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The effects of uncoupling protein 3 haplotypes on obesity phenotypes and very low-energy diet–induced changes among overweight Korean female subjects

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Abstract

Responses to energy restriction tend to vary within the population because of genetic differences. In this study, we have genotyped 6 uncoupling protein 3 (UCP-3) polymorphisms (−55C/T, Int2-143G/C, Tyr99Tyr, Int3-47G/A, Int4-498C/T, and Tyr210Tyr) among 214 overweight Korean female subjects recruited from an obesity clinic. Three major haplotypes, identified with frequencies in excess of 0.04, were constructed from 6 single nucleotide polymorphisms. Association studies were then undertaken, involving the measurement of anthropometric characteristics and body composition both before and after 1 month of a energy-restriction regimen. At baseline, haplotype 1 (ht1) [CGTACC] was associated with elevated anthropometric characteristics, including body weight, waist-hip ratio, and body mass index, as well as body components, including body fat mass and body fat–free mass. After the completion of the 1-month weight control program, which involved a very low-energy (2900 kJ/d) diet, we analyzed the outcomes according to the UCP-3 genetic polymorphisms. Among the 3 principal haplotypes, ht1 [CGTACC] was significantly associated with an increased reduction in body weight, in the codominant ($P = .022$), dominant ($P = .016$), and recessive ($P = .041$) models. Body mass index reduction was associated with the ht1 haplotype in a similar fashion. Among the body components, changes in body fat mass were significantly associated with ht1 [CGTACC] ($P = .028$), but changes in body fat–free mass were not significantly associated with the UCP-3 polymorphism.
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1. Introduction

Uncoupling protein 3 (UCP-3) is a member of a family of mitochondrial transporters, all of which are known to uncouple oxidative phosphorylation via an increase in proton leakage from the inner mitochondrial membrane [1], which results in the generation of heat rather than in energy storage. Reductions in the function or expression of UCP-3 tend to reduce energy expenditure and exacerbate the propensity to store energy as fat [2]. Uncoupling protein 3 has been determined to be predominantly expressed in mammalian skeletal muscle and brown adipose tissue and, albeit to a much lesser extent, within the heart [3]. Several studies have tentatively implicated UCP-3 in the regulation of obesity. Liu et al [4] reported that leptin-induced UCP-3 expression in the skeletal muscles of obese *ob/ob* mice resulted in significant weight losses. Transgenic UCP-3–overexpressing mice tend to be lean and exhibit a marked resistance to diet-induced obesity [5,6]. The additional phenotype change of transgenic UCP-3 was also associated with a reduction in the levels of fasting blood glucose and total cholesterol, as well as a 44% to 57% reduction in adipose tissue [5]. Therefore, UCP-3 appears to be an attractive candidate for studies regarding obesity phenotypes.

The human *UCP-3* gene is located on chromosome 11q13, adjacent to the *UCP2* gene [7-10]. Four single nucleotide polymorphisms (SNPs) in the coding region and 56 SNPs in the noncoding region, including the untranslated region and the intron, have been characterized previously (http://www.ncbi.nlm.nih.gov/SNP). In a previous study,

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Table 1
The characteristics of the subjects and the outcomes of weight control program

Characteristics	Mean ± SD
Age (y)	28.57 ± 9.63
Height (cm)	160.65 ± 5.49
Weight (kg)	80.40 ± 10.71
BMI (kg/m^2)	31.12 ± 3.67
Fat percentage (%)	40.26 ± 4.42
WHR	0.957 ± 0.064
SBP (mm Hg)	121.28 ± 12.91
DBP (mm Hg)	75.08 ± 10.86
Change in body weight (kg)	−7.03 ± 2.30
Change in body fat mass (kg)	−4.71 ± 2.66
Change in fat-free mass (kg)	−2.31 ± 2.37
Change in BMI (kg/m^2)	−2.71 ± 0.88
Change in WHR	−0.036 ± 0.021

Schrauwen et al [11] reported the identification of −55C/T SNP within the promoter region. A host of association studies have been conducted in a variety of populations in attempts to elucidate the role of this polymorphism with regard to obesity phenotypes. However, the results of these studies have varied markedly among different studied populations [11-17]. For example, the T allele of −55C/T polymorphism was associated with higher body mass index (BMI) in a French white population, but was associated with lower BMI in a US white population [12,13]. In a Danish population study, −55C/T SNP was not significantly associated with BMI [16,17]. Some studies concerning the exonic SNPs, Tyr99Tyr and Tyr210Tyr, have also yielded controversial results among different populations [13,18-20]. However, until the present study was conducted, most studies have focused on individual SNPs, whereas studies regarding the UCP-3 haplotypes have been quite limited, and there has been a decided paucity of studies conducted on the effects of UCP-3 haplotypes on the outcomes of a very low-energy diet (VLED) regimen.

In this study, we genotyped 6 of the SNPs of UCP-3, −55C/T (rs1800849), Int2-143G/C (rs2075576), Tyr99Tyr (rs1800006), Int3-47G/A (rs1685325), Int4-498C/T (rs2734827), and Tyr210Tyr (rs2075577), and constructed major haplotypes from them to characterize their association with obesity phenotypes and the outcomes of VLED among overweight Korean female subjects.

2. Materials and methods

2.1. Subjects

A total of 214 female subjects, all of whom had completed 1-month weight control programs, were recruited from an obesity clinic at the Kirin Oriental Medical Hospital (Seoul, Korea), and this study was approved by the Institutional Review Board of the Korea Institute of Oriental Medicine (Daejeon, Korea). Before and after a 1-month VLED weight control program, involving an intake of 2900 kJ/d, we measured anthropometric characteristics and body compositions in all of the enrolled subjects. Body compositions were evaluated via a bio-impedance analysis, using a commercial device (Inbody 2.0, Biospace, Seoul, Korea). The general characteristics of the subjects and outcomes of the weight control program are shown in Table 1.

