

Association of *UCP1* Genetic Polymorphisms with Blood Pressure among Korean Female Subjects

Recent studies have provided some clues with regard to the relationship existing between uncoupling protein 1 (*UCP1*) and blood pressure in animal experiments. In an attempt to determine the genetic polymorphisms that are associated with blood pressure in humans, we have analyzed genetic polymorphisms in *UCP1* gene. In this study, we assessed the association between *UCP1* genotypes and systolic blood pressure (SBP) and diastolic blood pressure (DBP), in a population comprised of 832 Korean female subjects, using a general linear model, which was adjusted for age and body mass index (BMI). Among 4 genetic polymorphisms and the haplotypes constructed from them, haplotype3 of *UCP1*, *UCP1-ht3[GAGA]*, evidenced significant associations with SBP ($p=0.005$) and DBP ($p=0.013$). However, this haplotype was not significantly associated with obesity phenotypes, including BMI or fat mass ($p>0.05$), thereby suggesting that its association with blood pressure was independent of obesity phenotypes.

Key Words : Mitochondrial Uncoupling Protein; Polymorphism, Genetic; Blood Pressure; Association

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INTRODUCTION

Uncoupling proteins (*UCPs*) are located within the inner mitochondrial membrane, and function as proton channels (1). Whereas mitochondria utilize energy derived from fuel combustion to create a proton electrochemical gradient across the mitochondrial inner membrane, *UCPs* uncouple proton gradient from ATP synthesis (2). The *UCP* family has been characterized in human, and most studies were conducted on three major members including *UCP1* (MIM 113730), *UCP2* (MIM 601693), and *UCP3* (MIM 602044) (1). The expression and activation of *UCPs* is normally mediated by the sympathetic nervous system, and is controlled by norepinephrine. Due to their significant roles in energy metabolism, many association studies have been conducted regarding the associations of *UCP* polymorphisms with metabolic phenotypes, including metabolic rate, energy expenditure, exercise efficiency, body mass index (BMI) and diabetes (3-7). Among *UCP* family members, *UCP1* is expressed primarily in brown adipose tissue (BAT), and has been reported to be responsible for thermogenesis (2).

Recently, the results of some animal studies have provided some clues regarding the relationship existing between respiratory uncoupling and blood pressure (8, 9). The uncoupling agent, dinitrophenol, has been demonstrated to induce

smooth muscle contraction (10), a hallmark of blood pressure elevation. *UCP1* is expressed primarily in brown adipose tissue, and periaortic brown adipose tissue is known to be present in both rodents and humans, in amounts sufficient to alter the temperature of the aortic blood (11, 12). Bernal-Mizrachi et al. demonstrated that *UCP1* expression in the aortic smooth muscle cells of transgenic mice increased the generation of aortic superoxide, coupled with a 46 mmHg increase in tail cuff systolic blood pressure (SBP), and a 21 mmHg increase in diastolic blood pressure (DBP) (8). The relationship between *UCP1* and blood pressure regulation was also suggested by the finding that blood pressure-regulatory hormones also modulate the expression of *UCP1*. Kraus et al. reported that aldosterone, a mineralocorticoid hormone that regulates blood pressure, also modulated the expression of *UCP1* in brown adipocytes (13). Porter et al. also determined that angiotensin II, one of the principal regulators of blood pressure, increased *UCP1* mRNA expression in brown adipose tissue (14).

Until now, however, the relationship of *UCP1* with blood pressure in human is not clear. In this study, the effects of genetic polymorphisms of *UCP1* on blood pressure were analyzed in a population consisting of 832 Korean female subjects.

MATERIALS AND METHODS

Subjects

The 832 female subjects who participated in this study were recruited from a women's health and obesity clinic in the Kirin Medical Foundation (Seoul, Korea). Mean age and BMI were 27.9 yr and 25.9 kg/m² respectively. Female subjects over 50 yr old with high possibility of menopause were only 2.2% of the total subjects (n=18), and exclusion of them caused no significant effects on the results. Individuals who were taking anti-hypertensive, anti-hyperlipidemic or anti-diabetic medications were excluded from this study. Individuals with hypertension but were not taking medicines at the time of blood pressure measurement were included in the study, and then they were advised for appropriate medical care.

