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# Association of *UCP2* and *UCP3* gene polymorphisms with serum high-density lipoprotein cholesterol among Korean women

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**Abstract**

Decreased serum high-density lipoprotein (HDL-C) cholesterol is a major risk factor for atherosclerosis and vascular disease. In this study, we assessed the association of 10 uncoupling protein (*UCP*) *2* and *UCP3* polymorphisms with serum HDL cholesterol levels and atherogenic indexes among 658 Korean women. Among the 10 single nucleotide polymorphisms (SNPs) in the *UCP2* and *UCP3* genes, 2 SNPs in *UCP2*, *−866G>A* and *+4787C>T* (*A55V*) that were tightly linked ($r^2$ = 0.97), were significantly associated with decreased HDL cholesterol levels after Bonferroni correction ($P$ = .003 in the recessive model). *+4589C>T* (*Y210Y*) in *UCP3*, a silent variation of *Tyr210Tyr* in exon 5, was also significantly associated with HDL cholesterol after multiple comparison correction. These 3 SNPs also exhibited some association with increases in the atherogenic index. Source-of-variation analysis revealed that *−866G>A* SNP accounted for 8.09% of the variation in serum HDL cholesterol levels independent of body mass index. We believe that our results may provide clues to the association of *UCP* genes with the risk of atherosclerosis through their effects on HDL cholesterol.


## 1. Introduction

Uncoupling proteins (*UCPs*) are a family of mitochondrial transporters, all of which are known to uncouple oxidative phosphorylation via proton leakage from the inner mitochondrial membrane. These mitochondrial proteins are implicated as potential regulators of thermoregulation and energy metabolism [1]. To date, more than 5 types of *UCPs* are known. *UCP1* is expressed exclusively in brown adipose tissue and is responsible for thermogenesis in mammalian neonates and rodents [2]. *UCP2* is expressed in almost all mammalian tissues, suggesting that it plays a functional role in global energy metabolism in the body [3-5]. *UCP3* is predominantly expressed in mammalian skeletal muscle and brown adipose tissue [6-8]. *UCP4* is mainly expressed in brain tissue [9] and *UCP5* is expressed with high abundance in the brain and testis [10].

In humans, the *UCP2* and *UCP3* genes form a cluster on chromosome *11q13*, a syntenic region that has been linked to hyperinsulinemia in mice [11-13]. The chromosomal location and tissue distribution patterns of *UCP2* and *UCP3* suggest that polymorphisms in these *UCPs* may contribute to metabolic disorders, via their major effects on energy metabolism. Genetic association studies have been conducted in efforts to elucidate the association of *UCP2* and *UCP3* gene polymorphisms with metabolic disorders. A recent study has demonstrated that the *−866G>A* single nucleotide polymorphism (SNP) of *UCP2* is significantly associated with asymptomatic carotid artery atherosclerosis in women [14]. It was also associated with the risk of coronary heart disease in a prospective study involving 2695 healthy men [15]. However, more studies are needed in other populations to confirm the relationship between *UCP2* and *UCP3* gene polymorphisms and metabolic disorders. It is well known that serum levels of HDL cholesterol are closely associated with metabolic disorders, and decreased HDL cholesterol is a major risk factor for atherosclerosis. In this study, we investigated 10 *UCP2* and

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Table 1
General characteristics of study subjects

| Variables | n | Mean ± SD |
|---|---|---|
| Age (y) | 658 | 27.72 ± 7.48 |
| Weight (kg) | 658 | 66.42 ± 10.89 |
| BMI (kg/m$^2$) | 658 | 25.69 ± 3.99 |
| SBP | 607 | 115.08 ± 12.71 |
| DBP | 608 | 72.20 ± 10.06 |

SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

*UCP3* gene polymorphisms and their association with serum HDL cholesterol in 658 Korean women.

## 2. Materials and methods

### 2.1. Subjects

The subjects in this study were recruited from the Women's Health and Obesity Clinic at the Kirin Oriental Medical Hospital (Seoul, Korea). Women with diabetes, hypertension, and liver/renal function disorders were excluded, as were women taking antihyperlipidemic medicine. The protocol of this study was approved by the institutional review board of the Korea Institute of Oriental Medicine (Daejon, Korea), and all blood samples were obtained with informed consent. A total of 658 women were included in the study; the subjects in this study partially overlap those in our previous study [16]. The general characteristics of the subjects are listed in Table 1.

