Abstract

Growth traits are under the control of multiple genes. Understanding the genetic information of related genes is helpful for the selection and breeding course through marker assisted selection. The aim of the current study was to investigate the association of A287G SNP in BMPR-1B gene with growth traits in chicken. A single nucleotide polymorphism was identified in 240 individuals using the PCR-RFLP technique and confirmed by sequencing. The allelic and genotypic frequencies were compared, using the Chi-squared test. Associations between the genotype of each polymorphism and the traits were analyzed using the General Linear Model of statistical software SAS. Three genotypes (AA, AG and GG) were detected in Fayoumi and Rhodes Island Red chicken. Sequencing revealed one mutation (287 A \rightarrow G) in the genotype AA in comparison to the genotype GG. The A287G SNP of BMPR-1B gene was associated significantly with body weight at 2nd (\(P=0.022\)), 3rd (\(P=0.034\)), 4th (\(P=0.011\)), 5th (\(P=0.035\)), 6th (\(P=0.001\)), 7th (\(P=0.008\)) and 8th (\(P=0.016\)) week of age. In conclusion, BMPR-1B gene may be associated with body weight in chicken and may be considered in Marker Assisted Selection program to improve chicken growth performance.

Keywords: Chicken, BMPR-1B, SNP, Growth traits

Tavuklarda Kemik Morfogenetik Protein Reseptörü 1B (BMPR-1B) Genindeki Tek Nükleotid Polimorfizmi ile Büyüme Özellikleri Arasındaki İlişki

Özet

Büyüme özellikleri çok sayıda genin kontrolü altında Oldur. İlgili genlerden genetik bilgileri anlamak, marker-destekli seleksiyon yoluya seçilme ve üreme sürecinde yardımcı olur. Bu çalışma amacı, tavuklarda BMPR-1B genindeki A287G SNP ile büyüme özellikleri arasındaki ilişi araştırmaktır. PCR-RFLP teknigi kullanılarak, 240 bireyde bir tek nükleotid polimorfizmi tespit edildi ve sekanslama ile doğrulandı. Allellik ve genotipik frekanslar Ki-kare testi kullanılarak karşılaştırıldı. Her polimorfizm genotipi ve özellikler arasındaki ilişikler SAS istatistiksel yazılıının Genel Lineer Modeli kullanılarak analiz edildi. Fayoumi ve Rhoid Adası Kırmızı tavuklarında üç genotipi (AA, AG ve GG) saptandı. Sıralama, genotip GG'ye kıyasla genotip AA'da bir mutasyon (287 A \rightarrow G) olduğunu gösterdi. BMPR-1B geninin A287G SNP'si, vücut ağırlığı ile 2. (\(P=0.022\)), 3. (\(P=0.034\)), 4. (\(P=0.011\)), 5. (\(P=0.035\)), 6. (\(P=0.001\)), 7. (\(P=0.008\)) ve 8. haftalarda (\(P=0.016\)) önemli ölçüde ilişkilidi idi. Sonuç olarak, BMPR-1B geni tavuklarda vücut ağırlığı ile ilişkilidi olabilir ve Markör Destekli Seçim programında tavukların büyüme performansını artırmak için dikkate alınabilir.

Anahtar sözcükler: Tavuk, BMPR-1B, SNP, Büyüme özellikleri

INTRODUCTION

Growth and egg production traits of chicken are controlled by a series of major genes and/or quantitative trait loci (QTL). Analyses of genetic markers in animals could lead to discernment of the genetic architecture of quantitative traits. There are two basic methods of QTLs identification: approach of the candidate gene and whole-genome scanning [1,2]. The candidate gene approach is an effective method for finding QTLs responsible for genetic variation in the traits of interest in agricultural animal species and calibrating whether specific genes are associated to economic traits in farm animals [3]. Several trials have been concerned in the fields of association

İletişim (Correspondence)
+20 122 3668785, Fax: +20 552283683
mahmoudtarabany2887@yahoo.com; mmohamedibrahim@zu.edu.eg
analysis between candidate gene SNPs with animal growth and body composition traits [3,7].

Fayoumi is a native chicken breed originated in Egypt, reared for meat and egg production. It is adapted to subtropical environmental conditions and performed well under intensive management conditions [8]. Rhode Island Red (RIR) is a dual-purpose breed of American class. It is well adapted to the local environmental conditions [9]. Genetic polymorphisms are playing an increment important role as genetic markers in many sectors of animal breeding. As the molecular genetic techniques developed, it has become possible to obtain a new class of gene markers based upon the variability at DNA sequence level. Application of these molecular genetic markers potentially will greatly enhance the intensity of selection and will most efficiently uncover the productive potential of birds. Additionally, marker-associated selection (MAS) based on the studies concerned candidate genes and their effect on the phenotypic manifestations. An important intent of modern breeding in the poultry industry is to synthesis high-performance poultry lines and breeds in two main directions of productivity, meat and eggs [10].

