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Relationship Between Combined Genotypes of *UCP* Gene and Growth Traits in Chickens

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Abstract: The uncoupling protein (UCP) is a member of the mitochondrial membrane transporter family, which plays an important role in energy metabolism. In the present study, the *UCP* gene was considered as a candidate gene for chicken growth traits, and the association of *UCP* gene SNPs with growth rate was investigated in the eighth generation of NEAUHLF broiler lines. Two SNPs were found in chicken *UCP* gene, and the association analysis results showed that both the individual and combination of chicken *UCP*gene SNPs were significantly associated with body weight of 7 weeks (BW7) and carcass weight (CW) (P<0.05), and the combination had much significant effects than the single SNP. This research suggested that the *UCP* gene could be a candidate gene or linked to a major gene which affected growth traits in chicken.

Key words: chicken, uncoupling protein gene, SNPs, genotype combination, growth traits

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Introduction

Defining the molecular genetic basis of economically important traits in agricultural species is an increasingly important goal. A large number of quantitative trait loci (QTL) and candidate gene analysis have revealed genomic regions and specific markers that are associated with traits such as growth, fat deposition in chicken (Abasht *et al*., 2006; Zhou *et al*., 2005; Wang *et al*., 2006; Leng *et al*., 2009; Tian *et al*., 2010). As we known, most economically important traits are controlled by multiple genes or loci, if many QTL are known, and favorable alleles are present in lines or breeds, genotype building strategies can be used to design new genotypes that combine favorable alleles at all loci to directly increase the quantity or quality of product or to reduce the cost at which the product can be produced (Dekkers and Hospital, 2002).

Among the genes involved in the development of growth, the uncoupling proteins (UCP) genes play important roles in the regulation of energy expenditure in animals (Gura, 1998). UCPs are members of the mitochondrial membrane transporter family, present in the mitochondrial inner membrane that mediates regulated discharge of the proton gradient that is generated by the respiratory chain and diminishing the resulting production of ATP and instead yielding dissipative heat. This energy-dissipater mechanism can be associated with the metabolism of fat and regulation of the energy expenditure (Ricquier, 2005). In mammals, UCPs family has four members,

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named UCP1, UCP2, UCP3 and UCP4 (Ricquier and Bouillaud, 2000). The predicted amino-acid sequence of avian UCP is 55%, 70% and 70% identical with mammalian UCP1, UCP2 and UCP3. Therefore, we deduce that the avian *UCP* gene plays the same role as mammalian *UCP*2 and *UCP*3 that relate to energy metabolism. The avian *UCP* gene is expressed only in the skeletal muscle and its abundance is increased 1.3-fold in a chicken line showing diet-induced thermogenesis, and 3.6- and 2.3-fold in cold acclimated and glucagons-treated ducklings developing muscle non-shivering thermogenesis respectively (Raimbault *et al*., 2001). The *UCP* gene, therefore, is potentially an excellent candidate gene for growth and body composition traits in chickens.

Two single nucleotide polymorphisms (SNP) of the *UCP* gene are detected. One is in chicken *UCP* gene intron2 (DNA C1240A, Accession No. AF 433170) and the other is in chicken *UCP* gene 3' UTR region (DNA C2594A, Accession No. AF 433170). The objectives of the present study were to genotype the polymorphisms in NEAUHLF broiler lines, and evaluate associations between individual and combination pattern of chicken *UCP* gene SNPs and growth and body composition traits.

Materials and Methods

Experimental populations and management

The Northeast Agricultural University high and lean fat broiler lines (NEAUHLF) have been selected divergently using abdominal fat percentage and plasma VLDL (very low-density lipoprotein) concentration as selection criteria since 1996. The abdominal fat weight and percentage of abdominal fat had significant difference between two lines after selection for four generations. The eighth generation of NEAUHLF broiler lines was used in the current study. All birds had access to feed and water ad libitum. Commercial corn-soybean-based diets that met all NRC requirements (National Ressearch Council, 1994) were provided in the study. From hatch to 3 weeks of age, birds received a starter feed (3 000 kal ME·kg^{-1} and 210 g·kg^{-1} CP) and from 3 to 7 weeks of age, birds were fed a grower diet (3 100 kal ME·kg^{-1} and 190 g·kg^{-1} CP).

