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Full Length Research Paper

Polymorphism in NPY and IGF-I genes associate with reproductive traits in Wenchang chicken

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Alleles of physiological candidate genes for reproductive traits, insulin-like growth factor-I (IGF-I) and neuropeptide Y (NPY) were assessed to determine the association with total egg production (NE), average days of continual egg-laying (ADCE) and number of double-yolked eggs (DYE) in Wenchang chicken (Chinese indigenous breed). PCR-RFLP method was used for genotypes identification. The frequency of restriction enzyme A/a alleles in the population was 0.46 (A) and 0.54 (a) for NPY. The frequency of restriction enzyme B/b alleles in the population was 0.53 (B) and 0.47 (b) for IGF-I, respectively. Four significant associations were found ($P < 0.05$): between NPY and NE (300 d) and between IGF-I polymorphism and NE (300 d), NE (400 d) and ADCE. Two significant effects were observed: for NPY and NE (300 d) and for IGF-I and NE (300 d). The current research supports the effects of NPY and IGF-I genes on reproductive traits of chickens.

Key words: Chicken, NPY, IGF-I, reproduction, SNP.

INTRODUCTION

The Wenchang chicken is a special indigenous breed in China. They are small in body size and dual purpose for meat and egg production. The chicken meat with especial flavor and good egg quality are accorded with Chinese consumers' taste. It is, therefore, necessary to study the Wenchang chicken by molecule marker method aimed to improve the reproductive traits quickly which will help to meet the large market demand for increased production.

Most traits with economic importance in farm animals show continuous variation. However, their underlying genetic nature is very complex. Molecular marker-assisted selection is efficient 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).

Reproduction is a comprehensive reflection of development of various parts of a chicken body and its final expression is the result of interaction among endocrinelogy, genetic, nutritional and environmental factors. Neuropeptide Y (NPY) is known to influence the release of gonadotropin-releasing hormone (GNRH) from the median eminence and is critical in controlling food intake in birds, possibly matching satiety to reproductive activity and the timing of puberty (Kuenzel and Fraley 1995). The NPY gene might produce markers for the age of the onset of lay and through its role in the control of ovulation, influence egg production rate. Numerous studies in mammals and more recently in teleost fish described IGF-I as potent paracrine modulator in a variety of tissues, regulating tissue-specific cell differentiation (Patino and Kagawa, 1999) and proliferation (Kadakia et al., 2005). IGF-I mediated effects *in vitro* characterize striking steps of the folliculogenesis such as steroidogenesis (Maestro et al., 1997) follicle differentiation (Kagawa et al., 2003) and the accumulation of vitellogenin (Tyler et al., 1987) and seem to be highly conserved differentiation among vertebrates (Lavoie et al., 1999). Based on findings in mammals, Adashi (1995) had therefore proposed IGF-I as paracrine regulator of

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Table 1. Gene's polymorphic loci and source.

Gene	Location	Diagnostic enzyme	Type of polymorphism
NPY	Transcription Start Site (TSS)	*Dra*I	4 bp deletion
IGF-I	5'-UTR	*PstI*	C/T transversion

follicular faith initiating the maturation of a follicle as intraovarian regulator.

Some understanding of the genetic architecture of quantitative traits may be gained by systematically analyzing of genetic markers in major physiological pathways. Several studies have shown that the NPY and IGF-I regulatory system affects reproductive traits (Myers, 1994; Blogowska et al., 2004). Thus, the NPY and IGF-I gene were chosen as candidate genes that might be associated with laying performance, or double-yolked egg.

The objective of the present study was to identify polymorphisms of NPY and IGF-I genes by developing PCR-RFLP methods to detect those DNA polymorphisms in Wenchang chicken (Chinese native breed). In particular, we searched for genotypic interaction between the two genes and analyzed the effects of genotypes on the relationship between these polymorphisms and reproductive 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. Data on egg production including total egg production, continual egg-laying and number of double-yolked eggs were collected daily using trap nests to identify individual birds. The data for individual hens were collected over 8 month of experimental period and recording commenced at 25-weeks age. All birds were raised in the same condition, fed commercial corn-soybean- based diets that met all NRC requirements ad libitum and fresh water access freely. DNA and trait data were obtained from 117 Birds.