### 2.2. Measurement of blood cholesterol levels

Blood samples were obtained from each subject after an overnight fast and centrifuged for 10 minutes at 2000 rpm to separate serum samples. Serum biochemical profiles including total cholesterol and HDL cholesterol were determined with an autobiochemical analyzer (SP-4410, ARKRAY, Kyoto, Japan). Atherogenic index was calculated using the equation: total cholesterol/HDL cholesterol [17].

### 2.3. Genotyping

The genomic DNA of each subject was extracted from whole blood using an Accuprep Genomic DNA Extraction Kit (Bioneer, Daejon, Korea) according to the manufacturer's instructions. To genotype the polymorphic sites, we designed amplifying primers and probes for TaqMan [18] as shown Appendix A. Primer Express (Applied Biosystems, Foster City, CA) was used in the design of both the polymerase chain reaction (PCR) primers and the TaqMan probes. One allelic probe was labeled with FAM dye and the other was labeled with VIC dye. Polymerase chain reaction was typically run in a TaqMan Universal Master mix (Applied Biosystems) with 900 nmol/L primers and 200 nmol/L TaqMan probes. The reactions were conducted in a 384-well format with a 5-$\mu$L total reaction volume containing 20 ng of genomic DNA. The plate was then placed in a thermal cycler (PE 9700, Applied Biosystems) and heated for 2 minutes at 50°C and 10 minutes at 95°C, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The TaqMan assay plate was then transferred to a Prism 7900HT (Applied Biosystems), and the fluorescence intensity of each well was read. Fluorescence data files from the plates were then analyzed using an automated software (SDS 2.1, Applied Biosystems). To genotype an insertion/deletion polymorphism (+7941 45–base pair [bp] insdel of *UCP2*), a segment of the *UCP2* gene near exon 8 was amplified by PCR with forward 5′-GAGGTGCCCCCATGACC-3′ and reverse 5′-TCCTGCACTCCCCATGTTAGT-3′ primers. The PCR began with denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. The PCR products (691 or 646 bp) were separated by electrophoresis through a 1.5% agarose gel and visualized by ethidium bromide staining.

Hardy-Weinberg equilibrium tests were conducted to determine whether individual variants were at equilibrium at each locus within the population. Between all pairs of biallelic loci, we examined widely used measures of linkage disequilibrium, Lewontin's $D'$ ($|D'|$) and $r^2$ [19]. Haplotypes and their frequencies were inferred using the HapAnalyzer algorithm (http://hap.ngri.go.kr).

### 2.4. Statistical analysis

Association analyses were conducted using the general linear model procedures, in which the effects of the *UCP2* and *UCP3* genotypes, age, and body mass index (BMI) were included. Age- and BMI-adjusted univariate analyses of variance were performed to delineate the independent effects of the *UCP2* and *UCP3* genotypes on HDL cholesterol level and atherogenic index. A $P$ value of less than .05 was considered statistically significant. Three procedures were used to account for multiple comparisons carried out on the 10 SNPs and 3 haplotypes of *UCP2* and *UCP3* genes as described by Benjamini et al [20], namely, Bonferroni correction [21], Benjamini and Hochberg's [22] adaptive false discovery rate (FDR) procedure, and Benjamini and Liu's [23] adaptive FDR procedure. The source of variations in HDL cholesterol level and atherogenic index were computed using type III sum of squares, which can quantify the effect of an independent variable after adjustment for all other variables included in the model, as described by Robitaille et al [24]. All analyses were conducted using SAS for Windows, v. 9.1 (SAS, Cary, NC).

## 3. Results

In this study, we evaluated genetic polymorphisms in the *UCP2* and *UCP3* genes with regard to their associations with serum HDL cholesterol levels in a population of 658 Korean women. The locations and allele frequencies of 10 polymorphic sites in the *UCP2* and *UCP3* genes are listed in Table 2. Genotype distributions of all loci were in