Phenotypic measurements

Body weight (BW) was measured at hatch up to 7 weeks of age in 2-week interval. Body composition traits were recorded at 7 weeks of age. These measurements included carcass weight (CW), pectoralis major weight (PMaW), pectoralis minor weight (PMiW), leg muscle weight (LMW), abdominal fat weight (AFW), heart weight (HW) and liver weight (LW), etc. All traits were also expressed as percentage of BW at 12 weeks of age. All the adipose tissues around the abdomen and gizzard stomach were separated carefully and weighed as AFW. All data were collected following proper animal care protocol.

Genotyping

The eighth generation of NEAUHLF broiler lines and the breeding birds of the eighth generation of NEAUHLF broiler lines were genotyped for both C1240A and C2594A polymorphisms of *UCP* gene.

C1240A: the PCR primers F: 5' CAT CGG GCT CTA CGA CTC TG 3' and R: 5'-GAA CCG CAC CTT GAC CAC 3' were used to amplify the 680 bp fragment which contained the C1240A polymorphism. PCR reactions were performed with 50 ng chicken DNA, 1×PCR reaction buffer, 5 pmol of each primer, 400 μmol·L^{-1} of dNTP, and 1 U *Taq* polymerase in a 25-μL final reaction volume. The reaction was carried out in a Geneamp PCR system 9700 thermal cycler (Applied Biosystems) and PCR conditions were 94℃ for 7 min, followed by 35 cycles of 94℃ for 30 s; 56℃ for 30 s; 72℃ for 30 s, and an extension at 72℃ for 5 min. The PCR product was then incubated with 3 U *Eco*72 I restriction enzyme and its supplied buffer at 37℃ for 3 h. Restriction patterns were visualized by electrophoresing the digestion product in a 2% agarose gel stained with ethidium bromide.

C2594A: the following modifications to the protocol

described above were used. PCR primers F: 5' GCT CCT GGA ACG TGG TGA 3' and R: GCT GCC TTT GGT CCC TCT 3' were used to amplify the 206 bp fragment which contained the C2594A polymorphism. The restriction enzyme *Hae* III was used to reveal the polymorphism. Restriction patterns were visualized by electrophoresing the digestion product in a 14% PAGE. Silver stain method was developed to display the bands.

Statistical analysis

The association between the polymorphism sites and chicken growth and body composition traits was analyzed using the GLM of JMP (SAS Institute Inc., 2002). The model was fitted with the genotype (G) and Line (L) as fixed effects; Family nested within the Line (F (L)), Dam nested within the Family and Line (D (F, L)) as random effects, G×L as interaction of G by L, and BW at 7 weeks (BW7) as a covariate, as the followings:

$$Y=\mu+G+L+F(L)+D(F,L)+G\times L+BW_7+e \quad (1)$$

Where, Y was the dependent variable, μ was population mean, and e was the random error. Significant difference between least square means of the different genotypes was calculated using a contrast test. Significance was determined as $P<0.05$, unless otherwise specified.

Results

Sequence variation and PCR-RFLP analysis

Sequencing of multiple individuals showed two SNPs, C/A SNP at base 1 240 and C/A SNP at base 2 594 (Accession Number AF433170). The PCR-RFLP method was developed successfully for genotyping the C1240A SNP in intron 2 and the C2594A SNP in the 3' UTR region of the chicken *UCP* gene, and all of the individuals were screened. For the C1240A site, allele A was defined as fragments of 680 bp and allele B (C1240A) was defined as fragments of 570 and 110 bp (Fig. 1). For the C2594A site, Allele C was defined as fragments of 186 and 20 bp and allele D (C2594A) was defined as fragments of 168, 18 and 20 bp (Fig. 2).

