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ORIGINAL PAPER

Human uncoupling protein 2 and 3 genes are associated with obesity in Japanese

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Abstract Human uncoupling proteins (UCPs) are mitochondrial proteins that are involved in the control of energy metabolism and the pathophysiology of obesity. Although there have been several reports on the association between the UCP2/UCP3 locus and the obesity, there have been no haplotype-based case–control studies with gender-specific analysis. The aim of this study was to examine whether there is an association between the UCP2/UCP3 locus and the obesity in the Japanese population when using a single nucleotide polymorphism (SNP)-based and haplotype-based case–control study with gender-specific analysis. We examined a group consisting of 551 subjects, of which 369 were non-obese and 182 were overweight and/or obese. We selected one nonsynonymous SNP (rs660339: Ala55Val) as a genetic marker. Genotyping for all subjects was performed by the TaqMan® polymerase chain reaction (PCR) method. Although the overall distributions of genotype and allele were not significantly different between the non-obese and the obese groups, the overall distributions of the genotype were significantly different in men ($P = 0.030$). In the obese group, male subjects with the Val allele were significantly more frequent in both association studies. There was a significant difference in the overall distribution of the haplotype (UCP3 rs180049, UCP3 rs2075577, UCP2 rs660339) between the weight groups ($P = 0.010$), and in women, there was a significant difference ($P = 0.042$) in the overall distribution of the haplotype (UCP3 rs2075577, UCP2 rs660339). Nonsynonymous rs660339 in the human UCP2 gene in men, and the haplotype (UCP3 rs2075577–UCP2 rs660339) in women might be good obesity markers.

Keywords Single nucleotide polymorphism · Uncoupling protein 2 · Obesity · Association study

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Introduction

Obesity is the state of abnormal energy metabolism. Both systemically and at the cellular level, energy metabolism is controlled by a large variety of enzymatic and nonenzymatic proteins [1].

The cell's energy substrate, ATP, is synthesized within the mitochondria. Energy is derived from the oxidation of fuels and is used to create an electrochemical gradient across the inner mitochondrial membrane by the export of protons from the matrix. These protons re-enter the mitochondrial matrix via ATP synthase, which uses energy from the electrochemical gradient to convert ADP to ATP. A group of five proteins, which are known as the uncoupling proteins (UCPs), are located within the inner mitochondrial membrane [2, 3]. UCP1 is expressed exclusively in brown adipose tissue (BAT) and is responsible for thermogenesis in mammalian neonates and rodents [4]. UCP2 is expressed in almost all mammalian tissues, suggesting that it plays a functional role in global

energy metabolism in the body [1, 5, 6]. UCP3 is predominantly expressed in mammalian skeletal muscle and BAT [7–9]. UCP4 is mainly expressed in brain tissue [10] and UCP5 is expressed with high abundance in the brain and testis [11, 12].

A substantial role for UCP2 or UCP3 in thermogenesis in humans is unlikely, as these proteins are not upregulated by cold. It has been suggested that the main function of UCP2 is to provide protection from the damage caused by reactive oxygen species or oxidative stress [13–15].

UCP2 and UCP3 genes are located in tandem within 8 kb of each other on chromosome 11q13 [16]. In previous association analyses that examined variations in the UCP2–UCP3 gene cluster and obesity or diabetes traits [5, 17–21], it was reported that this locus is associated with a risk for these disorders. A linkage study reported that resting metabolic rate (RMR) and other obesity-related phenotypes are associated with markers at the UCP2/UCP3 locus [17, 18, 22]. Several UCP2 gene variants have been reported in human studies and include: a G/A single nucleotide polymorphism (SNP) in the promoter region (−866G/A) [23], a valine-for-alanine substitution at amino acid 55 in exon 4 (Ala55Val), and a 45-bp insertion/deletion in the 3′-untranslated region [24, 25]. The Ala55Val polymorphism has been linked to variations in the energy balance via effects on the RMR and nocturnal physical activity [26, 27], and to type 2 diabetes [28, 29].

The aim of this study was to examine whether there is an association between the UCP2/UCP3 locus and the obesity in the Japanese population when using an SNP-based and haplotype-based case–control study with a gender-specific analysis.

Subjects and methods

Subjects

We examined 551 subjects who visited various departments of the Nihon University School of Medicine Hospital, such as Endocrinology, Cardiology, Public Care, etc. The subjects typically underwent healthcare examinations that included tests for high blood pressure, high blood sugar, hyperlipidemia, and high serum uric acid, among others. In order to avoid any sampling bias, we recruited subjects by handing out documents that detailed the nature of the study, and if the subjects then agreed to take part, samples were collected once informed consent was obtained. Therefore the recruitment methods were not designed to specifically enroll subjects as members of the control or obese groups. After sample collection, we used the body mass index (BMI) to divide the subjects into two groups, which were based on the presence or absence of obesity. The obese group consisted of 182 obese and overweight subjects (BMI was $\geq$25) with a mean age of 50.1 $\pm$ 8.0 years. The non-obese group consisted of 369 subjects with weights considered to be normal or within the normal lean range (BMI was <25) and these subjects had a mean age of 50.7 $\pm$ 8.3 years. Samples were also matched by gender ($P = 0.120$). Informed consent was obtained from each subject in accordance with the protocol approved by the Ethics Committee of the Nihon University School of Medicine and the Clinical Studies Committee of Nihon University Hospital.