The general characteristics of the subjects are listed in Table 1. The SBP and DBP of each of the subjects were measured after at least 10 min of resting. Body fat mass was measured via bio-impedance analysis, using a commercial device (Inbody 2.0, Biospace Co., Seoul, Korea). Genomic DNA was obtained with the informed consent of all participants, and the proto-

Table 1. General characteristics of study subjects

Variables	N	Mean ± SD
Age (yr)	832	27.88 ± 7.80
Weight (kg)	832	66.86 ± 11.74
BMI (kg/m ²)	832	25.89 ± 4.27
SBP	832	115.38 ± 12.84
DBP	832	72.35 ± 10.23

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. Amplifying primers and taqman probes for genotyping of UCP1 polymorphisms

Gene	Loci		Sequence
UCP1	-3954A>G	Forward	CCCAGTGGTGGCTAATGAGAG
		Reverse	GGAGAGGGTGGTATCCCCAA
		Probe-1 (VIC)	GTTTGATCAAGTGCATT
		Probe-2 (FAM)	GTTTGATCGAGTGCATT
	-1766A>G	Forward	TCCACATCTAATCATTATAAAGCTGTATTGT
		Reverse	TTCATGTTATCACCTGTGTAGTGCTT
		Probe-1 (VIC)	CATTATGTGAGAGCACTT
		Probe-2 (FAM)	ATTTATGTGAGGGCACTT
	+940A>G (A64T)	Forward	CCGACGTCCAGTGTATTAGGTAT
		Reverse	AGTTTCATCCGCCCTTCTGTTTT
		Probe-1 (VIC)	AACAATCACCGCTGTGGT
		Probe-2 (FAM)	AACAATCACCACTGTGGT
+6537A>T (M229L)	Forward	GGCTCTTATCGCTGGATTTTGC	
	Reverse	GTCCTGGTGGAGAATTAATAAATCTGGTT	
	Probe-1 (VIC)	CAACAGCTATGTCCCTC	
	Probe-2 (FAM)	CAACAGCTTTGTCCCTC	

UCP1, uncoupling protein 1.

cols of this study were approved by the Institutional Review Board. Genomic DNAs from each subject were extracted from whole blood, using the Accuprep™ Genomic DNA Extraction Kit (Bioneer Co., Daejeon, Korea), in accordance with the manufacturer's instructions.

Genotyping

The genotyping of genetic polymorphisms was conducted via the TaqMan method. Primer Express (Applied Biosystems, Foster City, CA, U.S.A.) was employed in the design of both the polymerase chain reaction (PCR) primers and the TaqMan probes. The relevant primer information was listed in Table 2. One allelic probe was labeled with FAM dye, and the other with VIC dye. PCRs were run in TaqMan Universal Master Mix (Applied Biosystems) with PCR primer concentrations of 900 nM and TaqMan probe concentrations of 200 nM. Reactions were conducted in a 384-well format, in a total reaction volume of 5 μ L, using 20 ng of genomic DNA. The plates then were positioned in a thermal cycler (PE 9700, Applied Biosystems) and heated for 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. The assay mixtures were then transferred to a Prism 7900HT (Applied Biosystems), in which the fluorescence intensity in each well of the plate was determined.

Statistical analysis

Hardy-Weinberg equilibrium tests were utilized in order to determine whether or not individual genetic polymorphisms were in equilibrium at each locus within the population. |D'| and r² were evaluated in order to measure linkage disequilibrium between all single nucleotide polymorphism (SNP) loci (15), and haplotypes and their frequencies

were inferred using the algorithm, HapAnalyzer (<http://hap.ngri.go.kr>). Association analyses were performed using general linear model procedures, in which were included the effects of the *UCP1* genetic polymorphism, age, and BMI. Age and BMI-adjusted analysis of covariance (ANCOVA) was conducted in order to assess the independent effects of the *UCP1* polymorphisms on blood pressure. Statistical significance was established at a level of $p < 0.05$. All analyses were conducted using the SAS system for windows, version 8.2 (SAS Korea, Seoul, Korea).

