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Identification of Novel SNPs with Effect on Economic Traits in Uncoupling Protein Gene of Korean Native Chicken

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ABSTRACT : The avian uncoupling protein (avUCP) is a member of the mitochondrial transporter superfamily that uncouples proton entry in the mitochondrial matrix from ATP synthesis. The sequencing analysis method was used to identify nucleotide polymorphisms within the avUCP gene in Korean native chicken (KNC). This study identified ten single nucleotide polymorphisms (SNPs) in the avUCP gene. We analyzed the SNPs of the avUCP gene to investigate whether polymorphism in the gene might be responsible for quantitative variations in economic traits in KNC. Three significant polymorphic sites for economic traits were avUCP C+282T (mean body weight, $p<0.05$), avUCP C+433T (daily percent lay, $p<0.05$), and avUCP T+1316C (daily percent lay, $p<0.05$). The frequency of each SNP was 0.125 (C+282T in avUCP gene exon 1 region), 0.150 (C+433T in avUCP gene intron 1 region), and 0.15 (T+1316C in avUCP gene exon 3 region), respectively. Among the identified SNPs, one pair of SNPs (genotype CC, C+282T and TT, avUCP C+433T) showed the highest daily percent lay ($p<0.05$) and mean body weight ($p<0.05$) and the frequency was 0.067. This study of the avUCP gene could be useful for genetic studies of this gene and selection on economic traits for KNC. (**Key Words :** Uncoupling Protein (UCP) Gene, Single Nucleotide Polymorphism (SNP), Korean Native Chicken (KNC))

INTRODUCTION

The mitochondrial uncoupling protein UCP1 is expressed exclusively in brown adipose tissue (BAT). It is known to uncouple phosphorylation from oxidation and hence to be involved in energy metabolism and heat production, especially under cold exposure (Ricquier et al., 2000). UCP-1 transports fatty acid anions that can reenter the mitochondria after their protonation. The electrochemical gradient that is generated along the inner mitochondrial membrane by respiration dissipates as a result of UCP activation and heat is produced instead of chemical energy (Garlid et al., 1996). More recently, two other members of the UCP gene family, termed UCP-2 and UCP-3, have been identified (Boss et al., 1997; Fleury et al., 1997; Gimeno et al., 1997; Vidal-Puig et al., 1997). UCP-2 and UCP-3 exhibit structural homology to and share functional properties with UCP-1. UCP-2 gene is expressed in several human tissues including BAT, white adipose tissue, lung, liver, spleen, and macrophages (Fleury et al., 1997; Gimeno et al., 1997). UCP-3 gene is strongly expressed in skeletal muscle and, to a lesser extent, in heart, BATand white adipose tissue (Boss et al., 1997; Vidal-Puig et al., 1997).

Birds are also known to regulate their body temperature by elevating their heat production under low temperatures, partially by activating non-shivering thermogenesis (NST) in skeletal muscle (Duchamp et al., 1999). The expression of this protein is especially enhanced in cold-acclimated ducklings and in cockerels from an energy inefficient laying strain (Gabarrou et al., 1997). However, avUCP expression is not affected in skeletal muscle of chickens submitted to a 4-h cold stress (Raimbault et al., 2001).

The human UCP-1 gene expression and activity is regulated by sympathetic nervous system through 3-adrenergic receptor (Cassard et al., 1990; Clement et al., 1996; Bouillaud et al., 2001; Shihara et al., 2001). Polymorphism in the 5'-flanking region of the UCP-1 gene

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Table 1. Primers for sequencing analysis of avUCP gene

Name	Sequence	Region
avUCP_1F	ggcctcagtttccctaag	96-103 bp
avUCP_1R	cactggactcactctcagc	716-734 bp
avUCP_2F	gtagggaccgactacctca	1,097-1,115 bp
avUCP_2R	catccctacactcatgcc	1,540-1,557 bp
avUCP_3F	gcaactccatcattaactgc	2,048-2,066 bp
avUCP_3R	ccttctcctctctccattct	2,718-2,737 bp

was found and shown to be associated with increased body weight and body fat gain over time in the Quebec Family Study (Oppert et al., 1994; Kogure et al., 1998; Bouillaud et al., 2001). Several other studies reported associations of UCP-1 polymorphism with obesity and related metabolic disorders (Fogelholm et al., 1998; Pihlajamaki et al., 1998; Hayakawa et al., 1999; Sivenius et al., 2000; Heilbronn et al., 2000; Proenza et al., 2000; Herrmann et al., 2003).

