Uncoupling protein 2 gene (*UCP2*) 45-bp I/D polymorphism is associated with adiposity among Malaysian women

Yee-How Say[1,*], Zi-Lian Ban[1], Yogambigai Arumugam[1], Trishal Kaur[1], Mee-Lay Tan[1], Phee-Phee Chia[2] and Sook-Ha Fan[1]

[1]*Department of Biomedical Science, Faculty of Science, and* [2]*Department of Science and Engineering, Centre for Foundation Studies, Universiti Tunku Abdul Rahman (UTAR) Perak Campus, Kampar, Perak, Malaysia*

**Corresponding author (Fax, +605-4661676; Email, sayyh@utar.edu.my)*

This study investigated the association of Uncoupling Protein 2 gene (*UCP2*) 45-bp I/D polymorphism with obesity and adiposity in 926 Malaysian subjects (416 males; 265 obese; 102/672/152 Malays/Chinese/Indians). The overall minor allele frequency (MAF) was 0.14, while MAFs according to Malay/Chinese/Indian were 0.17/0.12/0.21. The polymorphism was associated with ethnicity, obesity and overall adiposity (total body fat percentage, TBF), but not gender and central adiposity (waist–hip ratio, WHR). Gender- and ethnicity-stratified analysis revealed that within males, the polymorphism was not associated with ethnicity and anthropometric classes. However, within females, significantly more Indians, obese and those with high TBF carried I allele. Logistic regression analysis among females further showed the polymorphism was associated with obesity and overall adiposity; however, when adjusted for age and ethnicity, this association was abolished for obesity but remained significant for overall adiposity [Odds Ratio (OR) for ID genotype =2.02 (CI=1.18, 3.45; p=0.01); I allele =1.81 (CI=1.15, 2.84; p=0.01)]. Indeed, covariate analysis controlling for age and ethnicity also showed that those carrying ID genotype or I allele had significantly higher TBF than the rest. In conclusion, *UCP2* 45-bp I/D polymorphism is associated with overall adiposity among Malaysian women.

[Say Y-H, Ban Z-L, Arumugam Y, Kaur T, Tan M-L, Chia P-P and Fan S-H 2014 Uncoupling protein 2 gene (*UCP2*) 45-bp I/D polymorphism is associated with adiposity among Malaysian women. *J. Biosci.* **39** 867–875] **DOI** 10.1007/s12038-014-9488-y

1. Introduction

The prevalence of obesity is rising at an alarming rate worldwide, including Malaysia, where its 4th National Health and Morbidity Survey (NHMS IV) in 2011 reported the prevalence of overweight and obesity at 29.4% and 15.1%, respectively (Institute of Public Health Malaysia, 2011). Obesity is a multifactorial disorder which involves interplay between genetic and environmental factors (Kopelman 2000). The current obesity pandemic is the result of an obesogenic environment with energy-dense foods and lack of physical activity in individuals who have a genetic susceptibility towards developing obesity (Hurt *et al.* 2011). There are more than 120 candidate genes which have been linked with obesity-related phenotypes in the 2005 version of the human obesity gene map (Rankinen *et al.* 2006), and the uncoupling protein (UCPs) genes are a family of them.

UCPs are approximately 32 kDa proteins that belong to a family of mitochondrial carrier proteins present in the inner mitochondrial membrane (Yonezawa *et al.* 2009). UCPs dissipate the proton gradient by allowing the re-entry of protons into the mitochondrial matrix during oxidative ATP generation, resulting in the uncoupling of the respiratory chain and heat production (Aquila *et al.* 1985). The human *UCP2* gene is mapped to chromosome 11q13 and the protein has the widest tissue expression of all UCPs – in white adipose tissue, skeletal muscle, spleen, lung, pancreatic β-cells, and isolated macrophages (Fleury *et al.* 1997;

Keywords. Adiposity; genetic polymorphism; Malaysia; obesity; uncoupling protein 2

Published online: 20 October 2014

Gimeno *et al.* 1997; Arsenijevic *et al.* 2000). UCP2 plays a role in regulating ATP synthesis, generation of reactive oxygen species and glucose-stimulated insulin secretion in β-cells (Ricquier and Bouillaud, 2000).

