Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 3 [6] May 2014: 140-143

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Growth Hormone Receptor Gene Polymorphism and its Associations with Some Growth traits in West-Azarbaijan Native chicken

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ABSTRACT
The chicken growth hormone (GHR) gene has an important function in chicken growth and reproduction. Polymorphisms (are the most frequently found DNA sequence variations in the animal genome and can be used as genetic markers for association analysis with economic traits. The primary aim of this study was to identify polymorphisms in GHR as potential candidate genes for growth and production traits in Iranian chicken. In this study 200 blood samples were collected form West-Azarbaijan Native chicken (100 hens and 100 roosters) to investigate GHR gene polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). According to the data, HindIII was able to produce 2 final alleles: A and B. the A allele was in 290 and 428 bases while B allele located in 170, 258 and 290 bases. Also, AA genotype was in 290 and 428 bases whereas BB founded in 170, 258 and 290 bases, respectably. Furthermore, in this study no heterozygote identified. Mutation on AA genotype was in the 294 nucleotide. Also, the observed mutation for BB genotype located in the 541 nucleotide. In both genotypes the mutation was A→G transition. Genotype and allele frequencies for BB and AA genotypes were 84.85 and 15.15, respectively. According to the data, there was no significant correlation between polymorphism in intron 2 gene in GHR with treats (P>0.05).
***Key words**: Polymorphism, GHR, West-Azerbaijan Native chicken*

Received 20.02.2014 Revised 04.03.2014 Accepted 29.03. 2014

INTRODUCTION

As in mammals, the growth and development of chickens are primarily regulated by the somatotropic axis. The somatotropic axis, also named neurocrine axis or hypothalamus-pituitary growth axis, consists of essential compounds such as growth hormone (GH), growth hormone releasing hormone (GHRH), insulin-like growth factors (IGF1 and 2), somatostatin (SS), their associated carrier proteins and receptors, and other hormones like insulin, leptin and glucocorticoids or thyroid hormones [1]. The Growth Hormone (GH) and Insulin-like Growth Factor 1 (IGF-1) genes are candidates for growth in animals, since they play a key role in growth regulation and development. Genetic polymorphism in native breeds is a major concern considering the necessity of preserving genetic resources. It is very important to characterize genetically indigenous breeds [2]. The genes of somatotropic axis play a central role in the regulation of growth and development. Previous studies showed that variation of these genes affected gene expression at the transcription and translation levels [3, 4]. Variation in the genes of somatotropic axis could function as candidates for the evaluation of their effects on chicken growth and development traits. Previous studies have shown that some single nucleotide polymorphisms (SNP) of the somatotropic axis genes indeed affected growth traits significantly. Chicken growth hormone gene is located in the long arm of chromosomal 1q4. It is about 4 kb in size, and consists of five exons and four introns. Chicken growth hormone is an important hormone which secretes from the anterior pituitary gland, and plays a crucial role in chicken growth and development. Chicken growth hormone also has an effect on egg production and disease resistance. For instance, it can increase egg production and improve resistance to Marek's disease and avian leucosis [5]. Nevertheless, the genetic potential in almost all chicken breeds has not yet been much revealed. Based on the literatures, the chicken GHR are considered to be the most important candidate genes that can influence chicken performance traits including growth,

body measurement, carcass and reproduction. So, the hypothesis of the current study was to determine GHR 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 province, 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 in the 718 bases were used to propagation intron 2 of GHR chicken using fragment as Forward: 5' GGCTCTCCATGGGTATTAGGA 3' and Reverse: 5' GCTGGTGAACCAATCTCGGTT 3' (Fermentas, Germany) and accuracy of primers checked using blast procedure from NCBI database (AC188373). 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 $MgCl_2$, 2 µL of 1 mM deoxynucleotide triphosphate mixture, and 1 U of Taq DNA polymerase; cycle parameters were 94 °C for 5 min then 35 cycles of 94 °C for 45 sec, 60 °C for 45 sec, and 72 °C for 60 sec, with a final extension step for 10 min at 72 °C; the PCR products with length 776 bp were digested at 37 °C overnight with 10 U of Hind III. This 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.

