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Association of transforming growth factor-ß3 gene polymorphism with growth and body composition traits in Iranian commercial broiler lines

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Transforming growth factor-β (TGF-β) polypeptides are members of a large superfamily of growth and differentiation factors that regulate the proliferation and differentiation of a great variety of cell types. The current study was designed to investigate the associations of TGF-β3 gene polymorphism on chicken growth and body composition traits. Genomic DNAs were extracted from 400 chickens from four different commercial broiler lines. Genotyping for the TGF-β3 gene using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and *BsII* restriction endonuclease showed a mutation in 294-bp fragment located on the fourth intron of chromosome 5. Polymorphism in TGF-β3 gene was significantly (P < 0.1) associated with breast muscle weight (BMW), abdominal fat weight (AFW), wing weight (WINW), percentage of carcass weight (%CW), percentage of drumstick weight (%DW), percentage of breast muscle weight (%BMW), percentage of back weight (%BAKWT) and percentage of wing weight (%WINW). This research suggests that TGF-β3 gene could be a candidate gene that can affect some body composition traits in chicken.

Key words: Broiler lines, body composition traits, transforming growth factor beta 3 (TGF-β3), PCR-RFLP.

INTRODUCTION

Quantitative genetic methods effectively regard the animal as a black box with many genes contributing to

the expression of all traits under selection. Molecular genetics opens this black box by elucidating the effect of single genes on the phenotypic expression of traits (Bumstead and Palyga, 1992). The candidate gene approach is a powerful method for finding the quantitative trait loci (QTL) responsible for genetic variation in the traits of interest in agricultural animal species (Rothschild and Soller, 1997). Although, traditional selection for phenotypic values of broiler chickens has made significant improvements in growth rates and meat yields during the past half century, the high selection intensity for growth rate has caused many physiological disorders such as obesity, ascites, leg problems, as well as a reduction in overall immunocompetence (Dunnington and Siegel, 1996; Deeb and Lamont, 2002). Marker-assisted selection (MAS) can be used to increase selection

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Abbreviations: MAS, Marker-assisted selection; **QTL,** quantitative trait loci; **TGF-β3,** transforming growth factor beta 3; **BMPs,** bone morphogenetic proteins, **GDFs,** growth and differentiation factors; **PCR-RFLP,** polymerase chain reaction-restriction fragment length polymorphism; **BW,** live body weight; **CW,** carcass weight; **BMW,** breast muscle weight; **DW,** drumstick weight; **BAKW,** back weight; **WINW,** wing weight; **AFW,** abdominal fat weight; **SNP,** single nucleotide polymorphism.

efficiency and make further improvements in production traits. Genetic markers linked with QTL allow for direct selection of genotype (Lamont et al., 1996). Understanding the genetic control of growth in chickens will provide an opportunity for genetic improvement of production performance and physiology (Li et al., 2003).

Transforming growth factor beta 3 (TGF-β3) is a type of protein, known as a cytokine, which is involved in cell differentiation, embryogenesis and development (Sanders and Wride, 1997; Jakowlew et al., 1991). It belongs to a large family of cytokines called the transfor-ming growth factor beta superfamily, which includes the TGF-β family, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) as well as the inhibins and activins (Piek et al., 1999). The chicken TGF-β subfamily consists of four identified members: TGF-β1, TGF-β2, TGF-β3 and TGF-β4 (Burt and Law, 1994). Chicken TGF-β3 maps to chromosome 5 (Groenen et al., 2000). The biological activities of chicken TGF-β isoforms appear to be similar to those of mammals (Cogburn et al., 2000). The chicken TGF-ß3 gene consists of 7 exons, 6 introns and spans 16-kb of the chicken genome. Comparison of human and chicken TGF-ß3 genes reveals two regions of sequence conservation in the 5' end. First, an 87-bp region is centered on the major transcription start site within the human gene. Second, a 162-bp region is more strongly conserved than the first and located in a region corresponding to the reported human 5'-UTR (Burt et al., 1995).

This study was designed to elucidate the TGF-ß3 gene polymorphism in Iranian commercial broiler lines and to determine the associations between TGF-ß3 SNP with growth and body composition traits based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods.

MATERIALS AND METHODS

Chicken populations

Four different Iranian commercial broiler lines were used in the current study. All birds had free access to feed and water. The individuals were raised in floor pens and fed commercial corn-soybean diets that met the National Research Council (1994) requirements. Fifteen generation individuals (n = 400) was used in the present study. Live body weight (BW) was measured at 6 wk of age. Chickens were slaughtered, carcasses were eviscerated and dissected. Carcass weight (CW), breast muscle weight (BMW), drumstick weight (DW) back weight (BAKW), wing weight (WINW) and abdominal fat weight (AFW) traits were determined. All traits were expressed as percentage of BW at 6 weeks of age.

DNA extraction

Whole blood samples were collected from 400 chickens at 6 weeks of age. They were obtained from four different commercial broiler lines, which were selected for production and reproduction traits for 15 generations. Genomic DNA were extracted using salting-out method with some modifications (Javanrouh et al., 2006).