In the present study, we identified ten novel SNPs in avUCP gene in Korean native chicken (KNC) population. This study was conducted to investigate the effect of the ten SNPs in avUCP gene on economic traits in the KNC population.

MATERIALS AND METHODS

Animal and genomic DNA extraction

60 KNC population used in this study for analysis. The analysis traits were body weight at age 270 days, mean egg weight at age 270 days and daily percent lay at age 270 days in 60 KNC population. DNA samples were extracted from blood by some modification of the method used by Miller et al. (1988).

PCR amplification

Three primer sets for the PCR amplification, to sequencing analysis, primers were designed based on GenBank sequences (Accession. no. AF433170)(Table 1). The Polymerase Chain Reaction was conducted in 10 μl volumes, each containing 100 ng of genomic DNA, 10× PCR buffer (100 mM Tris pH 8.9, 50 mM KCl, 15 mM MgCl, 0.01% gelatin, 0.1% Triton X-100, 10 mg/ml BSA),

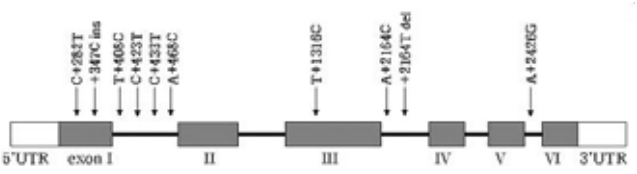

Figure 1. Identification of sequence polymorphism in avUCP gene (Gene Bank accession no. AF433170). Coding exons are marked by shaded blocks and 5' and 3' UTR by white blocks. The first base of translational start site is denoted nucleotide +1.

10 pmole of each primer, 40 μM of dNTPs and 0.5 unit Taq DNA polymerase (Promega, USA). The condition of PCR was a first denaturation step of 4 min at 94°C followed by 35 cycles, each consisting of 40 sec at 94°C, 30 sec at 61.3°C, 2 min at 72°C and then, a final step of 10 min at 72°C using Peltier Thermal Cycler 200 (MJ Research, USA).

Sequencing

Direct sequences were generated from both stands with ET terminator Cycle Sequencing Kit on a PTC 200 peltier thermal cycler (MJ Research, USA). The extension reaction in a 10 μl volume was performed and extension products were electrophoresed on MegaBACE DNA Analysis System (Amersham Biosciences, USA.) Searching sequence mutation was using the seqMAN II software(DNA Star Inc.)

Statistical analysis

For each trait, the following linear covariate model was used using SAS (SAS 9.2).

$$y_{jk} = \mu + G_j + e_{jk}$$

where y_{jk} = a phenotypic record;
μ = overall mean;
G_j = genotype;
e_{jk} = random residual.

Table 2. Novel single nucleotide polymorphisms (SNPs) in UCP gene in Korean native chickens

Locus	Region	No. of variants/No. of chr.	Frequency*	Heterozygosity
C+282T	exon 1	15/120	0.125	0.108
+347C ins	exon 1	7/120	0.058	0.058
T+408C	intron 1	10/120	0.083	0.033
C+423T	intron 1	6/120	0.050	0.025
C+433T	intron 1	18/120	0.150	0.083
A+468C	intron 1	14/120	0.116	0.116
T+1316C	exon 3	19/120	0.158	0.143
A+2164G	intron 4	2/40	0.050	0.050
+2166T del	intron 4	8/40	0.200	0.200
A+2426G	intron 5	6/40	0.150	0.150

* Frequencies of rare alleles.

Table 3. Effect of avUCP gene polymorphism on economic traits of Korean native chicken

Locus	Region	Traits	Genotype		
			CC	CT	TT
C+282T	exon 1	Body weight	1,669.78±35.197[a]	1,513.07±66.210[ab]	1,420.00±238.723[b]
C+433T	intron 1	Daily percent lay (%)	80.80±1.619[b]	87.70±3.473[ab]	92.25±5.492[a]
T+1316C	exon 3	Daily percent lay (%)	80.03±2.471[b]	79.93±2.962[ab]	83.61±1.729[a]

[a, b] Different superscripts within rows are significantly differ, $p<0.05$.

