A *UCP1-412A > C* polymorphism is associated with abdominal fat area in Korean women

MIN HO CHA[1], KIL SOO KIM[2], DONGCHUL SUH[3], SANG-IN CHUNG[3] and YOOSIK YOON[3]

[1]*Department of Medical Research, Korea Institute of Oriental Medicine, Daejeon, Republic of Korea*
[2]*Obesity Clinic, Kirin Oriental Hospital, Seoul, Republic of Korea*
[3]*Department of Microbiology, College of Medicine, Chung-Ang University, Seoul, Republic of Korea*

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We used genomic sequencing of Korean subjects to identify the uncoupling protein-1 (*UCP1*) polymorphism *-412A > C* (rs3811787 in the dbSNP database). This study is the first to associate this polymorphism with a human phenotype. The frequency of the major *A* allele was 0.53 and that of the minor *C* allele was 0.47. The *-412A > C* polymorphism was not linked to the well-known *-3826A > G* (rs1800592; $|D'| = 0.60$ and $r^2 = 0.33$), yielding four predicted haplotypes from these two polymorphisms. We associated *-412A > C, -3826A > G* and their haplotypes with computed tomography-measured body fat areas from 367 Korean female subjects. The *G* allele of *-3826A > G* and the *C* allele of *-412A > C* were significantly associated with larger areas of abdominal subcutaneous fat in a dominant model ($p = 0.001$ and $p = 0.0004$, respectively); combining them together (*ht2[GC]*) enhanced this significance ($p = 0.00005$). In contrast, presence of the *A* allele in both polymorphisms (*ht1[AA]*) was significantly associated with smaller areas of abdominal subcutaneous fat ($p = 0.003$). We observed no significant associations between these *UCP1* genetic polymorphisms and thigh fat areas, visceral fat areas, or blood biochemical profiles, suggesting that this polymorphism might differentially affect fat accumulation in different parts of the human body, even though further study is needed to elucidate the mechanism of it.

Yoosik Yoon, Department of Microbiology, College of Medicine, Chung-Ang University, 221 Heukseok-Dong, Dongjak-Gu, Seoul, 156-756, Republic of Korea. E-mail: thanks@cau.ac.kr

Recent work has profiled numerous candidate genes in order to identify novel genetic contributors to body fat accumulation and obesity. The gene product of one of these candidates, uncoupling protein (UCP), uncouples the proton gradient from ATP synthesis by promoting leakage in the inner mitochondrial membrane, thus playing a major role in thermogenesis (GARLID et al. 1996). *UCP1* was the first identified member of the *UCP* family, and it was once thought to be exclusive to brown adipose tissue, a specialized fat involved in energy expenditure and heat generation in rodents and human infants. More recently, however, many reports have suggested significant roles for *UCP1* in human adults. One study detected *UCP1* mRNA and protein in the white adipose tissue of adult humans in addition to infants (GARRUTI and RICQUIER 1992). Upon directly measuring *UCP1* mRNA levels in white adipose tissue obtained from fat biopsies, OBERKOFLER et al. observed significantly lower expression levels in obese subjects than in lean subjects, implicating *UCP1* in fat accumulation (OBERKOFLER et al. 1997). Various studies reported *UCP1* expression in adult humans: in the perirenal fat of adult pheochromocytoma patients (BOUILLAUD et al. 1988), around the neck arteries and in the pericardium of outdoor workers and long-term alcohol consumers (HUTTUNEN et al. 1981; HUTTUNEN and KORTELAINEN 1990).

The human *UCP1* gene is located on a long arm of chromosome 4 (CASSARD et al. 1990), and the first genetic polymorphism in *UCP1*, a *Bc*l I restriction fragment length polymorphism (RFLP), was identified in the 1994 Quebec family study (OPPERT et al. 1994). This RFLP was subsequently characterized as an A→G single nucleotide polymorphism (SNP) at −3826 bp upstream of the TATA box in the 5′-flanking region of the *UCP1* gene (*−3826A > G)* (CASSARD-DOULCIER et al. 1996). Until now, most *UCP1* genetic variation studies have focused on *−3826A > G*, including many association studies in various populations investigating whether this polymorphism affects obesity phenotypes. Some studies suggested the *−3826A > G* polymorphism correlates with the obesity phenotype (FUMERON et al. 1996; HEILBRONN et al. 2000; MATSUSHITA et al. 2003; NAGAI et al. 2003), but other did not (URHAMMER et al. 1997; GAGNON et al. 1998; SCHAFFLER et al. 1999; KIEC-WILK et al. 2002). These conflicting reports suggest that the 5′-flanking region of the *UCP1* gene may contain other undiscovered polymorphisms that contribute to the effects of this genomic region on the obesity phenotype.

