Association of the melanocortin-3(MC3R) receptor gene with growth and reproductive traits in Mazandaran indigenous chicken

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Abstract  Melanocortin-3 receptor (MC3R) plays an important role in the central control of energy homeostasis, and several functional polymorphisms of this gene have been detected. We have studied MC3R as a candidate gene responsible for variation in economically important traits in the chicken. To determine the association between MC3R polymorphism and phenotypic variation, a total of 190 individuals from breeding station of Mazandaran indigenous chicken was genotyped using a modified PCR-RFLP method. The association of growth and reproductive traits was studied using a generalized linear model. The association analysis suggested a positive effect of genotype AA with average egg weight at ages 28 (EW28), 30 (EW30) and 32 (EW32) weeks compared with the GG genotype (P<0.05). The association results also showed a positive effect of genotype AG with average egg weight at age 30 (EW30) weeks compared with the GG genotype (P<0.05). In addition, the breeding values of AG genotype for average egg weight at age 32 (EW32) weeks and average egg weight at ages 28 (EW28), 30 (EW30) and 32 (EW32) weeks were higher than the GG genotype (P<0.05). The findings form a basis for further analysis of the relation between genetic variations in MC3R gene and economically important traits, and application of molecular markers in poultry breeding.

Keywords: production traits, chicken, MC3R, PCR-RFLP


Introduction

Genetic diversity in indigenous breeds is a major concern considering the necessity of preserving what may be the precious and irreplaceable richness, regarding new productive demands. Conservation should be based on a deep knowledge of the genetic resources of a specific breed. Therefore, it is important to try to characterize genetically indigenous breeds (Shojaei et al., 2011). A species without enough genetic diversity is thought to be unable to cope with the changing environment or evolving competitors and parasites, and the ability of a population to respond adaptively to environmental changes depends on the level of genetic variability or diversity it contains. Thus, studies of population genetic diversity and the structure of population within and between species may not only illustrate the evolutionary process and mechanism but also provide information useful for biological conservation of animals (Notter, 1999; Askari et al., 2011). Molecular markers are increasingly used for the study of genetic diversity of populations in recent years (Zietkiewicz et al., 1994; Zamani et al., 2013). The leptin–melanocortin system is an important regulator of energy balance through its effect on energy intake and energy expenditure (Coll et al., 2007; Seeley et al., 2004). The melanocortins, a family of peptides produced from the post-translational processing of proopiomelanocortin (POMC), regulate the ingestive behavior and energy expenditure, and elicit diverse biological effects by binding to a distinct family of G protein-coupled receptors with seven transmembrane domains (Cone, 2005). From the five cloned melanocortin receptors, two (MC3R, MC4R) have been identified as important downstream effectors regulating energy homeostasis in response to neuropeptides secreted by POMC and the agouti-related peptide (AgRP) neurons (Cone, 2005). All melanocortin receptors (MC3R) have been isolated in the chicken, and each chicken MCR subtype has a different pattern of tissue expression and function. Recently, several studies in an-
Some researchers reported that MC3R homozygous for knockout mutations of the MC3R gene had increased body fat with a reciprocal decrease in lean mass, not caused by increased food intake but arose from increased feed efficiency (Butler et al., 2000; Chen et al., 2000). The chicken MC3R is a protein with 325 amino acids sharing 75.3-76.8 identity with the mammalian MC3R (Takeuchi and Takahashi, 1999). Association between polymorphism in MC3R gene and obesity has been detected in humans (Civanova et al., 2006).

Archaeological excavations confirmed the presence of the domestic fowl in the territory of Iran at the ancient times (Mohammadabadi et al., 2010). It is known that Persian chickens from the Gilan Province took part in the origin of the Russian Orloff breed (Mohammadabadi et al., 2010). Since 1981, twelve chicken breeding centers were established for reproducing native poultry varieties, and a total number of chickens they maintain are about 8000 birds. Currently, there are eight breeding centers in Fars, West Azarbaijan, Isfahan, Mazandaran, Khorasan, Yazd, Zanjan and Khuzestan provinces (Mohammadabadi et al., 2010). Research on native chicken populations of Iran has been initiated, and the data on the genetic variability of different loci in these populations have been published (Esmailkhalian et al., 2004; Mohammadabadi et al., 2010; Mohammadifar et al., 2013 ; Moazeni et al., 2016). However, data on genetic variability of MC3R locus in Iranian native chickens, especially in Mazandaran indigenous chicken have not been published. Therefore, the objective of this study was to identify the single nucleotide polymorphism (SNP) in MC3R in Mazandaran indigenous chicken, which would form a solid basis for further study on associating them with reproductive traits.