Table 4. The interaction analysis between polymorphism C+282T and T+1316C effect on mean egg weight in Korean native chicken

C+282T	T+1316C	Frequency	Mean egg weight (g)
CC	CC	0.017	46.00±3.16
CC	TC	0.183	48.45±0.95
CC	TT	0.566	49.11±0.54[a]
CT	TC	0.067	45.50±1.58[b]
CT	TT	0.150	48.00±1.05
TT	TT	0.017	49.00±3.16

[a, b] Different superscripts within columns are significantly differ, $p<0.05$.

Table 5. Interaction analysis of C+433T and T+1316C polymorphism effect on no. of egg production in Korean native chicken

C+433T	T+1316C	Frequency	Daily percent lay (%)
CC	CC	0.017	85.00±11.13
CC	TC	0.183	78.81±3.35[b]
CC	TT	0.566	81.32±1.90[b]
CT	TC	0.050	80.33±6.42
CT	TT	0.117	90.85±4.20[a]
TT	TC	0.017	91.00±11.13
TT	TT	0.050	92.66±6.42

[a, b] Different superscripts within columns are significantly differ, $p<0.05$.

RESULTS AND DISCUSSION

To investigate SNPs of avUCP gene, we used the PCR method to amplify the regions by using the specific primers (Table 1). The first PCR product (avUCP_1F and avUCP_1R) was 638 bp, involving the whole exon 1, 2, intron 1, and partial intron 2. The second PCR product (avUCP_2F and avUCP_2R) was 460 bp, involving the whole exon 3 and partial intron 2, 3. The third PCR product (avUCP_3F and avUCP_3R) was 689 bp, involving the whole exon 4-6, intron 4, 5, partial intron 3 and 3' untranslated region (UTR). The sequencing analysis method was used to identify nucleotide polymorphisms within the avUCP gene in Korean native chicken (KNC). This study identified ten SNPs; two in exon 1, three in intron 1, one in exon 3, two in intron 4 and one in intron 5 (Figure 1). The frequencies of identified each SNPs were 0.125 (C+282T), 0.058 (+347C ins), 0.083 (T+408C), 0.050 (C+423T), 0.150 (C+433T), 0.116 (A+468C), 0.158 (T+1316C), 0.050 (A+2164G), 0.200 (+2166T del), and 0.150 (A+242G) in 60 unrelated KNC (Korean Lviestock Research Institute, Korea), respectively (Table 2). The positions of individual polymorphisms were calculated and named according to the sequence available in the GeneBank (accession no. AF33170).

We next investigated the effect of the ten SNPs in avUCP gene on economic traits in KNC population. As shown Table 3, the analysis results showed significant three polymorphic sites on daily percent lay and mean body weight. Significant three SNPs on economic traits were C+282T, C+433T and T+1316C. C+282T is located in avUCP exon 1 region, C+433T is located in avUCP intron 1 region, and T+1316C is located in avUCP exon 3 region. As shown in Table 3, genotype CC of avUCP C+282T had a significant higher body weight (+249.8 g) than genotype TT ($p<0.05$). Genotype TT of avUCP C+433T showed had a significant higher daily percent lay (+11.4%) than genotype CC ($p<0.05$). SNP avUCP T+1316C had significant daily percent lay and mean body weight. Genotype TT of avUCP T+1316C had a significant higher daily percent lay (+3.6%) than genotype CT ($p<0.05$).

Among detected SNPS, genotype CC of avUCP C+282T and genotype TT of avUCP C+433T showed the highest mean body weight (1,669.78 g) and daily percent lay (92.25%), respectively. The two SNPs may be useful for selection on mean body weight and daily percent lay trait in KNC.

We next analyzed the association between the major SNPs, avUCP C+282T, C+433T and T+1316C. The results of association analysis between SNPs C+282T and T+1316C are shown in Table 4. The combination genotype CC (C+282T) and TT (T+1316C) had a significant higher mean egg weight (+3.6 g), as compare to the combination genotype CT (C+282T) and TC (T+1316C) ($p<0.05$). The frequencies of genotype CC (C+282T) and TT (T+1316C), CT (C+282T) and TC (T+1316C) were 0.566 and 0.065, respectively. Therefore, the genotype CC of C+282T and TT of T+1316C is presumed to exert good effect on mean egg weight trait.

