

Detection of Polymorphism at the Insulin Like Growth Factor-I Gene in Mazandaran Native Chicken using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Method

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Abstract: Problem statement: Molecular genetics selection on individual genes is a promising method to genetically improve economically important traits in chickens. The Insulin like Growth Factor-I (IGF1) gene may play important roles in growth of multiple tissues, including muscle cells, cartilage and bone. **Approach:** In the present study polymorphism of the promoter and 5' untranslated region of IGF-1 gene of Mazandaran native fowls was investigated. In order to evaluate the IGF-1 gene polymorphism we have used a Restriction Fragment Length Polymorphism (RFLP) method. Blood samples were collected from randomly chosen 100 Mazandaran native fowls. Genomic DNA was extracted using modified salting-out method and used amplified polymerase chain reaction technique. The promoter and 5' untranslated region of the fowl IGF-1 gene was amplified to produce a 621 bp fragment. The PCR products were electrophoresed on 2.5% agarose gel and stained by etidium bromide. **Results:** Then, they were digested of amplicon with PstI and revealed two alleles A and B. Data were analyzed using Pop Gene 32 package. In this population, AA, AB, BB genotype have been identified with the 25.88, 50.23, 23.89% frequencies. A and B alleles frequencies were 0.51, 0.49, respectively. The Chi-square (χ^2) test was significant and the population was in Hardy-Weinberg equilibrium ($p < 0.05$). **Conclusion:** The PCR technique amplified a DNA fragment of IGF-1 with 621 bp. The results of the RFLP analysis showed two fragment 257 and 354bp after restriction with enzyme with PstI that identify changes in 5' untranslated region. In according to action modes and importance of IGF-1, its polymorphisms can be related to economical traits such as body weight, muscle cells and bone.

Key words: Insulin-like Growth Factors (IGF1), native chicken, Average Daily Gain (ADG), gene polymorphism, molecular genetics, untranslated region, Hardy-Weinberg equilibrium

INTRODUCTION

Insulin-Like Growth Factors (IGF) consist of a family of polypeptide hormones structurally associated with insulin with multiple metabolic and anabolic functions (McMurtry *et al.*, 1997). The IGF1 gene may play important roles in growth of multiple tissues, including muscle cells (myocyte differentiation cell multiplication), cartilage (chondrocyte colony formation, alkaline phosphatase activity) and bone (osteoblast division and proliferation) (Zapf and Froesch, 1999). Intense genetic selection of broilers has successfully increased growth rate and breast muscle percentage. However, physiological disorders are occurring, such as increased obesity and decreased skeletal integrity (Deeb and Lamont, 2002). The IGF-I and IGF-II stimulate the proliferation, differentiation

and metabolism of myogenic cell lines from different species (Florini *et al.*, 1996). To simultaneously improve production and fitness traits, molecular markers associated with one or both sets of traits may be useful (McMurtry *et al.*, 1997). The IGFs have been shown to regulate body and muscle growth in chickens (Duclos, 1998). Several studies have shown that circulating IGF-I affects growth rate in poultry (Goddard *et al.*, 1988; Scanes *et al.*, 1989; Ballard *et al.*, 1990). In chickens divergently selected for high or low growth rates, mRNA levels in the high growth rate line than in the low there were significantly higher IGF1 growth rate line (Beccavin *et al.*, 2001). Tomas *et al.* (1998) showed that recombinant human IGF-I infusion in chickens enhanced growth and decreased carcass fat content. Associations of an IGF1 promoter polymorphism with Average Daily Gain (ADG) and

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feed efficiency were found in 2 genetically diverse Black Penedescenca chicken strains (Amills *et al.*, 2003). QTL by a genome scan, a QTL affecting BW at 6 wk has been found at 160 cM (confidence interval 114 to 180 cM) on chromosome 1 (Sewalem *et al.*, 2002). A QTL at 150 cM (confidence interval 100-182 cM) on chromosome1 affecting abdominal fat weight (AFW) has been detected in the same F2 cross (Ikeobi *et al.*, 2002). Duclos (1998) indicated that IGF-I stimulated glucose uptake, amino acid uptake and protein synthesis and inhibited protein degradation by satellite cell derived myotubes. In another experiment, a quality line selected for increased breast yield and decreased fatness had significantly higher circulating IGF-I concentration than the unselected control line (Tesseraud *et al.*, 2003). The IGF1 gene, therefore, was selected as a biological candidate gene to investigate growth, body composition, metabolic and skeletal traits in chickens. The objective of this study was to detection of polymorphism of the insulin like growth factor-I (IGF-1) Gene in Mazandaran native chicken using PCR-RFLP method.

