



Growth Hormone Gene Polymorphism in Two Iranian Native Fowls (Short Communication)

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Abstract

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Biochemical polymorphism study is a method of determination of genetic variation. This variability could be a basis for selection and subsequent genetic improvement in farm animals. The polymorphism in the intron 1 of chicken growth hormone (cGH) gene was investigated in the Iranian native fowls by using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method. The genomic DNA was extracted from 217 samples (129 samples from the native fowls of Isfahan province and 88 samples from the native fowls of Mazandaran province) by using modified salting out technique. The DNA fragment of the growth hormone gene with 776 bp was amplified by PCR using specific primers. Then the PCR products were digested with MspI restriction enzyme and analyzed on 2.5% agarose gel. The allelic frequency of intron 1 locus for A1, A2 and A3 alleles in Isfahan native fowls were 0.60, 0.21 and 0.19 and those in Mazandaran native fowls were 0.28, 0.05 and 0.67, respectively. The results of current study indicated that the intron 1 of cGH is polymorphic in Iranian native fowls and could be exploited as a candidate gene for marker-assisted selection for growth-related traits.

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Introduction

Polymorphism was defined as the co-occurrence of two or more varieties in the same population at the same time in such proportions that the rarest of them cannot be maintained by mutation alone (Osterhoff, 1964). Moreover the biochemical polymorphism is the occurrence of varieties attributed to the biochemical differences, which are under genetic control. It has created a way for the genetic improvement in farm animals. This kind of diversity can be used as a useful tool for characterization of farm animals. Moreover it is possible to determine the degree of differences or similarities between and within breeds and therefore they are very useful raw materials for genetic improvement of farm animals.

cGH is a polypeptide hormone that is involved in a wide variety of physiological functions, such as growth, egg production, aging and reproduction. The cGH gene is located on the tip of the long arm of the chromosome 1 (Shaw *et al.*, 1991). It consists of 5 exons and 4 introns with an overall length of 4.1 kb (Ip *et al.*, 2001; Kansaku *et al.*, 2008). This gene encodes a mature growth hormone protein with 191 amino acids and a signal peptide with 25 amino acids (Tanaka *et al.*, 1992). Chicken growth hormone is an important hormone which is secreted from the anterior pituitary gland and plays a crucial role in growth and development of the chickens. Polymorphism in the animal growth hormone gene has been reported previously. Studies on artificially inseminated (AI) bulls have revealed that restriction fragment length polymorphisms (RFLPs) of GH gene were associated with AI bull reproductive performance (Lechniak *et al.*, 1999). PCR-RFLPs were also investigated in various populations of Chinese native chickens and it was suggested that an allele present in the intron 1 might be linked to laying performance (Ip *et al.*, 2001). Su *et al.* (2014) confirmed that polymorphism of the GH gene and its haplotypes is related to chicken egg production traits. RFLPs have been identified in the cGH gene showing that these polymorphisms are associated with egg production traits, resistance to Marek's disease and avian leukosis (Kuhnlein *et al.*, 1997; Feng *et al.*, 1997). It has been reported that the RFLPs of cGH gene are significantly related to the chicken abdominal fat content (Fotouhi *et al.*, 1993). A significant correlation was reported between GH and meat quality in Anka and Rugao hens (Sheng-Long *et al.*, 2008). Moreover researches on the intron 1 of GH showed that this gene can affect some body composition traits in Arian broiler chickens (Ghelghachi *et al.*, 2013).

The objective of present study was to investigate the cGH gene polymorphism in Iranian native fowls by using PCR-RFLP technique.

Materials and Methods

The blood samples were collected randomly from two indigenous chicken flocks of Breeding Centre of Iran: 129 blood samples from native fowls of Isfahan province and 88 samples from native fowls of Mazandaran province.

The genomic DNA was extracted from the blood samples by using modified salting out technique (Miller *et al.*, 1988). The quantity and quality of the extracted DNA was checked by spectrophotometer and agarose gel electrophoreses. The intron 1 region of chicken growth hormone gene was amplified by a set of primers (5'-ATCCCCAGGCAAACATCCTC-3' or PM3 forward and 5'-CCTCGACATCCAGCTCACAT-3' or PM3 reverse) earlier used by Ip *et al.* (2001). The specificity of primer pairs was confirmed by BLAST with all the nucleotide sequences available for chicken at the National Center for Biotechnology Information (NCBI). The reaction mixture was subjected to initial denaturation of 95°C for 4 min followed by 30 cycles of 94°C for 30 s, annealing at 63°C for 120 s and extension at 72°C for 90 s. Final extension was given for 5 min at 72°C.

The PCR products were separated on 1.5% agarose gels containing 1X Tris-Borate-EDTA (TBE). The gels were stained with ethidium bromide and the images were obtained in UV gel doc documentation systems (UK). RFLPs were used for analysis of cGH gene polymorphisms. The PCR products of GH gene were digested with 0.5 µl of MspI restriction enzyme and 2 µL buffers 10X in a final reaction volume of 20 µL. The reaction mixture was incubated at 37°C for 4 hrs. The resulting fragments were separated by horizontal electrophoresis (80 V, 2 hrs) on 2.5% agarose gel, stained with ethidium bromide and were visualized under UV light.

The observed number of alleles and genotypes, the observed and expected heterozygosity for each locus and the average of heterozygosity over all loci were used to assess the genetic variability of two investigated populations (Krasnopiorova *et al.*, 2012). The Hardy Weinberg equilibrium was evaluated in studied populations.