Consistent with this idea, through the genomic sequencing of the Korean population, we identified a *−412A > C* polymorphism in the 5′-flanking region of

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the *UCP1* gene, about 3.4 kb downstream from the well-known $-3826A>G$ polymorphism. Herein, we analyzed the associations of these two *UCP1* polymorphisms with the areas of abdominal adipose tissue among Korean female subjects.

MATERIAL AND METHODS

Subjects

We recruited 367 Korean female subjects from the Obesity Clinic at Kirin Oriental Medical Hospital (Seoul, Korea); their general characteristics are listed in Table 1. Genomic DNA was obtained with informed consent, and the study protocol was reviewed by the Institutional Review Board of the Korea Institute of Oriental Medicine. Fat and fat-free masses of the subjects were measured by bio-impedance analysis using a commercially-available device (Inbody 2.0, Biospace Co., Korea). For almost all of the subjects ($n=358$), the areas of abdominal subcutaneous fat and visceral fat were measured from computed tomography (CT) cross sectional pictures of the abdominal region, as previously described (MATSUZAWA et al. 1995). The subcutaneous fat areas on the thighs of each subject were also measured using CT (Hispeed CT/e, GE, USA). Blood samples obtained from subjects after they had fasted more than 12 h were centrifuged at 2000 rpm for 10 min, serum concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and glucose were measured with an auto-biochemical analyzer (SP-4410, Arkray Co., Japan).

DNA preparation and polymorphism discovery

Genomic DNA from each subject was extracted from peripheral blood using Accuprep™ Genomic DNA Extraction Kits (Bioneer Co., Korea) according to the manufacturer's protocol. Concentrations of 200 randomly-selected DNA samples were measured using Picogreen (Molecular Probes, Eugene, OR, USA), and equal amounts were mixed to prepare pooled DNA. The pooled DNA was diluted to a working concentration of 2.5 ng μl^{-1}.

Table 1. *General characteristics of the subjects used in this study.*

		Total subjects ($n=367$)
Age	(year)	27.01 ± 8.12[1)]
Weight	(kg)	68.77 ± 13.02
BMI	($kg\ m^{-2}$)	26.50 ± 4.50
Fat mass	(kg)	24.72 ± 8.73
Fat-free mass	(kg)	44.05 ± 5.69
Total cholesterol	($mg\ dl^{-1}$)	224.69 ± 71.67
LDL-cholesterol	($mg\ dl^{-1}$)	165.73 ± 70.24
HDL-cholesterol	($mg\ dl^{-1}$)	49.53 ± 18.57
Triglycerides	($mg\ dl^{-1}$)	79.55 ± 40.57
Glucose	($mg\ dl^{-1}$)	100.65 ± 29.76

[1)]mean ± SD

Screening for genetic variations was performed by PCR amplification and direct sequencing. A working draft of the *UCP1* genomic sequence (NT_034706.2) was obtained through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/ >) and used to design the primers. Primer sequences are available upon request. Forward and reverse sequences of pooled and individual DNA samples were analyzed and compared to the working draft of the *UCP1* genomic sequence. Sequencing was carried out using a Dynamic™ ET Dye Terminator Kit (Amersham Biosciences, UK) and a MegaBACE 500 Genetic Analyzer (Amersham Biosciences) according to the manufacturers' instructions. Base calling of the sample files was performed using Cimarron Base Caller software (Amersham Biosciences), ver 3.12. ClustalX alignment software ver. 1.8 (THOMPSON et al. 1997) was used to mark potential heterozygote positions and display them for evaluation.