MATERIAL AND METHODS

Animals and extraction of DNA: In this study whole blood samples were collected from 100 randomly chosen chickens, in Native fowls breeding station of Mazandaran located in the North of Iran. Approximately, 5 ml blood sample was gathered in EDTA tube and was transferred to -20 °C freezer. Genomic DNA was isolated by using DNA Extraction Kit and was based on Miller *et al.* (1988) method. Exon and intron region from 5' untranslated region of the IGF-1 gene amplified to a product of 621 bp using primers based on the sequence of the fowl. Spectrophotometer was used for investigating quality and quantity of DNA. The purity and concentration of DNA samples was estimated using UV-visible range spectrophotometer. DNA concentration was adjusted to 50 ng μL^{-1} before PCR amplification. All the DNA samples had the 260/280 OD ratios in the range of 1.8 to 2, indicating high purity. DNA was also examined by loading samples on 2.5% agarose gel and visualizing the band under gel documentation system.

PCR-RFLP for IGF-1 gene: The PCR primers (forward: 5'-GACTATACAGAAAGAACCAC-3'; reverse: 5'-TATCACTCAAGTGGCTCAAGT-3') for chicken IGF1 gene were used (Nagaraja *et al.*, 2000). The polymerase chain reaction for the IGF1 gene was performed using a buffer PCR1X, 200 μM dNTPs, 2

μM MgCl_2 , 10 pmol each primer, 0.15 U taq DNA polymerase, 200 ng genomic DNA and H₂O up to a total volume of 25 μL . 35 cycle of preliminary denaturation at 94° for 5 min, denaturation at 94°C for 45 s, annealing at 57°C for 45 s, extension at 72°C for 1 min and with a final extension step for 10 min at 72°C. The PCR products were separated by 2.5% (w/v) agarose gel electrophoresis. The amplified fragment of IGF-1 gene was digested with Pst I, 8 μL of PCR production with 1.5 μL buffer, 1U (0.5) of PstI and 11.5 μL H₂O up to a total volume of 15 μL , following the manufacturers instruction for 24 h at 37°C. The digestion products were electrophoresis on 2.5% agarose gel in 1X TBE and visualized by ethidium bromide staining for 1 h at 100 V. Individual PCR-RFLP fragment sizes in each sample were determined, based on standard DNA molecular weight markers for each gene, by viewing the banding pattern under UV light.

Statistical analysis: Pop Gene 32 package was used to calculate genotypic and allelic frequencies and to detect the state of population about Hardy-Weinberg equilibrium.

RESULTS

In order to study of polymorphism of the amplified fragment, we need the restricted enzyme that has cutting cite on the fragment. When, we have changes on the DNA fragment, we will show a different fragment in length or if we have a mutation on the cutting cites we would see the different number of fragment. There was one polymorphic PstI site in the 621 bp PCR product of IGF-1 gene. Sequence analysis of PstI site in the IGF-1 gene revealed a mutation at position 621 that was a C to T transition. If the enzyme cut the segment, two alleles (A and B) were observed, resulting in three genotypes. The PstI digests the allele A amplimer, but not allele B. The animals with both alleles were assigned as AB genotype, whereas those possessing only A or B alleles as AA or BB genotypes, respectively. Genotype AA showed two fragments of 364 and 257 bp, Genotype BB-one- band pattern fragment 621 bp and while AB animals displayed a pattern with all three-band fragments 621, 364 and 257bp (Fig. 1). The digestion of all samples showed breeder hens Mazandaran native fowls were polymorph for IGF-1 gene. Allele and genotype frequencies and the average heterozygosity in native fowls Mazandaran populations for the IGF-1 loci were resulted (Table 1).