Material and methods

Experimental population and sampling

Breeding station of Mazandaran Indigenous Chicken is located at 28 km far from Sari, the provincial capital of Mazandaran state, located in the north of Iran. In 1986, around 5000 cocks and hens were purchased from rural regions across the Mazandaran province and kept in a quarantine farm for one year. From those, about 2500 birds of two sexes were kept to produce hatching eggs and the chicks produced from these eggs were transferred to the station in 1988. Since then the birds have been individually tagged and trap nest has been used for pedigree recording (Moazeni et al., 2016). Parents of each generation (about 100 cocks and 800 hens) are selected among 6000 pedigreed and performance recorded birds produced each generation. In August 2009, a total of 205 blood samples from Mazandaran indigenous chicken including 10 males and 195 females were collected. Individuals were reared in native chicken breeding station of Mazandaran and they belonged to generation 17 of the breeding station pedigreed animals. Individuals of this generation were developed by crossing 80 sires and 751 dams from generation 16. Approximately 1 mL blood per chick from the wing vein was collected and kept in a tube containing anticoagulant EDTA (ethylenediaminetetraacetic acid). All samples were transferred to the laboratory in an ice box. The genomic DNA was extracted from white blood cells using a standard salting out procedure described by Mohammadabadi et al. (2009). The DNA samples were dissolved in TE (Tris-EDTA) buffer which was made from 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA (pH 8.0) and stored at 20°C until use.

Primer synthesis and PCR–RFLP reactions

The primers were designed on the basis of DNA sequence of the MC3R (accession number: AB017137) using the oligonucleotide design tool Primer 5.0 software (F: 5’-CATGATTGCAATCTGTAGCACC-3’ and R: 5’-GATGCAGAGATCCCCGATGAG-3’). PCR reactions were performed in a 20 µl mixture containing 10 pmol primers, 200 IM dNTP (deoxyribonucleotide triphosphate), 2 µl 10X reaction buffer which contained 1.5 mM MgCl₂, 1 unit of Taq-DNA polymerase (Promega, Madison, WI), and 50 ng genomic DNA as template. PCR method was used to optimize the reaction accuracy: 94°C for 5 min, 35 cycles of 94°C for 30 S, annealing at 60°C for 60 S, 72°C for 60 S, and a final extension at 72°C for 7 min. PCR products were electrophoretically separated on 2% agarose gel (5 V/cm) and stained with ethidium bromide. PCR products were digested by 10 units of MSPI restriction enzymes (Fermentase, Lithuania), 6 ml of PCR product, 1.4 ml of Tango buffer and 2 ml nuclelease-free water. The final volume of 10 ml was incubated in 37°C for 12h. The fr-
of frequency was calculated as \((p(1-p)/2n)^{1/2}\), where \(n\) is the sample size, \(p\) is the frequency of A or G allele. Marker-trait association analyses were conducted using model 2 in GLM procedure of SAS9.1 software. The significant differences of least squares means were tested with Tukey–Kramer’s multiple range tests, and a P-value of \(\leq 0.05\) was considered statistically significant.

\[ Y_{ijk} = \mu + M_i + e_{ijk} \] (2)

where, \(Y_{ijk}\) is the estimated breeding values of the trait, \(\mu\) is the population mean, \(M_i\) is the fix effect of genotype, and \(e_{ijk}\) is the residual random error.

**Results**

**Genotyping results**

Table 1 shows the genotypic and gene frequency of \(MC3R\) gene and statistical description of data set is presented in Table 2. Genotypes of individuals were investigated by PCR-RFLP (Figure 1).