Biochemical analysis

The Clinical Laboratory Department of Nihon University Hospital used standard laboratory methods to measure the serum total cholesterol, HDL cholesterol, creatinine and uric acid concentrations, fasting blood sugar, and HbA1c in blood [30].

Genotyping

Using information on SNP allelic frequencies from the website of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=7351) and the SNP browser software 3.0 (Applied Biosystems, Branchburg, NJ, USA), SNPs on the human UCP2 and UCP3 genes with minor allele frequencies >30% (in Japanese subjects) were selected. SNPs with relatively high minor allele frequencies have been shown to be very useful as genetic markers for genetic association studies. We selected three SNPs for the human UCP2 and UCP3 genes. The SNP accession numbers were rs1800849, rs2075577, and rs660339 (Fig. 1). rs1800849 is a C-to-T substitution in the 5′-untranslated region of exon 1 of the UCP3 gene. rs2075577 is a synonymous C-to-T substitution in exon 5 of UCP3, with a change of Tyr to Tyr at codon 210 (Tyr210Tyr). rs660339 is a nonsynonymous C-to-T substitution in UCP2 exon 4 at position 544 of the putative mRNA, with a change of alanine to valine at codon 55 (Ala55Val). Genotypes were determined using Assay-on-Demand kits (Applied Biosystems) with TaqMan® PCR. When allele-specific fluorogenic probes hybridize to the template during the PCR, the 5′-nuclease activity of *Taq* polymerase can discriminate alleles. Cleavage results in increased emission of a reporter dye that is otherwise quenched by the dye TAMRA. Each 5′-nuclease assay requires two unlabeled PCR primers and two allele-specific probes. Each probe is labeled with a reporter dye (VIC and FAM) at the 5′-end and TAMRA at the 3′-end. PCR amplification was performed using 2.5 µl of TaqMan® Universal Master Mix, No AmpErase® UNG (2×) (Applied Biosystems) in final reaction volumes of 5 µl, along with

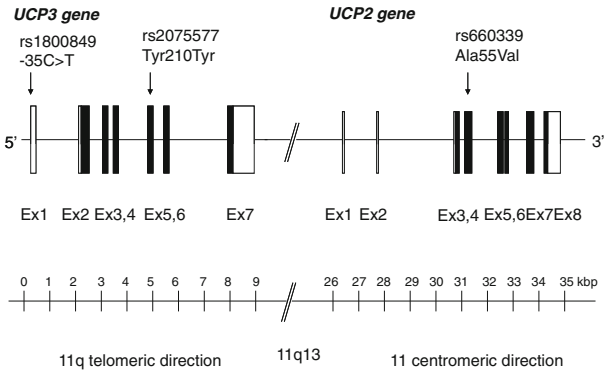

Fig. 1 Locus of the human UCP3 and UCP2 genes in 11q13. The UCP3 gene consists of seven exons separated by six introns, and the UCP2 gene consists of eight exons separated by seven introns. *Boxes* indicate exons, while *lines* indicate introns and intergenic regions. *Filled boxes* indicate coding regions. *Arrows* mark the polymorphism locations

2 ng of DNA, 2.375 μl of ultrapure water, 0.079 μl of Tris–EDTA (TE) buffer (1×), 0.046 μl of TaqMan® SNP Genotyping Assay Mix (40×) containing a 331.2 nM final concentration of primers, and a 73.6 nM final concentration of probes. Thermal cycling conditions were 95°C for 10 min, followed by 50 cycles of 92°C for 15 s, and 60°C for 1 min. Thermal cycling was performed using the GeneAmp 9700™ system.

All 96-well plates contained 80 samples of an unknown genotype, and four reactions with reagents but no DNA. Control samples without DNA were required for the SDS 7700® signal processing, as outlined in the TaqMan® Allelic Discrimination Guide (Applied Biosystems). PCR plates were read on an SDS 7700® instrument in the end-point analysis mode of the SDS version 1.6.2 software package (Applied Biosystems). Genotypes were visually determined by comparison with dye-component fluorescent emission data shown in the *XY* scatterplot of the SDS software. Genotypes were also automatically determined by the signal processing algorithms in the software. The results of both scoring methods were saved to two output files for later comparison.

Statistical analysis

Data are presented as means ± SD. Allele frequencies were calculated from the genotypes of all subjects. Hardy–Weinberg equilibrium was assessed by χ^2 analysis. Differences in clinical data between obesity and non-obesity groups, and genotypes were assessed by Mann–Whitney and Kruskal–Wallis tests. The distributions of genotypes between obesity patients and non-obesity subjects were tested by a two-sided Fisher's exact test. In addition, logistic regression analysis was performed to assess the contribution of the major risk factors.

Statistical significance was established at $P < 0.05$. Statistical analyses were performed using SPSS software for Windows, version 12 (SPSS Inc., Chicago, IL, USA).

Linkage disequilibrium and haplotype-based case–control study

SNPAlyze software for Windows, version 3.2.3 (Dynacom Co., Ltd., Yokohama, Japan) was used to perform the linkage disequilibrium (LD) and the haplotype-based case–control study. This software is available at: http://www.dynacom.co.jp/products/package/snpalyze/index.html.