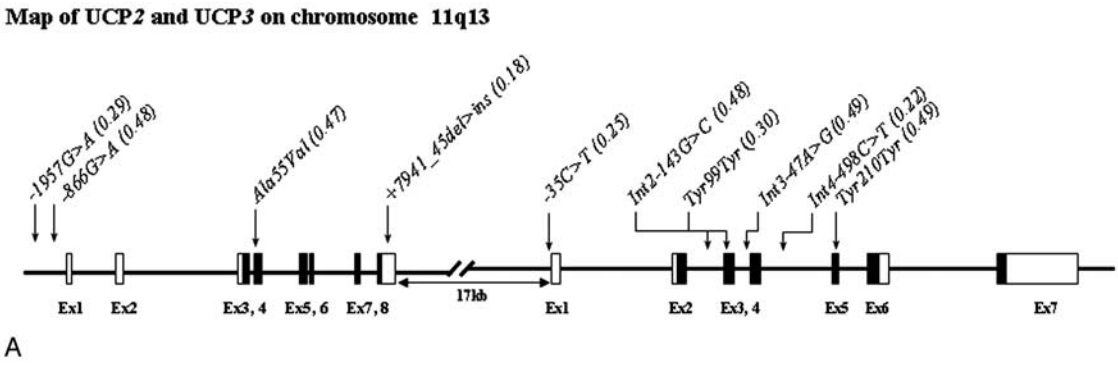
Table 2
Polymorphisms of *UCP2* and *UCP3* genes used for genotyping of the subjects

| Gene | *Locus* | Position | *Locus from start codon* | Minor allele frequency | HWE | rs no. |
|---|---|---|---|---|---|---|
| *UCP2* | *−1957G>A* | Promoter | *−6422G>A* | 0.29 | 0.80 | rs649446 |
| | *−866G>A* | Promoter | *−5331G>A* | 0.48 | 0.16 | rs659366 |
| | *Ala55Val* | Exon 4 | *+320C>T* | 0.47 | 0.05 | rs660339 |
| | *+7941_45del>ins* | 3′ UTR | *+3473 45del>ins* | 0.18 | 0.08 | – |
| *UCP3* | *−35C>T* | Promoter | *−2078C>T* | 0.25 | 0.99 | rs1800849 |
| | *Int2-143G>C* | Intron 2 | *+521G>C* | 0.48 | 0.13 | rs2075576 |
| | *Tyr99Tyr* | Exon 3 | *+834C>T* | 0.30 | 0.99 | rs1800006 |
| | *Int3-47A>G* | Intron 3 | *+1063A>G* | 0.49 | 0.37 | rs1685325 |
| | *Int4-498C>T* | Intron 4 | *+1811C>T* | 0.22 | 0.74 | rs2734827 |
| | *Tyr210Tyr* | Exon 5 | *+2546C>T* | 0.49 | 0.16 | rs2075577 |

HWE indicates Hardy-Weinberg equilibrium; rs, reference no. of each SNP for NCBI's database; UTR, untranslated region.

Hardy-Weinberg equilibrium ($P \geq .05$). Because the *UCP2* and *UCP3* genes form a cluster on chromosome 11q13 (Fig 1A), linkage disequilibrium analysis and haplotype construction were performed together. The linkage disequilibrium coefficients between the 10 polymorphisms of the *UCP2* and *UCP3* genes were $|D'|$ of 0.54 to 1 and $r^2$ of 0.09 to 0.97 (Fig. 1B). Haplotypes were constructed as shown in Fig. 1C, and 3 common haplotypes, *ht1*, *ht2*, and *ht3*, with frequencies of greater than 0.05 were used for subsequent association analyses.

After adjusting for the subjects' ages and BMI values, 10 SNPs, as well as the 3 common haplotypes constructed from them, were analyzed for their possible associations with serum HDL cholesterol levels. Among these, *−866G>A* in the promoter and *Ala55Val* (*+320C>T* from translation start site) in exon 4 of *UCP2* exhibited the most significant associations with *HDL* cholesterol levels (Table 3). The mean HDL cholesterol levels according to *−866G>A* genotypes were as follows: 54.40 mg/dL in the *GG*-type subjects, 54.46 mg/dL in the *GA*-type subjects,




