Research Article

Importance of Single Nucleotide Polymorphisms (SNPs) of *Insulin-like Growth Factor-1* (*IGF-I*) and *Ovocalyxin-32* (*OCX-32*) Genes for Production Traits in Mazandaran Indigenous Chicken

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Abstract

We suggested two important proteins (insulin-like growth factor-I (IGF-I) and ovocalyxin-32 (OCX-32) have crucial effects on muscle differentiation, growth, and reproductive traits in chicken production. On the association between *IGF-I* gene polymorphism and production traits, the evaluation of the associations between SNPs with reproductive traits suggests a positive effect of genotype AC with average egg weight at age of 30 (EW30) ($P <$ 0.05) compared with genotype CC. We confirmed that the g.570 C > A polymorphism is significantly associated with average egg weight at age of 30 (EW30). On the other hand, for the association between *OCX-32* gene polymorphism and production traits, two single nucleotide polymorphisms (SNPs) c.381G > C and c.494 A > C, were confirmed. Associations of *OCX-32* genotypes with egg number (EN) were significant (p<0.05). From these findings we concluded that these markers should be considered for growth and production traits in chicken.

Keywords: Production traits, Chicken, IGF-I, OCX-32, PCR-RFLP

Introduction

Economically important livestock products are dependent on production and reproductive traits in domestic animals, which are under the control of multiple genes, mapping and analyzing polymorphisms of genes involved in the main metabolic pathways related to animal growth and distribution of nutrients to different tissues [1,2]. Understanding the genetic information of related genes is helpful for the selection and breeding course through marker assisted selection (MAS) in domestic animals. Candidate genes have well-known biological functions related to the development or physiology of important traits [3]. Such genes can encode structural proteins or a member in a regulatory or biochemical pathway affecting the expression of the trait [4] and can be tested as putative QTLs [5].

For poultry industry, meat and eggs are main products, for which some genes impact on such as insulin-like growth factor (IGF-I) and ovocalyxin-32 (OCX-32) have crucial roles on muscle differentiation, growth and reproduction. The presence of IGF-I in blood was detected as one of skeletal growth factors that produced in the liver tissue [6]. Therefore, insulin-like growth factor 1 is a key growth factor involved in a variety of biological processes [7,8]. Some study has shown that IGF-I mRNA levels were significantly lower in the low growth rate line than in the high growth rate line [9].

Recent studies on a single nucleotide polymorphism (SNP) in the chicken *IGF-I* gene have reported that there are significant associations between a polymorphism in this gene and its promoter in production and reproduction traits in poultry [10-12]. And the other hands, one group of detected eggshell matrix proteins related to major proteins of the egg white. Ovalbumin was the first egg white protein that explained in the matrix of the eggshell [13]. A second group contains proteins such as osteopontin that are also found in other tissues [14]. Finally, a third group of proteins includes those specific to the uterine tissue and to the eggshell that have only been detected in extracts of eggshell. Recent studies have shown associations between single nucleotide polymorphism of *ovocalyxin-32* gene its family genes and egg production traits [15-18]. The eggshell contains some eggshell-specific matrix proteins such as ovocleidin-17, ovocleidin-116, ovocalyxin-32 and ovocalyxin-36 [14,19]. It was demonstrated that ovocalyxin-32 (OCX-32) is a 32 kDa protein that found in the outer region and cuticle of the shell [20]. Dunn *et al.* [16] reported that a single nucleotide polymorphism in the intron of the *OCX-32* gene was associated with the thicknesses of the mammillary layer. Some studies have shown that low egg production strains expressed more transcripts of the *OCX-32* gene in comparison of high strains at egg-laying stages and offered that the *OCX-32* gene is a crucial marker that associated with egg production [21,22].

The objectives of the present study was 1) to detect SNPs of *IGF-I* and *OCX-32* genes by developing PCR-RFLP methods, and 2) to investigate and analyze associations between those SNPs and growth and egg production traits in Mazandaran indigenous chicken.

Material and Methods

Growth and Egg Production Traits

The evaluated traits and their descriptions were presented in Table 1.