2.2. Measurement of serum biochemical profile

Blood samples obtained from each of the subject after an overnight fast were centrifuged for 10 minutes at 2000 rpm, and serum biochemical parameters were assessed with an autobiochemical analyzer (SP-4410, ARKRAY, Kyoto, Japan).

2.3. Genomic DNA extraction

Genomic DNA was prepared from each of the blood samples using the LaboPass Genomic DNA Extraction Kit (COSMO, Seoul, Korea), in accordance with the manufacturer's protocols. Informed consent was acquired from all subjects.

2.4. Genotyping of SNP and haplotype estimation

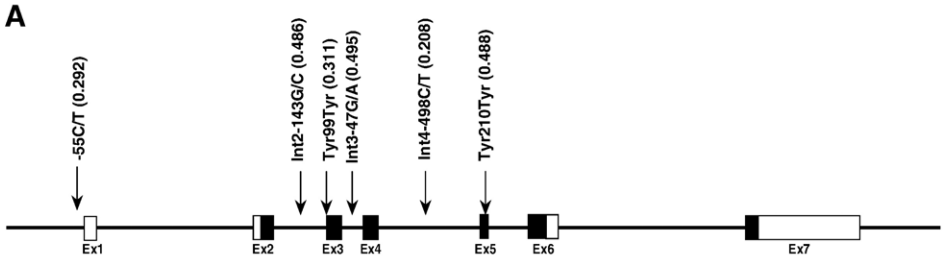
To genotype the polymorphic sites, we designed amplifying primers and probes for TaqMan [21]. Both the polymerase chain reaction (PCR) primers and the TaqMan probes were designed using Primer Express (Applied Biosystems, Foster, CA), and the sequences of the primers and probes were designed using the published sequence of the *UCP-3* gene (GenBank accession no. AP003717). One allelic probe was labeled with FAM dye and the other with VIC dye. The PCRs were run in TaqMan Universal Master mix (Applied Biosystems) with PCR primer concentrations of 900 nmol/L and TaqMan probe concentrations of 200 nmol/L. The reactions were conducted in a 384-well format, in a total 5-μL reaction volume using 20 ng of genomic DNA. The plates were then positioned 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 assay mixtures were then transferred to a Prism 7900HT instrument (Applied Biosystems), where the fluorescence intensities were read for each of the wells in the plates. The fluorescence data files from each plate were analyzed using automated allele-calling software (SDS2.1). Information regarding the primers is available on our Web site (http://www.kiommed.re.kr/ucp-3.html).

We used χ^2 tests to determine whether individual variants were at equilibrium at each locus within the population (Hardy-Weinberg equilibrium). We examined widely used measures of linkage disequilibrium between all pairs of biallelic loci, Lewontin's D' ($|D'|$) and r^2 [22]. Haplotypes and their frequencies were inferred using the algorithm, HapAnalyzer (http://hap.ngri.go.kr).

2.5. Statistical analyses

All values are expressed as means ± SD. Age-adjusted univariate analyses of variance were conducted via the general linear model procedure to determine the independent

A



B

		|D'|				
		-55C/T	Int2-143G/C	Tyr99Tyr	Int3-47G/A	Int4-498C/T	Tyr210Tyr
	-55C/T	-	1	1	1	0.929	0.918
	Int2-143G/C	0.468	-	1	0.990	0.518	0.939
r^2	Tyr99Tyr	0.978	0.479	-	1	0.929	0.919
	Int3-47G/A	0.451	0.945	0.461	-	0.490	0.910
	Int4-498C/T	0.100	0.074	0.102	0.064	-	0.655
	Tyr210Tyr	0.356	0.795	0.365	0.776	0.107	-

C

	-55C/T	Int2-143G/C	Tyr99Tyr	Int3-47G/A	Int4-498C/T	Tyr210Tyr	Frequency
ht1	C	G	T	A	C	C	0.423
ht2	T	C	C	G	C	T	0.295
ht3	C	C	T	G	T	T	0.149
Others							0.133

Fig. 1. Gene maps and haplotypes of the *UCP-3* gene. A, Polymorphisms of UCP-3. Minor allele frequencies are shown in parentheses. B, Linkage disequilibrium coefficient, $|D'|$ and r^2, among 6 SNPs. C, Haplotypes of UCP-3. Common haplotypes with frequencies of more than 0.04 are shown.

effects of the UCP-3 polymorphism on the obesity phenotype. A P value of .05 or less was considered to be statistically significant. All statistical analyses were conducted using SPSS ver. 10.0 (SPSS, Chicago, IL).

3. Results

The *UCP-3* gene is located on chromosome 11q13 and spans approximately 8.5 kilobases. Six reported SNPs, −55C/T (−2078C/T), Int2-143G/C (+521G/C), Tyr99Tyr (+834T/C), Int3-47G/A (+1063G/A), Int4-498C/T (+1811C/T), and Tyr210Tyr (+2546T/C), in which the numbers in the parentheses are counted from the first "ATG," a start codon, were genotyped among the overweight Korean female subjects in the study (Fig. 1A). The general characteristics of the subjects, which are shown in Table 1, indicate that the subjects of this study were all either overweight or obese, with a mean BMI of 31.1, and had been recruited from an obesity clinic. The linkage disequilibrium coefficients between the 6 SNPs were $|D'|$ of 0.49 to 1.00 and r^2 of 0.074 to 0.978, as shown in Fig. 1B, and 3 common haplotypes (frequency >0.04) were constructed from the 6 SNPs (Fig. 1C). The distribution of 6 SNPs and 3 haplotypes was consistent with Hardy-Weinberg equilibrium ($P > .05$) as was shown in our Web site (http://www.kiommed.re.kr/ucp-3.html).