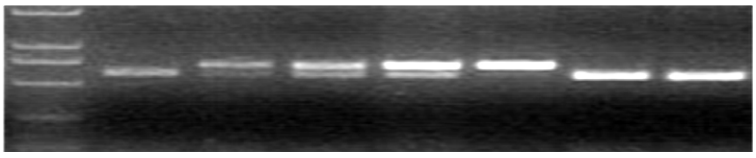

Fig. 1 PCR-RFLP pattern for *UCP* gene C1240A polymorphism with *Eco*72 I digestion

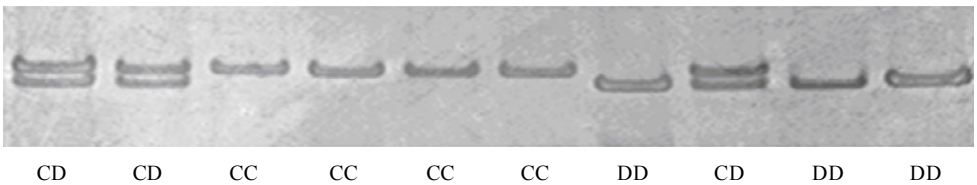

Fig. 2 PCR-RFLP pattern for *UCP* gene C2594A polymorphism with *Hae* III digestion

Association and effects of single *UCP* gene SNP with growth traits

The *UCP* gene C1240A and C2594A polymorphisms were predominantly related to BW7 and CW ($P<0.05$) (Table 1). However, there were no significant differences on AFW, PMiW, PMaW, LMW, HW and LW among individuals with different genotypes of *UCP*

gene.

For the C1240A SNP of the *UCP* gene, there were significantly higher BW and CW of 7 weeks in the birds that were homozygous for the B alleles than in those homozygous for the A alleles (P<0.05) (Table 2). For the C2594A SNP of the *UCP* gene, there were significantly higher BW and CW of 7 weeks in the birds that were homozygous for the C alleles than in those homozygous for the D alleles (P<0.05) (Table 2).

Table 1 Effects of individual and combination of *UCP* C1240A and C2594A polymorphisms on chicken growth traits and body composition traits (*P* value)

Traits	*P* value		
	C1240A	C2594A	Combination of C1240A and C2594A
BW7	0.0326	0.0083	0.0040
CW	0.0337	0.0072	0.0071
PMaW	NS	NS	NS
PMiW	NS	NS	NS
LMW	NS	NS	NS
AFW	NS	NS	NS
HW	NS	NS	NS
LW	NS	NS	NS

BW7, Body weight of 7 weeks; CW, Carcass weight; HW, Heart weight; PMaW, Pectoralis major weight; LMW, Leg muscle weight; AFW, Abdominal fat weight; HW, Heart weight.

Table 2 Effects of individual and combination of *UCP* C1240A and C2594A polymorphisms on chicken growth traits and body composition traits (LSM)

Polymorphisms	Genotype	BW7 (g)	CW (g)
UCP C1240A	AA (69)	2 250.04±25.90b	1 989.02±23.17b
	AB (178)	2 315.72±18.20a	2 048.78±16.23a
	BB (125)	2 324.99±20.74a	2 054.72±18.53a
UCP A1197C	CC (176)	2 300.48±18.59a	2 039.92±16.76a
	CD (166)	2 325.23±16.61a	2 055.81±14.97a
	DD (50)	2 216.64±32.19b	1 955.67±28.92b
Combination of C1240A and 2594A	AACC (7)	2 245.14±71.68abc	1 999.64±64.38abc
	ABCC (63)	2 348.36±34.06a	2 080.69±30.51a
	BBCC (98)	2 311.48±23.00a	2 046.08±20.51a
	AACD (26)	2 290.11±38.05ab	2 029.20±34.12ab
	ABCD (107)	2 317.06±21.48a	2 050.51±19.14a
	BBCD (27)	2 389.84±38.51a	2 098.96±34.49a
	AADD (36)	2 219.40±35.24bc	1 957.59±31.54bc
	ABDD (9)	2 039.32±98.12c	1 804.72±88.18c
	BBDD (0)	/	/

a and b means within a row with no common superscripts are different (P<0.05). The same as below.