RESULTS

In this study, genetic polymorphisms in *UCP1* gene were evaluated with regard to their association with blood pressure in a population composed of 832 Korean women. Sixty five male subjects were also recruited, and their numbers were not enough for parametric statistical analyses. Because the number of male subjects was less than 8% of the number of female subjects, the statistical analyses were confined to female subjects for the controlling of the sex-related phys-

iological factors. Inclusion of male subjects in statistical analyses showed similar results (data not shown).

Among the 37 *UCP1* polymorphisms listed in the NCBI database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>), four SNP found in promoter and exon regions were selected for genotyping of all 832 subjects. The gene map of *UCP1* and locations of the selected SNPs are shown in Fig. 1A, and characteristics of them were summarized in Table 3. The genotypic distributions of four selected SNPs were all determined to be in Hardy-Weinberg equilibrium (HWE) ($p > 0.05$). As shown in Fig. 1B, four common haplotypes with frequencies more than 0.05 were constructed from four selected SNPs of *UCP1*. Among them, *UCP1-ht1*[AAGA] was tagged by $-3954A > G$ with a frequency in excess of 95%. Likewise, *UCP1-ht2*[GGGA] and *UCP1-ht4*[GAAT] were tagged almost completely by $-1766A > G$ and $+6537A > T$, respectively. These tagged haplotypes were not analyzed further, as their effects were almost identical to their tagging SNPs.

Four SNPs and *UCP1-ht3*[GAGA] haplotype were further analyzed with regard to their association with SBP and DBP after adjusting for age and BMI in the 832 Korean female subjects. *UCP1-ht3* evidenced a significant association with