Analyses of anthropometric characteristics with the SNPs of UCP-3 indicated that no SNP studied here was significantly associated with any of the anthropometric characteristics (Table 2). Among the 3 haplotypes, ht1 [CGTACC] was significantly associated with increased body weight ($P = .006$ and .001), waist-hip ratio (WHR) ($P = .039$ and .014), and BMI ($P = .006$ and .002) in the codominant and recessive models. When the subjects' body compositions were assessed via bio-impedance analysis, and analyzed in conjunction with the UCP-3 polymorphisms, ht1 [CGTACC] evidenced a significant association with increased fat mass ($P = .017$ and .004) and with fat-free mass ($P = .029$ and .008) in both the codominant and recessive models (Table 3). Body protein mass, a component of the fat-free mass, was also shown to have increased in the subjects exhibiting the ht1 haplotype in the codominant, dominant, and recessive models ($P = .002$, .043, and .002, respectively). Ht2 [TCCGTC] and ht3 [CCTGTT] were not associated with anthropometric characteristics or body compositions. The results of association analyses of body minerals and water mass with the UCP-3 polymorphisms can be viewed at www.kiommed.re.kr/ucp-3.html.

Table 2
Association analyses of anthropometric characteristics with UCP-3 polymorphisms among overweight Korean female subjects

Phenotype	Locus	C/C	C/R	R/R	*P*		
					Codominant	Dominant	Recessive
Body weight (kg)	−55C/T	99 (81.34 ± 11.57)	99 (79.15 ± 9.61)	16 (82.29 ± 11.47)	.234	.254	.357
	Int2-143G/C	55 (82.44 ± 10.70)	110 (79.82 ± 11.13)	49 (79.40 ± 9.62)	.268	.106	.502
	Tyr99Tyr	97 (81.55 ± 11.58)	101 (78.99 ± 9.60)	16 (82.29 ± 11.47)	.153	.159	.357
	Int3-47G/A	52 (82.69 ± 10.69)	112 (79.83 ± 11.11)	50 (79.27 ± 9.62)	.212	.081	.435
	Int4-498C/T	134 (81.24 ± 10.54)	71 (78.43 ± 10.25)	9 (83.37 ± 15.36)	.109	.111	ND
	Tyr210Tyr	54 (79.17 ± 9.55)	111 (79.96 ± 10.98)	49 (82.73 ± 11.16)	.223	.363	.092
	Ht1	71 (79.48 ± 10.23)	105 (79.19 ± 10.60)	38 (85.43 ± 10.73)	**.006**	.434	**.001**
	Ht2	101 (81.22 ± 11.49)	100 (79.23 ± 9.60)	13 (83.02 ± 12.33)	.234	.324	.264
	Ht3	153 (80.76 ± 10.36)	56 (79.24 ± 11.64)	5 (82.26 ± 11.75)	.574	.392	ND
Waist/hip ratio	−55C/T	99 (0.962 ± 0.064)	99 (0.947 ± 0.063)	16 (0.981 ± 0.071)	.115	.188	.227
	Int2-143G/C	55 (0.962 ± 0.064)	110 (0.955 ± 0.068)	49 (0.954 ± 0.058)	.706	.422	.635
	Tyr99Tyr	97 (0.963 ± 0.064)	101 (0.947 ± 0.062)	16 (0.981 ± 0.071)	.091	.148	.227
	Int3-47G/A	52 (0.964 ± 0.064)	112 (0.955 ± 0.067)	50 (0.955 ± 0.059)	.621	.334	.66
	Int4-498C/T	134 (0.962 ± 0.067)	71 (0.947 ± 0.056)	9 (0.960 ± 0.087)	.443	.23	ND
	Tyr210Tyr	54 (0.952 ± 0.056)	111 (0.955 ± 0.068)	49 (0.965 ± 0.065)	.487	.465	.257
	Ht1	71 (0.957 ± 0.061)	105 (0.949 ± 0.065)	38 (0.979 ± 0.066)	**.039**	.851	**.014**
	Ht2	101 (0.962 ± 0.064)	100 (0.948 ± 0.063)	13 (0.985 ± 0.076)	.131	.215	.212
	Ht3	153 (0.958 ± 0.065)	56 (0.956 ± 0.063)	5 (0.938 ± 0.049)	.813	.859	ND
BMI (kg/m^2)	−55C/T	99 (31.35 ± 3.89)	99 (30.70 ± 3.41)	16 (32.26 ± 3.68)	.224	.38	.227
	Int2-143G/C	55 (31.63 ± 4.04)	110 (30.93 ± 3.75)	49 (30.97 ± 2.99)	.481	.226	.715
	Tyr99Tyr	97 (31.42 ± 3.89)	101 (30.65 ± 3.40)	16 (32.26 ± 3.68)	.162	.268	.227
	Int3-47G/A	52 (31.71 ± 4.10)	112 (30.90 ± 3.73)	50 (30.99 ± 2.99)	.395	.173	.747
	Int4-498C/T	134 (31.51 ± 3.83)	71 (30.31 ± 3.03)	9 (31.74 ± 4.97)	**.048**	.051	ND
	Tyr210Tyr	54 (30.81 ± 2.89)	111 (30.96 ± 3.75)	49 (31.83 ± 4.18)	.283	.458	.117
	Ht1	71 (31.05 ± 3.25)	105 (30.57 ± 3.56)	38 (32.78 ± 4.26)	**.006**	.805	**.002**
	Ht2	101 (31.29 ± 3.87)	100 (30.80 ± 3.42)	13 (32.23 ± 3.85)	.37	.492	.301
	Ht3	153 (31.26 ± 3.75)	56 (30.73 ± 3.50)	5 (31.14 ± 2.88)	.677	.394	ND

Data are expressed as number of cases (mean ± SD). *P* values of age-adjusted general linear model are shown. *P* values <.05 are shown in boldface type. C/C, C/R, and R/R represent homozygotes for common allele, heterozygotes, and homozygotes for rare allele, respectively. ND indicates that *P* value was not determined because case number was too small to be statistically analyzed.