D' with $P > 0.5$ were considered to be haplotype blocks. In this haplotype-based case–control study, haplotypes with a frequency <0.01 were excluded. The distribution of haplotype frequencies was calculated using the χ^2 test. A probability level of $P < 0.05$ was considered to indicate statistical significance.

Results

Clinical characteristics are shown in Table 1. SBP, DBP, BMI, and serum concentrations of uric acid and total cholesterol were significantly higher, while serum concentrations of HDL cholesterol were significantly lower, in the obese group as compared to the non-obese group. There were no significant differences between the groups with regard to serum concentrations of creatinine. Age was not significantly different between the groups.

We succeeded in genotyping 369 non-obese subjects and 182 obese patients. The observed and expected heterozygosities in the non-obese group were 53.9 and 49.9%, respectively, which were in good agreement with predicted Hardy–Weinberg equilibrium values ($P = 0.126$). Genotype frequencies were 25.4% Val/Val, 44.2% Ala/Val, and 30.9% Ala/Ala in obese patients, and 21.4% Val/Val, 53.9% Ala/Val, and 24.7% Ala/Ala in the non-obese subjects. The overall distribution of alleles was not significantly different between the two groups ($P = 0.085$, Table 2). When limited to men, the genotype frequencies were 23.3% Val/Val, 44.2% Ala/Val, and 32.6% Ala/Ala in obese patients, and 20.3% Val/Val, 57.8% Ala/Val, and 21.9% Ala/Ala in the non-obese subjects. The overall distributions of genotypes and Val/Val + Ala/Val versus Ala/Ala were significantly different between the two groups, and the frequency of Ala/Ala was significantly higher than that of Val/Val + Ala/Val in obese men ($P = 0.033$, Table 2).

In the logistic regression analysis, the confounding factors showing the significant difference in Table 1 were used. SBP and DBP were associated with hypertension, fasting blood sugar and HbA1c were associated with

Table 1 Characteristics of study participants

	Total			Men			Women		
	Non-obese	Obese	*P*-value	Non-obese	Obese	*P*-value	Non-obese	Obese	*P*-value
No. of subjects	369	182		237	129		132	53	
Age (years)	50.7 ± 8.3	50.1 ± 7.9	0.299	50.7 ± 7.0	49.3 ± 8.0	0.060	50.6 ± 10.3	51.8 ± 7.5	0.365
BMI (kg/m^2)	21.9 ± 2.2	27.8 ± 2.9	<0.001	22.0 ± 2.1	27.8 ± 2.7	<0.001	21.7 ± 2.2	28.1 ± 3.3	<0.001
SBP (mmHg)	139 ± 34	155 ± 32	<0.001	140 ± 33	152 ± 30	0.001	139 ± 36	164 ± 35	<0.001
DBP (mmHg)	85 ± 21	96 ± 20	<0.001	86 ± 21	95 ± 20	<0.001	83 ± 21	98 ± 21	<0.001
Pulse (beats/min)	76 ± 15	76 ± 12	0.349	76 ± 16	76 ± 12	0.252	76 ± 12	76 ± 13	0.799
Fasting blood sugar	103.7 ± 17.4	119.1 ± 40.2	0.049	104.9 ± 19.7	122.8 ± 44.5	0.077	101.5 ± 12.0	106.1 ± 12.6	0.390
HbA1c (%)	5.2 ± 0.8	5.8 ± 1.3	0.007	5.2 ± 0.9	6.1 ± 1.4	0.011	5.0 ± 0.4	5.2 ± 0.4	0.368
Creatinine (mg/dl)	0.83 ± 0.23	0.86 ± 0.23	0.189	0.91 ± 0.22	0.92 ± 0.21	0.628	0.70 ± 0.18	0.70 ± 0.18	0.896
Total cholesterol (mg/dl)	204 ± 43	212 ± 42	0.004	198 ± 42	208 ± 43	0.005	214 ± 43	222 ± 37	0.183
HDL cholesterol (mg/dl)	60 ± 17	49 ± 15	<0.001	58 ± 16	46 ± 12	<0.001	64 ± 18	57 ± 16	0.010
Uric acid (mg/dl)	5.3 ± 1.7	6.5 ± 5.1	<0.001	5.8 ± 1.4	6.5 ± 1.4	<0.001	4.4 ± 1.7	6.7 ± 9.3	<0.001
Hyperlipidemia	33.6%	48.9%	0.001	28.7%	47.3%	<0.001	42.4%	52.8%	0.199
Hypertension	46.3%	69.2%	<0.001	47.3%	65.9%	0.001	44.7%	77.4%	<0.001
Diabetes mellitus	6.5%	17.0%	<0.001	8.4%	18.6%	0.004	3.0%	13.2%	0.008
Drinking (%)	64.1%	64.8%	0.919	80.0%	76.3%	0.472	35.2%	37.5%	0.857
Smoking (%)	46.3%	53.9%	0.122	56.5%	64.1%	0.192	28.0%	29.2%	>0.999

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HDL* high-density lipoprotein

