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Full Length Research Paper

Association between single nucleotide polymorphism of apoVLDL-II gene with growth and body composition traits in Iranian commercial broiler line

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Very low density apolipoprotein-II (apoVLDL-II) is a major polypeptide component of avian VLDL. The function of apoVLDL-II is the transport of neutral lipids (triacylglycerol) in the form of VLDL in the plasma. The apoVLDLII gene is dormant in embryos, chicks and roosters but can be activated by estrogen. The current study was designed to investigate the association of an apoVLDL-II 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 apoVLDL-II gene by using PCR-RFLP method and *SfcI* restriction endonuclease showed a mutation in 492-bp fragment located on the first intron. Polymorphism in apoVLDL-II gene was significantly ($P < 0.05$) associated with body weight at 6 week (BW6), carcass weight (CW), breast muscle weight (BMW), drumstick weight (DW) and wing weight (WINW). No significant difference was observed in the back weight (BAKW) and abdominal fat weight (AFW). This research suggests that apoVLDL-II gene could be a candidate gene that can affect some growth traits in chickens.

Key words: Broiler lines, growth traits, apoVLDL-II gene, association, polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP).

INTROUDUCTION

For the last 30 to 40 years, one of the business objectives of primary breeders was to select birds that deliver the best commercial performance (growth rate, feed conversion and meat yield) and economic return for their customers.

Although traditional selection for phenotypic values of broiler chickens has made significant improvements in growth rates and meat yields during the past half century; but now, the high selection intensity for growth rate has caused many physiological disorders such as obesity, ascites and 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 efficiency and make further improvements in production traits. Genetic markers linked with QTL allow for direct selection of genotype (Lamont et al., 1996). To improve production and fitness traits simultaneously, molecular markers associated with one or both sets of traits may be useful. Understanding the genetic control of growth in chickens will provide an opportunity for genetic

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Abbreviations: VLDL, very low density protein; **apoVLDL-II,** very low density apolipoprotein-II; **PCR-RFLP,** polymerase chain reaction-restriction fragment length polymorphism; **BW6,** 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; **MAS,** Marker-assisted selection; **QTL,** quantitative trait locus; **SNP,** single nucleotide polymorphism.

Table 1. Genotype and gene frequency apoVLDL-II gene in chicken populations.

Population	Genotype frequency			Allele frequency		Chi-square value
	AA	AB	BB	A	B	
Line A	0.00	0.247	0.752	0.1238	0.8762	P < 0.05
Line B	0.00	0.252	0.747	0.1262	0.8738	
Line C	0.00	0.000	1	0.0000	1	
Line D	0.00	0.260	0.739	0.1301	0.8699	

improvement of production performance and physiology (Li et al., 2003).

ApoVLDL-II is a major apolipoprotein (apo) in plasma very low density lipoprotein (VLDL) in laying hens and in estrogen-treated cockerels and roosters (Codina-Salada et al., 1983). It is produced exclusively in the liver and, in response to estrogen treatment, the mRNA for this protein increases markedly (Codina-Salada et al., 1983). ApoVLDL-II was the first vertebrate apolipoprotein studied by modern recombinant DNA techniques (Chan et al., 1980). It contains 82 amino acid residues with a single cysteine at residue number 75 (Jackson et al., 1977). ApoVLDL-II is an avian apolipoprotein with no mammalian counterpart. However, the structure of apoVLDL-II indicates that it is evolutionarily related to the mammalian apolipoproteins (Jackson et al., 1978). The apoVLDL-II gene has the same 4 exon / 3 intron structure and the protein contains the typical 11- and 22-residue internal repeats characteristic of the mammalian apolipoproteins (Li et al., 1988).

To explain the genetic control of growth of rapidly growing chickens, apoVLDL-II gene may be used as candidate gene for growth and body composition traits. A few studies have examined association between single nucleotide polymorphism of apoVLDL-II gene with growth and body composition traits in the chicken (Li et al., 2005; Musa et al., 2007). Therefore, the present study was developed to detect apoVLDL-II gene polymorphism in Iranian commercial broiler lines and evaluate the associations between apoVLDL-II SNP with growth and body composition traits based on PCR-RFLP methods.

MATERIALS AND METHODS

Phenotypic measurements

Live BW was measured at 6 weeks 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.

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 for15 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. (2005) as follows: (5' CCT CTA TGA CAT GGT TGC CT 3' and 5' ATG GGT TTG ACC CTG CTA TG 3'). Fifteen µl of each PCR reaction contained: 1X PCR buffer; 5 mM $MgCl_2$; 0.25 µM primers; 200 µM dNTPs; 1 unit of Taq polymerase; 150 ng/reaction genomic DNA and ddH_2o. Thermal cycling included initial denaturation at 94°C for 3 min, 35 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min and final extension at 72°C for 8 min. A single nucleotide polymorphism (SNP) of the apoVLDL-II gene was detected by digesting 10 µl of the 492-bp PCR product with *SfcI* restriction endonuclease at 37°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 apoVLDL-II 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 the GLM precedures of JMP (SAS Institute, 2000) with genotype (G), line (L), sex (S) and genoype nested within line [G (L)] as fixed effects according to the model:

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

here Y was the reponse variable, µ was population mean and e was the random error. Significant differences between least-squares means of the different genotypes were calculated using a contrast test. Significance was determined as $P < 0.05$.

RESULTS

Allele frequency

The genotype and allele frequencies at apoVLDL-II loci calculated by PopGene.S2 software are shown in Table1.

