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Novel single nucleotide polymorphism in intron 4 of TGF-β3 gene and it's association with production trait in Isfahan native fowl

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ABSTRACT

Transforming growth factor-β (TGF-β3) belong to the large family multifunctional growth factors that have prominent role in growth and development and homeostasis tissues. The object of this study was to recognition of single nucleotide polymorphism at fourth intron of TGF-β3 gene and studies its association with breeding value of some production trait in Isfahan native fowl. The traits were included12-week body weight, age at first egg, egg number and average egg weight. Breeding value of these traits were used to study relationship between new mutations and traits. One hundred and forty blood samples were collected from randomly chosen pedigree herd of Isfahan native hens and the DNA extraction was conducted. Amplification conducted by PCR reaction after designing primers. The PCR products were sequenced. Markers were detected by align and compare sequenced PCR product with the sequence obtained from gene bank (accession number X60091). Twelve new mutations were recognized for intron 4 of TGF-β3 in Isfahan native fowls involving four substitutions, four insertions, two inversion and two deletions. All of substitution, insertion and deletion were showed one-nucleotide alteration (SNP) but two inversions include one two-nucleotide and one four-nucleotide alteration. Without three nucleotide substitution in nucleotides numbers 2182, 2693 and 2706, all of other mutations were fixed at this population. The lowest frequency (%14.29) obtained at one thymine substitution in return for cytosine in nucleotide number 2182. Any significant difference was not observed between breeding values of traits at three unfixed SNP.

Key words: TGF-β3, Single nucleotide polymorphisms, association, native fowls

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INTRODUCTION

In recent decade, quantitative genetics approach with molecular genetics and advanced statistical procedures made a significant change in animal breeding. Molecular marker assisted selection may be required to increase selection efficiency and make further improvements in production performance [1]. Genetic markers linked with QTL allow for direct selection on genotype [2]. The candidate gene approach is a powerful method for finding the QTL responsible for genetic variation in the traits of interest in agricultural animal species [3]. Single nucleotide polymorphism (SNP) is one of the best genetic markers for this purpose. Many of these markers have recognized in growth related genes similar to insulin like growth factors (IGFs), chicken growth hormone (cGH) and so on [4-6].
Transforming growth factor β (TGF-β) subfamily, that also named cytokines, contains a group of structurally related polypeptide growth factors that have a prominent role in growth and development and homeostasis tissues in organisms. TGF-β subfamily molecules consist of four identified members in poultry that include TGF-β1, TGF-β2, TGF-β3 and TGF-β4 [7]. This group has many biological effect similar to effect on differentiation, proliferation and growth of cells, extra cellular matrix formation [8]. They also have immune functions such as myogenesis, chondrogenesis, osteogenesis, hematopoiesis, epithelial cell function [9]. Chicken *TGF*-β3 locus was physically located on chromosome 5. This gene contains seven exons and six introns spanning 16kb of the chicken genome

[10, 11]. This demonstrate that TGF-β3 stimulates the chondrogenic differentiation of chick limb mesenchymal cells in vitro, and is most effective during early stages of limb chondrogenesis [12] .

Study of polymorphism at TGF-β2, TGF-β3 and TGF-β4 genes were carried out by PCR-RFLP method. The TGF-β2 and TGF-β4 polymorphisms were associated with traits of skeletal integrity, such as tibia length, bone mineral content, bone mineral density, and the percentage of each measure to body weight. The TGF-β3 polymorphism was associated with traits of growth and body composition such as body weight, average daily gain, weight of breast muscle, abdominal fat pad and spleen, as well as the percentage of these organ weights to body weight and the percentage of shank weight and length to body weight [1]. In another study association of TGF-β2, TGF-β3 and three other genes were studied for response to salmonella entritidis in poultry with PCR-RFLP method. The result of this paper was shown that TGF-β3 polymorphism has significant effect on cecum bacterial load [13].

The objective of present study was to identify mutations in fourth intron of TGF-β3 gene and investigate their association with the growth and production traits of Isfahan native fowls.

MATERIALS AND METHODS

Experimental flock and sampling

The total of 140 blood samples was collected from randomly chosen native chickens from breeding station of Isfahan at central of Iran. This population has been established since 1980 to objective of conservation and extension the endangered population of native fowls in rural areas. Genetic improvement also conducted at this population for increasing productivity. For this purpose, the best of 100 cocks and 800 hens have selected among 6000 pedigreed and performance recorded birds and was used as parent of next generation. The extension part is continuously producing and distributing eight-week-old chicken among rural communities with the aim of increasing the population of native fowls in central provinces of Iran.