Establishment of a PCR 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 240-base pair (bp) fragment of the NPY gene was amplified by polymerase chain reaction (PCR) using forward (5'-TCTCAGAGCTCCAACGTATGA-3') and reverse (5'-ATATTTCTGTGCTGAACAACA-3') primers (IC Dunn et al., 2004). Cycles applied were: denaturation 95℃, 5 min; followed by 36 cycles. Each cycle consisted of 45 s at 95℃, 45 s at 59℃, 60 s at 72℃ and final synthesis 72℃ , 10 min.

A 621-base pair (bp) fragment of the IGF-I gene was amplified by polymerase chain reaction (PCR) using forward (5'-GACTATACA GAAAGAACCAC-3') and reverse (5'-TATCACT CAAGTGGCTCA AGT-3') primer (Nagaraja et al., 2000). Cycles applied were: denaturation 94℃, 5 min; followed by 35 cycles; Each cycle consisted of 45 s at 94℃, 45 s at 60℃, 60 s at 72℃ and final synthesis 72℃ , 10 min.

Statistical analysis

Data for 300-day egg production (NE 300 d), 400-day egg production (NE 400 d), average days of continual egg-laying (ADCE) and the number of double-yolked (DYE), were obtained from the farm records. Statistical calculations were performed using SPSS procedures. Frequencies of distribution of alleles within the lines were compared with Chi-square test. The effects of NPY and IGF-I 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 2 lines, μ = overall mean, G_i = fixed effect of the NPY marker genotypes, I_k = fixed effect of the IGF-I marker genotypes, B_{ik} = the interaction between the 2 genotypes, and E_{ijk} = random error.

As the interaction term was not significant for any of the traits analyzed, the model was subsequently reduced to $Y_{ijk} = \mu + G_i + I_k + E_{ijk}$.

Screening for restriction-enzyme-detectable single nucleotide polymorphisms

A PCR of DNA from each bird was performed according to the conditions described above. For NPY, the PCR product was digested using 5U *Dra* I enzyme at 37℃ overnight. The digestion products were separated by horizontal electrophoresis (50 volts, 60 min) in 2% agarose gels in 1 × TBE and 1.0 µM ethidium bromide. And for IGF-I gene, 10 U *Pst* I was used to digested at 37℃ 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).

RESULTS

Sequence variation and PCR-RFLP analysis

For NPY, the 240-bp product was sequenced for individuals of Wenchang chicken. The following DNA restricttion fragments were obtained for NPY-*Dra*I polymorphism: 79 bp/161 bp for the aa genotype, 79 bp/161 bp/240 bp and for the AA genotype and 240 bp for the AA genotype (Figure 1). The genotypes were not differerent 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 have fragments of 257, 364 bp for the bb genotype and 257, 364, 621 bp for the Bb genotype and 621 bp (no digestion) for the BB genotype (Figure 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 NPY and IGF-I genes.

Genes	Genotype frequencies			Allele frequencies		χ^2
NPY	0.21 (AA)	0.50 (Aa)	0.29 (aa)	0.46 (A)	0.54 (a)	45.07
IGF-I	0.32 (BB)	0.41 (Bb)	0.27 (bb)	0.53 (B)	0.47 (b)	3.64

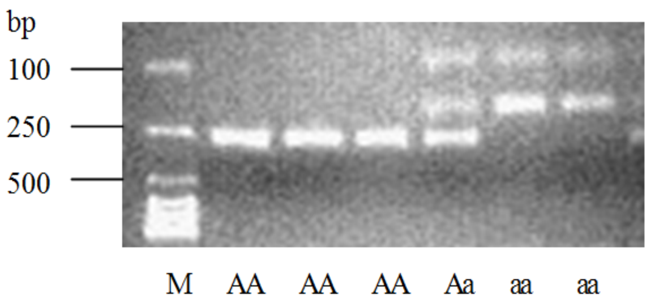

Figure 1. PCR-RFLP pattern for NPY transcription start site region with *Dra*I digestion.

