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Analysis on Association of a SNP in the Chicken *OBR* Gene with Growth and Body Composition Traits

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ABSTRACT : Leptin receptor (OBR) is a member of the class I cytokine receptor family. It signals mainly via the JAK/STAT pathway and plays an important role in regulating body energy storage and metabolism. This study was designed to investigate the effects of the *OBR* gene on chicken growth and body composition. Broiler lines selected divergently for or against abdominal fat were used. Primers for the exon9-region in the *OBR* gene were designed using chicken genomic sequences from the public genome domain. A C/A single nucleotide polymorphism (SNP) was found and its three genotypes (AA, AB and BB) were identified in this population. The results showed that the *OBR* polymorphism was associated with fatness traits, such as abdominal fat weight and abdominal fat percentage. This research suggests that *OBR* or a linked gene has effect on fat deposition in the chicken. (**Key Words** : Chicken, *OBR* Gene, Single Nucleotide Polymorphism (SNP), Abdominal Fat)

INTRODUCTION

Accompanying selection for rapid growth, meat-type chickens exhibit an increase in physiological disorders such as obesity. Production performance and fitness traits were negatively correlated in chicken (Martin et al., 1990; Pinard et al., 1998). Multitrait selection to simultaneously improve fitness and increase production is, therefore, difficult to achieve by direct selection. Molecular marker-assisted selection (MAS) may be required and the integration of traditional genetic selection and modern molecular methods may be preferred for breeding chickens in the future (Li et al., 2003).

The gene encoding leptin (obese gene) was identified using a positional cloning approach in mice in 1994 (Zhang et al., 1994). This finding has helped to define a newly emerging endocrine role for adipocyte and to understand how food intake and energy metabolism are regulated. Leptin is a peptide hormone mainly secreted by adipose tissues (Zhang et al., 1994) and is an adipocyte-specific protein that functions as an "adipostat" to sense and regulate body energy stores in mammals (Houseknecht and Portacarrero, 1998). The mouse leptin-receptor (*OBR*) was first isolated from the mouse choroids-plexus using an expression cloning assay. The *OBR* gene was mapped on the mouse chromosome 4 (Tartaglia et al., 1995). OBR, a member of the class I cytokine receptor family, signals via a pathway of janus-activated kinases (JAK)/signal transducers and activators of transcription (STAT) and mitogen-activated protein kinase (MAPK) (Houseknecht and Portacarrero, 1998). The gene expresses in most tissues such as brain, heart, liver and muscle in mammals (Ohkubo et al., 2000). OBR has a single membrane spanning domain and exists in five different isoforms (OB-Ra, OB-Rb, OB-Rc, OB-Rd and OB-Re) which are derived from alternative splicing of the mRNA. All isoforms have an identical ligand-binding domain but differ at the C-terminus (Fei et al., 1997). Only the OB-Rb contains a long intracellular domain and carries two JAK-binding protein motifs, which are necessary for the activation of the JAK-STAT pathway (Tartaglia et al., 1997). OB-Ra is the prevalent isoform. It has a short intracellular domain that contains only one of the two JAK-binding domains and cannot activate the JAK-STAT pathway. Its transducers signal through the activation of the MAPK pathway (Houseknecht and Portacarrero 1998). OB-Re is the only soluble receptor among all the isoforms. The functions of OB-Rc and OB-Rd had not been studied in depth.

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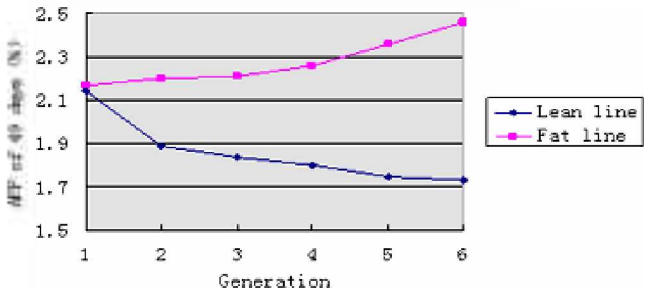

Figure 1. Effects of divergent selection on abdominal fat deposition. The two lines (Lean and Fat) differed significantly in abdominal fat percentage (AFP) of 49 days from the 4th generation onwards.

