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Original article

ALLELIC POLYMORPHISM OF INSULIN-LIKE GROWTH FACTOR I GENE AND ITS ASSOCIATION WITH PRODUCTION TRAITS IN NATIVE CHICKENS

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Summary

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Insulin-like growth factor 1 (IGFI) is an essential regulator of growth, cell proliferation/differentiation and protein synthesis in a variety of cell types. *IGFI* is considered as one of the most important candidate genes controlling production and reproduction traits in chickens. This locus could be linked to the highly effective genes affecting egg production and growth traits. The aim of present study was to investigate the *IGFI* gene polymorphism and its association with growth and egg production traits in Iranian native chicken. A total of 313 blood samples were collected from the Native poultry breeding centre, Khorasan province, Iran. Single nucleotide polymorphism (SNP) of the IGFI 5'-UTR was detected by PCR-RFLP method and PstI restriction endonuclease enzyme. Finally, the SNP was confirmed by sequencing of RFLP profiles. Association between *IGFI* alleles and production traits was evaluated using multivariate regression analysis and GLM procedures. Two alleles A (PstI –) and B (PstI +) and three genotypes (A/A, A/B and B/B) were identified for the *IGFI* gene. Allele B was the most frequent (60.1%) and considered as reference allele for association study. A/B genotype was significantly correlated with lower first egg weight and higher egg laying intensity compared to B/B and A/A genotypes. No significant association was observed between *IGFI* genotypes and other production traits including egg weight, weight of sexual maturity and body weight. These results suggest that there is a possibility of *IGFI* genotypes acting as a genetic marker for selecting some egg production traits in native chickens.

Key words: chicken, IGFI, Iran, production traits

INTRODUCTION

Preserving native breeds is an essential concern for conservation of domestic animal genetic resources. Native breeds are usually small-size with low productivity, but have specific characteristics such as resistance to different diseases as a

result of exposure to pathogens and adaptation to the environment (Schou *et al.,* 2010; Chang *et al.,* 2011).

Production traits are multifactorial and controlled by the additive effects of multiple genes. Furthermore, health events and environmental factors could also influence these phenotypic traits (Li *et al.,* 2010; Bulut *et al.,* 2013). Nevertheless, the candidate gene approach could be used as a powerful method for evaluating the effect of contributing genes which are involved in the expression of quantitative differences between individuals in the population (Kwon & Goate 2000; Nagaraja *et al.,* 2000).

Candidate genes that are extensively studied in chickens are those of the growth hormone axis, because of the wide range of biochemical and physiological processes that they regulate. This axis mainly comprises growth hormone (GH), growth hormone releasing factor (GRF), GH receptor, insulin-like growth factor-I (IGFI) and insulin-like growth factor-II (IGFII) (Feng *et al.,* 1997).

IGFI is a single chain polypeptide comprising 70 amino acid residues, with three intra chain disulfide bridges. IGFI is an essential regulator of cell proliferation and differentiation, DNA/protein synthesis and growth stimulation in different species. It is associated with several phenotypic features in chicken including production and reproduction traits. *IGFI* gene could be linked to the quantitative trait loci (QTL) that control egg production, egg quality, body weight and reproductive traits in chicken (Nagaraja *et al.,* 2000; Kim *et al.,* 2004; Li *et al.,* 2010; Tang *et al.,* 2010). The purpose of this study was to evaluate the *IGFI* gene polymorphism and its association to production traits (body weight, egg production and sexual maturity) in Iranian native chickens.

