Research Journal of Poultry Sciences 3 (2): 19-22, 2010
ISSN: 1993-5285

Effects of the Polymorphisms of GHR Gene and IGF-1 Gene on Egg Quality in Wenchang Chicken

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Abstract: Alleles of physiological candidate genes for reproductive traits, Growth Hormone Receptor (GHR) and Insulin-like Growth Factor-I (IGF-I) were assessed to determine the association with Haugh Unit (HU), egg weight EGW, Egg Shell Weight (ESW), Egg Shell Strength (ESS) and Egg Shell Thickness (EST) at 55-week age of Wenchang chicken (Chinese indigenous breed). PCR-RFLP technique was applied to analyze the polymorphisms of GHR intron 5 and IGF-1 of Wenchang chickens. The effects of GHR intron 5 and IGF-1 on the egg quality were analyzed. The results showed that the allele frequencies of C, D were 0.19, 0.81 for GHR-Intron 5 and the allele frequencies of A, B were 0.53, 0.47 for IGF-1, respectively. Three significant were observed for GHR and EST; for IGF-1 and ESW and for IGF-1 and ESS.

Key words: Chicken, GHR, IGF-1, egg quality, allele, reproductive traits

INTRODUCTION

The Wenchang chicken is a special indigenous breed in Hainan province of China. They are small in body size and dual purpose for meat and egg production. Compared to the good meat flavor of Wenchang chicken, the egg quality can not meet the consumers demand very well. Egg quality has been defined by Stadelman (1977) as the characteristics of an egg that affect its acceptability to the consumers.

Egg quality is the more important price contributing factor in table and hatching eggs. Therefore, the economic success of a laying flock solely depends on the total number of quality eggs produced. It is generally agreed that all characteristics of egg quality have a genetic basis. Therefore, it is necessary to study the Wenchang chicken by molecule marker method aimed to improve the egg quality traits efficiently which will ensure the large need of market.

Most traits with economic interest in farm animals show continuous variation. However, their underlying genetic nature is very complex. Molecular marker-assisted selection is efficiency and makes further improvements in production performance. A candidate gene approach is a powerful method for understanding the direct genetic basis involved in the expression of quantitative difference between individuals (Rothschild and Soller, 1997; Nagaraja *et al*., 2000).

Some understanding of the genetic architecture of quantitative traits may be gained by systematically analyzing of genetic markers in major metabolic pathways. In addition to this major endocrine pathway mediated by the hypothalamus, pituitary gland and liver, other tissues that produce GH and IGF-I have been identified, indicating that these hormones together with their receptors and binding proteins provide a complex regulatory network that coordinates a multitude of traits (Harvey and Hull, 1997). In terms of egg production and egg shell quality, associations have been found for polymorphisms in the putative candidate genes IGF-1, GH and GHR in the growth hormone endocrine pathway (Feng *et al*., 1997; Kuhnlein *et al*., 1997; Nagaraja *et al*., 2000). In this study, single nucleotide polymorphisms of two candidate genes of GHR and IGF-I genes of Wenchang chicken (Chinese indigenous breed) were detected by PCR-RFLP method. In particular, for genotypic interaction between the two genes was searched and analyzed the effects of genotypes on the relationship between these SNPs and egg quality traits of Wenchang chicken.

MATERIALS AND METHODS

Experimental chickens and traits: A total of 120 Wenchang chickens which were purebred introduced from Hainan Province were bred in testing center of poultry quality, Ministry Agriculture of China.

All birds were raised in the same condition, fed commercial corn-soybean based diets that met all NRC (1994) requirements *ad libitum* and fresh water access freely. Five egg quality performance including Haugh Unit (HU), Egg Weight (EGW), Egg Shell Weight (ESW), Egg Shell Strength (ESS) and Egg Shell Thickness (EST) at 55 weeks age were measured. DNA and egg quality data were obtained from 117 Birds.

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Establishment of a PCR-RFLP assay: Blood was sampled from plumage veins and sampled into test tubes containing an anticoagulant solution. Genomic DNA was isolated from it and eluted into 350 μL of TE. A 740-Base Pair (bp) fragment of the GHR gene intron 5 was amplified by Polymerase Chain Reaction (PCR) using forward (5'-ACGAAAAGTGTTTCAGTGTTGA-3') and reverse (5'-TTTATCCCGTGTTCTCTTGACA-3') primers. Cycles applied were denaturation 94°C, 5 min followed by 35 cycles. Primers used to detect the NspI RFLP located near the GHR gene were (forward) and (reverse). Each cycle consisted of 45 sec at 94°C, 45 sec at 56°C, 60 sec at 72°C and final synthesis 72°C, 10 min (Dunn *et al*., 2004).