Development of PCR-RFLP assay

Genotyping was done using primers according to Li et al. (2003) as follows: (5ʹ TCA GGG CAG GTA GAG GGT GT 3ʹ; 5ʹ GCC ACT GGC AGG ATT CTC AC 3ʹ). 15 µl of each PCR reaction contained: 1X PCR buffer, 2 mM MgCl$_2$, 0.25 µM primers, 200 µM dNTPs, 1 unit of Taq polymerase, 150 ng/reaction genomic DNA and ddH$_2$O. The reaction conditions were 94°C for 3 min, 35 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min and extension of 72°C for 8 min. A single nucleotide polymorphism (SNP) of the TGF-ß3 gene was detected by digesting 10 µl of the 294-bp PCR product with *Bsl*I restriction endonuclease at 55°C overnight. Restriction patterns were visualized by agarose gel electrophoresis and ethidium bromide staining. Restrictied fragments were run on agarose gel and visualized by ethidium bromide staining.

Statistical analysis

Allele and genotype frequencies

The TGF-ß3 allele frequencies were calculated by simple allele counting (Falconer and Mackay, 1996). The possible deviations of allele and genotype frequencies from the Hardy–Weinberg equilibrium were examined with PopGene.S2 softwear by a Pearson's Chi-square test.

Association analysis

Data were subjected to ANOVA analysis using JMP performed by SAS 9.0 software with genotype (G), line (L) and sex (S) as fixed effects according to the model:

$$Y = \mu + G + L + S + e$$

Where, Y is the response variable, µ is the population mean and e is the random error. Significant differences between least-squares means of the different genotypes were calculated using a contrast test.

RESULTS

Allele frequency

The genotype and allele frequencies at TGF-ß3 loci calculated by PopGene.S2 software are shown in Table 1. The B allele was more frequent than A allele in three broiler lines (A, B and C), and therefore, most of the birds were homozygous for the B allele. The Chi-square test (P < 0.05) indicated that the genotype distributions were not in Hardy–Weinberg equilibrium.

Identification of polymorphism and PCR-RFLP analysis

The transition of C into A SNP at base 2833 from the fourth intron TGF-ß3 gene creates a restriction site for *Bsl*I endonuclease. The 294-bp fragment was digested with *Bsl*I restriction enzyme. The restriction enzyme *Bsl*I-digested PCR product had fragments of 145, 75 and 74

Table 1. Genotype and gene frequency of TGF-β3 gene in chicken population.

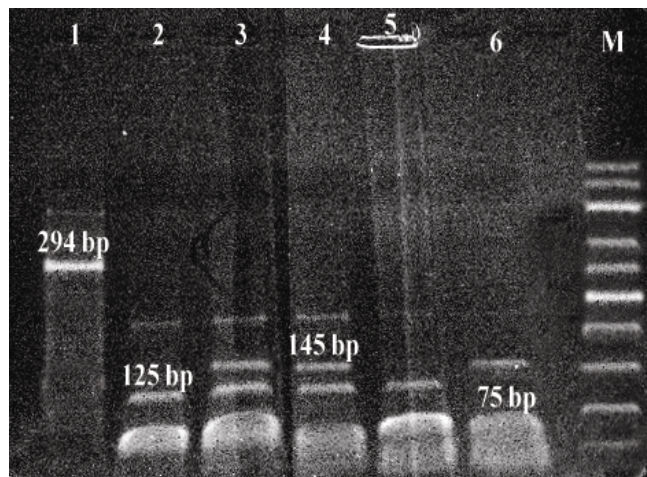| Population	Genotype frequency			Allele frequency		Chi square
	AA	AB	BB	A	B	
Line A	0.09	0.545	0.365	0.364	0.636	
Line B	0.053	0.617	0.329	0.361	0.639	P<0.05
Line C	0.255	0.480	0.265	0.495	0.505	
Line D	0.50	0.143	0.357	0.571	0.429	

Figure 1. PCR-RFLP pattern for TGF-β3 gene with *Bsl*I digestion. Lane 1, Undigested 294 bp PCR product; Lanes 2 and 5, BB genotype; Lanes 3 and 4, AB genotype; Lane 6, AA genotype and Lane M, molecular marker size.

bp for AA homozygotes, fragments of 145, 125, 75 and 74 bp for AB heterozygotes and 125 , 75, 74 and 20 bp for BB homozygotes (Figure 1).

Association of TGF-ß3 Gene SNP with body composition

The genotypes of the SNP of the chicken TGF-ß3 gene were used in the genetics analysis of the population. There were significant associations (P < 0.1) between the SNP and body composition traits (AFW, BMW, WINW, %CW, %DW, %BMW, %BAKW and %WINW). There were no significant associations (P > 0.1) between the SNP and BW6, CW, DW, BAKW and %AFW.

DISCUSSION

Identifying the QTL responsible for the economic important traits will facilitate poultry breeding programs. Molecular genetic information is required to enhance genetic improvement of animal species. The candidate gene approach is a very powerful method to investigate asso-

ciations of gene polymorphisms with economically important traits in farm animals (Rothschild and Soller, 1997). Many studies have examined growth, skeletal and immunity traits using the candidate gene approach in chickens (Zhou and Lamont, 2001; Amills et al., 2003; Li et al., 2005).