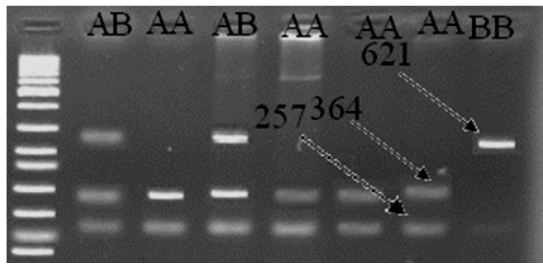


Fig. 1: Results of analysis PCR-RFLP for IGF-1 gene by restriction enzyme PstI on 2.5% agarose gel on ladder 100 bp (fermentas)

Table 1: Genotype distribution and allelic frequencies at the IGF-1 gene in breeder hens of Mazandaran native fowls breeding station (N=100).

Loci	Genotypes frequencies			Allelic frequencies		Average heterozygosity	χ^2
	AA	AB	BB	A	B		
IGF-1	25.88	50.23	23.89	0.51	0.49	0.491	2.71

DISCUSSION

A main goal of the animal breeder is to select superior animals for breeding. Screening favorable alleles for selection at the DNA level provides an ideal tool for marker-assisted selection. This result showed that there were polymorphisms in IGF-1 segment, as previously observed by Li *et al.* (2010). A and B allele frequencies were 0.51 and 0.49, respectively. The genotype frequencies within 100 chickens examined were 25.88 for AA, 23.89 for BB and 50.23 for AB. χ^2 test signify Hardy-Weinberg equilibrium in this population ($p < 0.05$). The observed and expected heterozygosity was 0.42 and 0.50, respectively. To confirm of accuracy of digestion, this process was performed twice. The low diversity and the different between effective and true allele number is due to more frequency of allele A compared to allele B that reduced frequency in this locus. This number is more, if there are more loci with same combination of alleles. Amelioration of Inducible Nitric Oxide Synthases, Insulin like Growth Factor-1 Gene Expression and Insulin Receptor Substrate-1 in Liver Amelioration of Inducible Nitric Oxide Synthase (Hala, 2010), shows The administration of L carnitine to rats fed high fructose diet mitigated the adverse effects of fructose load (insulin resistance) through the regulation of studied genes expression as well as insulin receptor substrate-1. Insulin Like Growth Factor-I Gene Polymorphism Associations with Growth, Body Composition, Skeleton Integrity and Metabolic Traits in Chickens (Zhou *et al.*, 2005). They showed The IGF1

gene was selected as a candidate gene to investigate associations of gene polymorphisms with growth, body composition, skeletal integrity and metabolic factors in F2 broiler-inbred line crosses. Effects of the polymorphism IGF-1 gene were surveyed on egg quality in Wenchang chicken (Li *et al.*, 2010). They showed that change in restriction site of PstI generated different restricted segments. Three genotypes and two alleles were seen in Wenchang fowls. The allelic frequencies of IGF-1 gene in Wenchang fowls were 0.53 and 0.47 for alleles A and B and genotypic frequencies were 0.32, 0.41 and 0.27 for genotypes AA, AB and BB, respectively. Results showed significance from Hardy-Weinberg equilibrium in population of Wenchang fowls. Association study revealed allele A had positive effect than allele B on economical traits. Obtained results from Mazandaran native fowls are approach with Wenchang fowls. The frequency of mutant allele is higher than wild type allele in Mazandaran native fowls. It could be due to physiological role of allele A in Iranian fowls. Inbreeding and family selection can be one of the major factors for enhancing of AA genotype in Mazandaran native fowls. Reared chickens in Mazandaran native fowls breeding station are prepared from state center and in addition it is a closed population and therefore open for disequilibrium factors. Inbreeding coefficient is high in the closed population that, in turn, caused to decreases of diversity in population. Increasing effective population size, controlling mating and preparing independent populations with large number of primitive individuals are necessary for preventing decrease of diversity in Mazandaran native fowls.

CONCLUSION

The PCR technique amplified a DNA fragment of IGF-1 with 621 bp. The results of the RFLP analysis showed two fragment 257bp, 354bp after restriction with enzyme with PstI that identify changes in 5' untranslated region. In according to action modes and importance of IGF-1, its polymorphisms can be related to economical traits such as body weight, muscle cells and bone.

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