**Table 1. Genotypic and allelic frequency of \(MC3R\) gene**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
<th>Frequency</th>
<th>Allele</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>175</td>
<td>0.92</td>
<td>G</td>
<td>0.95</td>
</tr>
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<td>AG</td>
<td>10</td>
<td>0.05</td>
<td>A</td>
<td>0.05</td>
</tr>
<tr>
<td>AA</td>
<td>5</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>190</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Figure 1. The electrophoretic gel patterns of the MSPI PCR-RFLP. Lane 1 (M) is Ladder, lane 2 (PCR) is the amplified gene fragment, lanes 3 and 4 are homozygote GG, lanes 5 and 6 are heterozygote and lanes 7 and 8 are homozygote AA.](image)
**Polymorphism in chicken MC3R gene**

The entire nucleotide coding regions of MC3R, consisting of a single exon, amplified by using direct PCR, was polymorph (3 genotypes GG, GA and AA were observed). In the MC3R gene, there is a silent substitution (i.e., a substitution of a base that causes no change in amino acid coding) in the coding region; Ser183Ser resulted from G > A substitution at position 1424 in the MC3R genomic DNA sequence.

**Association results**

The AG genotype had higher average egg weight at 30 weeks of age (EW30) compared with GG genotype ($P < 0.05$). The AA genotype also recorded higher average egg weight at 28 weeks of age (EW28), 30 weeks of age (EW30) and 32 weeks of age (EW32) compared with GG genotype ($P < 0.05$) (Table 3). In addition, AG genotype was significantly associated with breeding value for average egg weight at 28 weeks of age (EW28), 30 weeks of age (EW30) and 32 weeks of age (EW32) and average egg weight at 30 weeks of age (EW30) compared with GG genotype (Table 4). There was no significant interaction between the gene additive effects.

**Discussion**

Using a candidate gene approach, we identified polymorphism in the exon of the MC3R gene in Mazandaran chickens. The SNP in the exon included synonymous and non-synonymous polymorphisms. At any given position in a DNA sequence, a nucleotide can be substituted by any of the 4 nucleotide bases and may result in a silent mutation of the gene or the frame shift mutation of the genetic mutation.
on might be able to change the amino acid sequence, or terminate producing peptide synthesis of complete peptide chains, because of the genetic code with degeneracy, some alkali gene replacement may not cause amino acid sequence change. Our results showed synonymous variations, that is code base sequence change with no amino acid sequence change. The reason why the mutation with same amino acid sequence affected the traits in other studies is still unclear. Hence, the aim of this study was to analyze the association of the SNP genotypes of MC3R gene with chicken growth and reproductive traits. The results of association analysis between single SNP of chicken MC3R gene and growth and reproductive traits substantiated our conjecture that the genotypes of this SNP were significantly associated with the economically important traits in chicken. In summary, commercial breeding programs of chickens have become more and more complex, thus it would be important for the breeders to use molecular methods such as marker assisted selection (MAS) method to improve the economically important traits, while maintaining the overall fitness. The results of this study indicated that SNP markers were associated with growth and reproductive traits, thus it could be concluded that MC3R gene plays an important role in the regulation of reproductive traits in chickens. In the other words, the MC3R gene shows great potential for use in molecular MAS programs to control growth and reproductive traits.

References


**MC3R gene and growth and reproductive traits**


همبستگی ژن گیرنده ملانوکورتین-3 (MC3R) با صفات رشد و تولید مثل در ماکیان بومی مازندران

چکیده

گیرنده ملانوکورتین-3 (MC3R) نقش مهمی در کنترل مرکزی هموستاتیک ای ژنربی ی ای می کند و چندین چندشکلی عملکردی از این ژن شناسایی شده است. در این پژوهش، ژن کاندیدا MC3R، که مسئول تغییر در صفت هیپوسحل بین ژنی در طول زمان می باشد تعیین شد. برای تعیین همبستگی بین چندشکلیه ژنی MG3R و تغییر فنوتیپی، پرندی از این ژن با روش PCR-RFLP تعیین شدند. همبستگی ژنی MC3R با صفات رشد و تولید مثل با معنی تا حدودی پذیرش یافته شد. آماری همبستگی، اثر مثبتی زنیتی از ژنی پرستی پرندگان در سنین 28 و 30 هفتگی را در مقداری با زنیتی GG مثبت داد (P<0.05). تابع همبستگی همچنین اثر مثبتی زنیتی از ژنی AG با مانگیز ون در سنین 30 و 32 هفتگی را در مقداری با زنیتی GG مثبت داد (P<0.05). این پژوهش نشان داد که اثراتی چنین این ژنی باعث بهبود صفاتی مثل یرا پرستی و در نتیجه ای اساسی را برای ایانالز بهبودی همبستگی بین تغییرهای زنیتی در زنیتی MC3R و صفاتی مثل ایانالز و کاربرد نشانگر زنیتی یافته در بهبودگی ماکیان را تشکیل می دهد.