Results

The PCR products with 776 bp length which run on 1.5% agarose gel were presented in Figure 1.



Figure 1. Representative result of Agarose gel electrophoresis of PCR products of intron 1 chicken growth hormone gene. A commercial DNA marker was used for size analysis.

M: Molecular weight marker.

The PCR products were digested with MspI restriction enzyme (5U), which recognizes the 5'- C↓C G G -3' sequence. In total, six restriction digestion profiles were found in the intron 1. There were three homozygous and three heterozygous genotypes (Figure 2).

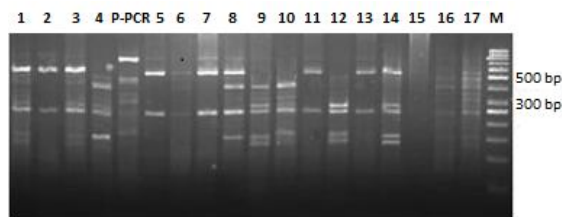


Figure 2. The electrophoresis patterns of intron 1 of the growth hormone (GH) gene of poultry obtained by PCR-RFLP.

Lanes 1, 2, 3, 5, 7, 11, 13: genotype A3A3; Lanes 4, 10: genotype A1A1; Lane 8 genotype: A1A3; Lane 9 genotype: A1A2; Lane 14 genotype: A2A3; Lanes 6, 15-17: no banding; Lane PCR: PCR product; Lane M: Molecular weight marker.

The genotypic frequencies were estimated by considering the presence of various RFLP patterns and the results were presented in Table 1. The highest frequency was observed in Isfahan native fowls for A1A1 genotype (0.581). Whereas the highest frequency in Mazandaran native fowls was observed for A3A3 genotype (0.614). The lowest frequency in Isfahan native fowls was found for A2A3 genotype and in Mazandaran native fowls was found for A1A2 genotype. Moreover, no A2A3 genotype were found in Mazandaran native fowls. The genotypic frequencies were tested for Hardy-Weinberg equilibrium by using a chi-square test and the result were shown in Table 1.

Table 1. Genotypic frequencies for Hardy-Weinberg equilibrium from intron 1 of the cGH gene in Iranian native fowl

Population	Isfahan native fowls		Mazandaran native fowls		
	Genotype	No. of birds	Frequency	No. of birds	Frequency
A ₁ A ₁		75	0.581	19	0.216
A ₂ A ₂		25	0.194	4	0.046
A ₃ A ₃		22	0.170	54	0.614
A ₁ A ₂		2	0.016	1	0.011
A ₁ A ₃		4	0.032	10	0.113
A ₂ A ₃		1	0.007	0	0
Sum		129	1	88	1
Cal. X ² value		209.8 **		111.6**	

** P ≤ 0.01

The differences among genotypes for Isfahan and Mazandaran native fowls were found to be significant. It indicated that two populations were not in Hardy-Weinberg equilibrium with respect to the marker locus, which is probably because of the genetic selection for growth related traits in these populations. Based on the genotypic frequencies, the allelic frequencies were calculated for each variety (Table 2).

Table 2. Allelic frequencies in intron 1 of the cGH gene in Iranian native fowl

Breeding station	A ₁	A ₂	A ₃
Isfahan	0.60	0.21	0.19
Mazandaran	0.28	0.05	0.67

Discussion

The PCR-RFLP analysis of the GH gene is effective in selection programs of poultry and also to estimate the genetic similarities and differences among different poultry breeds. The molecular data emerged as a useful tool to investigate birds with the great phenotypic plasticity. In this research, a 776 bp fragment was amplified in the GH locus. After digestion of this fragment with MspI restriction enzyme, A1 (414, 217, 125 bp), A2 (125, 147, 137, 267 bp) and A3 (237, 539 bp) alleles were revealed. Our findings were concordance to Ip *et al.* (2001) study.

Data obtained on gene and genotypic frequencies through the polymorphism study makes it not only possible to compare the gene stocks of animals, the possible effects of the genes on reproductive and performance traits, but also to study genetic variability under different environmental conditions of selection (Egena and Alao, 2014). The allele frequencies in the chicken flocks of breeding center of Isfahan and Mazandaran are shown in Table 2. In Isfahan native fowls, A1 allele showed the highest allele frequency (0.60) and A3 allele showed the lowest allele frequency (0.19). However, in Mazandaran native fowls, allele A3 showed the highest allele frequency (0.67) and the lowest frequency (0.05) was observed for allele A2. The differences in observed allelic frequencies between two native fowl populations could be due to difference in the selection pressure.

Ip *et al.* (2001) observed a significant difference between the allele frequency of Leghorn Hy-Line strain and that of other native Chinese chickens. A high frequency of allele A3 (0.95) and low frequency of allele A2 (0.00) was observed in Hy-Line chickens. Since the Leghorn Hy-Line strain has been selected for egg laying performance, they have suggested that either the absence of allele A2 or a high allele frequency of allele A3 could be linked to the laying performance.

The results of the current study indicated that intron 1 of cGH is polymorphic in Isfahan and Mazandaran native fowls. Therefore, the cGH gene could be exploited as a candidate gene for marker-assisted selection (MAS) in Iranian native fowls. Further studies are required to evaluate the association of production traits with cGH gene polymorphism in Iranian native fowl.

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