Blood samples were collected from each individual from wing vein in EDTA treated tube as an anticoagulant agent and were kept at -20°C in a freezer to be used in due course.

DNA Extraction

Genomic DNA was isolated by using DNA extraction Kit, which was based on Miller et al. (1988) method [14]. The quantity and quality of extracted DNA was checked by spectrophotometer and agarose gel electrophoreses, respectively. The extracted DNA samples were stored at -20°C until further use.

TGF-β3 gene amplification and SNP detection

Following primers were designed based on exon 3-6 TGF-β3 gene sequenced (Gene Bank accession number X60091) with help of Gene Runner software.

Forward: 5'- GGTGATAAGGAGCTGGGTT -3'
Reverse: 5'- CCCACGGCCATAGTCATC -3'

The PCR was performed in a total volume of 25 µl of the following mixture: 20 ng of genomic DNA, 2mM $MgCl_2$, 0.25 µM of each primer, 1 unit of Taq DNA polymerase, 200 µM of the mix of dNTP and standard reaction buffer. The thermal cycling conditions included an initial denaturation at 95°C for 5 min, followed by 30 cycles of 1min at 95°C, 1 min at 62°C and 1 min at 72°C, with a final extension step for 5 min at 72°C. PCR product was examined by loading in 1% agarose gel and observing the band under gel documentation system. PCR products were purified and sequenced (Macrogen co. Seoul, Korea).

Mutations were recognized by comparing sequenced samples and basic gene sequence that retrieved from Gene bank (accession number X60091) at BLAST program at web of NCBI* (National Center for Biotechnology Information) and clustalX2 software.

Statistical Analyzes

Allele of recognized SNP and breeding values of each trait for each of sampling animals arrived in EXCEL software from office 2010. Allelic frequencies were calculated by simple counting in excel (2010) software. Independent sample t-test method in SPSS program ver.19 was used for checking association between breeding value at these traits and SNP alleles.

* - www.ncbi.nlm.nih.gov

RESULTS AND DISCUSSION

SNP Detection

We successfully amplified a fragment of intron 4 of the TGF-β3 gene with 842 bp in length in Isfahan native chicken. We used the sequencing method in order to assess and detect mutations. In compare with obtained sequence at NCBI (Gene Bank accession number X60091), we identified 12 mutations at this region. These mutations include four one-nucleotide substitutions, four one-nucleotide insertions, two one-nucleotide deletions and two inversions. The inversions include one four-nucleotide inversion (4IV) from nucleotide number 2334 until 2337 and one two-nucleotide inversion (2IV) in nucleotides number 2461 and 2462. At marker of 4IV one cytosine and one thymine simultaneously was translocate from nucleotide numbers 2334 and 2335 to nucleotide numbers 2336 and 2337 and two guanidine from nucleotide number 2336 and 2337 was transferred to nucleotide number 2334 and 2335.this means that GGCT converted to CTGG. At marker of 2IV one cytosine and one guanidine were simultaneously translocate at nucleotide number 2461 and 2462 (CG→GC). Frequencies of these markers have shown in table no.1. We have fixed mutations at 7 SNP that respectively include: one guanidine deletion in nucleotide number 2190, one guanidine insertion in nucleotide number 2443, one guanidine substitution in return for adenosine in nucleotide number 2446, one adenosine insertion in nucleotide number 2448, one guanidine deletion in nucleotide number 2450, one guanidine insertion in nucleotide number 2456, one cytosine insertion in nucleotide number 2731 and also the mutations of 4IV and 2IV were observed in all individuals. We were observed three unfixed SNP that respectively include: one thymine substitution in return for cytosine in nucleotide number 2182 (S2182T/C), one adenosine substitution in return for guanidine in nucleotide number 2693 (S2693A/G) and one cytosine substitution in return for guanidine in nucleotide number 2706 (S2706C/G).

Table1-Single nucleotide polymorphism (SNP) detected by sequencing in intron 4 of chicken TGF-β3

SNP[1]	C→T	D G	4IV	I G	A→G	I A	D G	I G	2IV	G→A	G→C	I C	
Location	2182	2190	2334	2443	2446	2448	2450	2456	2461	2693	2706	2731	
%Frequency	14.29	100	100	100	100	100	100	100	100	23.81	52.38	100	

1- D means deletion, I mean insertion and IV means Inversion.

Association markers with breeding value of traits

We only calculated association of 3 unfixed SNP that include S2182T/C, S2693A/G and S2706C/G, with breeding value of traits. Association with other markers cannot calculate because frequency of new allele at these SNPs was fixed.