Experimental Population and Sampling

Chickens were raised in Native Chicken Breeding Station of Mazandaran, and they belonged to generation 19 of the breeding station pedigreed animals. Blood sample (1 mL) was taken in an EDTA-containing tube and all samples were freeze. Whole DNA was extracted by using DNA Extraction Kit [23]. The DNA samples were stored at -20ºC for use.

Primer Synthesis and PCR–RFLP Reactions

IGF-I analysis: Promoter region of the *IGF-I* gene amplified to a product of 361 bp using the oligonucleotide design tool Primer 5.0 software based on the *IGF-I* gene sequence of the fowl (Accession number: M74176). Primers were F: 5'-CTCTGCCACGAATGAAATGTGC-3' and R: 5'-GGGAGCATTTGCCTTCTCTC-3' for *IGF-I* gene. PCR method was used to optimize the reaction accuracy: 94ºC for 2 min, 30 cycles of 98ºC for 30 s, annealing at 55ºC for 30 s, 68ºC for 40 s, and a final extension at 72ºC for 7 min. Finally, PCR products were electrophoretically separated on 2% agarose gel (5 V/cm) and stained with ethidium bromide.

The fragment was amplified for PCR–restriction fragment length polymorphism (RFLP) analysis. An amplified fragment was digested by *Hinf*I for detecting the g.570C > A genotypes. The restriction enzyme digestion was incubated at 37°C for 4 h.

Table 1: Statistical description of data set for growth and egg production traits.

Traits	No. of animal	Mean	Coefficient of variation
BW1 (gr)	34,277	34.53	8.13
BW8 (gr)	42,057	553.7	17.12
BW12 (gr)	37,207	943.9	14.51
WSM (gr)	30,137	1684	11.92
ASM (day)	30,339	155.5	9.25
EN (number)	30,349	36.66	39.76
EW1 (gr)	26,284	40.21	15.77
EW28 (gr)	16,215	45.91	8.43
EW30 (gr)	18,021	47.12	8.52
EW32 (gr)	17,945	48.22	8.31
EW12 (gr)	17,837	48.62	9.34
AV (gr)	27,715	45.84	13.34
EM (gr)	27,725	1758	39.13
EINT (%)	30,339	53.07	33.33

BW1, BW8, BW12 = Body Weight at Birth, 8, 12 weeks of age, WSM = Body Weight at sexual maturity, ASM = Age at first egg, EN = Egg Number, EW1 = Weight of First Egg, EW28, EW30 and EW32 = Average Egg Weight at 28, 30 and 32 weeks of age respectively, EW12 = Average egg weight for First 12 weeks of production, AV = Average for EW28, 30 and 32, EM = Egg Mass (=EN×EW12), EINT = Egg Production Intensity (=Egg Number/Days Recording)×100).

OCX-32 Analysis

With forward primer 5'-CTCCAAACGTATGCTTCACTTA-3' and reverse primer 5'-ATTCTTGTGTTCGGTTACTTGT-3', approximately 342 bp covering complete exon-3 was obtained. As well as forward primer 5'-TGTTTCTGATGAAGAGCCAGA-3' and reverse primer 5'-CTTTGCCACTCTGTAGGCTGT-3', approximately 250 bp covering exon-4 was obtained. Two fragments containing the *OCX-32* polymorphisms (NM_204534: c.381G>C; and NM_204534: c.494A>C) were amplified for PCR-RFLP analysis. An amplified fragment was digested with *Nco*I for detecting the c.381G>C and c.494A>C genotypes, respectively. The restriction enzyme digestions were incubated at 37°C for 4 h.

Statistical Analyses

The relationship between genes polymorphism and related traits were calculated using general linear model of SAS 9.0 (SAS Inc., Cary, NC, USA). The used models in matrix notation were as follows:

$$Y = Xb + Za + e$$

Where, Y is the vector of observations; b the vector of fixed effects of generation, sex and hatch; a the vector of random direct genetic effects; e the vector of random residual effects; X and Z are incidence matrices relating the observations to the respective fixed and direct genetic effects.

Results

The Production Traits

The means and SD of the traits measured in the chickens are shown in Table 1.

