

*Full Length Research Paper*

# Detection of polymorphism of the insulin-like growth factor-I (IGF-I) gene in Mazandaran native chicken using PCR-RFLP method

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**Molecular genetic selection on individual genes is a promising method to genetically improve economically important traits in chickens. The insulin-like growth factor-I (IGF-I) gene may play important roles in growth of multiple tissues, including muscle cells, cartilage and bone. In the present study, polymorphism of the promoter and 5' untranslated region of IGF-I gene of Mazandaran native fowls was investigated. In order to evaluate the IGF-I gene polymorphism, we used the 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 amplified polymerase chain reaction technique. The promoter and 5' untranslated region of the fowl IGF-I gene was amplified to produce a 621 bp fragment. The PCR products were electrophoresed on 2.5% agarose gel and stained by ethidium bromide. Then they were digested of amplicons with PstI, which revealed two alleles A and B. Data were analyzed using Pop Gene 32 software package. In this population, AA, AB, BB genotypes were identified with 25.88, 50.23 and 23.89% frequencies, respectively. Allele frequencies (A and B) were 0.51 and 0.49, respectively. The Chi-square ( $\chi^2$ ) test did not show deviation from Hardy-Weinberg equilibrium ( $P < 0.05$ ).**

**Key words:** Insulin-like growth factor-I (IGF-I) gene, polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), polymorphism, native chicken.

## INTRODUCTION

Insulin-like growth factors (IGF) belong to a family of polypeptide hormones structurally related to insulin with multiple metabolic and anabolic functions (McMurtry et al., 1997). The IGF-I 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 bones (osteoblast division and proliferation) (Zapf and Froesch, 1999). Intense genetic selection of broilers 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). Improving production and fitness

traits simultaneously, molecular markers associated with one or both sets of traits may be useful (McMurtry et al., 1997). The IGFs showed body and muscle growth regulation in chickens (Duclos et al., 1998). Furthermore, circulating IGF-I affects growth rate in poultry (Goddard et al., 1988; Scanes et al., 1989; Ballard et al., 1990). In chickens selected for high or low growth rates, mRNA level in the high IGF-I growth rate line was significantly higher than in low growth rate line (Beccavin et al., 2001). In addition, recombinant human IGF-I infusion in chickens enhanced growth and decreased carcass fat content (Tomas et al., 1998). Associations of an IGF-I promoter polymorphism with average daily gain (ADG) and feed efficiency were found in two genetically diverse Black Penedesenca chicken strains (Amills et al., 2003). A QTL affecting body weight at 6 weeks has been found at 160 cM (confidence interval 114 to 180 cM) on chromosome 1 (Sewalem et al., 2002). Another QTL at 150 cM (confidence interval 100 to 182 cM) on chromosome 1 affecting abdominal fat weight (AFW) has also been

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detected (Ikeobi et al., 2002). Duclos (1998) indicated that IGF-I stimulated glucose and amino acid uptake and protein synthesis, and inhibited protein degradation by satellite cell derived myotubes. Furthermore, 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). Therefore, the IGF-I gene 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 investigate the polymorphisms of the insulin-like growth factor-I (IGF-I) gene in Mazandaran native chickens using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method.

## MATERIALS AND METHODS

### Animals and extraction

In this study, whole blood samples were collected from 100 randomly chosen native chickens from the breeding station of Mazandaran, located in the North of Iran. Approximately, 5 ml blood per animal was gathered in EDTA and kept at -20°C in a freezer until use. Genomic DNA was isolated by using DNA extraction Kit, which was based on Miller et al. (1988) method. Exon and intron region from 5' untranslated region of the IGF-I gene amplified a 621 bp product using primers based on the sequence of the fowl. A 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<sup>1</sup> 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-I gene

The PCR for the IGF-I gene was performed using a buffer PCR 1X, 200 μM dNTPs, 2 μM MgCl<sub>2</sub>, 10 pmol each primer, 0.15 U Taq DNA polymerase, 200 ng genomic DNA and H<sub>2</sub>O up to a total volume of 25 μl. The PCR cycle profile comprised 35 cycles of preliminary denaturation at 94°C 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 a final extension for 10 min at 72°C. The PCR primers (forward: 5'-GACTATACAGAAAGAACCAC-3'; reverse: 5'-TATCACTCAAGTGGCTCAAGT-3') for chicken IGF-I gene were used (Nagaraja et al., 2000). The PCR products were separated by 2.5% (w/v) agarose gel electrophoresis. The amplified fragment of IGF-I gene was digested with PstI, 8 μl of PCR production with 1.5 μl buffer, 1 U (0.5) of PstI and 11.5 μl H<sub>2</sub>O up to a total volume of 15 μl, following the manufacturer's instruction for 24 h at 37°C. Products were visualized on 2.5% agarose gel and allele sizes in each sample were determined.