When the serum biochemical parameters were analyzed with the UCP-3 polymorphisms, some associations were uncovered. Tyr210Tyr was significantly associated with increased glucose levels in the codominant and recessive models (P = .037 and .13, respectively). However, Tyr210Tyr was also associated with reduced levels of serum triglycerides in the codominant and dominant models (P = .044 and .21, respectively). The results of association studies between the serum biochemical parameters and the UCP-3 genetic polymorphisms can be viewed at www.kiommed.re.kr/ucp-3.html.

The subjects of this study all completed a 1-month weight control program, involving a VLED with an intake of 700 kcal/d. The mean body weight change was −7.03 kg, which included a fat mass change of −4.71 kg (67%) and a fat-free mass change of −2.31 kg (33%). The anthropometric and body composition changes resulting from this weight control program were analyzed in conjunction with the UCP-3 genetic polymorphisms. Ht1 [CGTACC] evidenced a significant association with increased reductions in body weight and BMI, in a gene-dose–dependent manner (Table 4). The means of body weight changes in the homozygotic subjects (ht1/ht1), the heterozygotic subjects (−/ht1), and the noncarriers were −7.72, −7.14, and −6.67 kg, respectively. These mean changes were statistically significant in the codominant (P = .022), dominant (P = .016), and recessive (P = .041) models. Changes in the BMI were detected as follows: −2.98 kg/m^2 in the ht1/ht1 homozygotes, −2.73 kg/m^2 in the −/ht1 heterozygotes, and −2.54 kg/m^2 in the noncarriers.

Among the 6 SNPs, Int3-47G/A was significantly associated with changes in body weight and BMI (P = .020 and .037 in the recessive model, respectively). The mean changes in body weight in the subjects harboring the GG and GA types of Int3-47G/A were −7.45 and −7.12 kg, and were thus more profound changes than were seen in subjects harboring the AA type (−6.37 kg). Changes in the BMI were as follows: −2.87 kg/m^2 in the GG type, −2.74 kg/m^2 in the GA type, and −2.49 kg/m^2 in the AA type, a pattern similar to that observed with the changes in body weight. Tyr210Tyr (+2546T/C) was also significantly associated with changes in body weight and BMI in the recessive model (P = .031 and .030, respectively). The mean change in the body weights of the CC type subjects in Tyr210Tyr (+2546T/C) was −7.65 kg, which is higher than was seen in the TC-type (−7.14 kg) and TT-type (−6.54 kg) subjects. The mean BMI changes were as follows: −2.95 kg/m^2 in the CC type, −2.69 kg/m^2 in the TC type, and −2.55 kg/m^2 in the TT type, a pattern similar to that of the body weight changes (Table 4).

Table 3
Association analyses of body composition with UCP-3 polymorphisms among overweight Korean female subjects