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Association and effects of combination of *UCP* gene C1240A and C2594A polymorphisms on chicken growth traits and body composition traits

Eight combination genotypes of C1240A and C2594A polymorphisms of *UCP* gene were found in the NEAU divergent broiler lines, for there was no DDBB genotype bird in this population. The combination of *UCP* C1240A and C2594A polymorphisms had significant effect on BW7 and CW (Table 1). The multiple comparison results showed that for BW7 and CW, the birds with AADD genotype were the lowest and with BBCC genotype were the highest among all the homogenous individuals which were AACC, BBCC, AADD and BBDD genotypes (P<0.05) (Table 2).

Difference comparison between homozygote individuals in single marker analysis and in combination genotype

For BW of 7 weeks, the differences between two homogenous individuals of C1240A (AA and BB) and C2594A (CC and DD) polymorphisms were 74.95 g and 83.84 g, respectively, and for CW of 7 weeks, the differences between two homogenous individuals of C2594A and C2594A polymorphisms were 65.7 and 84.25, respectively (Table 3). The difference between the combination homogenous (BBCC and AADD) individuals was 92.08 g for BW of 7 weeks, and 88.49 g for CW, which was much higher than the difference between single SNP homogenous individuals.

Table 3 Difference comparison between homozygote individuals in single marker analysis and in combination genotype analysis

Polymorphisms	Genotype	BW7 (g)	CW (g)
UCP C1240A	AA (69)	2250.04±25.90b	1989.02±23.17
	BB (125)	2324.99±20.74a	2054.72±18.53
	Difference	74.95	65.7
UCP C2594A	CC (176)	2300.48±18.59b	2039.92±16.76
	DD (50)	2216.64±32.19a	1955.67±28.92
	Difference	83.84	84.25
Combination of C1240A and C2594A	BBCC (98)	2311.48±23.00	2046.08±20.51
	AADD (46)	2219.40±35.24	1957.59±31.54
	Difference	92.08	88.49

Discussion

Growth rate is of great economic importance in commercial broiler production. Hence, growth rate received considerable interest in chicken breeding programmes. With the advent of molecular genetics tools, substantial advances have been made over the past decades through the application of molecular genetics in the identification of loci and chromosomal regions that contain loci affected traits of importance in livestock production (Andersson, 2001). This has enabled opportunities to enhance genetic improvement programs in livestock by direct selection on genes or genomic regions that affect economic traits through marker-assisted selection (Dekkers and Hospital, 2002).

The chicken *UCP* gene was first discovered by Raimbault in 2001. The nucleic acid sequence of avian

UCP is highly homologous to both mammalian UCP2 and UCP3 (Ricquier and Bouillaud, 2000). Mice *UCP*2/3 genes are on the 7 chromosome, and genomic linkage analysis showed that there is a QTL controlling heat production and food conversion efficiency on this chromosome (Moody *et al*., 1999). It is reported that mice *UCP*2/3 genes are associated with obesity, the regulation of body weight (Fleury *et al*., 1997). Liu *et al*. (2007) found that the SNP of *UCP* gene exon 3 was associated with muscle and fatness traits in chicken.

In most cases, a single gene of small effect is not sufficient to produce a clinical symptom, but their combined effects may confer additive genetic contributions (Xu *et al*., 2003). Similar to this, the combination of two SNPs of one gene may have more significant effects on traits than single site, of course, the precondition is that the two SNPs have coincident effects on the same traits. According to the research on the *UCP* gene in mammals and birds (Gura, 1998; Raimbault *et al*., 2001; Liu *et al*., 2007), we postulated the avian *UCP* gene as the candidate gene to affect growth and body composition traits in chickens. The current study was aimed at studying the association between the SNPs of *UCP* gene and growth and body composition traits. Results showed that both the individual and combination of chicken *UCP* gene C1240A and C2594A polymorphisms were predominantly related with BW7 and CW, and the combination genotype had higher effects than the single SNP, and the birds with BBCC genotype had higher BW7 and CW than birds with AADD genotype. Furthermore, the difference between combination homogenous (BBCC and AADD) individuals was higher than that of the single polymorphism site.