Map of *UCP1* on chromosome 4q28-q31

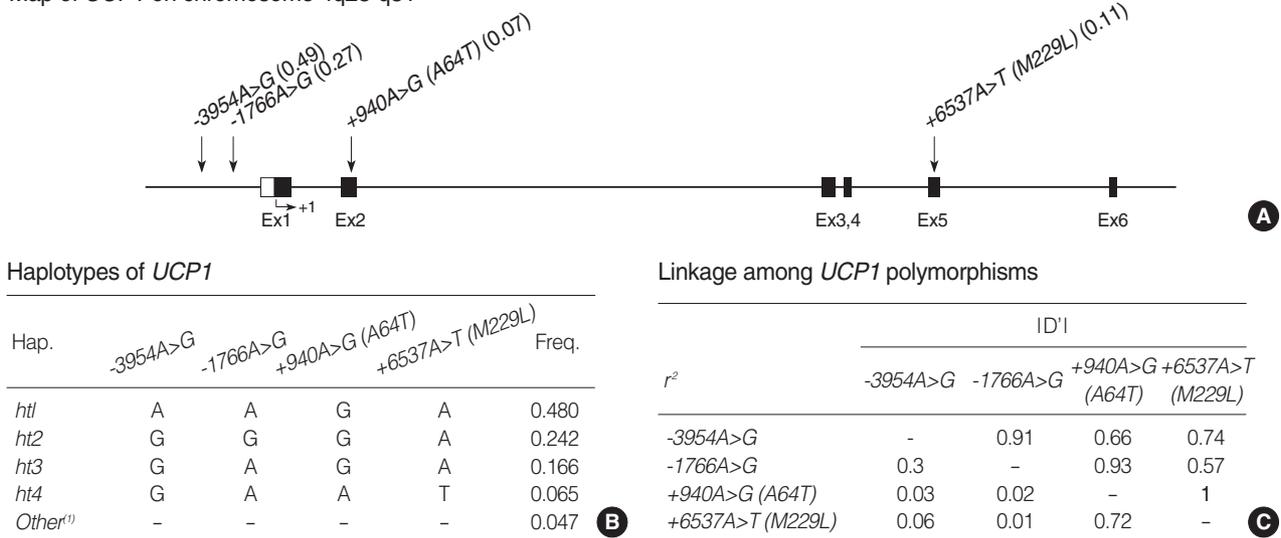


Fig. 1. Genetic polymorphisms of the *UCP1*. (A) Gene map of *UCP1* on chromosome 4q28-q31 (Ref. Genome Seq. NT_016354). Coding exons are marked by black blocks and 5' UTRs are marked by white blocks. (B) Common haplotypes constructed from four SNPs. (C) Linkage disequilibrium coefficients among four SNPs.

Table 3. Characteristic of polymorphisms used for genotyping of the whole subjects

Gene	Locus	Position	Amino acid change	Minor allele frequency	HWE	rs#
<i>UCP1</i>	$-3954A > G$	Promoter		0.49	0.98	rs1800592
	$-1766A > G$	Promoter		0.27	0.99	rs3811791
	$+940A > G$ (A64T)	Exon2	A64T	0.07	0.55	rs45539933
	$+6537A > T$ (M229L)	Exon5	M229L	0.11	0.91	rs2270565

HWE, Hardy-weinberg equilibrium; *UCP1*, uncoupling protein 1.

SBP (Table 4). The SBP distributions were as follows: 114.63 ± 0.50 mmHg ($n=553$) among *UCP1-ht3* non-carriers; 117.36 ± 0.75 mmHg ($n=248$) among heterozygotes; and 112.98 ± 2.11 mmHg ($n=31$) among homozygote ($p=0.005$ in codominant model). Similar associations were determined to exist between *UCP1-ht3* and DBP. The DBP distributions were as follows: 71.84 ± 0.42 mmHg among *UCP1-ht3* non-carriers; 73.80 ± 0.62 mmHg among heterozygotes; and 69.98 ± 1.77 mmHg among homozygote ($p=0.013$ in the codominant model) (Table 5). As five independent statistical tests were conducted in Table 4, the significance level is $0.05/5=0.01$ after Bonferroni correction, which is the most conservative method of multiple comparison correction (16). The results showed that the association of *UCP1-ht3* with SBP is highly significant: significant even after multiple comparison correction ($p<0.01$).

As blood pressure was closely associated with obesity phenotypes, the observed associations between *UCP1-ht3* and blood pressure might have been mediated by its effects on obesity phenotypes, in spite of the fact that BMI had been adjusted for in the statistical analyses. In order to ascertain this, the BMI and body fat mass of all subjects were compared with regard to *UCP1-ht3* (Table 6). Our findings indicated that *UCP1-ht3* had no statistically significant effect on either BMI or fat mass among the subjects. This suggests that the association of the *UCP1-ht3* with blood pressure may be independent on its effects on obesity phenotypes.

DISCUSSION

The association pattern of *UCP1-ht3* with blood pressure

Table 4. Analyses of covariance of *UCP1* polymorphisms with SBP controlling for age and BMI among Korean female subjects

Gene	Locus	C/C	C/R	R/R	<i>p</i>		
					Co-dominant	Dominant	Recessive
<i>UCP1</i>	-3954A>G	216 (113.97±0.80)	406 (116.08±0.59)	209 (115.48±0.82)	0.058	0.042	0.883
	-1766A>G	458 (115.27±0.56)	307 (115.73±0.68)	63 (114.77±1.49)	0.794	0.727	0.660
	+940A>G (A64T)	706 (115.34±0.45)	111 (115.20±1.13)	5 (113.56±5.31)	0.940	0.740	0.856
	+6537A>T (M229L)	673 (115.47±0.46)	149 (115.33±0.97)	8 (112.29±4.19)	0.749	0.773	0.454
	<i>UCP1-ht3</i>	553 (114.63±0.50)	248 (117.36±0.75)	31 (112.98±2.11)	0.005	0.010	0.249

C/C, C/R, and R/R represent homozygotes for the common allele, and heterozygotes and homozygotes for the rare allele, respectively. Genotype and haplotype distributions (mean±SE), and *p* values of three alternative models (co-dominant, dominant, and recessive) of analysis of covariance (ANCOVA) are shown. *p* values less than 0.05 were marked by bold fonts.