Phenotypic Analysis and Sequence Analysis

IGF-I

The single nucleotide polymorphisms was located in the promoter region and produced an A→C substitution at base 570 (accession number M74176), which was the same mutation identified in previous studies [10,11,24]. Genotypes of chickens were investigated by PCR-RFLP (Figure 1). Genotype and allele frequencies of the SNP in chickens are shown in Table 2. In this population, the AA genotype
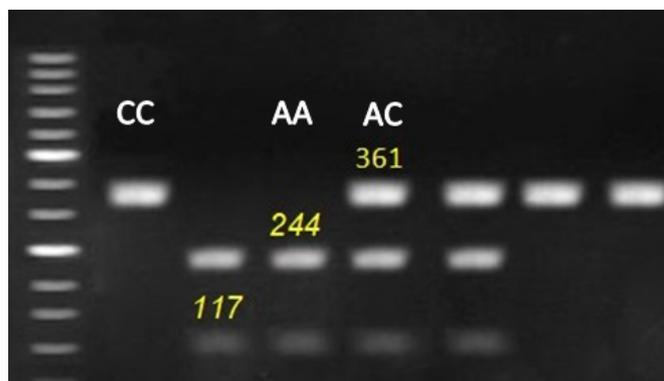

Figure 1: Representative genotyping of *IGF-1* gene at locus g.570C > A. by agarous gel electrophoresis. Strands with 361 for CC genotype, 117, 244 and 361 for AC genotype and 117 and 244 for AA genotype appeared at this locus.

Table 2: Genotypic and gene frequency of *IGF-I* gene.

IGF-I				
Genotype	No.	Frequency	Allele	Frequency
AA	113	0.62	A	0.78
AC	59	0.32	C	0.22
CC	11	0.06		
Total	183	1		

had the highest frequency (0.62), followed by AC (0.32) and CC (0.06), and the A and C allele frequencies were 0.78 and 0.22, respectively.

OCX-32

The SNPs in exon 3 and 4 were a C→G substitution at base 381 (c.381 G > C; accession number NM_204534) in exon 3, and C→A substitution at base 494 (accession number NM_204534) in exon 4, respectively, which was the same mutation reported in the previous study [21].

We observed 3 band on gel for *OCX-32* exon 3 (Figure 2). Three genotypes in this segment GG, GC and CC had the genotypic frequencies of 0.61, 0.26 and 0.13, respectively (Table 3). Two band

Table 3: Genotypic and gene frequency of *OCX-32* gene.

SNP	Frequency genotype			Frequency allele	
c.381G>C	GG	GC	CC	G	C
	0.61	0.26	0.13	0.74	0.26
c.494A>C	AA	AC	CC	A	C
	0.11	0.36	0.53	0.29	0.71

Table 4: Association of the *IGF-I* genotypes at the growth and egg production traits (Mean ± S.E.).

EW28	44.30 ± 0.52	45.51 ± 0.46	43.10 ± 1.01
EW30	48.44 ± 0.49 [ab]	48.41 ± 0.45 [a]	46.27 ± 0.96 [b]
AV	51.91 ± 0.44	51.38 ± 0.40	51.03 ± 0.83
EM	1929.45 ± 446.35	2019.28 ± 41.43	1958.00 ± 87.54
EINT	59.29 ± 2.64	61.26 ± 1.49	61.03 ± 3.07

[a,b]Values with different superscripts within the same row differ significantly (P<0.05).

patterns for *OCX-32* exon-4 (Figure 3) were observed. Three genotypes in this segment AA, AC and CC had the genotypic frequencies of 0.11, 0.36 and 0.53, respectively (Table 3).

Associations between Genotypes and Production Traits or Breeding Values

IGF-I

Results on the effects of *IGF-I* SNP on production and growth traits are shown in Table 4. The g.570 C > A genotype was significantly associated with average egg weight at age of 30 (EW30) ($P < 0.05$) in this population. No significant association was found between the *IGF-I* SNP and other traits (Table 4).