Number of alleles and effective alleles

Number of alleles and effective alleles were determined using PCR-RFLP. Genotype information processed in excel and determined using Pop Gene Version 1.31 software as described by Yeh et al., [6]. Also, the hardy-Weinberg equilibrium test was performed using $\varkappa$ square test through Pop Gene Version 1.31 software [6].

$\varkappa$ square test $=\sum_u \sum_{v \pm u} \frac{2_u D_{uv}^2}{p_u p_v}$ $X_T^2 = \sum_u \frac{nD_{uu}^2}{p_u^2}$

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 *HindIII*-RFLP in intron 2 of GHR gene

In this study 718 base in intron 2 before the 3 exon was propagated. In current study, RFLPs of terminated by *HindIII* was an index for DNA. *HindIII* was able to produce 2 final alleles: A and B. the A allele was in 290 and 428 bases while B allele located in 170, 258 and 290 bases. Also, AA genotype was in 290 and 428 bases whereas BB founded in 170, 258 and 290 bases, respectably. Furthermore, in this study no heterozygote identified (figure 1). In this regard, Li et al., (2008) on native Chinese poultry A_1A_1 and A_2A_2 were detected. In similar study, on Mazandaran native birds (Mazandaran, Iran), Enayati et al., [7] reported that these was a polymorphism in intron 2 o GHR gene. Our result was similar to their observations on number of segments but not in segment sizes. Genotype +/+ with 3 segment (157, 247 and 314) and genotype -/- with 314 and 404 bases.

GHR sequence

To investigate accuracy of the obtained results, samples GHR sequences was determined by BIONEER Company, Germany and the result is presented in figures 2 and 3. As seen, mutation on AA genotype was in the 294 nucleotide. Also, the observed mutation for BB genotype located in the 541 nucleotide. In both genotypes the mutation was A→G transition.

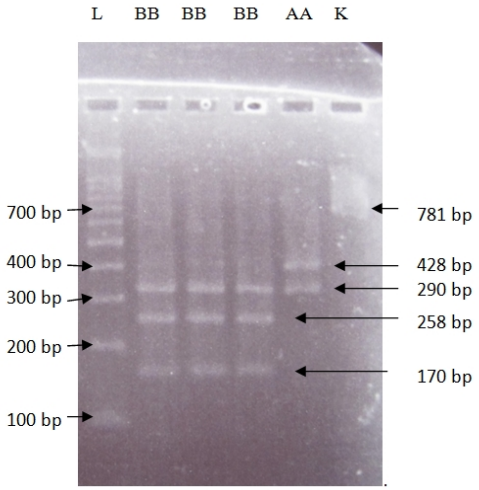

Figure 1. The *HinfIII* enzyme products on 718 fragment base on intron 2 of GHR gen. L: molecular weight, BB: genotype BB, AA: genotype AA, K: PCR product.