Genotyping assay

Genotyping of the $-412A>C$ polymorphism was based upon analysis of primer extension products, generated from the genomic DNA, using a chip-based matrix assisted laser desorption/ionization time of flight (MALDI-TOF) MassARRAY system (SEQUENOM, Inc., San Diego, CA, USA) according to the manufacturer's standard protocol. PCR and extension primers were designed using Primer3 (http://frodo.wi.mit.edu/). PCR primers were 5′ACGTTGGATG ATTGGTACTGTCACTGAGCTTG3′ and 5′ACG TTGGATGGAGCCTGCAAATCCATTATGA3′, and the extension primer was 5′ATTAAATCTGAC ATTTATGTGAG3′. After the overall automatic analyses, assays with bad peaks were checked and tested again manually. To verify the genotyping method used in this study, 21 randomly-selected samples were analyzed by direct sequencing to determine the precise nature of the sequence variation; no discrepancies were found.

The $-3826A>G$ polymorphism was genotyped by the RLFP, method as previously described (VALVE et al. 1998). PCR was conducted to amplify a genomic DNA fragment containing $-3826A>G$ position of *UCP1* gene, as follows: forward primer (5′CCAG TGGTGGCTAATGAGAGAA3′), reverse primer (5′ GCACAAAGAAGAAGCAGAGAGG3′), 3 μl dNTP mix (1 mM), 0.2 μl Taq DNA polymerase (1 unit), and

3 μl PCR buffer (10 ×) were combined and adjusted to a total volume of 30 μl with distilled water. The amplification protocol consisted of 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. The size of the amplified PCR products was verified (279 bp) by electrophoresis in a 3% agarose gel. The PCR products were subsequently digested with the restriction enzyme *Bc*l *I* for 1 h at 50°C and were subjected to electrophoresis in a 3% agarose gel. Genotypes were identified based on the resulting band patterns, as follows: GG type, single band of 279 bp; AG type, three bands of 279, 157, and 122 bp; AA type, two bands of 157 and 122 bp.

Statistical analysis

All values are presented as means ± standard deviation (SD). Age-adjusted univariate analysis was performed with a general linear model procedure to examine the independent effects of the genetic polymorphisms on fat mass, fat-free mass and body mass index (BMI). Age and fat mass-adjusted univariate analysis was performed to examine the genetic effects on CT-measured adipose tissue areas and blood biochemical parameters. Statistical significance was established at the level of $p < 0.05$. All analyses were performed by using SPSS ver. 10.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Simultaneous sequencing of the 5′ flanking region of *UCP1* using the pooled and individual genomic DNAs of Korean subjects revealed the *-412A > C* polymorphism at 412 base pairs upstream of the transcription start site (Fig. 1). A search of the dbSNP database (< http://www.ncbi.nlm.nih.gov/SNP/ >) identified this genetic polymorphism as rs3811787, located at position 141709869 on chromosome 4. Using MALDI-TOF-based methods, we genotyped this *−412A > C* (rs3811787) polymorphism among the 367 Korean female subjects to elucidate its effects on human phenotypes; this study is the first association study conducted on *−412A > C* (rs3811787). The genotypes *AA*, *AC* and *CC* comprised 25.9% (n = 95), 54.8% (n = 201), and 19.3% (n = 71) of the study subjects, respectively, consistent with Hardy-Weinberg equilibrium (p = 0.16).

The frequency of the major *A* allele was 0.53, and that of the minor *C* allele was 0.47. According to the international HapMap project, as shown at <www.hapmap.org>. (The International HapMap consortium 2003; Thorisson et al. 2005), the respective frequencies of the *A* and *C* alleles were 0.75 and 0.25 in Europeans, 0.62 and 0.38 in the Japanese, 0.47 and 0.53 in the Chinese, and 0.31 and 0.69 in Africans. The results of the present study showed that the allele frequencies of this SNP in Koreans were between those of the Japanese and Chinese populations. The allele frequency reports from the international HapMap project were derived from genotypes of 42–58 individuals from each population. Since we genotyped 367 Korean subjects, the present study provides much more accurate allele frequencies for this genetic polymorphism within the Asian population.

Alongside *-412A > C*, we also genotyped the more well-known *-3826A > G* SNP (rs 1800592). The frequency of the *A* allele was 0.51 and that of the *G* allele was 0.49, consistent with Hardy-Weinberg equilibrium (p = 0.85). Upon testing for linkage between these two polymorphisms, we obtained low linkage disequilibrium (LD) coefficients, demonstrating that *-412A > C* and *-3826A > G* are not in the same LD block in the Korean population: $|D'| = 0.60$ and $r^2 = 0.33$. Using the Expectation–Maximization algorithm (Devlin and Risch 1995; Qin et al. 2002), we predicted four haplotypes from these two polymorphisms, all of which satisfied Hardy-Weinberg equilibrium (Table 2).