OCX-32

Results on the effects of *OCX-32* SNP on growth and production traits are shown in Table 5. At exon 3, genotype GC had higher egg number compared with genotype GG ($P < 0.05$, Table 5). At exon 4, genotype CC was significantly associated with egg number ($P < 0.05$, Table 5) compared with genotype AC.
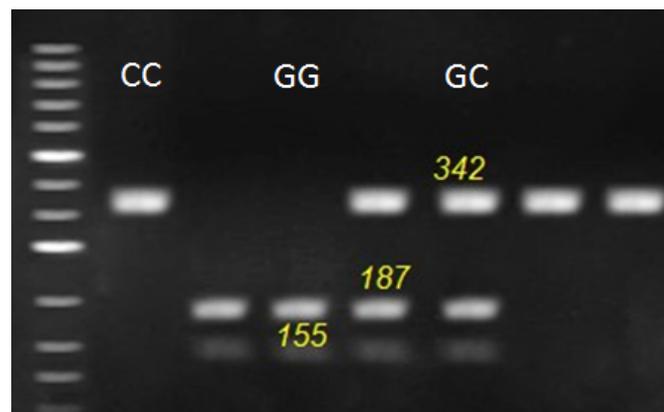

Figure 2: Representative genotyping of *OCX-32* gene at locus c.381 G>C. by agarous gel electrophoresis. Strands with 342 for CC genotype, 155, 187 and 342 for GC genotype and 155 and 187 for GG genotype appeared at this locus.
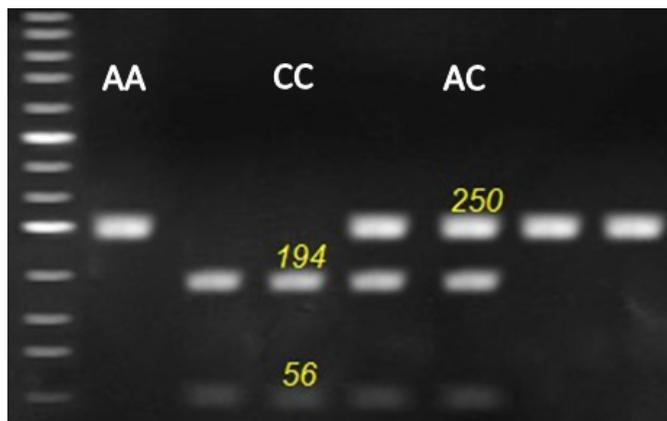
Table 5: Effects of c.381G>C and c.494A>C SNPs of the *OCX-32* gene on growth and production traits (least squares means ± SE).

SNP	c.381G>C			c.494A<C		
Genotype	**GG**	**GC**	**CC**	**AA**		**CC**
BW12 (gr)	751.33 ± 14.36	733.25 ± 16.24	707.71 ± 29.69	688.82 ± 26.03	732.47 ± 14.62	756.56 ± 13.50
WSM (gr)	1738.36 ± 21.54	1729.77 ± 26.07	1706.98 ± 53.00	1685.13 ± 46.32	1713.00 ± 24.07	1761.97 ± 23.05
ASM (day)	175.37 ± 1.66	177.76 ± 1.10	179.32 ± 3.25	172.34 ± 1.83	173.24 ± 1.99	172.42 ± 1.90
EN (number)	41.30 ± 0.79 [b]	43.64 ± 0.96 [a]	43.42 ± 1.95 [ab]	41.83 ± 1.72 [ab]	43.54 ± 0.89 [a]	341.39 ± 0.85 [b]
EW28 (gr)	44.56 ± 0.45	44.62 ± 0.57	45.51 ± 1.16	43.29 ± 0.98	44.53 ± 0.50	45.18 ± 0.50
EW30 (gr)	48.25 ± 0.35	47.84 ± 0.44	49.31 ± 1.01	46.81 ± 0.82	48.65 ± 0.49	48.13 ± 0.49
EW32 (gr)	52.56 ± 0.43	52.01 ± 0.52	49.70 ± 1.06	51.10 ± 0.97	52.06 ± 0.49	52.54 ± 0.47
EW84 (gr)	51.68 ± 0.49	51.46 ± 0.60	49.71 ± 1.21	49.38 ± 1.10	50.31 ± 0.56	51.81 ± 0.53
AV (gr)	50.22 ± 0.38	50.19 ± 0.46	48.19 ± 0.94	48.26 ± 0.83	50.03 ± 0.43	50.47 ± 0.41
EM (gr)	1947.08 ± 40.96	2070.16 ± 49.21	1987.59 ± 100.22	1907.50 ± 88.20	2061.73 ± 45.84	1958.06 ± 44.32

[a,b]Values with different superscripts within the same row differ significantly (P<0.05).