Although *OBR* has been intensively studied in mammals, limited progress had been made on chicken *OBR* gene. In 2000, the chicken *OBR* gene was cloned (Horev et al., 2000) and mapped on chicken chromosome 8 (Dunn et al., 2000). OBR plays an important role in leptin functioning signal transduction and it may have direct effect on the growth and development of the adipose tissues. The *OBR* gene is, therefore, a logical candidate gene for investigating effects on growth and development of chicken adipose tissue. In a previous study, we detected a C/A (cDNA C1167A, Accession No. AF169827) single nucleotide polymorphism (SNP) within chicken *OBR* gene Exon9 and found that there was a significant difference in the frequency of the genotypes among breeds and lines (Gu et al., 2002). The objectives of this study are to develop PCR-SSCP methods to detect this DNA polymorphism in a specific selected population and to evaluate associations between *OBR* polymorphism and growth and body composition.

MATERIALS AND METHODS

Experimental populations and management

The NEAU (Northeast Agricultural University) divergent broiler lines were used. The lean and fat broiler lines have been selected divergently using abdominal fat weight and plasma VLDL (very low-density lipoprotein) concentration as selection criteria since 1996. There were significant differences in abdominal fat weight and abdominal fat percentage between the two lines from the fourth generation onwards. The selection progress is shown in Figure 1. Male birds (228 birds, 148 in the lean and 80 in the fat line) derived from the sixth generation of this population were used in the present study.

The birds were raised in coops on plastic nets and had access to feed and water *ad libitum*. The birds were fed on commercial corn-soybean-based diets that met all the *NRC* requirements (National Research Council 1994). From hatch to 21 days old, the birds received a starter feed with 21% protein, from 22 to 42 days old they were on a diet with 19% protein, and from 43 days old to slaughter at 7 week of age on a diet with 17% protein.

Trait measurements

Measurements were taken at slaughter, including body weight (BW), carcass weight (CW), abdominal fat weight (AFW), heart weight (HW), liver weight (LW), spleen weight (SW), gizzard weight (GW), and glandular stomach weight (GSM). Abdominal fat percentage (AFP) was calculated as the ratio of AFW to BW.

Development of PCR-SSCP assays and screening the population

Genomic DNA was isolated from venous blood colleted in EDTA. The PCR primers (5' AAC CCA GAG CGT AGC TTC CA 3'; 5' TGA TGG CAA CAG TAC AAT GCG 3') were designed to amplify a 171-bp *OBR* gene exon9-region fragment by Primer 5.0 (PREMIER Biosoft International, 3786 Corina Way) according to chicken genomic sequences in the GenBank database (Accession no. AF169827). The 25 μl reaction volume included 50 ng of template, 1×PCR reaction buffer, 4 pmol of each primer, 0.4 mM dNTP, 1.5 mM $MgCl_2$ and 1 U Taq polymerase (Takara Biotechnology (Dalian) Co., Ltd., Dalian China). The reaction conditions were 94°C for 5 min, 35 cycles at 94°C for 40 s, 62°C for 50 s, 72°C for 40 s, and an extension at 72°C for 8 min. 1 μl PCR product was mixed with 5 μl loading buffer (98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol, 10 mM EDTA, 10% glycerol). The mixture was then denatured at 98°C for 10 min, placed on ice for 5 min, and electrophoresed for 8 to 9 h at 10 $V \cdot cm^{-1}$ on a 14% polyacrylamide gel with 5% glycerol. The silver staining method was used to visualize the bands. Individual PCR-SSCP banding patterns were determined under visible light.

Statistical analysis

The frequencies of the alleles and genotypes were calculated. The association between the genotypes and the traits was analyzed using the GLM (General Linear Model) of the JMP (John's Macintosh Product) software (SAS Institute 2002). The model was fitted with the genotype (G) and Line (L) as fixed effects, sire nested within line (S (L)) and dam nested within the combination of (line and sire, D (L, S)) as random effects, and BW as a covariate, as follows.

$$Y = \mu + G + L + BW + S(L) + D(S, L) + e \quad (1)$$

Where Y is the dependent variable, μ is population mean, and e is the random error. The interaction of G by L was not significant for all traits and, therefore, was not included in the final model. The interactions of G by S (L)