**LDs among UCP2 and UCP3 polymorphisms**

|D'|

| $r^2$ | -1957G>A | -866G>A | Ala55Val | +7941_45del>ins | -35C>T | Int2-143G>C | Tyr99Tyr | Int3-47A>G | Int4-498C>T | Tyr210Tyr |
|---|---|---|---|---|---|---|---|---|---|---|
| -1957G>A | - | 1 | 1 | 1 | 0.83 | 0.84 | 0.84 | 0.84 | 0.91 | 0.94 |
| -866G>A | 0.44 | - | 0.99 | 0.98 | 0.84 | 0.83 | 0.84 | 0.83 | 0.55 | 0.93 |
| Ala55Val | 0.45 | 0.97 | - | 0.99 | 0.84 | 0.83 | 0.84 | 0.83 | 0.54 | 0.93 |
| +7941_45del>ins | 0.10 | 0.23 | 0.24 | - | 0.81 | 0.81 | 0.82 | 0.80 | 0.81 | 0.91 |
| -35C>T | 0.68 | 0.31 | 0.31 | 0.06 | - | 1.00 | 1.00 | 0.99 | 0.97 | 0.95 |
| Int2-143G>C | 0.30 | 0.68 | 0.66 | 0.15 | 0.43 | - | 0.99 | 0.99 | 0.62 | 0.92 |
| Tyr99Tyr | 0.70 | 0.32 | 0.32 | 0.07 | 0.97 | 0.43 | - | 0.99 | 0.97 | 0.95 |
| Int3-47A>G | 0.30 | 0.67 | 0.65 | 0.15 | 0.42 | 0.95 | 0.42 | - | 0.62 | 0.90 |
| Int4-498C>T | 0.10 | 0.09 | 0.09 | 0.54 | 0.11 | 0.11 | 0.11 | 0.11 | - | 0.62 |
| Tyr210Tyr | 0.37 | 0.83 | 0.80 | 0.19 | 0.38 | 0.81 | 0.38 | 0.80 | 0.11 | - |

B

**Haplotypes of UCP2 and UCP3**

| Hap. | -1957G>A | -866G>A | Ala55Val | +7941_45del>ins | -35C>T | Int2-143G>C | Tyr99Tyr | Int3-47A>G | Int4-498C>T | Tyr210Tyr | Freq. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *ht1* | G | G | C | del. | C | G | T | A | C | C | 0.425 |
| *ht2* | A | A | T | del. | T | C | C | G | C | T | 0.256 |
| *ht3* | G | A | T | ins. | C | C | T | G | T | T | 0.147 |
| *ht4* | G | G | C | del. | C | G | T | A | T | C | 0.032 |
| *ht5* | G | G | C | del. | T | C | C | G | C | T | 0.020 |
| *ht6* | A | A | T | del. | C | G | T | A | C | T | 0.014 |
| *Others*[a] | - | - | - | - | - | - | - | - | - | - | 0.105 |

C

Fig. 1. Gene maps of the *UCP2* and *UCP3* on chromosome *11q13*. Coding exons are marked by black blocks and 5′and 3′ UTRs are marked by white blocks. A, Polymorphisms in *UCP2* and *UCP3* genes. The minor allele frequencies shown in parentheses were determined via the genotyping of 658 subjects. B, Linkage disequilibrium coefficients among *UCP2* and *UCP3* polymorphisms. C, Haplotypes constructed from 10 polymorphisms of *UCP2* and *UCP3* and their frequencies.

Table 3
Analyses of covariance of *UCP* polymorphisms with HDL cholesterol controlling for age and BMI among Korean female subjects

| Gene | Locus | C/C | C/R | R/R | Genetic power | *P* | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Codominant | Dominant | Recessive |
| *UCP2* | *−1957G>A* | 330 (54.16 ± 17.60) | 266 (53.05 ± 16.31) | 55 (49.75 ± 15.48) | 0.350 | .198 | .177 | .121 |
| | *−866G>A* | 186 (54.40 ± 16.40) | 313 (54.46 ± 17.54) | 159 (49.74 ± 16.02) | 0.781 | **.014** | .295 | **.003** |
| | *Ala55Val* | 192 (54.72 ± 17.07) | 306 (54.29 ± 17.23) | 157 (49.56 ± 15.94) | 0.814 | **.011** | .133 | **.003** |
| | *+7941_45del>ins* | 433 (53.48 ± 15.82) | 175 (53.71 ± 18.44) | 30 (46.07 ± 12.13) | 0.549 | .104 | .490 | **.033** |
| *UCP3* | *−35C>T* | 327 (54.20 ± 17.48) | 175 (52.41 ± 15.51) | 53 (52.83 ± 20.76) | 0.165 | .358 | .169 | .979 |
| | *Int2-143G>C* | 184 (53.60 ± 15.58) | 312 (54.38 ± 17.19) | 158 (50.84 ± 18.02) | 0.471 | .118 | .693 | **.041** |
| | *Tyr99Tyr* | 319 (54.24 ± 17.58) | 280 (52.34 ± 15.48) | 54 (53.09 ± 20.65) | 0.214 | .300 | .146 | .924 |
| | *Int3-47A>G* | 175 (53.66 ± 15.65) | 318 (54.39 ± 16.76) | 160 (50.79 ± 18.66) | 0.493 | .108 | .715 | **.038** |
| | *Int4-498C>T* | 402 (54.10 ± 16.81) | 223 (52.00 ± 16.33) | 31 (52.00 ± 22.75) | 0.262 | .215 | .081 | .752 |
| | *Tyr210Tyr* | 178 (54.07 ± 16.17) | 310 (54.62 ± 17.05) | 166 (49.96 ± 17.43) | 0.753 | **.017** | .417 | **.005** |
| Haplotype | *UCP2-UCP3-ht1* | 222 (51.50 ± 17.27) | 276 (53.72 ± 15.77) | 126 (55.37 ± 16.73) | 0.462 | .111 | .087 | .077 |
| | *UCP2-UCP3-ht2* | 356 (53.97 ± 17.32) | 228 (52.74 ± 15.24) | 40 (49.93 ± 16.52) | 0.261 | .356 | .226 | .268 |
| | *UCP2-UCP3-ht3* | 465 (53.55 ± 16.24) | 142 (53.12 ± 17.82) | 17 (46.53 ± 12.73) | 0.303 | .205 | .378 | .083 |