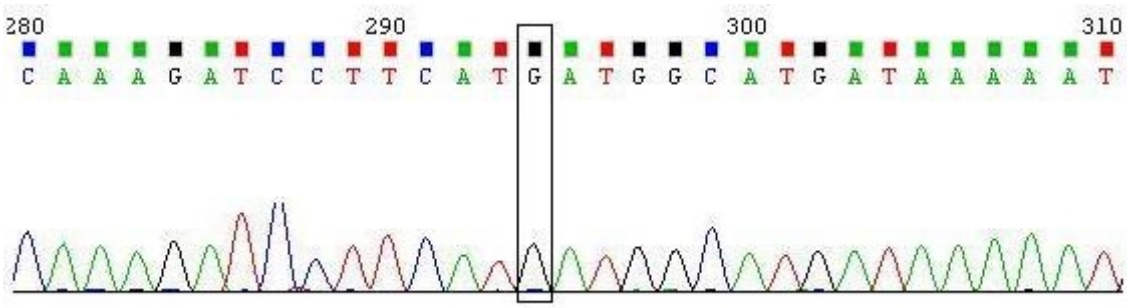

Figure 2. genotype AA for intron 2 in GHR

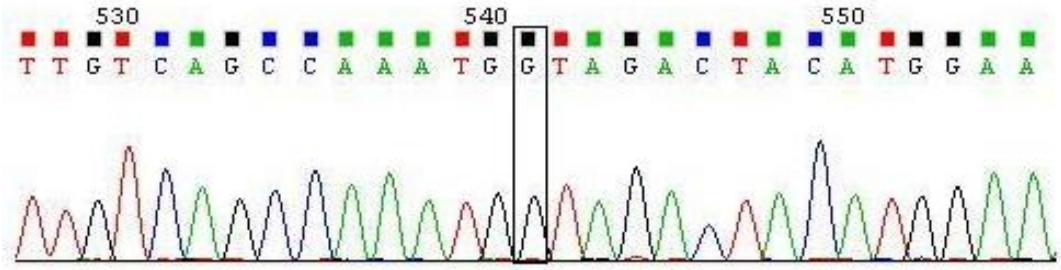

Figure 3. genotype BB for intron 2 in GHR

Genotype and allele frequencies and hardy-Weinberg test for intron 2 of GHR gene

Genotype and allele frequencies result is presented in table 1. In this study, Genotype and allele frequencies for BB and AA genotypes were 84.85 and 15.15, respectively. Formerly, Li et al., (2008) was reported that allele frequencies for allele A_1 and A_2 were 0.06 and 0.94, respectively. Also, genotype frequencies for A_1A_1 and A_2A_2 were 0.06 and 0.94, respectively. Also, $\varkappa_2$ was 89.97. In this regard, frequencies for allele + was 0.9935 while for allele – 0.0065 as reported by Enayati et al., [7]. In addition, they claimed that genotype frequencies for +/+ and -/- were 0.9935 and 0.0065, respectively. They reported GHR gene was out of Hardy-Weinberg equilibrium. Similarly, genotype frequencies for +/+ and -/- were reported 0.52 and 0.48 [8]. To our knowledge because of lack in heterozygosis, this population was out of Hardy-Weinberg equilibrium. It seems, these un-equilibrium might related to high selection rate.

Table 1. Genotype and allele frequencies in intron 2 in GHR.

Genotype Frequencies		Allele Frequencies	
BB	AA	A	B
84.85	15.15	84.85	15.15

Correlation of polymorphism in intron 2 gene in GHR with treats

Result of correlation of polymorphism in intron 2 gene in GHR with treats is seen in table 2. According to the data, there was no significant correlation between polymorphism in intron 2 gene in GHR with treats (P>0.05). Previously, Li et al., (2008) reported that there was no significant correlation on polymorphism in intron 2 gene GHR with number of two yolk eggs in Velchang poultry (P>0.05). In this study, no significant correlation observed on polymorphism GHR and total Egg Production trait (P>0.05). Also, Feng, [8] reported that there was a correlation but not significant among polymorphism in GHR gene and egg production age and Juvenile Body Weight (P>0.05). It has been reported that selection for body weight, feed efficiency and egg production over nine generations have led to an increase of the incidence of the *HindIII* + allele [8].

Table 2. Least Squares Means (LSM) of GHR gens.

Treat	AA	BB	P-value
Body weight on 12 weeks of age	164.34±10.85	162.06±5.23	0.85
Puberty age	-12.55±1.37	-12.26±.066	0.82
Mean egg production	1.56±.023	1.52±.011	0.87
Mean egg weight	-0.04±0.11	-0.09±0.05	0.69

The chicken growth hormone (cGH) gene is considered as one of the most important candidate genes that can influence chicken performance traits because of its crucial function in growth and metabolism. Genomic DNA from four divergent chicken breeds was screened for single nucleotide polymorphisms (SNPs) in the cGH gene using denaturing high-performance liquid chromatography and sequencing. It has been reported an SNP with G to A substitution of GH gene was significantly associated with abdominal fat pad weight, abdominal fat pad ratio, and crude fatty content of the breast muscle [7].

In recent years, DNA polymorphisms have been widely studied in the GH gene of various animals. In this study, we detected SNPs in cGH gene and analyzed their association with economic traits in Fars indigenous chicken. Eleven point mutations were identified in interon 1 of the cGH gene. Nie et al. [1] also detected 11 SNPs in intron 1, but in other studies only a few cGH gene SNPs in this region had been found [9]. This suggested that the GHR 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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