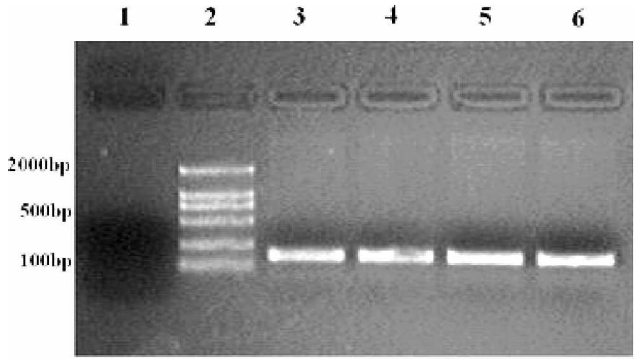

Figure 2. PCR products of the chicken *OBR* exon 9. Lane 1 is a negative control, Lane 2 is DL2000 Ladder, Lane 3-6 are PCR products.

and G by D (S, L) were not included due to missing data. Significant differences between least-squares means of the genotypes were calculated using a contrast test. Significance was determined as $p<0.05$.

The additive and dominance effects of the *OBR* gene were estimated as follows:

$$\text{Additive} = (AA-BB)/2 \quad (2)$$

$$\text{Dominance} = AB-(AA+BB)/2 \quad (3)$$

Where AA, AB and BB are the least squares means of genotype AA, AB and BB groups, respectively.

The genetic and phenotypic percentage contributions of *OBR* genotypes to the total variance were also estimated using the MTDFREML (Multiple Trait Derivative-Free Restricted Maximum Likelihood) package (Boldman and Vleck, 2002).

RESULTS

PCR-SSCP analysis

A PCR-SSCP method was successfully developed for screening the individuals of the population. The amplification product of the *OBR* exon9 was 171 bp (Figure 2). The polymorphism resulted in three genotypes defined as AA, AB and BB.

Table 3. Least-square means of AFP and AFW in *OBR* genotypes (Additive and dominant effects of *OBR* were also shown)

Genotype	AFW (g)	AFP (%)
AA	$61.92^{a}\pm3.34$	$0.023^{a}\pm0.00114$
AB	$50.49^{b}\pm1.61$	$0.019^{b}\pm0.00054$
BB	$53.88^{b}\pm1.44$	$0.020^{ab}\pm0.00048$
Additive	4.02	0.0015
Dominance	-7.41	-0.0025

[a,b] Means within a column with no common superscript differ significantly ($p<0.05$).

Frequency of genotypes and alleles

The frequencies of the genotypes and alleles are shown in Table 1. The frequency of allele A was lower than that of allele B. The frequency of allele B in the Lean line was higher than that in the Fat line. The frequencies of the genotypes in chickens belonging to the Lean and Fat lines differed significantly ($p<0.001$).

Effects of the *OBR* gene on growth and body composition

The effects of the main factors in the statistical model on the tested traits are listed in Table 2. There were significant associations between the *OBR* genotypes and fatness traits (AFW and AFP) ($p<0.05$). BW and Line had significant effects on all traits ($p<0.01$).

As is shown in Table 3, birds carrying allele B (homozygous OBR-BB and the heterozygous OBR-AB) had significantly lower AFW than those homozygous birds carrying allele A (OBR-AA) ($p<0.05$). OBR-AB birds had a significant lower AFP than OBR-AA birds ($p<0.05$). There were no significant differences among the *OBR* genotypes in BW, CW, HW, LW, SW, MSW and GSW ($p>0.05$). At the same time the *OBR* gene had an overdominant effect on both AFW and AFP. The effects of the *OBR* gene explained 8.9% of the genetic variance and 3.8% of the phenotypic variance of AFW, and 4.5% of the genetic variance and

Table 1. Allele and genotype frequencies of the *OBR* gene in the broiler lines for divergent selection on abdominal fat

Line	N[1]	Genotype frequency			Allele frequency		X^2
		AA	AB	BB	A	B	
Lean	148	0.0405	0.3716	0.5878	0.2264	0.7736	13.96
Fat	80	0.175	0.4125	0.4125	0.3813	0.6187	(0.001)[2]

[1] Number of individuals. [2] X^2 test and p value.

Table 2. Significance of effects of *OBR* genotype, line and body weight on chicken growth and body composition traits

	CW[1]	AFW	AFP	HW	LW	SW	MSW	GSW
Genotype	NS[2]	0.0201	0.0174	NS	NS	NS	NS	NS
BW	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Line	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

[1] CW = Carcass weight; AFW = Abdominal fat weight; AFP = Percentage of abdominal fat; HW = Heart weight; LW = Liver weight; SW = Spleen weight; MSW = Muscular stomach weight; GSW = Glandular stomach weight.
[2] Not significant at $p>0.05$.