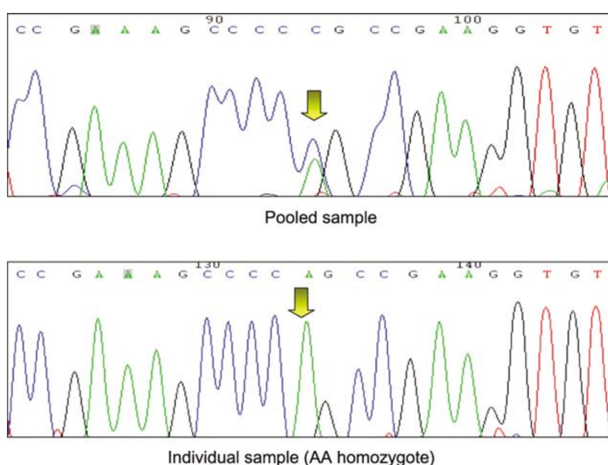

Fig. 1. Identification of the *-412A > C* polymorphism in Korean subjects. Shown are sequence trace views from positions −423 to −401 within the 5′ flanking region of *UCP1* for pooled and individual DNA samples. The vertical arrow indicates nucleotide position −412.

Table 2. *Predicted haplotypes from −3826A > G and −412A > C.*

Haplotype	*−3826A > G*	*−412A > C*	Frequency	HWE[1)]
Ht1[AA]	A	A	0.43	0.62
Ht2[GC]	G	C	0.38	0.14
Ht3[GA]	G	A	0.11	0.29
Ht4[AC]	A	C	0.08	0.59

[1)] p-values of deviation from Hardy-Weinberg equilibrium.

When we analyzed the two SNPs and their four haplotypes for associations with generally-used obesity indices, such as BMI and fat mass, we did not observe any significant associations (Table 3, $p > 0.05$); the same was true for fat-free mass. We then conducted CT scans of the majority of the subjects, in order to measure the cross-sectional fat areas at their abdomens and thighs, and to evaluate whether the *UCP1* genetic polymorphisms influenced body fat distribution (Fig. 2).

In our age and fat mass-adjusted analysis of covariance, the *UCP1* genetic polymorphisms showed significant associations with area of abdominal fat. The *G* allele in *-3826A > G* was significantly associated with larger abdominal fat areas ($p = 0.008$ in a codominant model and $p = 0.002$ in a dominant model), as was the *C* allele of *-412A > C* ($p = 0.005$ in a codominant model and $p = 0.001$ in a dominant model). *Ht2[GC]*, a combination of the *G* allele of *-3826A > G* and the *C* allele of *-412A > C*, showed the most significant association with larger abdominal fat areas ($p = 0.002$ in a codominant model and $p = 0.0004$ in a dominant model). In contrast, *ht1[AA]*, a combination of the *A* alleles from *-3826A > G* and *-412A > C*, significantly associated with smaller abdominal fat area ($p = 0.014$ in a codominant model and $p = 0.004$ in a recessive model). A possible association between *ht4[AC]* and smaller abdominal fat area ($p = 0.005$ in recessive model) is uncertain, because only four subjects were *ht4* homozygotes, making it impossible to exclude sampling bias. Comparisons among p-values for codominant, dominant, and recessive models indicated that the *G* allele of *-3826A > G,* the *C* allele of *-412A > C*, and their combination, *ht2[GC]*, associated with larger abdominal fat areas in dominant mode. Likewise, each of the *A* alleles from *-3826A > G* and *-412A > C* and their combination, *ht1[AA]*, associated with smaller abdominal fat areas in recessive mode. In contrast, we did not observe significant associations between the *UCP1* genetic polymorphisms and fat area measured from the thighs of the subjects, suggesting that the *UCP1* genetic polymorphisms might exert differential effects on fat accumulation in the abdomen and thigh. It is possible that the *UCP1* gene has differential effects on fat accumulation in central and distal parts of the human body.

