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ORIGINAL ARTICLE

IGF-I Gene Polymorphism and its Associations with Some Growth traits in West-Azerbaijan Native chicken using PCR-RFLP Techniques

Mehdi Seyyed Babayi [1*], Hamidreza Seyedabadi [1], Abolfazl Gorbani [1], Nosratollah Zarghami [2]
1. Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran
2. The Umbilical Cord Stem Cell Research Center (UCSRC), Tabriz University of Medical Science, Tabriz, Iran.
* Correspondence email: m.seyyedbabayi@yahoo.com

ABSTRACT
The aim of the study was to investigate IGF1 gene polymorphism and its associations with some growth traits in West-Azarbaijan native broilers. Blood samples collected from 200 birds (100 hens and 100 roosters) to investigate IGF1 gene polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). According to the data, The HindIII enzyme digested products were C and A alleles. The allele A includes 3 segment 191, 244 and 378 bases. The C segment included with 2 segments 199 and 622 bases. Three genotype AA and CC (homozygote) and AC (heterozygote) were detected on 3, 2 and 4 bands, respectively. There was no CC homozygote genotype in west Azerbaijan native poultry. Also, frequency for AA genotype was 0.83 whereas for BB 0.17 in west Azerbaijan native poultry. There was no significant difference on polymorphism of IGF1 with the traits (P>0.05). Furthermore, thigh and breast weight in AC genotype was greater but not significant than AA genotype while the abdomen fat was less in AC than AA (P>0.05). In addition, was no significant difference between AA and AC on body weight and live weight gain (P>0.05).
***Key words**: Genotype, IGF1, Polymorphism, West-Azerbaijan Native chicken*

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INTRODUCTION

The intensive application of selection methods in poultry farming has resulted in an increased growth rate and carcass yield but there are negative consequences to this process, including health problems, especially obesity and increased incidence of sudden death syndrome, immunosuppression, and diseases of the legs [1]. Growth controls by many genes are important and economic traits in the poultry industry. Integration of novel technologies with traditional methods and identification of effective genes have provided possibilities of more balanced selection [2]. Native poultry breeds are poor egg and growth performers but are well adapted to local environment and pathogenesis. In order to make native poultry commercially viable both genetic and nutritional interventions are required. Detection of molecular markers for selection of superior poultry stock for economically important traits and the incorporation of this information into breeding plans for improving native poultry performance offers means to incorporate genetic superiority for higher gains for egg and meat productivity [3]. The use of DNA markers to define the genotype and predict the performance of an animal is a powerful aid to animal breeding [2]. To improve production traits and health simultaneously it is appropriate to use molecular markers associated with one or two characteristics.

The insulin-like growth factor gene (IGF1) is a candidate gene for growth, body composition and metabolism, skeletal characteristics and growth of adipose tissue and fat deposition in chickens [4]. The insulin-like growth factor 1 receptor (IGFIR) is a membrane glycoprotein mediating most biological actions of IGF-1 and IGF-2, which have an important effect on chicken growth, carcass, and meat quality traits. Two receptors (IGF1R and IGF2R) were found in the mammals but only one (IGF1R) was found in the birds. IGF1R not only regulated the half-life time and activity of IGFs, but also played important roles

on the key developmental stage and adult stage such as the cell life cycle, transplantation, metabolism, subsistence, proliferation and differentiation [5].

The growth hormone is the main constituent of the somatotropic axis and plays crucial role in the postnatal growth and metabolism regulation. Additionally, GH affects indirectly by controlling the secretion of other hormones including IGF1, which interacts with insulin-like growth factor 1 receptors (IGF1R) in target tissues [6].

Many variations in the genome affected gene expression at the transcription and translation levels. Variations in the genes of somatotropic axis could function as candidates for the evaluation of their effects on animal growth and development traits. In humans, mutations at important regulatory sites of the IGF1R gene were associated with growth. Such mutations resulted in the failure of processing of proIGF1R to mature IGF1R and caused dysfunction and short stature of IGFR. These variations affected partly the expression and physiological functions of the IGF1R gene, and subsequently affected growth. However, few studies on associations of the IGF1R gene with growth and carcass traits were reported in chickens [5]. Nevertheless, the genetic potential in almost all chicken breeds has not yet been much revealed. Based on the literatures, the chicken IGFs are considered to be the most important candidate genes that can influence chicken performance traits including growth, body measurement, carcass and reproduction. So, the hypotheses of the current study IGF-I Gene Polymorphism and its Associations with Some Growth traits in West-Azarbaijan Native chicken.