MATERIALS AND METHODS

Experimental birds

Three hundred and thirteen whole blood samples were collected from the Native poultry breeding center, Khorasan province, Iran. Blood samples were taken from the ulnar vein and stored in vacuum tubes containing EDTA at –20 °C before examination. This population has been under selective breeding to improve egg production traits for nine generations. Twelve production traits measurements were recorded for each birds including: body weight at 1 day of age (BW1), body weight at 8 weeks of age (BW8), body weight at 12 weeks of age (BW12) , first egg weight (EW1), average egg weight at 28, 30 and 32 weeks of age (EW 28, EW30, EW32), average egg weight during the 84-day recording period (EW84), total egg number laid during the 84-day recording (EGGNO), egg laying intensity (EL-Inten), age and weight of sexual maturity (ASM, WSM). All applicable national and institutional guidelines for the care and use of animals were followed. Genomic DNA was extracted from whole blood using DNA Extraction Kit (Bioneer, Korea) and checked with spectrophotometry and electrophoresis.

PCR amplifications and genotyping of IGFI

A 621 bp fragment of 5' untranslated region (5'-UTR) of the *IGFI* gene was amplified using forward 5'-GACTATACA GAAAGAACCCAC-3' and reverse 5'-TCACTCAAGTGGCTCAAGGT-3' primers. The PCR reaction was carried out in a final volume of 25 μL containing: 50 ng of template DNA, 2 mM $MgCl_2$, 10 μM of each primer and 1 U Taq DNA polymerase (Fermentas, Germany). The applied cycles were denaturation at 94 °C

for 5 min, followed by 35 cycles of 45 s at 94 °C, 45 s at 57 °C, 60 s at 72 °C, and final synthesis at 72 °C for 10 min (Nagaraja *et al.,* 2000).

Single nucleotide polymorphism of the *IGFI* gene was identified by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) and PstI restriction endonuclease enzyme. As a final point, the IGFI SNP was confirmed by direct sequencing of each allele (ABI 3130 DNA sequencer, Applied Biosystems, Foster City, CA, USA).

Population genetic/statistical analysis

The observed (Na) and effective (Ne) allele numbers, genotype and allele frequencies, observed and expected heterozygosity (Ho and He, respectively) were estimated using POPGENE software version 1.32.0 (Levene, 1949; Yeh *et al.,* 1997). Genetic diversity was also measured by unbiased expected heterozygosity and number of alleles (Nei, 1973). Deviation from Hardy-Weinberg equilibrium (HWE) was also estimated using Fisher's exact test (Guo & Thompson, 1992).

Association between *IGFI* alleles and production traits including body weight (BW1, BW8 and BW12), egg weight (EW1, EW 28, EW30, EW32 and EW84), total egg number (EGGNO), age and weight of sexual maturity (ASM, WSM) and egg laying intensity (EL-Inten) were analysed using the following model:

$$Y_i=\mu+\Sigma\, b_j f_{ij}+\varepsilon_i$$

where Y_i: the dependent variable for specific trait in i^{th} chicken, μ: a general mean, f_{ij}: the copy number of the *IGFI:* j^{th} allele in the i^{th} chicken; b_j: half the substitution effect for the *IGFI* j^{th} allele; and ε_i: the residual effect for the i^{th} chicken with variance. For each allele, all individuals were considered as either non-carrier (0) or carrier (1) and then single band analysis was performed to determine the coefficient effect of each allele on each phenotype. The most frequent allele was considered as reference and the association study was evaluated using multivariate regression analysis and GLM procedures (SPSS v. 21).

RESULTS

The PCR-RFLP analysis with PstI enzyme revealed two alleles A (PstI –) and B (PstI +) and three genotypes: A/A (621 bp), A/B (621+ 257+ 364) and B/B (257+ 364) in 621 bp fragment of *IGFI* 5'-UTR (Fig. 1). Allele B and genotype A/B had the highest frequency in this population (60.1% and 61.98% respectively).