Phenotype	Locus	C/C	C/R	R/R	*P*		
					Codominant	Dominant	Recessive
Body fat mass (kg)	−55C/T	99 (33.38 ± 8.07)	99 (31.73 ± 6.92)	16 (34.07 ± 7.60)	.191	.222	.328
	Int2-143G/C	55 (33.65 ± 7.88)	110 (32.53 ± 7.85)	49 (31.89 ± 6.39)	.059	.279	.455
	Tyr99Tyr	97 (33.48 ± 8.12)	101 (31.67 ± 6.87)	16 (34.07 ± 7.60)	.145	.164	.328
	Int3-47G/A	52 (33.81 ± 7.88)	112 (32.48 ± 7.87)	50 (31.90 ± 6.35)	.438	.221	.450
	Int4-498C/T	134 (33.25 ± 7.63)	71 (31.45 ± 6.71)	9 (33.60 ± 11.52)	.193	.113	ND
	Tyr210Tyr	54 (31.71 ± 6.29)	111 (32.54 ± 7.82)	49 (34.02 ± 8.11)	.322	.311	.169
	Ht1	71 (32.05 ± 6.95)	105 (31.95 ± 7.51)	38 (35.83 ± 8.06)	**.017**	.456	**.004**
	Ht2	101 (33.30 ± 8.01)	100 (31.79 ± 6.90)	13 (34.50 ± 8.32)	.200	.276	.260
	Ht3	153 (32.82 ± 7.48)	56 (32.33 ± 7.80)	5 (32.05 ± 7.74)	.868	.596	ND
Body fat percentage (%)	−55C/T	99 (40.63 ± 4.54)	99 (39.77 ± 4.32)	16 (41.05 ± 4.19)	.245	.287	.332
	Int2-143G/C	55 (40.42 ± 4.81)	110 (40.35 ± 4.41)	49 (39.91 ± 4.02)	.850	.791	.574
	Tyr99Tyr	97 (40.63 ± 4.58)	101 (39.78 ± 4.28)	16 (41.05 ± 4.19)	.242	.208	.332
	Int3-47G/A	52 (40.51 ± 4.80)	112 (40.27 ± 4.45)	50 (39.98 ± 3.98)	.867	.667	.663
	Int4-498C/T	134 (40.55 ± 4.56)	71 (39.82 ± 3.95)	9 (39.43 ± 5.86)	.387	.171	ND
	Tyr210Tyr	54 (39.80 ± 3.96)	111 (40.29 ± 4.47)	49 (40.71 ± 4.82)	.631	.416	.458
	Ht1	71 (39.99 ± 4.08)	105 (39.98 ± 4.46)	38 (41.57 ± 4.79)	.138	.6	**.047**
	Ht2	101 (40.61 ± 4.50)	100 (39.80 ± 4.30)	13 (41.13 ± 4.66)	.280	.321	.328
	Ht3	153 (40.26 ± 4.53)	56 (40.42 ± 4.18)	5 (38.54 ± 4.12)	.667	.94	ND
Fat-free mass (kg)	−55C/T	99 (47.95 ± 5.08)	99 (47.42 ± 4.43)	16 (48.22 ± 5.32)	.645	.539	.601
	Int2-143G/C	55 (48.79 ± 4.76)	110 (47.29 ± 4.80)	49 (47.50 ± 4.75)	.159	.058	.738
	Tyr99Tyr	97 (48.07 ± 5.06)	101 (47.31 ± 4.46)	16 (48.22 ± 5.32)	.47	.347	.601
	Int3-47G/A	52 (48.87 ± 4.83)	112 (47.35 ± 4.75)	50 (47.37 ± 4.77)	.146	**.050**	.575
	Int4-498C/T	134 (47.99 ± 4.72)	71 (46.97 ± 4.90)	9 (49.76 ± 4.71)	.134	.277	ND
	Tyr210Tyr	54 (47.45 ± 4.77)	111 (47.42 ± 4.74)	49 (48.70 ± 4.93)	.280	.657	.111
	Ht1	71 (47.43 ± 4.60)	105 (47.25 ± 4.80)	38 (49.60 ± 4.83)	**.029**	.562	**.008**
	Ht2	101 (47.90 ± 5.05)	100 (47.44 ± 4.44)	13 (48.53 ± 5.67)	.613	.632	.466
	Ht3	153 (47.94 ± 4.66)	56 (46.90 ± 5.11)	5 (50.21 ± 4.86)	.182	.273	ND
Body protein mass (kg)	−55C/T	99 (11.49 ± 1.78)	99 (11.38 ± 1.39)	16 (10.69 ± 1.45)	.222	.382	.094
	Int2-143G/C	55 (11.87 ± 1.72)	110 (11.20 ± 1.53)	49 (11.22 ± 1.50)	**.030**	**.008**	.461
	Tyr99Tyr	97 (11.50 ± 1.80)	101 (11.37 ± 1.38)	16 (10.69 ± 1.45)	.205	.308	.094
	Int3-47G/A	52 (11.92 ± 1.73)	112 (11.23 ± 1.50)	50 (11.15 ± 1.53)	**.019**	**.005**	.270
	Int4-498C/T	134 (11.45 ± 1.55)	71 (11.20 ± 1.70)	9 (11.82 ± 1.24)	.358	.394	ND
	Tyr210Tyr	54 (11.14 ± 1.60)	111 (11.31 ± 1.45)	49 (11.80 ± 1.84)	.095	.214	**.037**
	Ht1	71 (11.06 ± 1.60)	105 (11.32 ± 1.47)	38 (12.16 ± 1.69)	**.002**	**.043**	**.002**
	Ht2	101 (11.49 ± 1.77)	100 (11.37 ± 1.41)	13 (10.63 ± 1.35)	.237	.382	.105
	Ht3	153 (11.41 ± 1.58)	56 (11.23 ± 1.63)	5 (12.06 ± 1.55)	.472	.609	ND

Data are expressed as number of cases (mean ± SD). *P* values of age-adjusted general linear model are shown. *P* values <.05 are shown in boldface type.

When we assessed changes in body composition, change in fat-free mass could not be significantly associated with any of the UCP-3 polymorphisms, but changes in fat mass were significantly associated with the ht1 haplotype ($P = .028$) (Table 5). Mean changes in body fat mass in the ht1/ht1 homozygotes, −/ht1 heterozygotes, and −/− homozygotes were −5.54, −4.67, and −4.32 kg, respectively, and exhibited a tendency toward a gene-dose–dependent pattern, with *P* values of .054, .096, and .028 in the codominant, dominant, and recessive models, respectively. Among the 6 SNPs, only Tyr210Tyr (+2546T/C) was significantly associated with changes in body fat mass. Changes in fat-free mass, body proteins, and mineral and water mass were not associated with any of the UCP-3 polymorphisms. These results can be viewed at www.kiommed.re.kr/ucp-3.html.

When changes in body composition were measured among 246 nonobese female subjects (BMI ≤ 25), the change in fat mass was also significantly associated with ht1 haplotype in the dominant model ($P = .008$). Mean change in body fat mass was −3.58 kg in subjects with ht1 allele and −2.78 kg in subject without ht1 allele, whereas the changes in anthropometric characteristics and fat-free mass were not associated with ht1 haplotype in nonobese Korean female subjects (data not shown).