This single nucleotide change at the -412 position upstream from the transcription start site could affect transcription factor binding affinity. A transcription factor binding site search using TFSEARCH site (<http://www.cbrc.jp/research/db/TFSEARCH.html>) revealed that the *-412A > C* polymorphism (rs3811787) is located adjacent to the binding site for the c-Rel

Table 3. *Age-adjusted association analyses of the UCP1 polymorphisms with BMI, fat mass, and fat-free mass in Korean female subjects. Shown are numbers of cases (mean ±SD) and p-values in the co-dominant model, adjusted for age. C/C, C/R, and R/R represent homozygotes for the common allele, and heterozygotes and homozygotes for the rare allele, respectively. Ht1, Ht2, Ht3 and Ht4 were classified as rare alleles because of their frequencies less than 0.5 as shown in Table 2. For each haplotype (ht), C/C means non-carrier (−/−); C/R means one-allele carrier (−/ht); R/R means two allele carrier (ht/ht).*

Locus	BMI				Fat mass				Fat-free mass			
	C/C	C/R	R/R	p	C/C	C/R	R/R	p	C/C	C/R	R/R	p
-3826A > G	97(26.39±4.11)	178(26.55±4.53)	92(26.51±4.87)	0.976	97(25.01±8.22)	178(24.58±8.72)	92(24.66±9.31)	0.947	97(44.07±5.00)	178(43.88±5.52)	92(44.36±6.69)	0.816
-412A > C	95(25.89±4.22)	201(26.67±4.71)	71(26.81±4.25)	0.341	95(24.05±7.96)	201(24.94±9.26)	71(24.99±8.20)	0.650	95(43.97±5.19)	201(44.07±5.59)	71(44.08±6.65)	0.996
Ht1[AA]	118(26.77±4.76)	188(26.47±4.42)	61(26.03±4.24)	0.615	118(25.06±9.21)	188(24.55±8.51)	61(24.55±8.52)	0.849	118(44.32±6.60)	188(43.94±5.33)	61(43.86±4.90)	0.851
Ht2[GC]	131(26.20±4.14)	191(26.74±4.81)	45(26.30±4.20)	0.579	131(24.53±7.91)	191(24.98±9.47)	45(24.12±7.74)	0.764	131(44.10±5.18)	191(44.02±5.77)	45(44.03±6.84)	0.975
Ht3[GA]	294(26.56±4.37)	65(26.20±5.07)	8(26.60±5.06)	0.815	294(24.79±8.53)	65(24.55±9.80)	8(23.43±7.05)	0.91	294(44.00±5.62)	65(43.96±5.76)	8(46.51±7.95)	0.469
Ht4[AC]	309(26.35±4.58)	54(27.19±4.06)	4(28.00±4.35)	0.356	309(24.48±8.82)	54(25.85±8.07)	4(27.60±10.20)	0.463	309(44.05±5.71)	54(43.73±5.57)	4(48.35±5.72)	0.353