UCP1, uncoupling protein; SBP, systolic blood pressure; BMI, body mass index.

Table 5. Analyses of covariance of UCP polymorphisms with DBP controlling for age and BMI among Korean female subjects

Gene	Locus	C/C	C/R	R/R	<i>p</i>		
					Co-dominant	Dominant	Recessive
<i>UCP1</i>	-3954A>G	216 (71.55±0.67)	407 (73.25±0.49)	209 (71.40±0.68)	0.035	0.169	0.113
	-1766A>G	458 (72.51±0.46)	308 (72.25±0.56)	63 (71.97±1.25)	0.885	0.654	0.733
	+940A>G (A64T)	707 (72.43±0.37)	111 (71.42±0.94)	5 (70.84±4.41)	0.576	0.742	0.297
	+6537A>T (M229L)	674 (72.59±0.38)	149 (71.59±0.81)	8 (69.75±3.50)	0.398	0.208	0.448
	<i>UCP1-ht3</i>	553 (71.84±0.42)	248 (73.80±0.62)	31 (69.98±1.77)	0.013	0.033	0.173

C/C, C/R, and R/R represent homozygotes for the common allele, and heterozygotes and homozygotes for the rare allele, respectively. Genotype and haplotype distributions (mean±SE), and *p* values of three alternative models (co-dominant, dominant, and recessive) of analysis of covariance (ANCOVA) are shown. *p* values less than 0.05 were marked by bold fonts.

UCP1, uncoupling protein; SBP, systolic blood pressure; BMI, body mass index.

Table 6. Analyses of covariance of *UCP1-ht3* with obesity phenotypes controlling for age among Korean female subjects

Gene	Phenotype	C/C	C/R	R/R	<i>p</i>		
					Co-dominant	Dominant	Recessive
<i>UCP1-ht3</i>	BMI (kg/m ²)	553 (26.04±0.17)	248 (25.64±0.24)	31 (25.16±0.77)	0.242	0.129	0.263
	Fat mass (kg)	553 (23.72±0.33)	248 (22.87±0.45)	31 (22.24±1.47)	0.224	0.095	0.374

C/C, C/R, and R/R represent homozygotes for the common allele, and heterozygotes and homozygotes for the rare allele, respectively. Haplotype distributions (mean±SE), and *p* values of three alternative models (co-dominant, dominant, and recessive) of analysis of covariance (ANCOVA) are shown.

BMI, body mass index; *UCP1*, uncoupling protein 1.

can not be explained by classical mendelian inheritance, because heterozygote showed higher blood pressure than *UCP1-bt3* homozygote. Another form of inheritance was suggested which is neither dominant nor recessive and in which only heterozygote is affected (17). In this situation, homozygosity for the normal allele and the mutant allele give a normal phenotype. Only heterozygote produces an abnormal phenotype because the two alleles, when present together, interact to produce a harmful effect. Pedigree studies provided possible evidences for the harmful interaction of two alleles in human diseases (18). *UCP1* polymorphism study conducted on Japanese males suggested similar two allele interaction. It was reported that *AG* heterozygote of the *A-3826G* polymorphism of *UCP1* has higher risk than *AA* and *GG* homozygote suggesting a harmful interaction of two alleles in *UCP1* heterozygote (19). Even though more study should be conducted to get clear evidence of the two allele interaction, our results suggest that heterozygosity of *UCP1-bt3* haplotype was associated with elevated blood pressure in Korea female subjects.

The findings of this study should be interpreted within the context of its limitations. The subjects of this study were young females, and our findings might not be appropriate for extrapolation to general population. However, the significant associations observed in this study provide some evidence of the involvement of *UCP1* in the blood pressure of human.

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