The bone morphogenetic proteins (BMPs) are members of the transforming growth factor b (TGFb) superfamily. They are multifunctional proteins that regularize growth and differentiation in many cell types and play fundamental functions during embryogenesis and the fertility in mammals [11,12]. BMPs are potent inducers of cartilage and bone formation and has an important role in the bone healing process and in improving therapeutic efficacy [11]. In multipotential mesenchymal cells derived from equine adipose cells, BMP-2 increase under magnetic field conditions which affect the osteogenic properties of the cells and enhance vascularization process [13]. A non-conservative substitution (Q249R) in the BMPR-1B sequence was related with the proliferation characteristics of some ewe breeds [14]. In the chicken ovary, granulosa cells are major target for BMPs and it was proposed that mRNA levels for BMPR-1B in granulosa cells are higher than in theca cells [15].

The objectives of the present study were to detect single nucleotide polymorphisms (SNPs) of BMPR-1B gene in Fayoumi and RIR chicken by PCR-RFLP and sequencing. In addition, investigating the association between these SNP and growth traits in chicken.

**MATERIAL and METHODS**

This study was carried out in accordance with the Zagazig University Animal Ethics Committee guidelines (ANWD-206), at the Biotechnology unit belonging to Department of Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University.
minimize exposure of the DNA to UV light. The minimum agarose slice was transferred to a 1.5 ml micro centrifuge or screw cap tube and then purified by using a commercially available gel extraction kit (Fermentas, Thermo Fisher Scientific, USA). Samples were labeled and sent for sequencing. Sequencing was done by European Custom Sequencing Centre (GATC Biotech AG, Germany) using both forward and reverse primers of PCR amplification. The obtained sequences were edited manually using ChromasLiteVer. 2.01, (http://www.technelysium.com.au/chromas.html) and aligned with Clustal Omega software to identify nucleotide polymorphism.

**Statistical Analysis**

All statistical procedures were performed using SAS statistical system package V9.1 [19]. Allelic and genotypic frequencies of the single nucleotide polymorphism (SNP) were calculated and Chi-Square test was performed to examine Hardy-Weinberg equilibrium. Marker-trait association analysis was conducted using the one-way analysis of variance (ANOVA) through the general linear models (GLM) procedure. The comparison of means was carried out with Duncan's multiple range tests.

**RESULTS**

**PCR Amplification**

Genomic DNA of the two chicken breeds was amplified using specific primers for *BMPR-1B* gene. PCR products were detected by running a 1.5% agarose gel electrophoresis (Fig. 1). The amplified products (581 bp)
were consistent with the target fragments and had a good specificity, which could be directly analyzed by RFLP and sequencing.

**RFLP Analysis**

The PCR-RFLP method was developed successfully for genotyping the A287G SNP in intron 6 of the chicken *BMPR-1B* gene, where all individuals have been screened. Three genotypes of AA, AG, and GG were detected and confirmed by sequencing (Fig. 2 A,B). The fragment sizes of 581 bp for the GG genotype, 294/287 for the AA genotype, and a combination of 581, 294 and 287 bp for AG genotype.

**Genotyping and Frequencies**

Allele and genotype frequencies of *BMPR-1B* gene were calculated within each breed (Table 1). The frequencies of GG genotype in Fayoumi and RIR chickens were 0.36 and 0.28, respectively; which obviously greater than AA frequencies. Therefore, the allele G was predominant in the populations (0.63 and 0.58, respectively). Chi-test showed that two chicken breeds were not in Hardy-Weinberg equilibrium, in which genotype frequencies had been distorted by recent selection, mutation, or migration.

### Table 1. Frequency of genotypes and alleles of *BMPR-1B* gene in Fayoumi and RIR chicken breeds

<table>
<thead>
<tr>
<th>Breed</th>
<th>Genotype Frequency (n)</th>
<th>Allele Frequency</th>
<th>x²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
</tr>
<tr>
<td>Fayoumi</td>
<td>0.09 (11)</td>
<td>0.55 (66)</td>
<td>0.36 (43)</td>
</tr>
<tr>
<td>RIR</td>
<td>0.12 (14)</td>
<td>0.60 (72)</td>
<td>0.28 (34)</td>
</tr>
</tbody>
</table>

¹ Significant at level (P<0.05), ² Significant at level (P<0.01)

**Association of the *BMPR-1B* Genotypes with Growth Traits**

The least squares means of body weight according to different genotypes of *BMPR-1B* gene in chicken populations were presented in Table 2. The A287G SNP of *BMPR-1B* gene is associated significantly with body weight at 2nd (*P*=0.022), 3rd (*P*=0.034), 4th (*P*=0.011), 5th (*P*=0.035), 6th (*P*=0.001), 7th (*P*=0.008) and 8th (*P*=0.016) week of age. Heterozygous genotype AG had a higher body weight than GG genotype over the whole experimental period. The clear significant differences between heterozygous AG genotype and AA genotype were detected ultimately at 7th and 8th week of age.