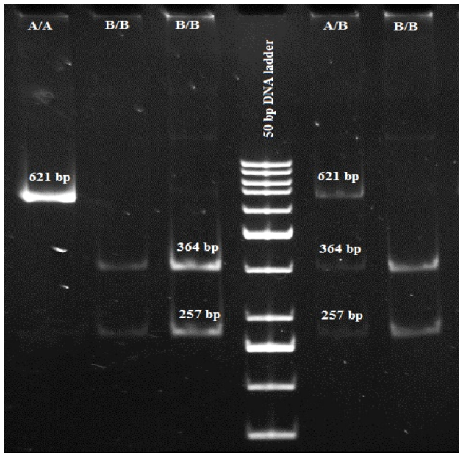

Fig. 1. PCR-RFLP pattern for 5´UTR of *IGFI* with PstI digestion on 10% polyacrylamide gel.

The allele and genotypic frequencies of IGFI-PstI are summarised in Table 1. Sequence analysis of three genotypes showed a C/T substitution at position 257, resulting in these polymorphisms. High

level of heterozygosity (62%) and deviation from Hardy-Weinberg equilibrium (P<0.0001) was also observed in this locus (Table 2).

Table 1. Genotype and allele frequency of IGFI-PstI in Iranian native chicken

Allele frequency (%)	Genotype frequency (%)	Genotype
A=39.9 B=60.1	8.95	A/A
	61.98	A/B
	29.07	B/B

Table 2. Observed and expected heterozygosity and hemozygosity in Iranian native chicken

Locus	IGFI
Sample size	626
Na	2
Ne	1.9245
Obs_Hom	0.3770
Obs_Het	0.6198
Exp_Hom	0.5188
Exp_Het	0.4812
HWE P-value	0.0000
Nei	0.4804

Na=observed number of alleles; Ne=effective number of alleles; Obs_Hom=observed homozygosity; Obs_Het=observed heterozygosity; Exp_Hom=expected homozygosity; Exp_Het =expected heterozygosity; Nei=expected heterozygosity as per Nei (1973). Expected homozygosity and heterozygosity were computed as per Levene (1949).

In association study, allele B was the most frequent allele and considered as a reference. Therefore, A/A and A/B genotypes were compared with B/B genotype for different production traits. The A/B genotype was significantly correlated with higher egg laying intensity (EL-Inten) (P=0.016) and lower first egg weight (EW1) (P=0.05). No significant association was found between IGFI genotypes and body weight at 1 day of age (BW1), body weight at 8 weeks of age (BW8), body weight at 12 weeks of age (BW12), average egg weight at 28, 30 and 32 weeks of age (EW 28, EW30, EW32), average egg weight during the 84-day recording period (EW84), total egg number laid for the 84-day recording period (EGGNO), age and weight of sexual maturity (ASM, WSM) (P>0.05) (Table 3).

DISCUSSION

Genetic diversity in chickens has evolved during domestication, selection for phenotypic traits, development of high productive breeds and interaction between host and pathogens (Weigend & Romanov, 2001; Izadi *et al.,* 2011). Selection based on the genetic factors has been considered as a practical approach for improving animal's production in recent breeding strategies. Linkage between a genetic marker and quantitative trait locus (QTLs) associated with special phenotypic trait could be used in marker assisted selection (MAS) programme. Based on it, genetic markers which are closely linked to these QTLs can be used in order to select and improve a special trait with high heritability (Chatterjee *et al.,* 2010; Bulut *et al.,* 2013).

Because of the important roles of insulin-like growth factors (IGFI and IGFII) in growth regulation and stimulation, these factors are considered as one of the most important candidate genes for controlling phenotypic traits (production and reproduction) in chickens (Nagaraja *et al.,* 2000; Kim *et al.,* 2004; Tang *et al.,* 2010).

IGFI is located on chromosome 1 within a linkage region where some QTLs controlling growth have been detected (Chatterjee *et al.,* 2010; Bulut *et al.,*

Table 3. Association of IGF1 genotypes with production traits in Iranian native chicken (vs the reference genotype B/B).