### Statistical analysis

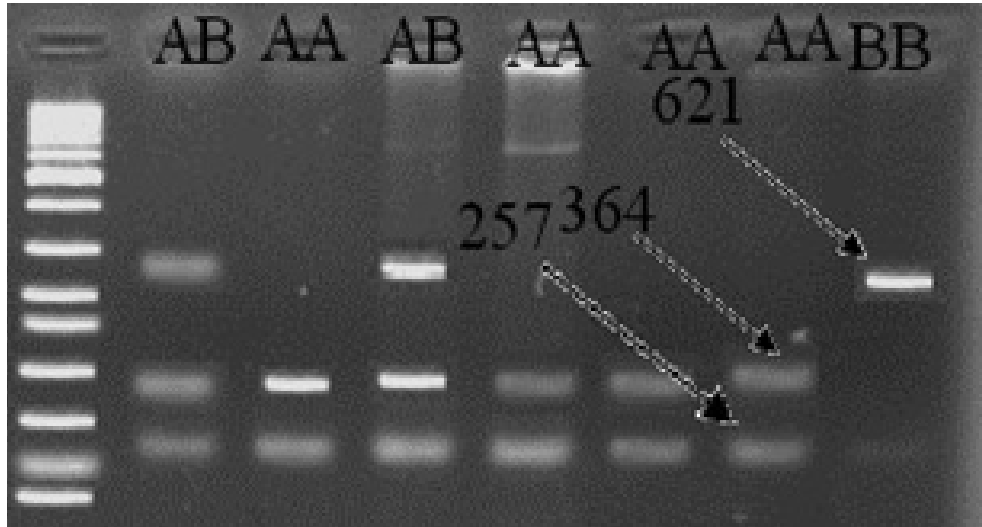
Pop Gene 32 software package (Yeh et al., 1999) was used to calculate genotypic and allele frequencies and to detect the state of population about Hardy-Weinberg equilibrium.

## RESULTS AND DISCUSSION

In order to study the polymorphism of the amplified fragment, we needed the restricted enzyme that has cutting site on the fragment. This is so that we could show a different fragment in length, or if we have a mutation on the cutting sites, we would see the different number of fragment. We found one polymorphic PstI site in the 621 bp PCR product of IGF-I gene. Sequence analysis of PstI site in the IGF-I gene revealed a mutation at position 621 (C/T) transition. When the enzyme was used to cut the segment, two alleles (A and B) were observed, resulting in three genotypes. The PstI digested the allele A amplicon, but not allele B. Those animals with both alleles were assigned the AB genotype, whereas those possessing only A the B allele were assigned the AA or BB genotypes, respectively. Genotype AA showed two fragments: 364 bp and 257 bp, genotype BB one band pattern fragment 621 bp, while AB animals displayed a pattern with all three band fragments 621 bp, 364 bp and 257 bp (Figure 1).

The digestion of all samples showed that the Mazandaran chickens were polymorph for IGF-I gene. Estimates of allele and genotype frequencies and the average heterozygosity for the IGF-I loci in Mazandaran native fowls populations are shown in Table 1. The results showed that there were polymorphisms in IGF-I segment, as previously observed by Li et al. (2010). The percentage of genotype frequencies were 25.88 for the AA, 23.89 for the BB and 50.23 for the AB. Gene frequencies for the A and B alleles were 0.51 and 0.49, respectively.  $\chi^2$  test confirmed Hardy-Weinberg equilibrium in this population ( $p < 0.05$ ). The observed and expected heterozygosity were 0.42 and 0.50, respectively. To confirm of accuracy of digestion, this process was performed twice. Hala (2010) showed that 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. IGF-I gene polymorphism was associated with growth, body composition, skeleton integrity, and metabolic traits in chickens (Zhou et al., 2005). They showed that the IGF-I 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.

Effect of the polymorphism IGF-I 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-I 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 show significance deviation from Hardy-Weinberg equilibrium in population of Wenchang fowls. Obtained results from Mazandaran native fowls are



**Figure 1.** PCR-RFLP results for IGF-I gene restricted by enzyme PstI on a 2.5% agarose gel and 100 bp allele ladder.

**Table 1.** Genotype distribution and allele frequencies of the IGF-I gene in the Mazandaran native fowls (N = 100).

Loci	Genotype frequency			Allele frequency		Average heterozygosity	$\chi^2$
	AA	AB	BB	A	B		
IGF-I	25.88	50.23	23.89	0.51	0.49	0.491	2.71

in approximation with Wenchang fowls. The frequency of mutant allele is however, higher than wild type allele in Mazandaran native fowls. This could be due to physiological role of allele A in Iranian fowls. Inbreeding and family selection could 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, thus leading to decrease 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-I with 621 bp. The results of the RFLP analysis showed two fragments (257 bp and 354 bp) after restriction with enzyme PstI that identified changes in 5' untranslated region. And according to the action modes and importance of IGF-I, its polymorphisms can be related to economical traits such as body weight, muscle cells and bone.

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