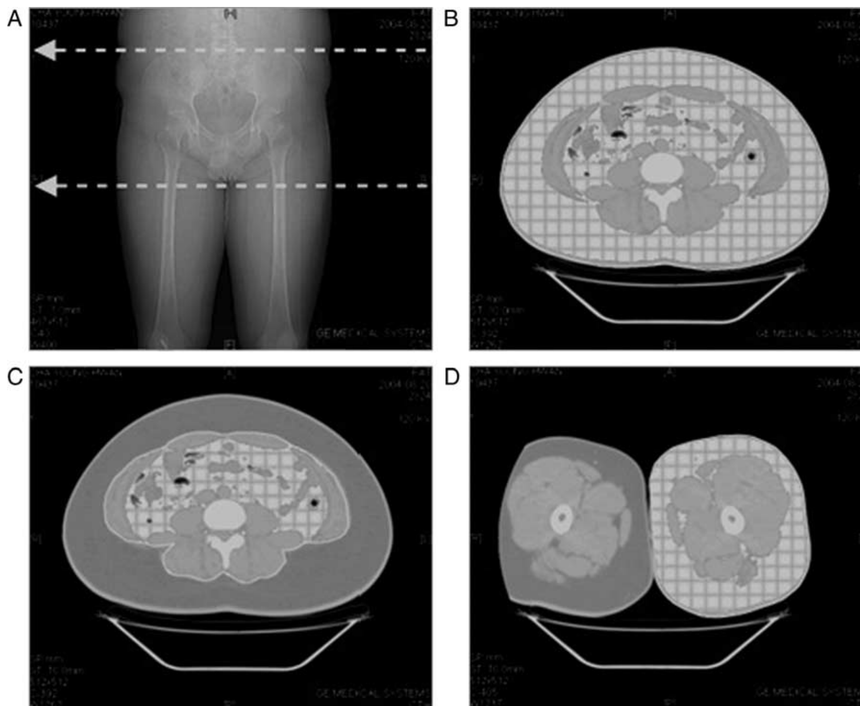

Fig. 2. Measurement of cross-sectional fat tissue areas using CT. (A) Dashed lines indicate the abdominal and thigh positions used for cross-sectional fat area measurement. (B) An example of total abdominal fat area. (C) An example of visceral fat area. (D) An example of thigh subcutaneous fat area.

transcription factor (Kunsch et al. 1992). An A/C polymorphism adjacent to the c-Rel binding site might influence its binding affinity and subsequent transcriptional activation. Reportedly, an important hormone involved in obesity, leptin, controls the intracellular localization of c-Rel, suggesting a possible mechanism by which this genetic polymorphism operates (Napoleone et al. 2007), although further studies will definitively answer this question.

Abdominal fat is divided into subcutaneous and visceral fat, and the abdominal subcutaneous fat area can be obtained by subtracting visceral fat areas from total abdominal fat areas shown in Fig. 2. Using this technique, we analyzed the effects of the *UCP1* genetic polymorphisms on the two components of abdominal fat, subcutaneous and visceral fat (Table 5). The *G* allele of $-3826A>G$ and the *C* allele of $-412A>C$ associated with larger areas of subcutaneous fat in a dominant model ($p=0.001$ and $p=0.0004$, respectively). Their combination (*ht2[GC]*) showed the most significant association with higher subcutaneous fat area in a dominant model ($p=0.00005$). Combining the *A* alleles from both polymorphisms, *ht1[AA]*, produced a significant association with smaller subcutaneous fat area in a recessive model ($p=0.003$). *Ht4[AC]* showed a p-value of 0.013 in a recessive model, but the number of subjects who were *ht4/ht4* homozygotes was too small to obtain statistical significance. In contrast, we observed no significant associations between the *UCP1* genetic polymorphisms and visceral fat area. In fact, the effects of the *UCP1* genetic polymorphisms on subcutaneous fat were similar to their effects on total abdominal fat, but with smaller p-values (Tables 4 and 5).

We could not identify significant associations between the *UCP1* genetic polymorphisms and blood biochemical profiles (data not shown). Visceral fat accumulation clearly correlates with aberrant biochemical profiles, but the relationship between subcutaneous fat and biochemical profiles is unknown (Björntorp 1991). Troglitazone, an anti-diabetic medication, reportedly increased subcutaneous fat area while improving biochemical profiles, providing evidence that subcutaneous fat accumulation is not related to aberrant biochemical profiles (Akazawa et al. 2000). Matsuzawa et al. suggested that the visceral fat to subcutaneous fat ratio (V/S ratio) is a better indicator of aberrant biochemical profiles (Matsuzawa et al. 1995). In the present study, the V/S ratio did not significantly differ with the *UCP1* genetic polymorphisms, providing a possible explanation for the similar biochemical profiles among *UCP1* genotypes (data not shown).

According to the results of this study, the first study to associate the *UCP1* $-412A>C$ polymorphism with

Table 4. *Age- and fat mass-adjusted association analyses of the UCP1 polymorphisms with abdominal and thigh fat areas in Korean female subjects. C/C, C/R, and R/R represent homozygotes for the common allele, heterozygotes and homozygotes for the rare allele, respectively. Ht1, Ht2, Ht3 and Ht4 were classified as rare alleles because of their frequencies less than 0.5 as shown in Table 2. For each haplotype (ht), C/C means non-carrier (−/−); C/R means one-allele carrier (−/ht); R/R means two allele carrier (ht/ht). Shown are numbers of cases (mean ± SD) and p-values in co-dominant, dominant, and recessive models, adjusted for age and fat mass for regression analyses.*