Number of subjects (mean ± SD) and *P* values of 3 alternative models (codominant, dominant, and recessive) are shown. *P* values of less than .05 are set in boldface type. C/C, C/R, and R/R represent homozygotes for the common allele, heterozygotes, and homozygotes for the rare allele, respectively.

and 49.74 mg/dL in the *AA*-type subjects ($P$ = .003 in the recessive model). In *Ala55Val* SNP, the mean HDL cholesterol levels were as follows: 54.72 mg/dL in the Ala/Ala-type subjects, 54.29 mg/dL in the *Ala/Val*-type subjects, and 49.56 mg/dL in the *Val/Val*-type subjects ($P$ = .003 in the recessive model). In *UCP3*, *Tyr210Tyr* (*+2546C>T* from translation start site) showed the most significant association with HDL cholesterol level ($P$ = .005 in the recessive model). In all 3 of these polymorphisms, mean HDL cholesterol levels of heterozygotes were similar to those of homozygotes of the major allele, suggesting a recessive nature for the minor alleles; this was also shown by the lowest $P$ value of the recessive model. No significant associations were found among the 3 common haplotypes (frequency >0.05). Haplotype 1, which carried *−866G* and *55Ala UCP2* alleles and *+2546C* (*Tyr210Tyr*) of *UCP3*, showed a weak tendency for association with HDL cholesterol levels ($P$ = .077 in the recessive model). One of the possible reasons for this weak tendency among common haplotypes compared to the significant association with individual SNPs might be the hidden effects of rare haplotypes. As shown in Fig 1C, the 3 common haplotypes (frequency, >0.05) together account for 82.8% of the subjects' alleles, with the remaining 17.2% belonging to rare haplotypes. The effects of these rare haplotypes are hard to evaluate because the numbers of subjects belonging to these rare haplotypes are too few to statistically analyze.

To correct the significance level according to multiple comparisons, we compared the observed $P$ values with the Bonferroni threshold and 2 FDR thresholds as described in Materials and methods. The observed $P$ value for *−866G>A* and *Ala55Val* in *UCP2* was lower than the Bonferroni threshold (0.0038) and the $P$ value for *Tyr210Tyr* in *UCP3* satisfied both FDR thresholds (0.0054 and 0.0115) (Table 4).

When the atherogenic index was analyzed with the 10 SNPs and 3 common haplotypes, only 3 SNPs showed

Table 4
Correction of the significance level according to multiple comparison

| Gene | *Polymorphisms* | Observed *P* values | Rank | Bonferroni threshold | FDR (BH) threshold | FDR (BL) threshold |
|---|---|---|---|---|---|---|
| *UCP2* | *−866G>A* | **.003** | 1 | 0.0038 | 0.0038 | 0.0038 |
| *UCP2* | *Ala55Val* | **.003** | 2 | 0.0038 | 0.0077 | 0.0045 |
| *UCP3* | *Tyr210Tyr* | **.005** | 3 | 0.0038 | 0.0115 | 0.0054 |
| *UCP2* | *+7941_45del>ins* | .033 | 4 | 0.0038 | 0.0154 | 0.0065 |
| *UCP3* | *Int3-47A>G* | .038 | 5 | 0.0038 | 0.0192 | 0.0080 |
| *UCP3* | *Int2-143G>C* | .041 | 6 | 0.0038 | 0.0231 | 0.0102 |
| Haplotype | *UCP2-UCP3-ht1* | .077 | 7 | 0.0038 | 0.0269 | 0.0133 |
| Haplotype | *UCP2-UCP3-ht3* | .083 | 8 | 0.0038 | 0.0308 | 0.0181 |
| *UCP2* | *−1957G>A* | .121 | 9 | 0.0038 | 0.0346 | 0.0260 |
| Haplotype | *UCP2-UCP3-ht2* | .268 | 10 | 0.0038 | 0.0385 | 0.0406 |
| *UCP3* | *Int4-498C>T* | .752 | 11 | 0.0038 | 0.0423 | 0.05 |
| *UCP3* | *Tyr99Tyr* | .924 | 12 | 0.0038 | 0.0462 | 0.05 |
| *UCP3* | *−35C>T* | .979 | 13 | 0.0038 | 0.05 | 0.05 |