MATERIAL AND METHODS

Animal and Blood Samples

West Azerbaijan native poultry lines which their reproductive characteristics studied for 12 generation were used in this study (Tala Tabeh, west Azerbaijan provnce, Iran). A 200 West-Azarbaijan native birds (100 hens and 100 roosters) were randomly selected, blood samples (2ml in EDTA containing tubes) collected from via wing vain using disposable syringes in all birds and stored at -20 C° until used at hematology laboratory.

Establishment of a PCR-RFLP assay

The PCR primers for the chicken IGF_1 were used as 813 bases. Forward: 5' CA TT GC GC AG GC TC TA TC TG 3' and Reverse: 5' TC AA GA GA AG CC CT TC AA GC 3' (Fermentas, Germany) and accuracy of primers checked using blast procedure from NCBI was used. The PCR was performed in a total volume of 15 μL, containing 5 μL of genomic DNA, 10 pmol of each oligonucleotide primer, 2 μL 25 mM MgCl2, 2 μL of 1 mM deoxynucleotide triphosphate mixture, and 1 U of Taq DNA polymerase; cycle parameters were 94 °C for 8 min then 35 cycles of 95 °C for 30 sec, 64 °C for 30 sec, and 72 °C for 5 min, with a final extension step for 2.07 min at 72 °C; the PCR products with length 776 bp were digested at 37 °C overnight with 10 U of Hinf I. This enzyme acts on 5'...G ↓A N T C ...3 and 3'...C T N A ↑G...5'. Hind III enzyme acts on 5'...A ↓A G C T T ...3' and 3'...T T C G A ↑A...5'.

Carcass characteristics

To investigate polymorphism, phenotypic characteristics of the birds at 12 weeks of age include maturity age, mean of number and weight of eggs were used.

STATISTICAL ANALYSIS

Obtained results was processed and analyzed using GLM procedure in SAS ver. 9.1 using Least Squares Means (LSM).

$$y_{ijk} = \mu + Genotype_i + Sex_j + e_{ijk}$$

RESULTS AND DISCUSSION

Polymorphism of HinfI-RFLP in 5' exon in IGF1 gene

In this study the untranslatable fragment was the 5'-UTR (177 base) and promoter was 636 base. *HindIII* enzyme cut position was after 192 and before the 632 nucleotide. The *HindIII* enzyme digested products were C and A alleles. The allele A includes 3 segment 191, 244 and 378 bases. The C segment included with 2 segments 199 and 622 bases. Three genotype AA and CC (homozygote) and AC (heterozygote) were detected on 3, 2 and 4 bands, respectively (figure 1).

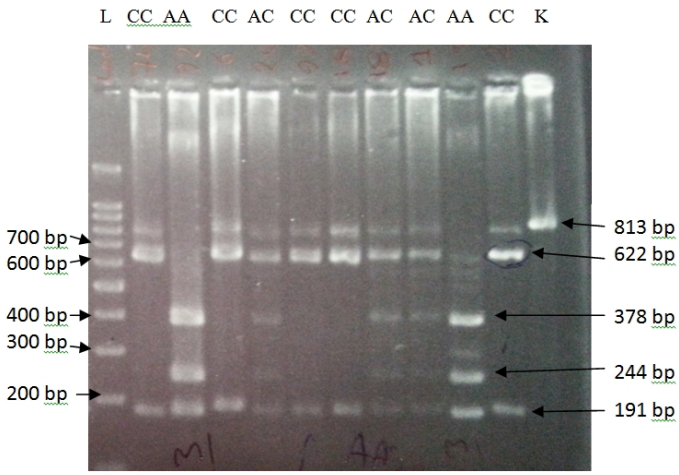

Figure 1. The *HinfI* enzyme products on 813 fragment base on IGF1 gen promoter. L: molecular weight, CC: genotype CC, AA: genotype AA, AC: genotype AC, K: PCR product.

Previously, Zhou et al., [4] using the current technique was reported that mutation on SAP (A→C) was on the 570 bases (M74176) in leghorns which our results was similar to their report. In addition, Kadlec et al., [1] in a study on Ross 300 and Cobb 500 (commercial meat type broilers) reported that in an 813 base fragment, A allele was in 191, 244 and 378 bases and C allele in 191 and 622. In addition no C genotype reported in their report.