### Table 2. Least squares means of body weight (g) according to genotypes at the SNP A287G of BMPR-1B gene

<table>
<thead>
<tr>
<th>Traits</th>
<th>Genotypes</th>
<th>RSD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW Day old (g)</td>
<td>AA 28.7</td>
<td>AG 28.6</td>
<td>GG 27.2</td>
</tr>
<tr>
<td>BW 1st wk (g)</td>
<td>71.3</td>
<td>73.9</td>
<td>68.3</td>
</tr>
<tr>
<td>BW 2nd wk (g)</td>
<td>150.1 ²</td>
<td>156.8 ³</td>
<td>126.6 ²</td>
</tr>
<tr>
<td>BW 3rd wk (g)</td>
<td>235.2 ³</td>
<td>242.9 ³</td>
<td>200.3 ²</td>
</tr>
<tr>
<td>BW 4th wk (g)</td>
<td>320.4 ³</td>
<td>328.7 ³</td>
<td>266.6 ²</td>
</tr>
<tr>
<td>BW 5th wk (g)</td>
<td>382.5 ⁴</td>
<td>398.6 ⁴</td>
<td>327.2 ²</td>
</tr>
<tr>
<td>BW 6th wk (g)</td>
<td>477.4 ³</td>
<td>499.2 ³</td>
<td>412.7 ³</td>
</tr>
<tr>
<td>BW 7th wk (g)</td>
<td>571.2 ³</td>
<td>677.4 ³</td>
<td>518.3 ³</td>
</tr>
<tr>
<td>BW 8th wk (g)</td>
<td>673.3 ³</td>
<td>773.8 ³</td>
<td>606.7 ³</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The primary objectives of the current study were to detect single nucleotide polymorphisms (SNPs) of *BMPR-1B* gene in Fayoumi and RIR chicken by PCR-RFLP and sequencing. Growth and body composition are an inclusive 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 [20]. Growth is under complex genetic monitoring, and uncovering the molecular mechanism of growth
results in more efficient selection for growth in broiler chickens [21]. Identifying the QTL will facilitate poultry breeding programs for the economic important traits. Molecular genetic information is required to be used to consolidate genetic improvement of animal species. The candidate gene approach is a very powerful method to examine associations of gene polymorphisms with economically important traits in farm animals [22]. Application of breeding programs that utilize marker-assisted selection requires advances in some areas like detection and estimation of associations of identified genes and their genetic markers with economic traits. Phenotypic evaluation is critical to establish marker-assisted associations or carry out the candidate gene validations required to conduct MAS [23]. Up to now, majority of association studies, especially in chicken, have been performed using phenotypic information.

The A/G transition at the base position of 287 in BMPR-1B gene was investigated in two chicken breeds. The allele frequency of G was higher than that of A in those two breeds and was in the range that reported by Zhang et al. [18] in Zang chickens. While the allele frequency of A was higher than that of G in three Chinese native chickens, a synthetic broiler line [18] and Mazandaran native chicken [24]. Our results showed that A287G SNP of chicken BMPR-1B gene is associated significantly with body weight from the 2nd till the 8th week of age. On the contrary, Zhang et al. [18] and Niknafs et al. [24] recorded non-significant association between BMPR-1B gene and growth traits. Zhang et al. [18] stated that A287G SNP of chicken BMPR-1B is associated with egg production from 47 to 56 weeks. Previous studies showed that BMPR-1B gene as a well known effective gene for reproductive traits [16,25].

Chicken BMPR-1B mRNA sequences were first identified by Sumitomo et al. [26] and Lim et al. [27]. Lim et al. [27] reported that BMP signaling, including BMPR-1B, is involved in chick diencephalic development, and the expression level of BMPR-1B reduced in the theca of chicken ovary from F1 to F3 follicles. Onagbesan et al. [17] proposed that BMPR-1B is possibly concerned in follicular differentiation and maintenance of the follicular hierarchy. Therefore, the expression level or the activity of BMPR-1B in the granulosa and/or theca of chicken ovary may be associated with oocyte maturation.

BMPR-1B as a member of the transforming growth factor β (TGF-β) receptor superfamily played the important actions in signal transduction. The current model of induction of signalling responses is at the cell surface, the ligand binds a complex of transmembrane receptor serine/threonine kinases (types I and II) and incites transphosphorylation of the Gly-Ser (GS) segments in the type I receptor by the type II receptor kinases. The consequently activated type I receptors phosphorylate selected Smads at C-terminal serines, and these receptor activated Smads (R-Smads) then form a complex with a common Smad4. Energetic Smad complexes translocate into the nucleus, where they regularize transcription of target genes, through physical interaction, CBP or p300 coactivators and functional cooperation with DNA-binding transcription factors (X) [28].

In conclusion, the broiler chickens have been subjected to 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 have been included in selection policies. In addition to difficulty of measurement of these traits, the correlations among them are complex. MAS can be a perfect option to improve selection programs. The results from the current study indicated that a SNP marker in the BMPR-1B gene was associated with growth traits in chickens, therefore, a potential marker for molecular MAS programs in chicken. However, the conclusion was only preliminary; it was worth increasing the number of chicken breeds, and expanding the number of samples to make in-depth study.

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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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