3.0% of the phenotypic variance of AFP.

DISCUSSION

The candidate gene approach is a powerful method for finding the QTL responsible for genetic variation in the traits of interest in agricultural animal species (Rothschild and Soller, 1997). And now it is widely used in the field of basic researches of broiler breeding. The polymorphism of insulin-like growth factor I (*IGF I*) in 6 chicken breeds was detected by PCR-RFLP (Wang et al., 2004), in which associations between *IGF-I* gene and chicken body weight have been revealed. The coding region of chicken *PPAR-α* gene was analyzed by PCR-SSCP, which suggested that *PPAR-α* gene may be a major gene or linked to the major genes that impact chicken fat metabolism and the SNPs could be used in molecular assistant selection (MAS) as a genetic marker for the chicken fatness traits (Meng et al., 2005). A SNP (G54C) was found in CDS region of chicken *MC4R* gene (Li et al., 2006), through which it can be deduced that *MC4R* gene may have great effects on body weight and skeletal development in chicken, and maybe a potential marker for use in Molecular-Assisted Selected programs.

The NEAU (Northeast Agricultural University) broiler lines have been selected divergently on abdominal fat. The fat and lean lines displayed significantly different abdominal fat deposition from the 4th generation onwards. These lines provided a valuable resource for studying candidate genes on fatness. In our previous study, a SNP was detected in the chicken *OBR* gene Exon9, the frequency of genotypes among various chicken breeds and lines differed significantly (Gu et al., 2002). That finding was confirmed in the present study, and the frequencies of genotype AA and allele A were significantly lower in the Lean line than those in the Fat line. The decrease in the frequency of *OBR* genotype AA in the Lean line might be the direct consequence of the selection against abdominal fatness, suggesting *OBR* as a casual gene for abdominal fat deposition.

Excessive fat in chickens should be limited in order to enhance production efficiency and product quality. OBR, a hypothalamic receptor of Leptin, is an integral component of a complex physiological system involved in regulating fuel stores and energy expenditure (Zhang et al., 1994). The main physiological function of OBR is to bind leptin so that the leptin can exert its effect on energy metabolism *in vivo*.

Several researchers have investigated associations of *OBR* polymorphisms with fatness traits in mammals. A SNP GLN223ARG was found in the human *OBR* gene. Genotypes at that locus were associated with differences in body mass index (BMI), fat mass and serum leptin levels in postmenopausal Caucasian women. This association indicated that functional variations in the *OBR* gene are important factors in the regulation of adiposity and BMI (Quinton et al., 2001). Four SNPs of porcine *OBR* gene intron2, exon2, 6, and 18 were found in Landrace, Yorkshire by MS-PCR and PCR-RFLP. Effects of exon6 and 18 polymorphisms on backfat thickness were significant ($p<0.05$) in Landrace and Yorkshire (Chen et al., 2004). Thus, extensively studying the chicken *OBR* gene may lead to a great breakthrough in the understanding of body weight regulation of poultry.

In the current study, a SNP in exon9 of the *OBR* gene was detected and the *OBR* gene was associated with AFW and AFP. OBR-AA birds had higher AFW than OBR-AB and OBR-BB ones, and had higher AFP than OBR-AB birds ($p<0.05$). It can be deduced that allele A may be related to higher AFW and allele B may lead to lower AFW. Therefore, allele B can be considered as a beneficial allele. A comparison of the mean values for all three genotypes suggests that *OBR* mainly acts in a dominant fashion on abdominal fat traits, with allele B contributing to lower fat deposition. Birds with different genotypes had no significant differences in growth and other body composition traits. The results point to the possible identification of *OBR* as a candidate gene of quantitative trait loci (QTL) useful for altering abdominal fat.

In summary, commercial breeding programs of broiler chickens have become more complex and challenging because so many objectives need to be simultaneously considered to reduce production costs, maintain health, and improve product quality. Breeding goals must include increased growth rate, increased breast muscle yield, decreased abdominal fat, maintenance of good development and overall fitness. The relationships of these traits are complex, and some of the traits are very difficult to measure. Therefore, molecular MAS can improve genetic selection programs. The results from the current study indicated that a SNP marker in the *OBR* gene is associated with fat deposition. As suggested by low AFW and AFP, birds which inherited allele B intended to be lean. The *OBR* gene showed a great potential for use in molecular MAS programs to control fat deposition.

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