4. Discussion

Recently, a variety of candidate genes have been searched in an attempt to determine the genetic factors involved in the pathogenesis of obesity and body fat accumulation. Uncoupling protein 3, which has been suggested as one such candidate, is primarily expressed in skeletal muscle, a major site of thermogenesis in humans. In this study, we have genotyped 6 SNPs (−55C/T in the promoter, Tyr99Tyr and Tyr210Tyr in the exon, and 3 others in the intron) and elucidated the associations of the 6 SNPs and 3 primary haplotypes with obesity phenotypes and the outcomes of a VLED regimen. Our association study

Table 4
Effects of UCP-3 polymorphisms on VLED-induced change in anthropometric characteristics among overweight Korean female subjects

Phenotype	Locus	C/C	C/R	R/R	*P*		
					Codominant	Dominant	Recessive
Changed body weight (kg)	−55C/T	99 (−7.22 ± 2.27)	99 (−6.93 ± 2.39)	16 (−6.43 ± 1.90)	.363	.246	.272
	Int2-143G/C	55 (−7.44 ± 2.15)	110 (−7.08 ± 2.15)	49 (−6.45 ± 2.70)	.087	.126	**.045**
	Tyr99Tyr	97 (−7.32 ± 2.16)	101 (−6.83 ± 2.46)	16 (−6.43 ± 1.90)	.180	.084	.272
	Int3-47G/A	52 (−7.45 ± 2.21)	112 (−7.12 ± 2.10)	50 (−6.37 ± 2.70)	**.047**	.124	**.02**
	Int4-498C/T	134 (−6.99 ± 2.30)	71 (−7.18 ± 2.28)	9 (−6.33 ± 2.61)	.562	.778	ND
	Tyr210Tyr	54 (−6.54 ± 2.67)	111 (−6.99 ± 2.14)	49 (−7.65 ± 2.11)	**.048**	.072	**.031**
	Ht1	71 (−6.49 ± 2.47)	105 (−7.14 ± 2.14)	38 (−7.72 ± 2.23)	**.022**	**.016**	**.041**
	Ht2	101 (−7.24 ± 2.25)	100 (−6.90 ± 2.38)	13 (−6.32 ± 1.99)	.290	.189	.250
	Ht3	153 (−6.96 ± 2.25)	56 (−7.29 ± 2.33)	5 (−6.02 ± 3.38)	.405	.517	ND
Changed waist/hip ratio	−55C/T	99 (−0.037 ± 0.022)	99 (−0.035 ± 0.020)	16 (−0.037 ± 0.023)	.773	.474	.863
	Int2-143G/C	55 (−0.038 ± 0.022)	110 (−0.036 ± 0.021)	49 (−0.036 ± 0.022)	.815	.522	.817
	Tyr99Tyr	97 (−0.038 ± 0.022)	101 (−0.035 ± 0.020)	16 (−0.037 ± 0.023)	.553	.278	.863
	Int3-47G/A	52 (−0.038 ± 0.022)	112 (−0.036 ± 0.020)	50 (−0.036 ± 0.022)	.765	.464	.782
	Int4-498C/T	134 (−0.035 ± 0.022)	71 (−0.039 ± 0.020)	9 (−0.032 ± 0.022)	.249	.188	ND
	Tyr210Tyr	54 (−0.036 ± 0.023)	111 (−0.035 ± 0.020)	49 (−0.038 ± 0.023)	.636	.979	.362
	Ht1	71 (−0.036 ± 0.021)	105 (−0.035 ± 0.020)	38 (−0.038 ± 0.024)	.745	.862	.444
	Ht2	101 (−0.037 ± 0.022)	100 (−0.035 ± 0.020)	13 (−0.038 ± 0.025)	.676	.387	.985
	Ht3	153 (−0.035 ± 0.021)	56 (−0.039 ± 0.022)	5 (−0.035 ± 0.022)	.413	.214	ND
Changed BMI (kg/m^2)	−55C/T	99 (−2.77 ± 0.86)	99 (−2.68 ± 0.91)	16 (−2.54 ± 0.79)	.502	.349	.353
	Int2-143G/C	55 (−2.86 ± 0.86)	110 (−2.73 ± 0.81)	49 (−2.52 ± 1.02)	.132	.150	.074
	Tyr99Tyr	97 (−2.81 ± 0.82)	101 (−2.65 ± 0.93)	16 (−2.54 ± 0.79)	.288	.141	.353
	Int3-47G/A	52 (−2.87 ± 0.88)	112 (−2.74 ± 0.79)	50 (−2.49 ± 1.02)	.076	.134	**.037**
	Int4-498C/T	134 (−2.71 ± 0.89)	71 (−2.76 ± 0.83)	9 (−2.41 ± 1.00)	.508	.878	ND
	Tyr210Tyr	54 (−2.55 ± 1.01)	111 (−2.69 ± 0.81)	49 (−2.95 ± 0.84)	.058	.108	**.030**
	Ht1	71 (−2.54 ± 0.94)	105 (−2.73 ± 0.80)	38 (−2.98 ± 0.91)	**.04**	**.039**	**.039**
	Ht2	101 (−2.78 ± 0.86)	100 (−2.68 ± 0.90)	13 (−2.49 ± 0.83)	.378	.279	.267
	Ht3	153 (−2.69 ± 0.88)	56 (−2.81 ± 0.84)	5 (−2.25 ± 1.24)	.342	.586	ND

Data are expressed as number of cases (mean ± SD). *P* values of age-adjusted general linear model are shown. *P* values <.05 are shown in boldface type.

indicated that any SNP among the −55C/T, Tyr99Tyr, and Tyr210Tyr did not show significant association with obesity phenotypes such as BMI and body fat mass. These results are consistent with the association studies of −55C/T in Danish population and Pima Indians [8,16]. Both SNPs of Tyr99Tyr and Tyr210Tyr were also not associated with BMI and body fat mass in a UK white population and in Quebec Family Study [13,23]. In contrast, Otabe et al [12] reported that −55C/T polymorphism was associated with BMI in obese French white subjects in which TT type was higher than CC or CT type and that Tyr99Tyr was associated with BMI in Pima Indians [20]. Therefore, it is evident that the effects of these SNPs on obesity phenotypes should be confirmed by multiple studies and also should be carefully examined among different populations.