Table 5 was summarized that the association analysis between SNPs avUCP C+433T and T+1316C. The combination genotype TT (C+433T) and TT (T+1316C), CT (C+433T) and TT (T+1316C) showed significant higher daily percent lay (+12.0%) than genotype CC (C+433T) and TC (T+1316C) ($p<0.05$). The combination genotype TT (C+433T) and TT (T+1316C) showed the highest daily percent lay (+13.8%) but it was not significant because of

Table 6. Interaction analysis of C+282T and C+433T polymorphism effect on economic traits in Korean native chicken

C+282T	C+433T	Frequency	Daily percent lay (%)	Mean egg weight (g)	Body weight (g)
CC	CC	0.567	81.35±1.90	49.32±0.52[a]	1,669.1±40.6[a]
CC	CT	0.133	87.00±3.93	48.25±1.08	1,638.7±83.7
CC	TT	0.067	92.25±5.56[a]	46.50±1.53	1,737.5±118.3[a]
CT	CC	0.183	78.27±3.35[b]	46.63±0.92[b]	1,460.9±71.3[b]
CT	CT	0.033	90.50±7.87	50.50±2.17	1,800.0±167.4
TT	CC	0.017	90.00±11.13	49.00±3.07	1,420.0±236.7

[a, b] Different superscripts within columns are significantly differ, $p<0.05$.

high standard deviation value. The frequencies of combination genotype TT (C+433T) and TT (T+1316C), CT (C+433T) and TT (T+1316C), genotype CC (C+433T) and TC (T+1316C) were 0.050, 0.117, and 0.183, respectively.

The allele T of C+433T and allele T of T+1316C are presumed to exert good effect on the daily percent lay trait. The combination genotype CC (C+282T) and CT or TT (C+433T) showed food effect on all traits investigated.

As shown Table 6, the combination genotype CC (C+282T) and TT (C+433T) showed a significant higher daily percent lay (+4%) than CT and CC ($p<0.05$). The frequencies of combination genotypes CC and TT, CT and CC were 0.067, 0.183, respectively. The combination genotype CC (C+282T) and CC (C+433T) showed a significant higher mean egg weight (+2.7 g) than CT and CC ($p<0.05$). The frequency of combination genotype CC and CC was 0.567. The combination genotype CC (C+282T) and TT (C+433T) showed a significant higher body weight (+263 g) than CT and CC ($p<0.05$). The allele C of C+282T and allele T of C+433T are presumed to exert good effect on all of the investigated traits and the SNPs could be useful as a selection marker for chicken breeding program.

There was difference of the expression level of avian UCP gene between to thermoneutral and cold exposed. Also expression level of avUCP gene influenced to body weight and body weight gain (Collin et al., 2003). Polymorphism of the human UCP-1 gene was found and shown to be associated with increased body weight and body fat gain over time in the Quebec Family Study (Oppert et al., 1994; Kogure et al., 1998; Bouillaud et al., 2001). This polymorphism was also related to the resistance to weight loss during a low-calorie diet (Fumeron et al., 1996). Recently, it has been reported (Esterbauer et al., 2001; Krempler et al., 2002.) that a common -866G/A polymorphism in the promoter of the human UCP2 gene, which enhances its transcriptional activity, resulting in increased UCP2 mRNA levels in human fat cells, is associated with a reduced risk of obesity but increased risk of type 2 diabetes in obese middle-aged subjects. Herrmann et al. (2003) reported that the frequencies of the UCP-1 Ala64Thr and UCP-3 C-55T alleles were 27.2% and 12.0%, respectively. And significant associations were observed between polymorphism and body mass index or obesity. According to the results of this study, polymorphism of the avUCP gene was influencing in several economic traits. Significant three polymorphic sites on economic traits were avUCP C+282T (mean body weight, $p<0.05$), avUCP C+433T (daily percent lay, $p<0.05$), and avUCP T+1316C (daily percent lay, $p<0.05$). The frequencies of each SNPs were 0.125 (C+282T in avUCP gene exon 1 region), 0.150 (C+433T in avUCP gene intron 1 region), and 0.15 (T+1316C in avUCP gene exon 3 region), respectively. The combination genotype CC (C+282T) and TT (C+433T) showed a significant higher daily percent lay and mean body weight than the other combination genotypes. It is more useful using two SNP markers on selection for improvement of economic traits than using one SNP marker. SNPs in avUCP gene may be suitable to do selection on economic traits of Korean native chicken. Recently, many researches about of UCP gene are going on, but research about polymorphism of the avUCP gene is rare. This study may be used usefully as basis data in research about UCP gene.

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