A 621-Base Pair (bp) fragment of the IGF-I gene was amplified by Polymerase Chain Reaction (PCR) using forward (5'-GACTATACAGAAAGAACCAC-3') and reverse (5'-TATCACTCAAGTGGCTCAAGT-3') primer (Nagaraja *et al*., 2000). Cycles applied were: denaturation 94°C, 5 min followed by 35 cycles.

Each cycle consisted of 45 sec at 94°C, 45 sec at 60°C, 60 sec at 72°C and final synthesis 72°C, 10 min. A PCR of DNA from each bird was performed according to the conditions described above. For GHR intron 5, 10 U NspI was used to digest at 37°C overnight and digested products were electrophoresed for 1 h at 80 V on a 2.5% agarose gel. And for IGF-I gene, 10 U Pst I was used to digested at 37°C overnight and digested products were electrophoresed for 1 h at 100 V on a 3.5% agarose gel. Individual PCR-RFLP fragment sizes for each gene were determined by visualizing the banding pattern under ultraviolet light (Table 1).

Statistical analysis: Statistical calculations were performed using SPSS12.0 procedures. Frequencies of distribution of alleles within the lines were compared with χ^2-test.

The effects of IGF-I and GHR genotypes on the egg production of chicken were analyzed using GLM procedure. The following model was used:

$$Y_{ijk} = \mu + G_i + I_k + B_{ik} + E_{ijk}$$

Y_{ijk} = Trait analyzed in two lines
μ = Overall mean
G_i = Fixed effect of the GHR marker genotypes
I_k = Fixed effect of the IGF-I marker genotypes
B_{ik} = The interaction between the two genotypes
E_{ijk} = Random error

Table 1: Gene's polymorphic loci and source

Gene	Location	Position	Diagnostic enzyme	Type of polymorphism
GHR	Intron 5	571	NspI	C/T transversion
IGF-I	5'-UTR	364	PstI	C/T transversion

As the interaction term was not significant for any of the traits analyzed, the model was subsequently reduced to:

$$Y_{ijk} = \mu + G_i + I_k + E_{ijk}$$

RESULTS

Sequence variation and PCR-RFLP analysis: For GHR intron 5, a 740 bp fragment was amplified and two SNPs were discovered that were linked both cytosine-thymidine transversions in it (Fig. 1). The genotypes differed from the expected Hardy-Weinberg equilibrium (Table 2).

For IGF-I, a 621 bp fragment of 5'-UTR (5'-untranslated region) was obtained. The restriction enzyme Pst I digested PCR products had fragments of 257, 364 bp for the C_2C_2 genotype and 257, 364, 621 bp for the C_1C_2 genotype and 621 bp (no digestion) for the C_1C_1 genotype (Fig. 2).

The observed distribution of genotypes was not different from the distribution expected under the assumption of Hardy-Weinberg equilibrium (Table 2).

Table 2: Frequencies of genotypes and alleles of the GHR and IGF-I genes

	Genes	
Results	GHR-Intron 5	IGF-I
Genotype	0.20 (B_1B_1)	0.32 (C_1C)
	0.80 (B_2B_2)	0.41 (C_1C_2)
Allele	0.20 (B_1)	0.27 (C_2C_2)
	0.80 (B_2)	0.53 (C_1)
	-	0.47 (C_2)
χ^2	45.07	3.64

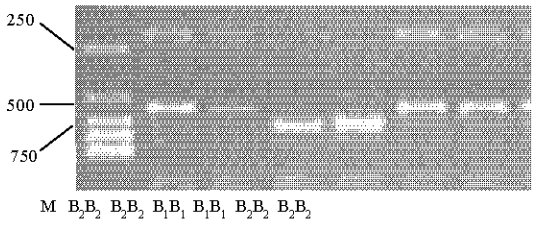

Fig. 1: PCR-RFLP pattern for GHR intron 5 with NspI digestion

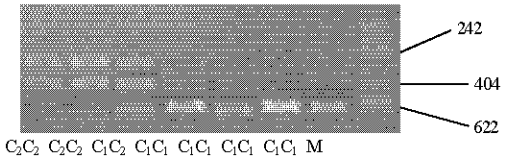

Fig. 2: PCR-RFLP pattern for 5'UTR of IGF-I with PstI digestion

Table 3: Correlation analysis between GHR genotypes and egg quality

	Genotype		
Traits	CC	DD	Additive
HU	66.86	69.91	0.105
EGW	48.06	49.21	0.113
ESW	6.20	6.37	0.093
ESS	3.73	4.07	0.129
EST	310.29^{a}	322.93^{b}	0.193*

^{a,b}Means within a row without a common superscript differ significantly ($p<0.05$), $^{*}p<0.05$

Table 4: Correlation analysis between IGF-1 genotypes and egg quality

	Genotype				
Traits	AA	AB	BB	Additive	Dominant
HU	69.25	68.32	71.19	0.061	-0.081
EGW	48.50	49.13	49.40	0.089	0.029
ESW	6.07^{A}	6.55^{B}	6.32^{AB}	0.149	0.256**
ESS	4.01^{ab}	4.23^{b}	3.66^{a}	-0.120	0.184*
EST	317.99	323.21	319.91	0.034	0.086

^{a,b}Means within a row without a common superscript differ significantly ($p<0.05$), ^{A,B}Means within a row without a common superscript differ significantly ($p<0.01$), $^{*}p<0.05$, $^{**}p<0.01$

Associations between genotypes and traits: Associations of genotypes with egg quality traits were initially analyzed using a linear model that included terms for the GHR genotype, the IGF-I genotype and the interaction between the two genotypes. However, the interaction term was not significant ($p>0.05$) so, it was removed from the model.