Three major haplotypes were constructed from the 6 SNPs in this study, and the major haplotype, ht1 [CGTACC], was significantly associated with body weight, WHR, and BMI (Tables 2 and 3). Recently, Liu et al [13] reported an association of the UCP-3 haplotype with obesity. They constructed 3 major haplotypes from 3 SNPs (−55C/T, Tyr99Tyr, and Tyr210Tyr) and demonstrated that ht1 [CTC] was associated with BMI. The ht1 [CTC] constructed by Liu et al had an equal base at same position with the ht1 [CGTACC] assessed this study, thereby providing consistent results with regard to the effects of the UCP-3 haplotype on obesity phenotypes.

The expression of UCP-3 is known to be modulated by both fasting and energy restriction [24,25]. Millet et al [26] observed a significant up-regulation of UCP-3 expression in skeletal muscle and adipose tissue in individuals undergoing fasting, and Schrauwen et al [27] suggested that the expression of UCP-3 was related to increased plasma free fatty acid levels and fat oxidization during fasting. It has also been reported that the expression of UCP-3 messenger RNA (mRNA) can be correlated positively with the plasma levels of free fatty acids, which are known to increase under fasting conditions because of the lysis of stored fat [1,28-30]. These previous studies indicated that UCP-3 is involved in the utilization of stored fat under energy-restriction conditions, suggesting the possibility that its genetic polymorphisms may have some effect on the efficiency with which fat is utilized, as well as the loss of stored fat during periods of energy restriction.

Until now, however, there has been a paucity of studies conducted regarding the effects of UCP-3 polymorphisms on the outcomes of energy-restriction regimens. Harper et al [18] divided 24 overweight women into 2 groups (diet-response and diet-resistant) after the completion of a 6-week weight control program involving VLED with an intake of 900 kcal/d and elucidated the distribution of 3 of the SNPs of UCP-3, including −55C/T, Tyr99Tyr, and Tyr210Tyr. The distributions of each of the SNPs between the diet-response and diet-resistant groups were similar. However,

Table 5
Effects of UCP-3 polymorphisms on VLED-induced change in body composition among overweight Korean female subjects

Phenotype	Locus	C/C	C/R	R/R	*P*		
					Codominant	Dominant	Recessive
Changed body fat mass (kg)	−55C/T	99 (−4.83 ± 3.24)	99 (−4.63 ± 2.09)	16 (−4.48 ± 1.76)	.706	.498	.517
	Int2-143G/C	55 (−5.08 ± 4.01)	110 (−4.68 ± 2.03)	49 (−4.38 ± 1.91)	.354	.218	.270
	Tyr99Tyr	97 (−4.91 ± 3.22)	101 (−4.56 ± 2.14)	16 (−4.48 ± 1.76)	.543	.301	.517
	Int3-47G/A	52 (−5.13 ± 4.09)	112 (−4.67 ± 2.02)	50 (−4.36 ± 1.93)	.288	.173	.237
	Int4-498C/T	134 (−4.80 ± 3.07)	71 (−4.62 ± 1.76)	9 (−4.13 ± 1.83)	.744	.644	ND
	Tyr210Tyr	54 (−4.36 ± 1.96)	111 (−4.61 ± 2.02)	49 (−5.34 ± 4.15)	.112	.215	**.047**
	Ht1	71 (−4.32 ± 1.83)	105 (−4.67 ± 2.05)	38 (−5.54 ± 4.64)	.054	.096	**.028**
	Ht2	101 (−4.82 ± 3.20)	100 (−4.63 ± 2.09)	13 (−4.47 ± 1.93)	.694	.492	.512
	Ht3	153 (−4.72 ± 2.93)	56 (−4.72 ± 1.78)	5 (−4.25 ± 2.24)	.920	.980	ND
Changed body fat percentage (%)	−55C/T	99 (−2.65 ± 4.16)	99 (−2.65 ± 2.25)	16 (−2.46 ± 1.38)	.832	.855	.544
	Int2-143G/C	55 (−2.83 ± 5.34)	110 (−2.58 ± 2.21)	49 (−2.54 ± 1.74)	.819	.552	.696
	Tyr99Tyr	97 (−2.70 ± 4.19)	101 (−2.60 ± 2.25)	16 (−2.46 ± 1.38)	.815	.724	.544
	Int3-47G/A	52 (−2.88 ± 5.46)	112 (−2.56 ± 2.20)	50 (−2.54 ± 1.79)	.771	.485	.697
	Int4-498C/T	134 (−2.68 ± 3.84)	71 (−2.59 ± 1.79)	9 (−2.25 ± 1.97)	.907	.925	ND
	Tyr210Tyr	54 (−2.48 ± 1.80)	111 (−2.53 ± 2.20)	49 (−3.04 ± 5.60)	.522	.588	.261
	Ht1	71 (−2.44 ± 1.73)	105 (−2.60 ± 2.24)	38 (−3.09 ± 6.32)	.515	.414	.300
	Ht2	101 (−2.63 ± 4.12)	100 (−2.65 ± 2.23)	13 (−2.47 ± 1.54)	.832	.876	.544
	Ht3	153 (−2.65 ± 3.64)	56 (−2.60 ± 1.84)	5 (−2.55 ± 2.35)	.997	.969	ND
Changed fat-free mass (kg)	−55C/T	99 (−2.39 ± 2.96)	99 (−2.30 ± 1.82)	16 (−1.94 ± 0.89)	.917	.748	.733
	Int2-143G/C	55 (−2.35 ± 3.71)	110 (−2.41 ± 1.66)	49 (−2.07 ± 1.76)	.764	.93	.472
	Tyr99Tyr	97 (−2.41 ± 2.98)	101 (−2.28 ± 1.81)	16 (−1.94 ± 0.89)	.871	.635	.733
	Int3-47G/A	52 (−2.32 ± 3.81)	112 (−2.45 ± 1.63)	50 (−2.01 ± 1.80)	.605	.958	.350
	Int4-498C/T	134 (−2.20 ± 2.72)	71 (−2.55 ± 1.58)	9 (−2.21 ± 2.10)	.718	.448	ND
	Tyr210Tyr	54 (−2.18 ± 1.71)	111 (−2.38 ± 1.67)	49 (−2.30 ± 3.92)	.903	.716	.889
	Ht1	71 (−2.17 ± 1.63)	105 (−2.47 ± 1.67)	38 (−2.16 ± 4.40)	.713	.64	.626
	Ht2	101 (−2.42 ± 2.94)	100 (−2.27 ± 1.80)	13 (−1.86 ± 0.91)	.864	.643	.701
	Ht3	153 (−2.25 ± 2.58)	56 (−2.55 ± 1.67)	5 (−1.77 ± 2.75)	.676	.578	ND
Changed body protein mass (kg)	−55C/T	99 (−0.638 ± 0.851)	99 (−0.581 ± 0.525)	16 (−0.606 ± 0.779)	.844	.624	.860
	Int2-143G/C	55 (−0.636 ± 1.013)	110 (−0.600 ± 0.486)	49 (−0.599 ± 0.736)	.957	.770	.968
	Tyr99Tyr	97 (−0.644 ± 0.858)	101 (−0.577 ± 0.522)	16 (−0.606 ± 0.779)	.779	.538	.860
	Int3-47G/A	52 (−0.632 ± 1.039)	112 (−0.610 ± 0.479)	50 (−0.584 ± 0.738)	.964	.820	.832
	Int4-498C/T	134 (−0.591 ± 0.802)	71 (−0.652 ± 0.522)	9 (−0.544 ± 0.549)	.873	.706	ND
	Tyr210Tyr	54 (−0.610 ± 0.700)	111 (−0.603 ± 0.489)	49 (−0.623 ± 1.071)	.988	.935	.923
	Ht1	71 (−0.579 ± 0.635)	105 (−0.628 ± 0.491)	38 (−0.613 ± 1.207)	.942	.741	.993
	Ht2	101 (−0.644 ± 0.844)	100 (−0.572 ± 0.521)	13 (−0.625 ± 0.860)	.753	.547	.766
	Ht3	153 (−0.594 ± 0.758)	56 (−0.662 ± 0.567)	5 (−0.477 ± 0.706)	.794	.684	ND