Table 2 Genotype and allele distributions in non-obese and obese subjects

			Total					Men					Women				
			Non-obese		Obese		*P*-value	Non-obese		Obese		*P*-value	Non-obese		Obese		*P*-value
			369	%	181	%		237	%	129	%		132	%	52	%	
rs1800849 UCP3 5′ near gene (C > T)	Genotype	C/C	172	46.6	88	48.6	0.626	109	46.0	64	49.6	0.618	63	47.7	24	46.2	0.918
		T/C	161	43.6	80	44.2		107	45.1	57	44.2		54	40.9	23	44.2	
		T/T	36	9.8	13	7.2		21	8.9	8	6.2		15	11.4	5	9.6	
		C/C	172	46.6	88	48.6	0.716	109	46.0	64	49.6	0.513	63	47.7	24	46.2	0.871
		T/T + C/T	197	53.4	93	51.4		128	54.0	65	50.4		69	52.3	28	53.8	
		C/T + C/C	333	90.2	168	92.8	0.344	146	61.6	136	105.4	0.837	117	88.6	47	90.4	1.000
		T/T	36	9.8	13	7.2		19	8.0	19	14.7		15	11.4	5	9.6	
	Allele	C	505	68.4	256	70.7	0.446	325	68.6	185	71.7	0.401	180	68.2	71	68.3	1.000
		T	233	31.6	106	29.3		149	31.4	73	28.3		84	31.8	33	31.7	
rs2075577 UCP3 Tyr210Tyr (C > T)	Genotype	T/T	94	25.5	49	27.1	0.572	58	24.5	31	24.0	0.555	36	27.3	18	34.6	0.633
		C/T	192	52.0	87	48.1		129	54.4	65	50.4		63	47.7	22	42.3	
		C/C	81	22.0	46	25.4		49	20.7	33	25.6		32	24.2	13	25.0	
		T/T + C/T	286	77.5	136	75.1	0.452	187	78.9	96	74.4	0.297	99	75.0	40	76.9	1.000
		C/C	81	22.0	46	25.4		49	20.7	33	25.6		32	24.2	13	25.0	
		T/T	94	25.5	49	27.1	0.757	58	24.5	31	24.0	1.000	36	27.3	18	34.6	0.379
		C/T + C/C	273	74.0	133	73.5		178	75.1	98	76.0		95	72.0	35	67.3	
	Allele	T	380	51.5	185	51.1	0.798	245	51.7	127	49.2	0.536	135	51.1	58	55.8	0.645
		C	354	48.0	179	49.4		227	47.9	131	50.8		127	48.1	48	46.2	
rs660339 UCP2 Ala55Val (C > T)	Genotype	Ala/Ala	91	24.7	56	30.9	0.085	52	21.9	42	32.6	0.030*	39	29.5	14	26.9	0.649
		Ala/Val	199	53.9	80	44.2		137	57.8	57	44.2		62	47.0	23	44.2	
		Val/Val	79	21.4	46	25.4		48	20.3	30	23.3		31	23.5	16	30.8	
		Ala/Ala	91	24.7	56	30.9	0.152	52	21.9	42	32.6	0.033 *	39	29.5	14	26.9	0.722
		Val/Val + Ala/Val	278	75.3	126	69.6		185	78.1	87	67.4		93	70.5	39	75.0	
		Ala/Ala + Ala/Val	290	78.6	136	75.1	0.331	189	79.7	99	76.7	0.507	101	76.5	37	71.2	0.355
		Val/Val	79	21.4	46	25.4		48	20.3	30	23.3		31	23.5	16	30.8	
	Allele	Ala	381	51.6	192	53.0	0.749	241	50.8	141	54.7	0.353	140	53.0	51	49.0	0.422
		Val	357	48.4	172	47.5		233	49.2	117	45.3		124	47.0	55	52.9	

P-values were calculated by Fisher's exact test. * $P < 0.05$

diabetes mellitus, and total and HDL cholesterol were associated with hyperlipidemia. Therefore, we selected uric acid, history of hyperlipidemia, hypertension, and diabetes mellitus as the confounding factors to be examined in the multiple regression analyses. These subsequent analyses revealed that the frequency of Ala/Ala was significantly higher than that of Val/Val ± Ala/Val in obese men ($P = 0.015$; odds ratio = 0.523; 95% confidence interval, CI = 0.310–0.882). The Ala/Ala genotype was determined to be an independent risk factor for obesity that was separate from the other confounding factors, including diabetes mellitus.

Table 3 shows the LD analysis for the three SNPs. The fact that all values of D' were >0.5 indicates that all SNPs were located in a haplotype block.

The results of the haplotype-based case–control study are shown in Table 4. Although the common haplotypes C–C–C, T–T–T, and C–T–T were not significantly different, the haplotypes C–T–C and C–C–T were significantly different between obese and non-obese in the combined total group (Table 4). The overall distributions of the haplotype (UCP3 rs2075577–UCP2 rs660339) differed significantly in women ($P = 0.042$). The frequency of the haplotype C–T in the obese patients was significantly higher than that seen in the non-obese subjects.