The list of the observed *P* values of the recessive models is sorted from smallest to largest. *P* values that pass multiple comparison thresholds are set in boldface type. The rightmost 3 columns demonstrate 3 different multiple comparison procedures: Bonferroni procedure, FDR controlling procedures of Benjamini and Hochberg (BH), and FDR of Benjamini and Liu (BL).

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

| Gene | Locus | C/C | C/R | R/R | Genetic power | *P* | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Codominant | Dominant | Recessive |
| *UCP2* | *−1957G>A* | 328 (3.63 ± 1.28) | 266 (3.62 ± 1.34) | 54 (3.94 ± 1.29) | 0.307 | .392 | .472 | .183 |
| | *−866G>A* | 185 (3.60 ± 1.24) | 312 (3.55 ± 1.24) | 158 (3.91 ± 1.48) | 0.733 | .060 | .518 | **.018** |
| | *Ala55Val* | 191 (3.59 ± 1.24) | 305 (3.55 ± 1.24) | 156 (3.91 ± 1.49) | 0.733 | .066 | .364 | **.020** |
| | *+7941_45del>ins* | 431 (3.64 ± 1.22) | 174 (3.61 ± 1.47) | 30 (4.23 ± 1.34) | 0.584 | .195 | .54 | .070 |
| *UCP3* | *−35C>T* | 325 (3.62 ± 1.27) | 276 (3.66 ± 1.34) | 52 (3.75 ± 1.34) | 0.088 | .746 | .449 | .910 |
| | *Int2-143G>C* | 183 (3.65 ± 1.21) | 311 (3.57 ± 1.26) | 157 (3.82 ± 1.49) | 0.395 | .225 | .823 | .093 |
| | *Tyr99Tyr* | 317 (3.62 ± 1.28) | 280 (3.66 ± 1.34) | 53 (3.74 ± 1.33) | 0.084 | .747 | .453 | .935 |
| | *Int3-47A>G* | 174 (3.64 ± 1.21) | 317 (3.55 ± 1.25) | 159 (3.83 ± 1.49) | 0.488 | .145 | .802 | .055 |
| | *Int4-498C>T* | 401 (3.59 ± 1.21) | 221 (3.74 ± 1.45) | 31 (3.86 ± 1.37) | 0.289 | .178 | .064 | .503 |
| | *Tyr210Tyr* | 177 (3.62 ± 1.22) | 309 (3.54 ± 1.25) | 165 (3.89 ± 1.48) | 0.704 | .050 | .604 | **.016** |
| Haplotype | *UCP2-UCP3-ht1* | 221 (3.78 ± 1.39) | 274 (3.62 ± 1.26) | 126 (3.52 ± 1.22) | 0.372 | .206 | .214 | .100 |
| | *UCP2-UCP3-ht2* | 354 (3.63 ± 1.25) | 228 (3.65 ± 1.38) | 39 (3.92 ± 1.33) | 0.199 | .679 | .576 | .414 |
| | *UCP2-UCP3-ht3* | 463 (3.64 ± 1.22) | 141 (3.65 ± 1.55) | 17 (4.20 ± 1.30) | 0.320 | .214 | .445 | .083 |

Number of subjects (mean ± SD) and *P* values of 3 alternative models (codominant, dominant, and recessive) are shown. *P* values of less than .05 are set in boldface type. C/C, C/R, and R/R represent homozygotes for the common allele, heterozygotes, and homozygotes for the rare allele, respectively.

associations: *−866G>A* and *Ala55Val* of *UCP2* and *Tyr210Tyr* of *UCP3* were associated with atherogenic index in the recessive model with $P = .018$, $P = .020$, and $P = .016$, respectively (Table 5). However, none of them were significant when corrected for multiple testing.