TGF-β is a multifunctional peptide that controls proliferation, differentiation and other functions in many cell types. The TGF-β genes influence the growth and differentiation of many different cell types and play an important role in processes such as myogenesis, chondrogenesis, osteogenesis, hematopoiesis, epithelial cell differentiation and adipogenesis (Burt and Law, 1994). Therefore, they are logical targets for investigation as candidate genes for economically important traits in chickens.

Previous studies showed a C/A mutation at base 2833 (GenBank accession no: X60091) in the fourth intron of the TGF-ß3 (Li et al., 2003). Although this mutation was not located at an identified protein-binding site, the polymorphism was associated with growth, gain, skeleton and body composition traits of growing birds. Therefore, using SNP, the fourth intron of the TGF-ß3 gene might have been closely linked with functional polymorphism in other regions of the TGF-ß3 gene or the other linked genes. Disagreement of the SNPs genotype frequencies with the Hardy-Weinberg equilibrium expectations tested indicated that TGF-ß3 gene frequency was non-significantly different between populations (P < 0.05). This may be due to the high selection program performed in population as meat chicken with similar gene frequency.

Growth and body composition are a comprehensive reflection of development of various parts of the chicken body, and its final expression is the result of interaction among genetic, nutritional and environmental factors (Scanes et al., 1984). Individuals with AB genotype showed significantly higher BMW, WINW, %BMW and %WINW than those of the AA and BB genotypes, and higher %CW, %DW and %BAKWT in birds with AA genotype than those of the AB and BB genotypes recorded. Also, higher AFW in BB genotype than those of the AA and AB genotypes was detected. This result was similar to results of Li et al. (2003). The allele association found in the present study was consistent with the selection history of broilers. Previouse study has not observed the association between the TGF-ß3 gene with WINW,

Table 2. Effect of TGF-β3 gene polymorphisms on growth and body composition.

Trait[1]	P-value	AA	AB	BB
BW6(g)	0.199	2462.4 ±29.95 a	2506.55±21 ab	2515.93±24.2 b
CW	0.61	1714.8±25.1 a	1725±17.3 a	1738.5±20.28 a
BMW	0.001	543.1± 10.05 a	579.3±6.9 b	570.12± 10.05 b
DW	0.135	505.16±7.9 a	489.55±5.43 a	496.53±6.35 a
WINW	0.03	195.23±2.7b	202.42±2.03a	200.86±2.37a
BAKW	0.39	381.39±5.8a	379.36±4a	384.23±4.76a
AFW	0.063	25.1±1.1a	25.33 ±0.83a	26.39±0.96b
%CW	0.076	0.69±0.003a	0.68±0.002b	0. 68±0.002b
%BMW	0.004	0.22±0.002b	0.23±0.001a	0.22±0.001b
%DW	0.0001	0.205±0.002a	0.195±0.001b	0.196±0.001b
%WINW	0.085	0.079±0.0007a	0.082±0.0004b	0.079±0.0005a
%BAKW	0.062	0.155±0.001a	0.152±0.001b	0.151±.001ab
%AFW	0.353	0.01±0.001a	0.01±0.001a	0.01±0.001a

[a,b]Means with no common superscripts differ significantly ($P < 0.1$); [1]BW6 (g), body weight at 6 week; CW, carcass weight; BMW, breast muscle weight; DW, drumstick weight; WINW, wing weight; BAKW, back weight; AFW, abdominal fat weight, %CW, carcass weight as percentage of BW at 6 wk of age; %BMW, breast muscle weight as percentage of BW at 6 wk of age; %DW, drumstick weight as percentage of BW at 6 wk of age; %WINW, wing weight as percentage of BW at 6 wk of age; %BAKW, back weight as percentage of BW at 6 wk of age; %AFW, abdominal fat weight as percentage of BW at 6 wk of age.

%WINW, %BAKWT and %CW. The current study is the first to report such a relationship between TGF-ß3 gene and these traits in the chicken. Results from the current study identified TGF-ß3 as a potential candidate gene of QTL that is useful in the selection of broilers to increase BMW, WINW, %BMW, %CW, %BAKWT, %WINW and %DW and reduce AFW of the chicken.

We showed that TGF-ß3 SNP has no significant ($P > 0.1$) effect on body weight at 6 weeks of age (BW6), CW, DW, BAKW and %AFW (Table 2). In contrast, Li et al. (2003) reported that SNP in TGF-ß3 gene significantly ($P < 0.1$) affected BW6 and %AFW.

In conclusion, the broiler chickens have undergone intensive breeding with so many objectives that should be simultaneously considered to reduce costs, improve health and product quality. Also, several traits such as fitness and growth traits have been included in selection indices. In addition to difficulty of measurement of these traits, the corrolations among them is complex. MAS can be an ideal option to improve selection programs. The results from the current study indicated that a SNP marker in the TGF-ß3 gene was associated with body composition traits in chickens growing to market weight and are therefore, a potential marker for molecular MAS programs in commercial broiler lines in Iran.

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