Discussion

Our study suggests that Japanese men having the Ala/Ala genotype of UCP2 Ala55Val are prone to obesity. Many association studies have examined the role of UCP2 in body weight homoeostasis using genetic variants such as SNPs. Ala55Val in the UCP2 gene is one of the most commonly studied variants in previous reports, as it is a nonsynonymous variant located in an exon region (exon 4). Furthermore, the heterozygosity rate is very high, with the rate reported to be 0.578. Urhammer et al. [31] first discovered Ala55Val by single-strand conformation polymorphism and direct sequencing. To date, this variant has been reported to be associated with the sleeping metabolic rate [17] and exercise efficiency while bicycling [32]. There have also been some positive association studies between UCP2 Ala55Val and obesity.

Recently, Wang et al. [22] reported that Val55 increased the risk of being overweight and becoming obese as compared with Ala55. It was also reported that the fasting insulin levels in subjects with the Val55 homozygosity were higher than those in subjects without the Val55 homozygosity in a Paiwan aboriginal obese/overweight group. Furthermore, Astrup et al. [26] reported that 24-h energy expenditure, adjusted for fat-free mass, fat mass and spontaneous physical activity, was lower in the Val/Val homozygotes than in the Ala/Ala and Ala/Val genotypes. Val/Val had an approximately 20% higher 24-h spontaneous physical activity, particularly at night. Energy expenditure due to higher spontaneous physical activity counteracted the Val/Val group's lower 24-h resting energy expenditure for a given body size and composition. In addition, the 24-h respiratory quotient adjusted for energy balance, age, sex, and spontaneous physical activity, was higher in the Val/Val homozygotes than in the Ala/Ala and Ala/Val groups. Therefore, subjects with the UCP2 Val/Val genotype exhibit enhanced metabolic efficiency and a lower fat oxidation than the Ala/Ala and Ala/Val genotypes. In the CARDIA study, it was demonstrated that the Val/Val genotypes were more likely to be diagnosed with diabetes mellitus. The relative risk of diabetes for individuals with the Val/Val genotype when compared with those having the Ala/Ala or Ala/Val genotypes was similar for consistently thin persons and for those with central obesity [28].

On the other hand, most studies have reported negative results for BMI and diabetes mellitus with Ala55Val, regardless of ethnicity [33]. Chen et al. [34] recently reported that morbidly obese patients with either Ala/Val or Val/Val genotype experienced greater weight loss when compared with patients with the Ala/Ala genotype after laparoscopic adjustable gastric banding. The current study is the first one to examine the association between UCP2 Ala55Val and obesity in Japanese subjects, and it revealed that subjects with the Ala/Ala genotypes were prone to obesity. Thus, these results might also indicate that development of obesity could be related to differences in ethnicity, heterozygosity rate, etc. Walder et al. [17] reported that Ala55Val heterozygotes had higher metabolic rates during sleep than homozygotes among the Pima Indians. Also, when individuals >45 years of age were considered, heterozygotes were found to have the lowest BMI.

In normal energy metabolism, oxidative phosphorylation in mitochondria captures energy from the resulting proton gradient, leading to an efficient formation of ATP. UCPs diminish the proton gradient, leading to less ATP

Table 3 Pairwise LD ($|D'|$ above diagonal) for the three SNPs

SNP	$\|D'\|$		
	UCP3 rs1800849	UCP3 rs2075577	UCP2 rs660339
UCP3 rs1800849		*0.953*	*0.757*
UCP3 rs2075577			*0.955*
UCP2 rs660339			

Italicized values are $|D'| > 0.5$

Table 4 Haplotype-based case–control study in obese and non-obese subjects

				Overall χ^2			Overall *P*-value			Frequency in total		χ^2	*P*-value	Frequency in men		χ^2	*P*-value	Frequency in women		χ^2	*P*-value
				Total	Man	Women	Total	Man	Women	Non-obese	Obese			Non-obese	Obese			Non-obese	Obese		
Haplotypes	rs1800849	rs2075577		0.541	3.508	3.000	0.763	0.320	0.392												
	UCP3 5' C > T	UCP3 Tyr210Tyr																			
H1	Mj C	Mn C								0.477	0.492	0.221	0.638	0.470	0.508	0.935	0.334	0.481	0.449	0.248	0.619
H2	Mn T	Mj T								0.314	0.292	0.541	0.462	0.305	0.283	0.391	0.532	0.319	0.305	0.046	0.831
H3	Mj C	Mj T								0.210	0.217	0.065	0.799	0.215	0.209	0.022	0.883	0.200	0.234	0.426	0.514
H4	Mn T	Mn C												0.011	0.000	2.752	0.097	0.000	0.012	2.507	0.113
Haplotypes	rs1800849		rs660339	2.427	4.482	0.748	0.489	0.214	0.862												
	UCP3 5' C > T		UCP2 Ala55Val																		
H1	Mj C		Mj C							0.482	0.482	0.006	0.940	0.478	0.493	0.160	0.689	0.489	0.453	0.403	0.526
H2	Mn T		Mn T							0.281	0.245	1.481	0.224	0.284	0.230	2.702	0.100	0.276	0.280	0.002	0.964
H3	Mj C		Mn T							0.203	0.225	0.616	0.433	0.208	0.224	0.325	0.569	0.193	0.230	0.650	0.420
H4	Mn T		Mj C							0.035	0.048	0.890	0.346	0.031	0.053	2.246	0.134	0.042	0.037	0.020	0.889
Haplotypes		rs2075577	rs660339	10.674	4.205	8.180	0.014	0.240	0.042												
		UCP3 Tyr210Tyr	UCP2 Ala55Val																		
H1		Mn C	Mj C							0.478	0.477	0.003	0.996	0.470	0.508	0.932	0.334	0.485	0.423	1.092	0.296
H2		Mj T	Mn T							0.482	0.458	0.523	0.470	0.483	0.445	0.966	0.326	0.469	0.489	0.137	0.711
H3		Mj T	Mj C							0.040	0.050	0.504	0.478	0.037	0.047	0.543	0.461	0.046	0.058	0.176	0.675
H4		Mn C	Mn T							0.000	0.014	9.964	0.001	0.011	0.000	2.709	0.100	0.000	0.030	7.419	0.006
Haplotypes	rs1800849	rs2075577	rs660339	14.998	7.048	10.540	0.010	0.133	0.061												
	UCP3 5' C > T	UCP3 Tyr210Tyr	UCP2 Ala55Val																		
H1	Mj C	Mn C	Mj C							0.475	0.480	0.029	0.866	0.472	0.508	0.859	0.354	0.481	0.430	0.706	0.401
H2	Mn T	Mj T	Mn T							0.285	0.249	1.460	0.227	0.289	0.242	1.749	0.186	0.277	0.270	0.003	0.957
H3	Mj C	Mj T	Mn T							0.200	0.214	0.277	0.599	0.202	0.206	0.011	0.917	0.199	0.219	0.122	0.727
H4	Mn T	Mj T	Mj C							0.029	0.046	1.740	0.187	0.023	0.044	2.087	0.149	0.043	0.044	0.026	0.873
H5	Mj C	Mj T	Mj C							0.011	0.000	4.008	0.045	0.014	0.000	3.329	0.068	0.000	0.016	5.048	0.025
H6	Mj C	Mn C	Mn T							0.000	0.012	8.075	0.004	–	–	–	–	0.000	0.021	5.048	0.025