Data are expressed as number of cases (mean ± SD). *P* values of age-adjusted general linear model are shown. *P* values < .05 are shown in boldface type.

the numbers of subjects proved insufficient for the detection of the ostensibly mild effects of genetic polymorphisms.

In this study, we have elucidated the effects of the UCP-3 polymorphisms on the outcomes of a VLED regimen, which in this case was a 1-month weight control program of 700 kcal/d, using 214 overweight Korean female subjects as a sample. Among the 3 major haplotypes, ht1 [CGTACC] was significantly associated with VLED-induced changes in body weight and BMI, and this occurred in a gene-dose–dependent manner, that is, most profound in homozygotes (ht1/ht1), intermediate in heterozygotes (−/ht1), and least pronounced in noncarriers (−/−). Among the body components, with regard to VLED-induced changes, only body fat mass could be significantly associated with UCP-3 polymorphisms, and ht1 also evidenced significant associations with VLED-induced changes in body fat mass ($P = .028$), which was consistent with the role of UCP-3 in the utilization of fat under energy restriction, as was mentioned above. Similar results were obtained from nonobese Korean female subjects in whom BMI was lower than 25 kg/m^2 (data not shown).

Two SNPs, Int3-47G/A and Tyr210Tyr, both of them were significantly associated with VLED-induced changes in body weight in this study, were intronic or silent SNPs, inducing no amino acid changes. It remains unclear as to the manner in which these intronic or silent SNP might exert their effects on phenotypes, but a host of other studies have shown associations between intronic or silent SNPs and certain phenotypes. Orloff et al [31] reported that the SNP located in intron 6 of the Wilms' tumor gene (rs2234591) was associated with focal segmental glomerulosclerosis in an African American population. Pinotti et al observed that a silent polymorphism in intron 7 of the factor VII gene (IVS7) was associated with reduced concentrations of factor VII, apparently occurring via the regulation of factor VII mRNA levels [32]. These results suggested that some types of intronic SNPs might exert effects on phenotypes via the regulation of mRNA levels, or by other, heretofore unknown, mechanisms. Several

studies have also associated silent SNPs with other phenotypes. Forton et al [33] reported that C707T, a silent polymorphism of osteopontin (GCC/GCT, alanine), was associated significantly with systemic lupus erythematosus, and Ohshiro et al [34] determined that a silent SNP located at codon 25 (CAA/CAG, glutamine) of leptin was linked with morbid obesity in a Japanese population. Silent SNP may also be linked with other unknown functional polymorphisms, although other possibilities exist as well.

In this study, for the first time, haplotypes were constructed from 6 SNPs of UCP-3, and their associations with VLED outcomes were analyzed in a large subject population. Among the 3 primary haplotypes, ht1 [CGTACC] was associated significantly with VLED-induced losses of body weight and fat mass among 214 overweight Korean female subjects. It is clear that the findings of this study should be interpreted within the context of its limitations. The subjects of this study were recruited from an obesity clinic, and our findings are not appropriate for extrapolation to the general population. The observed associations, then, will require further confirmations in other subject groups.

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