As serum cholesterol levels are generally related to obesity [25,26], the observed associations between HDL cholesterol levels and these 3 SNPs in *UCP2* and *UCP3* could be mediated by their effects on obesity phenotypes, although the BMI values had been adjusted in the statistical analyses (Table 3 and 4). However, when the obesity phenotypes of the subjects were compared according to genotype, none of the *UCP2* and *UCP3* polymorphisms examined in this study exerted any statistically significant effects on BMI or fat mass (data not shown). These results indicated that the associations of the *UCP2* and *UCP3* polymorphisms with HDL cholesterol levels are independent of their effects on obesity phenotypes.

To confirm the effect of the 3 SNPs on HDL cholesterol levels, we performed source-of-variation analysis, which includes BMI and age (Table 6). Because the 3 SNPs were highly linked as shown in Fig 1B, only *−866G>A* SNP was introduced in the model. In the recessive model, the *−866G>A* genotype accounted for 8.09% of the variation in HDL cholesterol levels independent of BMI and age among the study subjects ($P = .003$). Body mass index accounted for 32.21% of the variation in HDL cholesterol levels ($P < .0001$).

## 4. Discussion

High-density lipoprotein mediates cholesterol transport from nonhepatic tissues to the liver for conversion to bile acids, a process referred to as reverse cholesterol transport, and decreased plasma HDL cholesterol is a major risk factor for atherosclerosis and vascular disease [27]. According to twin and pedigree studies, approximately 50% of plasma HDL cholesterol variability is determined by genetic factors [28,29]. Mutations in genes including ABC transporter A1 (*ABCA1*), apolipoprotein A-I (*apoA-I*), and lecithin cholesterol transferase (*LCAT*) are implicated in rare mendelian forms of HDL deficiency and familial hypoalphalipoproteinemia [30,31]. However, the DNA sequence variants that contribute to variations in HDL cholesterol plasma levels in the general population are still largely unknown.

Most association studies of *UCP* polymorphisms and HDL cholesterol level have been conducted on the *−3826A>G* SNP of *UCP-1* with controversial results [32-35]. In this study, we investigated the associations of 10 SNPs of *UCP2* and *UCP3* with HDL cholesterol levels in a population of 658 Korean women. Two tightly linked SNPs, *−866G>A* and *Ala55Val* of *UCP2* ($|D'| = 0.99$ and $r^2 = 0.97$), were significantly associated with HDL cholesterol levels after Bonferroni correction ($P = .003$ in recessive model in both SNPs). They were also associated with atherogenic index ($P = .018$ and $P = .020$ in recessive models, respectively). *Ala55Val* SNP in exon 4 causes a conservative amino acid change, and until now, there has been no evidence that it causes a functional change in the protein. It is tightly linked with *−866G>A*, and its effects cannot be separated from those of the *−866G>A* SNP.

The *−866G>A* SNP, which is located in the promoter region of the *UCP2* gene, was first identified by Esterbauer et al [36]. The minor *A* allele is associated with enhanced *UCP2* messenger RNA (mRNA) expression in human adipose tissue in vivo. Computational analysis

Table 6
Source of variation in HDL cholesterol and atherogenic index among Korean female subjects

| | HDL cholesterol | | Atherogenic index | |
|---|---|---|---|---|
| | % of Variance | *P* | % of Variance | *P* |
| *−866G>A* | 8.09 | .003 | 4.94 | .018 |
| Age | 0.11 | .733 | 8.07 | .003 |
| BMI | 32.21 | <.0001 | 59.23 | <.0001 |

demonstrated that this SNP is involved in the putative binding sites for several transcription factors such as aryl-hydrocarbon receptor, hypoxia inducible factor 1a, insulin promoter factor 1, and paired box-containing protein 6. Preferential binding of some presumptive transcription factors to the G or *A* allele in the promoter sequence may confer tissue-specific advantage to either allele. Reporter gene assay experiments showed that the *A* allele has a 22% more effective transcriptional activity in differentiated adipocytes [36]. On the other hand, Oberkofler et al [14] reported that the *A* allele of the *−866G>A* polymorphism was implicated in decreased transcription rates in human endothelial cells and macrophages. They showed binding of cell type–specific protein complexes to the region encompassing the *−866G>A* SNP and suggested HIF1A involvement in the regulation of *UCP2* expression in endothelial cells and macrophages.