Figure 3: Representative genotyping of *OCX-32* gene at locus c.494 A > C. by agarous gel electrophoresis. Strands with 250 for AA genotype, 56, 194 and 250 for AC genotype and 56 and 194 for CC genotype appeared at this locus.

Discussion

In the present study, the g.570 A < C polymorphism of the *IGF-I* promoter was detected in Mazandaran indigenous chicken: allele frequencies for A and C were 0.78 and 0.22, respectively. The g.570 A < C polymorphism has been reported as a candidate mutation influencing growth and carcass traits [10,11,25]. Previous studies have shown that the g.570 A > C polymorphism is significantly related to body weight at 107 days of age [10], 2, 4, 6 and 8 weeks of age [11], and 5 weeks of age [25].

For production traits, however, no significant differences were found in the association between the g.570 A < C polymorphism and other traits. In contrast, the g.570 A < C polymorphism is strongly assumed to be involved in 30 weeks (EW30). These results support the notion that selection of average egg weight at age of 30 weeks (EW30), is a powerful method to increase production traits and the g.570 A<C polymorphism might become a marker for elevating average egg weight at age of 30 (EW30). In particular, we propose that the identification of g.570 A < C genotypes may be useful in the selection for reproductive traits. To make further progress, it is necessary to investigate the associations between the g.570 A<C genotypes and production traits in the other breed.

The relationship between the *IGF-I* promoter mutation and growth or carcass traits has been studied in some breeds of chicken and cattle. For example, effects of the polymorphism *IGF-I* gene were surveyed on egg quality in Wenchang chicken [12]. Some results showed single nucleotide polymorphisms 512-bp upstream from the start codon had significant associations with weight gain during the first 20 days after weaning and on-test weight in Angus cattle [26]. Furthermore, the same report indicated that the g.570 C > A substitution A→C in the promoter region involved the suppression of one potential CdxA transcription factor binding site. Hence, the different alleles detected in the present study might alter the transcription rate and the gene expression level of *IGF-I*, thereby affecting circulating IGF-I concentrations and muscle development.

An association between the single nucleotide polymorphisms of the *OCX-32* gene and the thicknesses of the mammillary layer was recently reported by Dunn *et al.* [16]. In the present study, the SNP of the *OCX-32* gene were significantly associated with body weight at age of 12 weeks, average of EW28, EW30 and EW32, egg number and average egg weight at age of 32 and 84 weeks. We did not find any significant association between these single nucleotide polymorphisms and the other investigated traits. In the case of c.494 A > C SNP in exon 4 implied that the chicken *OCX-32* gene may affect reproductive and production traits related traits simultaneously. Yang *et al.* [22] showed that the *OCX-32* expression levels and EPR were related. Therefore, to confirm the causal mutation derived by only a single SNP, we need to evaluate the effects in different breeds and lines of chickens and investigate the function of this gene in detail. Our results show that detection and utilization of candidate gene mutations and DNA markers obtained by whole-genome scanning may directly improve growth, production traits and other economic traits within the same breeds. In particular, we propose that the identification of genotypes may be useful in the selection for production and reproduction traits. To make further progress, it is necessary to investigate the associations between the genotypes and traits in the other breeds. This result shows that *OCX-32* gene can be used as a candidate marker in marker-assisted selection. Further functional analysis is essential to ascertain the effects of the *OCX-32* exon polymorphism.

Abbreviations

IGF-I: Insulin-like growth factor-1

OCX-32: Ovocalyxin-32

BW1, BW8, BW12: Body Weight at Birth, 8, 12 weeks of age

WSM: Body Weight at sexual maturity

ASM: Age at first egg

EN: Egg Number

EW1: Weight of First Egg

EW28, EW30 and EW32: Average Egg Weight at 28, 30 and 32weeks of age respectively

EW12: Average egg weight for First 12 weeks of production

AV: Average for EW28, 30 and 32

EM: Egg Mass

EINT: Egg Production Intensity

Acknowledgement

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