Genotype and allele frequencies and hardy-Weinberg gen promoter in IGF1

Genotype frequency for IGF1 was determined using PopGene 32 software and presented in table 1. Previously, Kadlec et al., [1] reported, in Ross 300 strain frequency of A and C alleles were 0.915 and 0.085, respectively. In this study, there was no CC homozygote genotype in west Azerbaijan native poultry. Also, frequency for AA genotype was 0.83 whereas for BB 0.17 in west Azerbaijan native poultry. In comparison, for Cobb 500 strain frequency for AA and CC genotypes were 0.93 and 0.07, respectively. In addition, observed frequency for AA and AC were 0.86 and 0.14, respectively. To our knowledge we think the observed differences might relate to animal population or selection methods. In this regard, Abbasi and Kazemi [7] using the *PstI* enzyme method on IGF1 promoter gene, reported the frequency for A allele was 0.51 while for C was 0.49. Furthermore, genotype frequencies for AA, AB and BB alleles were 25.88, 50.23 and 23.89, respectively. It seems, the observed differences may related to enzyme method.

Table 1. Genotype and allele frequencies in IGF1 gen promoter.

Genotype Frequencies			**Allele Frequencies**	
CC	AC	AA	C	A
34.37	14.58	51.04	0.4167	0.5833

Table 2. κ square test of genotype distribution in IGF gen.

population	κ_2	Pr>X2
West-Azarbaijan Native chicken	47.77	0.00

Interaction of IGF1 promoter gene polymorphism with the other traits is presented in table 3. According to the data, there was no significant difference on polymorphism of IGF1 with the traits (P>0.05). In a similar study, Kadlec et al., [1] revealed that in AC genotype of Cobb 500 hens, there was a correlation between IGF1 promoter gene polymorphism and liver weight (as percent of body weight). In these birds, thigh and breast weight in AC genotype was greater but not significant than AA genotype while the abdomen fat was less in AC than AA (P>0.05). There was no significant difference between AA and AC on body weight and live weight gain (P>0.05). Also, the promoter gene polymorphism in IGF1-SNP1 was in hardy- Weinberg equilibrium.

Previously, Li et al., [8] a significant correlation was observed between IGF1 polymorphism and egg production and average days of continual egg-laying (P<0.05). Also, growth traits, body weight and mean daily weight gain was significantly higher in IGF1-SNP1 homozygotes in comparison to heterozygotes (P<0.05) [4]. Interestingly, there was no significant correlation between growth traits and IGF1 polymorphism (P>0.05) but there was a positive correlation between IGF1-SNP1 polymorphism and

mean weight gain on day 107 as well as feed efficacy on days 44, 73 and 103 (P<0.05) [9]. In this study, there was a significant difference between observed and prospect animals for each genotype which claims that this population is out of hardy- Weinberg equilibrium.

Table 3. Least Squares Means (LSM) of IGF1 gens.

Treat	CC	AC	AA	P-value
Body weight on 12 weeks of age	178.11±0.92	173.77±5.94	173.16±9.12	0.81
Puberty age	-12.14±0.50	-13.73±0.93	-12.28±.060	0.31
Mean egg production	1.63±0.10	1.48±0.20	1.54±0.13	0.77
Mean egg weight	0.27	-0.12±0.06	-0.05±0.11	0.27

Reproduction is a composite of complex developments that are influenced by genetic, nutritional, and environmental factors. Although association studies cannot determine if the GHR and IGF-1 gene allele markers are responsible for the variation in a particular trait or whether the variation is due to a closely linked locus, we think that 2 genes would influence the traits in chickens [8]. In a prior study, Gouda and Essawy [10] analyzed the polymorphism of IGF-I gene among Egypt chicken breeds and indicated their effect on the growth traits of chicken was significant. IGFBP2 and STAT5b act as modulators for the biological action of IGF gene in various signaling pathway, so their expression level as well as SNPs play important role in the action of IGF protein. This suggested that the IGF1 gene is, therefore, a potential marker for use in marker-assisted selection programmers. This needs to be further confirmed by evaluating polymorphism and traits of growth in other native breed and lines of chickens.

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