Tissue-specific transcriptional regulation of the *−866G>A* SNP was also shown in its effects on phenotypes. Esterbauer et al [36] showed that the *A* allele was associated with decreased BMI in participants in the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR). However, Oberkofler et al [14] demonstrated that the *A* allele is significantly associated with carotid artery atherosclerosis in women in SAPHIR. The *A* allele was also associated with the risk of coronary heart disease in a prospective study involving 2695 healthy men. The risk of coronary heart disease doubled for *−866A/A* homozygotes compared with the *G/G* or *G/A* genotype [15]. Oberkofler et al [14] suggested that the *A* allele of *−866G>A* polymorphism may cause atherosclerosis through down-regulation of *UCP2* mRNA and concomitant reactive oxygen species (ROS) increase in endothelial cells and macrophages, and provided evidence for the relation between *UCP2* and ROS. *UCP2* has been shown to modulate the production of ROS by decreasing the mitochondrial membrane potential [37]. *UCP2* antisense oligonucleotide increased intracellular ROS generation in murine endothelial cells [38], and the disrupted expression of *UCP2* mRNA induced a concomitant increase in ROS generation in endothelial cells from *UCP2* null mice [39].

Low HDL cholesterol level, as well as ROS, is a major risk factor for atherosclerosis [40]. Our results indicate that *−866G>A* and 2 highly linked SNPs, *Ala55Val* of *UCP2* and *Tyr210Tyr* of *UCP3*, are associated with decreased serum HDL cholesterol and increased atherogenic index. Serum HDL has antioxidant activity and protects low-density lipoprotein against oxidative stress [41]. Kontush et al [42] showed that a low HDL cholesterol phenotype is accompanied by elevated systemic oxidative stress. They showed that systemic oxidative stress was elevated 2.3-fold in subjects with low HDL cholesterol, and their HDL subfractions displayed significantly lower antioxidant activity compared with healthy subjects. High-density lipoprotein subfractions from subjects with low HDL cholesterol have altered chemical composition, such as triglyceride enrichment and cholesterol deletion, which paralleled the reduced antioxidant activity. The association of *UCP2* polymorphisms with low HDL cholesterol levels, along with the role of *UCP2* in ROS generation, implies a significant role for *UCP2* in the process of atherosclerosis, as suggested by Oberkofler et al [14].

Among *UCP3* polymorphisms, only *+2546C>T*, a silent variation of *Tyr210Tyr* in exon 5, was significantly associated with HDL cholesterol after multiple comparison correction. As shown in Fig. 1C, the linkage disequilibrium coefficients between *−866G>A* in *UCP2* and *Tyr210Tyr* in *UCP3* were very high ($|D'|$ = 0.93 and $r^2$ = 0.83), indicating the effect of *Tyr210Tyr* in *UCP3* on HDL cholesterol level may be an effect of the highly linked *−866G>A* promoter polymorphism in *UCP2*.

Our analysis showed that none of the 10 SNPs are significantly associated with obesity phenotypes, including BMI and fat mass (data not shown). These results suggest that *UCP2* polymorphism may affect metabolic disorders independent of its effects on obesity; this could be explained by the differential transcription activity of the two *−866G>A* alleles in adipocytes and endothelial cells/macrophages. Some association studies also have reported obesity-independent association of *UCP2* polymorphism with metabolic disorders. As mentioned previously, Esterbauer et al [36] reported that the *A* allele of the *−866G>A* SNP was associated with decreased obesity in white participants in SAPHIR, although Oberkofler et al [14] showed the that *A* allele was associated with increased atherosclerosis in the same population. Ji et al [43] showed that the *−866G/A* polymorphism was not associated with obesity in Japanese, although it was significantly associated with hypertension, suggesting that the association of the polymorphism with hypertension is independent of obesity. They suggested that the differential effect of *−866G/A* on obesity and hypertension in white and Japanese populations may be caused by differences in environmental factors such as energy intake and salt consumption. It is possible that the association of the polymorphism with obesity is more clearly shown in populations with a high energy intake, such as in whites, whereas the association with hypertension is more pronounced in populations with a high salt intake, such as in the Japanese population.

The relationship between obesity and metabolic disorders reveals differences in various ethnic populations. In white populations, the risk of metabolic disorder is moderate at a BMI of 30 and severe at a BMI of 35. In East Asian populations, however, metabolic risk is moderate at a BMI of 25 and becomes severe at a BMI of 30 [44]. In this study, we determined that the frequency (0.48) of the risky *A* allele of *−866G>A* in the Korean population is similar to that of the Japanese population (0.46-0.47) [43,45], both of which are significantly higher than the frequencies (0.36-0.38) determined in white population studies [14,36,46]. The higher frequency of the risky allele

in the East Asian population may contribute, at least in part, to the higher susceptibility to metabolic disorders compared with white populations.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2007.01.023.

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