Haplotypes with frequency >0.01 were estimated using SNPAlyze software

P-values were calculated by χ^2 analysis. * $P < 0.05$

formation and thus, a smaller release of energy as heat [35, 36]. Uncoupling serves an important physiologic function by dissipating excess energy in the presence of a positive energy balance. In humans, the extent of uncoupling is partially regulated by the genetic polymorphism Ala55Val, in which the Val/Val genotype uncouples at a lower rate than the Ala/Ala genotype [28, 35]. Until now, there have been no reports that have demonstrated that the Ala55Val variant, which is located in exon 4 and which causes an amino acid change, affects the function of the UCP2 protein. However, our results for the variant are in contrast to previous reports, and thus, might suggest that the variant can act as a non-functional marker linked to a possible functional mutation.

The human UCP2 and UCP3 genes are located in tandem on 11q13. Thus, any study involving markers linked to this locus would test the hypothesis that either one or both genes can control a trait. Several studies have specifically examined linkage around the UCP2/3 gene locus. Bouchad et al. [18] reported positive evidence for a linkage with the RMR, while Wang et al. [22] reported that there was evidence for linkage to BMI in Paiwan aboriginal subjects. However, several other studies have reported that there was no evidence for linkage to BMI or any other measures of obesity (although none of the studies measured the RMR) [33]. Recently, Cha et al. [12] reported that Ala55Val in UCP2 and Tyr210Tyr in UCP3 were associated with decreased HDL cholesterol and an increased atherogenic index in Korean women. However, there was no association between these SNPs, HDL cholesterol, and the atherogenic index in our study (data not shown).

In this study, there were gender differences. The body weight control system could have a wider margin for environmental adaptation in female rodents [37]. In most rodent studies on obesity or on BAT and muscle UCPs, only male animals have been considered. Upon chronic cafeteria diet feeding, female rats have been shown to attain a larger excess of body weight than males, and their BAT, although hypertrophied, showed no signs of increased thermogenic potential per gram of tissue. This is the opposite of what happens in males, which have been found to exhibit BAT hypertrophy plus an increased BAT thermogenic capacity, including an enhanced expression of UCP1 and UCP2 [38].

In conclusion, this is the first study that has attempted to perform a haplotype-based case–control study using these three SNPs. Our results indicate that the nonsynonymous rs660339 in the human UCP2 gene might be a good obesity marker in men. Furthermore, the haplotype (UCP3 rs180049–UCP2 rs660339) might also be a good obesity marker in women. Further studies with other polymorphisms or isolated groups are needed in various populations in order to be able to determine whether there is an association between UCP2 gene and obesity.