For GHR gene, there was no association between the gene and HU, EGW, ESW and ESS. But a significant association between GHR-intron 5 polymorphism and the EST was found ($p<0.05$) (Table 3) as well as an additive effect of GHR-intron 5 on the EST trait ($p<0.05$). For IGF-I gene, there were no associations between the gene and HU, EGW and EST; however, significant associations were found between IGF-1 polymorphism and ESW and ESS (Table 4). Two dominant effects of IGF-1 on ESW and ESS were also observed.

DISCUSSION

Most traits with economic interest in animal production show continuous variation. However, their underlying genetic nature is very complex. It is possible to identify the chromosome regions containing genes affecting these traits because of today's availability of neutral polymorphisms scattered throughout the genome (Andersson *et al*., 1994). Chicken egg quality traits, like other economically important traits are controlled by a lot of minor modification genes and several major genes. The minor genes are small but their effects are large in most cases. Many successes have been claimed for the physiological candidate gene approach to explain trait variance (Rothschild *et al*., 1996; Short *et al*., 1997; Meng *et al*., 2002). In the present study, associations detected by the analysis with the single generation of hens of Wenchang chicken suggest that the GHR gene play a role in controlling EST and IGF-I gene had effects on ESW and ESS. The Growth Hormone Receptor (GHR), Insulin-like Growth Factor-I (GH-IGFI) system control the number of follicles in animals that are recruited to the rapid growth phase (Roberts *et al*., 1994; Monget *et al*., 2002). It is also known that the GH-IGFI system has been modified as a result of selection for improve growth rate (Ballard *et al*., 1990; Ge *et al*., 2001). There are obvious physiological connections between body weight homeostasis and the reproductive axis in both sexes. The rate of sexual maturation is much more closely associated with body growth than with chronological age (King, 2000). Thus, GH-IGFI system affected the chicken growth speed and body weight. In addition, it's known that body weight had significant genetic correlation to egg weight. Recently, Oke *et al*. (2004) also reported the significant relationship ($R^2 = 0.69$) was between egg weight and body weight at 44 weeks.

Indications that the IGFs may be involved in avian reproductive performance come from previous *in vivo* studies that used injections of GH, gonadotrophins or even IGFs. The injection of IGF-I in sex-linked dwarf chickens which lack GH receptors showed increased reproductive performance (Decuypere *et al*., 1992). Follicle numbers in laying hens increased after GH or gonadotrophin injections (Williams *et al*., 1992). The latter studies suggest that IGF is a local mediator of GH or gonadotrophin hormone action in the ovary. Recent *in vitro* studies using cell cultures have shown that IGF-I and -II have major roles to play in avian ovarian function. The amount and size of follicle affected egg performance and egg quality. Thus, changes in the GH/IGF axis may be associated with egg quality. Ankra-Badu and Aggrey (2005) reported that GHR gene was one of the most promising candidate genes for egg production and egg shell quality. Some studies have previously identified markers in the GH and GHR genes which are still segregating in many noninbred strains of White Leghorn chicken and have shown that they are associated with changes in body weight (Feng *et al*., 1998) and egg production rate (Kuhnlein *et al*., 1997). And Nagaraja *et al*. (2000) reveal a significant influence of the IGF-I genotype on egg weight and specific gravity while egg weight of PstI (+/-) genotype was heavier than PstI (-/-) genotype's. The same result of egg weight was found in the research. In addition, an additive effect of GHR-intron 5 on the EST trait and two dominant effects of IGF-1 on ESW and ESS were also observed. Different results were found between previous studies and the study which may be because the SNP identifies different alleles in these unrelated populations.

CONCLUSION

The study presents strong evidence of significant and simultaneous beneficial effects of GHR-SNP and

IGF-I-SNP associated with chicken egg quality. Whether the behavior of GHR and IGF-I variants is a paradigm for other genes to be determined. Further, the same genetic variants may have different effects in different genetic backgrounds.

ACKNOWLEDGEMENT

This research was supported by the national high technology research and development national public sector (Agriculture) special research program of P.R. China (No. nyhyzx07-039) and the earmarked fund for modern agro-industry technology research system.

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