Production traits	Overall mean	*IGF1* genotypes	Allele effect	Standard error	P value
EW1 (g)	40.59±7.3	A/A	–0.563	5.98	0.926
		A/B	–4.31	2.22	*0.050*
EW28 (g)	48.75±3.9	A/A	5.82	6.62	0.39
		A/B	–2.32	2.46	0.35
EW30 (g)	49.66±3.95	A/A	5.75	8.16	0.49
		A/B	–0.30	3.04	0.92
EW32 (g)	50.90±3.66	A/A	6.63	6.05	0.28
		A/B	–1.68	2.25	0.46
EW84 (g)	48.16±3.6	A/A	0.46	5.25	0.93
		A/B	–0.76	1.95	0.70
EGGNO	48.74±14.91	A/A	10.23	11.71	0.39
		A/B	4.98	4.36	0.27
EL-Inten (%)	69.24±17.5	A/A	13.07	9.38	0.18
		A/B	9.37	3.49	*0.016*
ASM (days)	157.76±11.5	A/A	7.46	12.85	0.56
		A/B	1.70	6.39	0.79
WSM (g)	1762.80±175.9	A/A	31.47	182.38	0.86
		A/B	122.84	90.79	0.18
BW1 (g)	35.80±3.8	A/A	2.49	2.57	0.33
		A/B	0.78	1.17	0.50
BW8 (g)	680.62±134.3	A/A	74.06	74.09	0.32
		A/B	43.38	33.78	0.20
BW12 (g)	1284.25±246.3	A/A	–61.77	140.26	0.66
		A/B	8.95	63.94	0.88

EW1: first egg weight; EW28; EW30; EW32: average egg weight at 28, 30, 32 weeks of age; EW84: average egg weight during the 84-day recording period, EGGNO: total egg number laid during the 84-day recording period, EL-Inten: egg laying intensity, ASM, WSM: age and weight of sexual maturity; BW1, BW8, BW12: body weight at 1 day, 8 weeks, 12 weeks of age.

2013). Several studies have shown that SNP within the 5' flanking region of IGFI (the promoter region), is significantly associated with body weight, egg production, egg shell weight and quality (Lei *et al.,* 2005; Tang *et al.,* 2010).

In the present study, the PstI-digested PCR products of the IGFI gene revealed polymorphic fragments (257, 364, and 621 bp), which was inconsistent with the first report by Nagaraja *et al.* (2000). The results of the allele and genotype frequency for the IGFI gene in native Iranian population were in close agreement with Silkies Chinese native breed and Mazandaran native chickens (another Iranian native breed), but somewhat different from that reported for Korean native Ogal chickens (KNOC) (Kim *et al.,* 2004; Tang *et al.,* 2010; Abbasi & Kazemi, 2011).

The association of IGFI B/B genotype with higher egg weight in the Iranian native population was in great agreement with white Leghorn chicken (Nagaraja *et al.,* 2000). No genetic association of IGFI alleles with body weight and age of sexual

maturity were found, neither in Iranian nor in Leghorns population. Association of B/B genotype with higher egg production has also been reported for Korean native Ogal chickens. The results of this research showed that allele B was associated with a higher expression of IGFII in ovarian follicles. So, B/B genotype could be a regulatory factor of follicular development and egg production in Korean native chickens (Kim *et al.,* 2004). Tang *et al.* (2010) studied the association between IGFI gene polymorphism and body weight, age of sexual maturity, egg weight and number in two Chinese (Beijing and Silkies) populations. The A/B genotype and A allele were significantly associated with higher BW8, BW10 and BW13 than B/B in Silkies population (Kim *et al.,* 2004; Tang *et al.,* 2010). The different population genetic backgrounds, selection intensity and breeding aims might be the main causes for such differences across the populations.

In conclusion, SNP in *IGFI* locus was significantly associated with egg laying intensity (EL-Inten) and first egg weight (EW1) in the Iranian native population. Based on these results, *IGFI* gene could be considered as a genetic marker for selecting and improving quantitative traits in local chicken. These data would be beneficial for breed conservation and improvement of phenotypic traits.

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