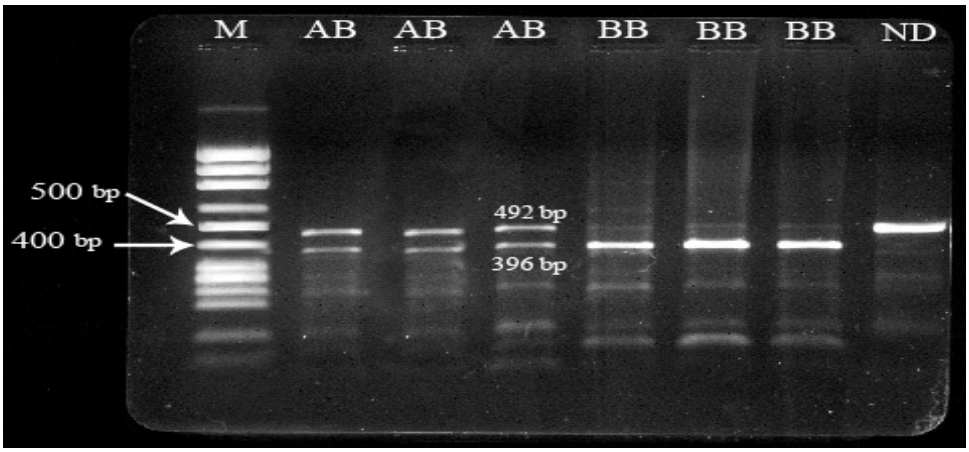

Figure 1. PCR-RFLP pattern for apoVLDL-II gene 5′-flanking region with *SfcI* digestion. AB = restriction fragment of 492 and 396 bp; BB = restriction fragment of 396 bp; ND = undigested 492 bp PCR product. M = 100 bp ladder.

Table 2. Effect of apoVLDL-II gene polymorphisms on growth and body composition.

Trait[1]	P-value	AB	BB
BW6(g)	0.0064	2601.3 ± 31.09 a	2519.14 ± 23.30b
CW	0.0019	1804.8 ± 15.8 a	1735.97 ± 11.20 b
BMW	0.0008	601.83 ± 5.2 a	575.02 ± 3.4 b
DW	0.0195	514.031 ± 3.5 a	496.7 ± 3.1 b
WINW	0.0028	208.424 ± 1.56 a	200.73 ± 1.2b
BAKW	0.0515	397.561 ± 2.8	386 ± 2.1
AFW	0.2668	27.93 ± 1.2	26.24 ± 0.86

[a,b]Means with no common superscripts differ significantly (P < 0.05). BW6(g) = Body Weight at 6 weekS; CW = carcass weight; BMW = breast muscle weight; DW = drumstick weight; WINW = Wing weight; BAKW = back weight and AFW = abdominal fat weight.

The B allele was more frequent than a allele in four broiler lines 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 G into A SNP in the position 5′-flanking region of the first intron apoVLDL-II gene creates a restriction site for *SfcI* endonuclease. The 492-bp fragment was digested with *SfcI* restriction enzyme. The homozygous genotype was defined as BB, heterozygous as AB (Figure 1). Genotype AA was not considered at analysis due to its very low frequency.

Association of apoVLDL-II Gene SNP with growth and body composition

Polymorphisms at apoVLDL-II loci were significantly (P < 0.05) associated with BW at 6 weeks of age and body composition traits (CW, BMW, DW and WINW) in these populations. It was high in the heterozygous genotype compared with the hemozygous. There were no significant associations (P > 0.05) between the SNP and BAKW and AFW in this population (Table 2). The interaction G X S and G X L were not significant for any trait and therefore were not included in the model.

DISCUSSION

Identifying the QTL which is responsible for the economic important traits will facilitate poultry breeding programs. Molecular genetic information is required to be used to enhance genetic improvement of animal species. The candidate gene approach is a very powerful method to investigate associations 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 (example, Zhou et al., 2001; Amills et al.,

2003; Li et al., 2003).

ApoVLDL-II is a major constituent of very low density lipoprotein and is involved in lipid transportation in chickens.

ApoVLDLII was the first apolipoprotein structural gene purified by molecular cloning (Wieringa et al., 1979; Chan et al., 1980).

Li et al. (2005) founded a G/A mutation at base 634 (GenBank accession no: V00448) in the first intron of the apoVLDLII gene. Although this mutation was not located at an identified protein-binding site, the polymorphism was associated with growth, gain and skeleton and body composition traits of growing birds. Therefore, SNP in the first intron of the apoVLDLII gene might, have been closely linked with functional polymprphism in other regions of the apoVLDLII gene or the other linked genes.

Disagreement of the of the SNPs genotype frequencies with the Hardy-Weinberg equilibrium expectations tested indicated that ApoVLDLII gene frequency was non-significantly different between populations ($P < 0.05$). This may be due to the high selection program done 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). Growth is under complex genetic control and considering the molecular mechanisms of growth will improve selection strategies in broiler chickens. In the present study, the apoVLDL-II SNP had an association ($P < 0.05$) with BW6 (g), CW, BMW and DW. This result was similar to results of Li et al. (2005) and Musa et al. (2007). In these populations, birds with AB genotype were significantly heavier than the BB genotypes ($P \leq 0.05$) for BW6 (g), CW, BMW, DW and WINW traits. The allele association is consistent with the selection history of broilers emphasized on increasing of market age. In this study, apoVLDL-II SNP was not significantly ($P > 0.05$) associated with BAKW and AFW. This was similar to results of Li et al. (2005). In contrast, Musa et al. (2007) reported that the studied SNP in apoVLDL-II gene significantly ($P < 0.05$) affect AFW.

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

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