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References

1. P. Kovacs, L. Ma, R.L. Hanson, P. Franks, M. Stumvoll, C. Bogardus, L.J. Baier, Genetic variation in UCP2 (uncoupling protein-2) is associated with energy metabolism in Pima Indians. Diabetologia **48**, 2292–2295 (2005)
2. O. Boss, T. Hagen, B.B. Lowell, Uncoupling proteins 2 and 3: potential regulators of mitochondrial energy metabolism. Diabetes **49**, 143–156 (2000)
3. T. Pawade, P.W. Ho, K.H. Kwok, A.C. Chu, S.L. Ho, D.B. Ramsden, Uncoupling proteins: targets of endocrine disruptors? Mol. Cell Endocrinol. **244**, 79–86 (2005)
4. D.G. Nicholls, A history of UCP1. Biochem. Soc. Trans. **29**(Pt. 6), 751–755 (2001)
5. C. Fleury, M. Neverova, S. Collins, S. Raimbault, O. Champigny, C. Levi Meyrueis, F. Bouillaud, M.F. Seldin, R.S. Surwit, D. Ricquier, C.H. Warden, Uncoupling protein–2: a novel gene linked to obesity and hyperinsulinemia. Nat. Genet. **15**, 269–272 (1997)
6. C.B. Chan, Endogenous regulation of insulin secretion by UCP2. Clin. Lab. **48**, 599–604 (2002)
7. P. Schrauwen, Skeletal muscle uncoupling protein 3 (UCP3) mitochondrial uncoupling protein in search of a function. Curr Opin Clin Nutr Metab Care **5**, 265–270 (2002)
8. D. Langin, D. Larrouy, P. Barbe, L. Millet, N. Viguerie Bascands, F. Andreelli, M. Laville, H. Vidal, Uncoupling protein-2 (UCP2) and uncoupling protein-3 (UCP3) expression in adipose tissue and skeletal muscle in humans. Int. J. Obes. Relat. Metab. Disord. **23**(Suppl. 6), S64–S67 (1999)
9. N. Tsuboyama Kasaoka, O. Ezaki, Mitochondrial uncoupling protein 3 (UCP3) in skeletal muscle. Front. Biosci. **6**, D570–D574 (2001)
10. W. Mao, X.X. Yu, A. Zhong, W. Li, J. Brush, S.W. Sherwood, S.H. Adams, G. Pan, UCP4, a novel brain-specific mitochondrial protein that reduces membrane potential in mammalian cells. FEBS Lett. **443**, 326–330 (1999)
11. X.X. Yu, W. Mao, A. Zhong, P. Schow, J. Brush, S.W. Sherwood, S.H. Adams, G. Pan, Characterization of novel UCP5/BMCP1 isoforms and differential regulation of UCP4 and UCP5 expression through dietary or temperature manipulation. FASEB J. **14**, 1611–1618 (2000)
12. M.H. Cha, I.C. Kim, K.S. Kim, B.K. Kang, S.M. Choi, Y. Yoon, Association of UCP2 and UCP3 gene polymorphisms with serum high-density lipoprotein cholesterol among Korean women. Metabolism **56**, 806–813 (2007)
13. D. Arsenijevic, H. Onuma, C. Pecqueur, S. Raimbault, B.S. Manning, B. Miroux, E. Couplan, M.C. Alves Guerra, M. Goubern, R. Surwit, F. Bouillaud, D. Richard, S. Collins, D. Ricquier, Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nat. Genet. **26**, 435–439 (2000)
14. C.B. Chan, M.C. Saleh, V. Koshkin, M.B. Wheeler, Uncoupling protein 2 and islet function. Diabetes **53**(Suppl. 1), S136–S142 (2004)
15. D.R. Gable, J.W. Stephens, J.A. Cooper, G.J. Miller, S.E. Humphries, Variation in the UCP2-UCP3 gene cluster predicts the development of type 2 diabetes in healthy middle-aged men. Diabetes **55**, 1504–1511 (2006)