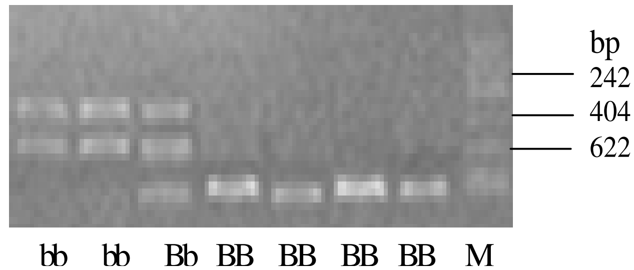

Figure 2. PCR-RFLP pattern for 5′UTR of IGF-1 with *Pst*I digestion.

Associations between genotypes and traits

Associations of genotypes with egg production traits were initially analyzed using a linear model that included terms for the NPY genotype, the IGF-I genotype and the interaction between the 2 genotypes. However, the interaction term was not significant (P > 0.05), so, it was removed from the model.

There were no associations between the NPY gene and NE (400 d), DYE, ADCE traits. But, a significant association between NPY polymorphism and the NE (300 d) (P < 0.05) was found (Table 3), as well as an additive effect of NPY on the NE (300 d) (P < 0.05).

There were no associations between the gene and DYE trait. But for NE trait, however, significant (P < 0.05) associations were found between IGF-I polymorphism and NE (300 d), NE (400 d) and ADCE. An additive effect of IGF-I on NE (300 d) was also observed (P < 0.05) (Table 4).

DISCUSSION

Reproduction is a composite of complex developments that result from endocrinology,genetic, nutritional and environmental factors. Although association studies cannot determine if the NPY and IGF-I gene allele markers are responsible for the variation in a particular trait or whether is due to a closely linked locus, we think that 2 genes would influence the traits in chickens.

NPY induces precocious puberty in chicks and controls feeding take (Kuenzel and Fraley, 1995). In mammals, the NPY neuronesare targets for leptin, which may be a mechanism that metabolic factors “gate” entry to puberty (Cheung et al., 1997). NPY also has an established role in controlling GNRH secretion during the preovulatary surge of gonadotrophins (Contijoch et al., 1993). 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. Hocking et al. (1994) and Bruggeman et al. (1997) found higher levels of IGF-I in the systemic blood of food-restricted broiler breeder hens during rearing than in those that were ad libitum fed. The injection of IGF-I in sex-linked dwarf chickens, which lack GH receptors, showed increased reproductive performance.

IC Dunn et al. (2004) found a dominance effect of NPY on age at first age in research of markers of alleles for 3 physiological candidate genes for reproductive traits. Nagaraja et al. (2000) reveal a significant influence of the IGF-I genotype on egg weight and specific gravity. Kim et al. (2004) reported that there is a possibility of IGF-I genotypes acting as a genetic marker for egg productivity of Korean Native Ogol Chicken. Ou yang et al. (2003) found that a dominance effect of NPY on 300 NE and 500 NE in Wang Zai Kang Le yellow chicken (Chinese native chicken) was found. In the present study the associations detected by analyzing a single generation of Wenchang hens suggest the the NPY gene plays a role in NE trait and the IGF-I gene afffects NE and ADCE. Differences exist between the results of previous studies and our study which may be due to the SNP identifies different alleles in these unrelated populations. But, the result of IGF-I gene afffecting NE was consistent with Kim and Ou yang’s researches.

In summary, the current study found strong evidence of significant and simultaneous beneficial effects of NPY-SNP and IGF-I-SNP associated with chicken reproductive traits. Whether or not the behavior of NPY 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.

Table 3. Correlation analysis between genotypes of NPY and reproductive traits.

Traits	NPY Genotype			Additive	Dominant
	AA	Aa	aa		
NE (300 d)	88.95^{a}	84.91^{ab}	81.55^{b}	-0.252*	-0.022
NE (400 d)	137.05	133.19	126.52	-0.158	-0.064
ADCE	3.26	3.14	2.75	-0.197	-0.071
DYE	0.24	0.35	0.42	0.102	-0.004

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

Table 4. Correlation analysis between genotypes of IGF-I and reproductive traits.

Traits	IGF-I Genotype			Additive	Dominant
	BB	Bb	bb		
NE (300 d)	82.61^{a}	82.94^{a}	89.03^{b}	0.199*	0.106
NE (400 d)	127.82^{a}	128.56^{ab}	137.84^{b}	0.177	0.089
ADCE	2.96^{a}	2.78^{a}	3.38^{b}	0.114	0.143
DYE	0.34	0.29	0.42	0.034	0.053

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

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