB genotype; Lanes 1, 3, 4, AB genotype; and Lane M is size marker of Roche company _ Germany.

Identification of Polymorphism and PCR-RFLP Analysis:

The transition of A into C SNP at base 570 from 5' 'UTR region IGF1 gene creates a restriction site for *HinfI* endonuclease. The 813-bp fragment was digested with *HinfI* restriction enzyme. The restriction enzyme *HinfI*-digested PCR product had fragments of 622 and 191 bp for AA homozygotes, fragments of 622, 378, 244 and 191 bp

for AB heterozygotes and 378, 244 and 191 bp for BB homozygotes (Figure 1). In addition, the IGF-1 genotypes were verified by DNA sequencing and they were presented in figure 2. There was a single nucleotide polymorphism of A570C. According to sequencing, AA bands represented A570A, AB bands represented A570C, BB bands represented C570C.

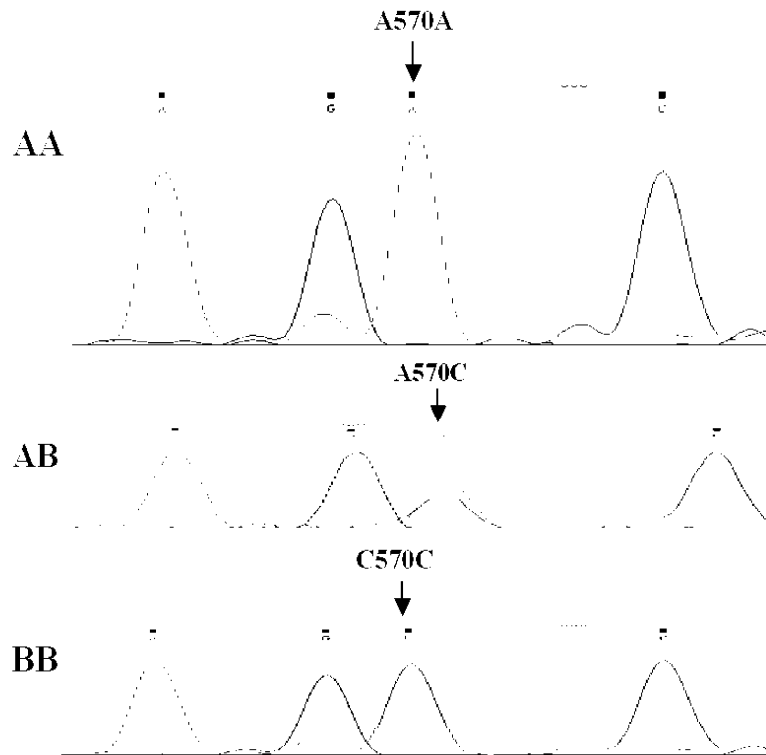


Fig. 2: According to sequencing, AA bands represented A570A, AB bands represented A570C, BB bands represented C570C

Association of IGF1 Gene SNP with Body Composition:

The genotypes of the SNP of the chicken IGF1 gene were used in the genetics analysis of the population. There were significant associations ($P < 0.05$) between the SNP and body composition traits (WINW, AFW, DW, %CW, %DW, %BMW and %WINW) (Table 2). There were lower ($P < 0.05$) AFW and WINW in birds that were of the AA genotype than those of the BB genotype. There were lower higher ($P < 0.05$) DW in birds that were of the AA genotype than those of the BB genotype. There were no significant associations ($P > 0.05$) between the SNP and BW6, BMW, CW, DW, BAKW, %BAKW and %AFW traits (Table 2).

DISCUSSION

As abdominal fat is positively correlated with BW, selection for rapid growth and meat yields in broilers over decades has been accompanied by increased abdominal fat and feed intake [14]. The traditional selection against abdominal fat involves difficulties in measuring the trait and limited number of samples. The emerging molecular markers that are closely associated with chicken fatness might provide better solutions for the problem.

The studied IGF1-SNP is in the promoter region. Multiple alignments among human, mouse, pig, cattle, goat and chicken IGF1 promoter sequences have shown that the promoter sequence is very conserved around the SNP location studied. The substitution A?C in the promoter region is involved the suppression of one potential CdxA transcription factor binding site [13]. Therefore, the studied mutation detected is hypothesized to affect the transcription rate of both alleles and, thus, the gene expression level of IGF1, as was confirmed by circulating IGF-I levels.

The IGF1 gene was selected as a candidate gene to investigate associations of gene polymorphisms with growth and body composition in Iranian broiler-inbred lines.

Disagreement of the of the SNPs genotype frequencies with the Hardy-Weinberg equilibrium expectations tested indicated that IGF1 gene frequency was non-significantly different between populations ($P < 0.05$). This may be due to the high selection program done in population as meat chicken with similar gene frequency.

Amills *et al.* [13], reported a A/C mutation in the 5_untranslated region of the IGF-I gene, near a putative

TATA box. They founded that a polymorphism of this SNP was related ($P<0.05$) with BW at 44 d in a commercial line chicken. Our genotypes were the same, but we didn't obtain any association between genotypes and BW(6). The current study, there were significantly higher AFW, %WINW, WINW and %BMW in birds that were of the BB genotype than those of the AA and AB genotypes; there was higher DW in birds that were of the AA genotype than those of the AB and BB genotypes and there was higher %CW in birds that were of the AB genotype than those of the AA and BB genotypes. This finding was similar to result of Zhou *et al.* [15]. Abdominal fat has been recognized as an undesirable trait. Infusion of IGF-I into chickens can increase circulating IGF-I concentration, stimulate growth, decrease insulin levels and lower consequent lipogenic activity, thereby reducing fatness [16]. The birds with AA genotype had significantly lower AFW than BB genotype. A comparison of the mean values for all three genotypes suggests that IGF-I mainly acts in a dominant fashion on abdominal fat traits, with allele A contributing to lower fat deposition. This specific gene SNP presents the opportunity to select at the molecular level, against the general tendency of broilers toward excess fat deposition and thereby overcoming a general negative correlation of growth and fat percentage. There were no significant associations ($P>0.05$) between the SNP and BW6, CW, BMW, BAKW, %BAKW, %CW and %AFW. In contrast, Zhou *et al.* [16] reported SNP in IGF-I gene significantly ($P<0.05$) affected BW6, BMW and %AFW birds. They observed there were significantly higher BW6, BMW in birds that were of the AA genotype than those of the AB and BB genotypes.

CONCLUSION

The results from the current study indicated that a SNP marker in the IGF-I gene was associated with fatness and body composition traits in chickens growing to market weight and is, therefore, a potential marker for molecular MAS programs in commercial broiler lines in Iran.

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