The association between these SNP and breeding values in 12-week Body weight (BVBW12), age at first egg (BVAFE), egg number (BVEN) and average egg weight (BVAEW) has calculated and inserted in table2 until table5, respectively. The statistical analyses were not showed significant difference between alleles at each locus for total traits. The maximum difference between alleles was shown in S2706C/G for BWAEW that new allele was shown higher breeding value but this difference was not significant (p-value= 0.092).

Table2-Association of SNPs in IGF1 gene with BVBW12 in Isfahan native hen

SNP	Allele	mean ± S.E	P-value
S2182T/C	T	230.25±3.80	0.874
	C	231.76±3.35	
S2693A/G	A	225.79±6.69	0.332
	G	233.34±3.78	
S2706C/G	C	235.24±5.76	0.241
	G	227.48±2.67	

Table3-Association of SNPs in IGF1 gene with BVAFE in Isfahan native hen

SNP	Allele	mean ± S.E	P-value
S2182T/C	T	-8.62±0.17	0.834
	C	-8.95±0.63	
S2693A/G	A	-9.53±0.8	0.518
	G	-8.71±0.66	
S2706C/G	C	-8.61±0.85	0.571
	G	-9.23±0.65	

Table4-Association of SNPs in IGF1 gene with BVEN in Isfahan native hen

SNP	Allele	mean ± S.E	P-value
S2182T/C	T	3.42±0.29	0.635
	C	3.1±0.27	
S2693A/G	A	3.04±0.28	0.805
	G	3.18±0.29	
S2706C/G	C	2.98±0.41	0.451
	G	3.33±0.20	

Table5-Association of SNPs in IGF1 gene with BVAEW in Isfahan native hen

SNP	Allele	mean ± S.E	P-value
S2182T/C	T	2.44±0.2	0.471
	C	2.70±0.14	
S2693A/G	A	2.85±0.13	0.401
	G	2.61±0.16	
S2706C/G	C	2.86±0.14	0.092
	G	2.43±0.2	

DISCUSSION

The candidate gene approach is one of the primary method for examine associations of gene polymorphisms with economically important traits in farm animals. In this study, we detected mutations in TGF-β3 gene and analyzed their association with economic traits in Isfahan native fowls. TGF-β is a multifunctional peptide that controls proliferation, differentiation and other functions in many cell types. Given the role that TGF-β super family genes play in growth and development, they are logical targets for investigation as candidate genes for economically important traits in chickens [1].

Twelve mutations were recognized in intron 4 of TGF-β3 gene with use of comparing sequenced data that involves 10 SNPs and two inversions. Only one SNP was detected in nucleotide number 2833 at fourth intron of TGF-β3 in other studies. At this mutation one adenosine was replaced with cytosine that was recognized by RFLP method [1, 13]. This approved that there was many difference between this native population and other breeds of chicken. The population of this study was a native population and this is possible that variation at this population was higher than eugenics population. Nie et al (2005) detested 46 SNPs in chicken growth hormone gene at four breeds. Thirty six of those SNPs were located on introns that eleven of these SNPs located on intron number 1 [15]. We recognized 9 fixed mutations that include 7 single nucleotide mutations and two inversions. Of course thirteen generation selection maybe fixed some alleles at this population. This maybe demonstrated that inbreeding increased at this population. The one detected marker with use of RFLP method (SNP at nucleotide number 2833) was fixed at one inbred synthetic white leghorn breed of poultry [16].

We also recognized three unfixed one-nucleotide mutation that any association was not obtained between these SNP and production trait at this study. Association between production traits and TGF-βs genes were studied in some researches [1, 13]. One detected SNP at nucleotide number 2833 was associated with some growth and body composition trait [1, 13]. Of course three of traits at this study were egg production traits and we can find any study about association TGF-β3 and egg production traits. This demonstrated for some animals that TGF-β superfamily are expressed by ovarian somatic cells and oocytes in a developmental, stage-related manner and function as intraovarian regulators of folliculogenesis [17]. TGF-β can simulate FSH receptor expression [18] and inhibin production [19]. Therefore, we suggested that TGF-β3 maybe affected on egg production traits. Xu et al (2011) have studied association between six candidate gene with age at first egg by use of PCR-RFLP method in one native chicken population of china and recognized significant effect at four markers[20].

CONCLUSION

In conclusion this study showed that there were rich polymorphisms in the intron 4 of chicken TGF-β3 gene. We also concluded alteration in fourth intron of TGF-β3 was not effected on egg production traits at this population. We suggest that alteration in other region of TGF-β3 gen and also other TGF-β genes at this population obtained and association of those with production trait are studied.

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