Locus	Abdominal fat area			p			Thigh fat area			p		
	C/C	C/R	R/R	Codominant	Dominant	Recessive	C/C	C/R	R/R	Codominant	Dominant	Recessive
-3826A > G	95(34004 ± 12924)	174(36268 ± 15485)	89(36628 ± 17010)	0.008	0.002	0.247	95(15548 ± 3859)	174(15211 ± 4178)	89(15137 ± 3747)	0.964	0.788	0.891
-412A > C	92(32342 ± 12171)	197(37002 ± 16512)	69(36753 ± 14696)	0.005	0.001	0.163	92(15267 ± 3964)	197(15340 ± 4173)	69 (15137 ± 3470)	0.710	0.521	0.493
Ht1[AA]	114(36929 ± 16616)	184(36116 ± 15335)	60(32426 ± 11626)	0.014	0.257	0.004	114 (15189 ± 3753)	184 (15221 ± 4079)	60 (15646 ± 4154)	0.481	0.504	0.244
Ht2[GC]	127(33545 ± 12997)	187(37191 ± 16693)	44(36043 ± 14408)	0.002	0.0004	0.111	127 (15298 ± 3791)	187 (15337 ± 4268)	44 (15003 ± 3276)	0.994	0.941	0.955
Ht3[GA]	288(35886 ± 14664)	63(35354 ± 18024)	7(34038 ± 13904)	0.76	0.479	0.68	288 (15362 ± 3989)	63 (14933 ± 4033)	7(15152 ± 3653)	0.768	0.562	0.799
Ht4[AC]	302(35420 ± 15253)	52(37941 ± 15687)	4(32740 ± 7136)	0.014	0.912	0.005	302 (15260 ± 4056)	52 (15427 ± 3675)	4(15051 ± 2757)	0.366	0.164	0.547

Table 5. *Age- and fat mass-adjusted association analyses of the UCP1 polymorphisms with abdominal subcutaneous fat and visceral fat areas in Korean female subjects. C/C, C/R, and R/R represent homozygotes for the common allele, heterozygotes and homozygotes for the rare allele, respectively. Ht1, Ht2, Ht3 and Ht4 were classified as rare alleles because of their frequencies less than 0.5 as shown in Table 2. For each haplotype (ht), C/C means non-carrier (−/−); C/R means one-allele carrier (−/ht); R/R means two allele carrier (ht/ht). Shown are numbers of cases (mean ± SD) and p-values in the co-dominant, dominant and recessive models adjusted for age and fat mass for regression analyses.*

Locus	Subcutaneous fat area			p			Visceral fat area			p		
	C/C	C/R	R/R	Codominant	Dominant	Recessive	C/C	C/R	R/R	Codominant	Dominant	Recessive
3826A > G	95(28147 ± 10144)	174(30154 ± 13120)	89(30374 ± 14101)	0.003	0.001	0.339	95(5856 ± 3472)	174(6113 ± 3350)	89(6253 ± 3691)	0.143	0.249	0.062
-412A > C	92(26838 ± 9759)	197(30796 ± 13883)	69(30263 ± 12007)	0.002	0.0004	0.187	92(5503 ± 3063)	197(6205 ± 3572)	69(6490 ± 3598)	0.848	0.566	0.831
Ht1[AA]	114(30557 ± 13893)	184(29995 ± 12756)	60(27025 ± 9336)	0.011	0.282	0.003	114(6372 ± 3610)	184(6120 ± 3470)	60(5401 ± 3100)	0.402	0.449	0.195
Ht2[GC]	127(27729 ± 10258)	187(30987 ± 14242)	44(29726 ± 11279)	0.0002	0.00005	0.174	127(5816 ± 3356)	187(6203 ± 3469)	44(6317 ± 3764)	0.348	0.569	0.149
Ht3[GA]	288(29776 ± 12119)	63(29433 ± 15181)	7(27761 ± 11446)	0.821	0.659	0.579	288(6110 ± 3466)	63(5920 ± 3556)	7(6276 ± 2797)	0.513	0.289	0.891
Ht4[AC]	302(29474 ± 12715)	52(31025 ± 12816)	4(27423 ± 5412)	0.046	0.509	0.013	302(5946 ± 3393)	52(6916 ± 3858)	4(5317 ± 2000)	0.002	0.043	0.021

a human phenotype, this polymorphism significantly associated with abdominal subcutaneous fat area, but not with visceral fat area and thigh fat area, suggesting that this polymorphism exerts a position-specific effect on body fat accumulation, even though further study is needed to elucidate the mechanism of it.

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