The 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 muscle yield, decreased abdominal fat, and 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 (Martin *et al*., 1990; Pinard-van *et al*., 1998). The results from the current study indicated that the *UCP* gene had considerable value for improved chicken growth rate, and the *UCP* gene could be a candidate gene or linked to a major gene which affected growth traits in chicken. Therefore, the DNA markers in this study had potential for usage in molecular marker-assisted selection programs.

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References

Abasht B, Dekkers J C M, Lamont S J. 2006. Review of quantitative trait loci identified in the chicken. *Poultry Science*, **85**(12): 2079-2096.

Andersson L. 2001. Genetic dissection of phenotypic diversity in farm animals. *Nature Review Genetics*, **2**: 130-138.

Dekkers J C, Hospital F. 2002. The use of molecular genetics in the improvement of agricultural populations. *Nature Review Genetics*, **3** (1): 22-32.

Fleury C, Neverova M, Collins S, *et al*. 1997. Uncoulping protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nature Gene*, **15** (3): 269-272.

Gura, T. 1998. Uncoupling proteins provide new clue to obesity's causes. *Science*, **280**(5368): 1369-1370.

Leng L, Wang S Z, Li Z H, *et al*. 2009. A polymorphism in the 3′-flanking region of insulin-like growth factor binding protein 2 gene associated with abdominal fat in chickens. *Poultry Science*, **88**(5): 938-942.

Liu S,Wang S Z, Li Z H, *et al*. 2007. Association of single nucleotide polymorphism of chicken uncoupling protein gene with muscle and fatness traits. *Journal of Animal Breeding and Genetics*, **124**(4): 230-235.

Martin A, Dunnington E A, Gross W B, *et al*. 1990. Production traits

and alloantigen systems in lines of chickens selected for high or low antibody responses to sheep erythrocytes. *Poultry Science*, **69**(6): 871-878.

Moody D E, Porop D. Nielsen M K, *et al*. 1999. Identification of influencing traits related to energy balance in selection and inbred lines of mice. *Genetics*, **152**(2): 699-711.

Pinard-van M H, Siegel P B, and Lamont S J. 1998. Lessons from selection experiments on immune response in the chicken. *Poultry Avian Biology Review*, **9**: 125-141.

Raimbault S, Dridi S, Denjean F, *et al*. 2001. An uncoulping protein homologue putatively involved in facultative muscle thermogenesis in birds. *The Biochemical Journal*, **353**: 441-444.

Ricquier D, Bouillaud F. 2000. Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance. *The Journal of Physiology*, **529**: 3-10.

Ricquier D, and Bouillaud F. 2000. The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *The Biochemical Journal*, **345**: 161-179.

Ricquier D. 2005. Respiration uncoupling and metabolism in the control of energy expenditure. *The Proceeding of Nutrition Society*, **64**(1): 47-52.

Tian J W, Wang S Z, Wang Q G, *et al*. 2010. A single nucleotide of chicken acetyl-CoA carboxylase A gene associated with fatness traits. *Animal Biotechnology*, **21**(1): 42-50.

Wang Q G, Li H, Li N, *et al*. 2006. Identification of single nucleotide polymorphism of adipocyte fatty acid-binding protein gene and its association with fatness traits in the chicken. *Poultry Science*, **85**(3): 429-434.

Xu Q, Jia Y B, Zhang B Y, *et al*. 2004. Association study of an SNP combination pattern in the dopaminergic pathway in paranoid schizophrenia: a novel strategy for complex disorders. *Molecular Psychiatry*, **9**(5): 510-521.

Zhou H, Mitchell A D, McMurtry J P, *et al*. 2005. Insulin-like growth factor-I gene polymorphism associations with growth, body composition, skeleton integrity, and metabolic traits in chickens. *Poultry Science*, **84**(2): 212-219.