16. D.W. Gong, Y. He, M.L. Reitman, Genomic organization and regulation by dietary fat of the uncoupling protein 3 and 2 genes. Biochem. Biophys. Res. Commun. **256**, 27–32 (1999)
17. K. Walder, R.A. Norman, R.L. Hanson, P. Schrauwen, M. Neverova, C.P. Jenkinson, J. Easlick, C.H. Warden, C. Pecqueur, S. Raimbault, D. Ricquier, M.H. Silver, A.R. Shuldiner, G. Solanes, B.B. Lowell, W.K. Chung, R.L. Leibel, R. Pratley, E. Ravussin, Association between uncoupling protein polymorphisms (UCP2-UCP3) and energy metabolism/obesity in Pima indians. Hum. Mol. Genet. **7**, 1431–1435 (1998)
18. C. Bouchard, L. Perusse, Y.C. Chagnon, C. Warden, D. Ricquier, Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans. Hum. Mol. Genet. **6**, 1887–1889 (1997)
19. R.E. Pratley, D.B. Thompson, M. Prochazka, L. Baier, D. Mott, E. Ravussin, H. Sakul, M.G. Ehm, D.K. Burns, T. Foroud, W.T. Garvey, R.L. Hanson, W.C. Knowler, P.H. Bennett, C. Bogardus, An autosomal genomic scan for loci linked to prediabetic phenotypes in Pima Indians. J. Clin. Invest. **101**, 1757–1764 (1998)
20. G. Argyropoulos, A.M. Brown, S.M. Willi, J. Zhu, Y. He, M. Reitman, S.M. Gevao, I. Spruill, W.T. Garvey, Effects of mutations in the human uncoupling protein 3 gene on the respiratory quotient and fat oxidation in severe obesity and type 2 diabetes. J. Clin. Invest. **102**, 1345–1351 (1998)
21. G. Solanes, A. Vidal Puig, D. Grujic, J.S. Flier, B.B. Lowell, The human uncoupling protein-3 gene. Genomic structure, chromosomal localization, and genetic basis for short and long form transcripts. J. Biol. Chem. **272**, 25433–25436 (1997)
22. T.N. Wang, M.C. Huang, H.L. Lin, C.H. Hsiang, A.M. Ko, W.T. Chang, Y.C. Ko, UCP2 A55V variant is associated with obesity and related phenotypes in an aboriginal community in Taiwan. Int. J. Obes. (Lond) **31**, 1746–1752 (2007) (Epub 2007)
23. H. Esterbauer, C. Schneitler, H. Oberkofler, C. Ebenbichler, B. Paulweber, F. Sandhofer, G. Ladurner, E. Hell, A.D. Strosberg, J.R. Patsch, F. Krempler, W. Patsch, A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. Nat. Genet. **28**, 178–183 (2001)
24. L.T. Dalgaard, O. Pedersen, Uncoupling proteins: functional characteristics and role in the pathogenesis of obesity and type II diabetes. Diabetologia **44**, 946–965 (2001)
25. R. Xu, K. Ishikawa, T. Katsuya, Y. Fu, H. Akasaka, M. Ikushima, Y. Chihara, N. Ura, K. Shimamoto, H. Rakugi, T. Ogihara, Association between senescence-related uncoupling protein 2 gene polymorphisms and abdominal obesity in Japanese subjects: The Tanno and Sobetsu study. Geriatr. Gerontol. Int. **6**, 15–19 (2006)
26. A. Astrup, S. Toubro, L.T. Dalgaard, S.A. Urhammer, T.I. Sorensen, O. Pedersen, Impact of the v/v 55 polymorphism of the uncoupling protein 2 gene on 24-h energy expenditure and substrate oxidation. Int. J. Obes. Relat. Metab. Disord. **23**, 1030–1034 (1999)
27. M. Klannemark, M. Orho, L. Groop, No relationship between identified variants in the uncoupling protein 2 gene and energy expenditure. Eur. J. Endocrinol. **139**, 217–223 (1998)
28. X. Yu, D.R.J. Jacobs, P.J. Schreiner, M.D. Gross, M.W. Steffes, M. Fornage, The uncoupling protein 2 Ala55Val polymorphism is associated with diabetes mellitus: the CARDIA study. Clin. Chem. **51**, 1451–1456 (2005)
29. K.A. Rance, A.M. Johnstone, S. Murison, J.S. Duncan, S.G. Wood, J.R. Speakman, Plasma leptin levels are related to body composition, sex, insulin levels and the A55V polymorphism of the UCP2 gene. Int. J. Obes. (Lond) **31**, 1311–1138 (2007)
30. N. Aoi, T. Nakayama, Y. Tahira, A. Haketa, M. Yabuki, T. Sekiyama, C. Nakane, H. Mano, H. Kawachi, N. Sato, M. Soma, K. Matsumoto, Two novel genotypes of the thiazide-sensitive Na–Cl cotransporter (SLC12A3) gene in patients with Gitelman's syndrome. Endocrine **31**, 149–153 (2007)
31. S.A. Urhammer, L.T. Dalgaard, T.I. Sorensen, A.M. Moller, T. Andersen, A. Tybjaerg Hansen, T. Hansen, J.O. Clausen, H. Vestergaard, O. Pedersen, Mutational analysis of the coding region of the uncoupling protein 2 gene in obese NIDDM patients: impact of a common amino acid polymorphism on juvenile and maturity onset forms of obesity and insulin resistance. Diabetologia **40**, 1227–1230 (1997)
32. B. Buemann, B. Schierning, S. Toubro, B.M. Bibby, T. Sorensen, L. Dalgaard, O. Pedersen, A. Astrup, The association between the val/ala–55 polymorphism of the uncoupling protein 2 gene and exercise efficiency. Int. J. Obes. Relat. Metab. Disord. **25**, 467–471 (2001)
33. N.A. Schonfeld Warden, C.H. Warden, Physiological effects of variants in human uncoupling proteins: UCP2 influences body-mass index. Biochem. Soc. Trans. **29**(Pt. 6), 777–784 (2001)
34. H.H. Chen, W.J. Lee, W. Wang, M.T. Huang, Y.C. Lee, W.H. Pan, Ala55Val polymorphism on UCP2 gene predicts greater weight loss in morbidly obese patients undergoing gastric banding. Obes. Surg. **17**, 926–933 (2007)
35. S. Enerback, A. Jacobsson, E.M. Simpson, C. Guerra, H. Yamashita, M.E. Harper, L.P. Kozak, Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature **387**, 90–94 (1997)
36. R.E. Gimeno, M. Dembski, X. Weng, N. Deng, A.W. Shyjan, C.J. Gimeno, F. Iris, S.J. Ellis, E.A. Woolf, L.A. Tartaglia, Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. Diabetes **46**, 900–906 (1997)
37. A.M. Rodriguez, A. Palou, Uncoupling proteins: gender dependence and their relation to body weight control. Int. J. Obes. **28**, 500–502 (2004)
38. P. Roca, A.M. Rodriguez, P. Oliver, M.L. Bonet, S. Quevedo, C. Pico, A. Palou, Brown adipose tissue response to cafeteria diet-feeding involves induction of the UCP2 gene and is impaired in female rats as compared to males. Pflugers Arch. **438**, 628–634 (1999)