Several gene polymorphisms of *UCP2* have been reported in human studies and the association between *UCP2* locus and vulnerability for obesity and type 2 diabetes have been investigated with specific attention being paid to -866G/A (rs659336) polymorphism in the promoter region; Ala55Val (C/T; rs660339) polymorphism in exon 4 and 45-bp insertion/deletion (I/D variant in the 3′-untranslated region of exon 8 (reviewed in Brondani *et al.* 2014). These three *UCP2* common polymorphisms have been variably associated with altered body mass index (BMI), changes in energy expenditure, and maintenance of body weight after overfeeding (Dalgaard and Pederson, 2001; Esterbauer *et al.* 2001). Previous studies showed that carriers of *UCP2* 45-bp I allele had significantly higher BMI and risk of developing obesity compared to D allele (Cassell *et al.* 1999; Evans *et al.* 2000; Marti *et al.* 2004; Lee *et al.* 2008; Brondani et al. 2014).

We previously performed a pilot study investigating the association of *UCP2* 45-bp I/D polymorphism with overweight among university students who were majority of the Chinese ethnicity (n =256), and found no association (Yiew *et al.* 2010). Due to the limitations of the study (small sample size, lack of other ethnic groups, lack of obese subjects), we expanded the study to a larger and more age- and ethnically-diverse sample population, in multiple cohorts of urban (Klang Valley) and sub-urban (Kampar, Perak) dwellings in Malaysia. Therefore, this study aimed to determine the prevalence of the *UCP2* 45-bp I/D polymorphism and its possible association with obesity (assessed by BMI), overall adiposity (assessed by total body fat percentage, TBF) and central adiposity (assessed by waist-to-hip ratio, WHR) in a representative sample of the multi-ethnic Malaysian population.

2. Materials and methods

2.1 *Subjects*

A convenience sampling method was adopted for this study. Questionnaire and sample collection was carried out among unrelated and non-overlapping 926 subjects comprising four cohorts: (1) 256 students of Universiti Tunku Abdul Rahman (UTAR) and Kolej Tunku Abdul Rahman (KTAR) at their Setapak, Kuala Lumpur campuses were recruited from Feb – Apr 2009, as described in our previous study (Yiew et al. 2010); (2) 180 UTAR Setapak Campus students and residents of Setapak and Petaling Jaya were recruited from Oct 2009 – Feb 2010 [83 M/97 F; 43 Malays(Y)/84 ethnic Chinese(C)/53 ethnic Indians (I); mean age 26.27 ±11.93 years]; (3) 194 patrons of the Kampar Health Clinic were recruited from June – Dec 2011 (75 M/119 F; 59 Y/97 C/38 I; mean age 54.29 ±13.61 years); (4) 296 UTAR Perak Campus students in Kampar were recruited from Jan – Apr 2013 (118 M/178 F; 250 C/46 I; mean age 21.34 ±1.57 years). The ethnicities of the subjects were self-identified. All subjects were pooled together for data analysis. This study has received ethical approvals either from the UTAR Scientific and Ethical Review Committee (SERC) or the Medical Research and Ethnics Committee, Ministry of Health Malaysia (NMRR-09-826-4266). All subjects signed informed consent forms, and the study was conducted in accordance with the Declaration of Helsinki (amended in Brazil, 2013).

2.2 *Questionnaire and anthropometric measurements*

Clinical and anthropometric measurements namely systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse rate, waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), weight, height, body mass index (BMI) and total body fat (TBF) were measured as described in our previous studies (Yiew *et al.* 2010; Chan *et al.* 2011). The cut-off points for obesity, overall adiposity (TBF) and central adiposity (WHR) were ≥25 kg/m^2 (WHO/IOTF/IASO, 2000), 20% (males) or 30% (females) (Omron, n.d.) and 0.90 (males) or 0.85 (females)(WHO, 2011), respectively.

2.3 *DNA extraction and genotyping*

The DNA extraction from mouthwash samples and Polymerase Chain Reaction- restriction fragment length polymorphism (PCR-RFLP) for genotyping of the *UCP2* 45-bp ID polymorphism was carried out according to our previous study (Yiew *et al.* 2010).

2.4 *Statistical analysis*

The IBM SPSS Statistics software was used to analyze the data of the study. Allelic frequencies were estimated by gene counting and the distribution of genotypes was tested for Hardy-Weinberg equilibrium using the Chi-square (χ^2) test. Data for continuous variables were presented as means ± standard deviations (SD) or adjusted means ± standard error of the mean (SEM) and as frequency for categorical variables. The normality of distributions of continuous variables was tested with the Kolmogorov-Smirnov test and variables that were not distributed normally were log-transformed prior to statistical analysis. Genotype and allele frequencies of the polymorphism were assessed for association with demographic and anthropometric classes using Pearson's χ^2 test in both overall and stratified analysis based on gender

and ethnicity. Logistic regression analysis (enter method) was performed with adjustment to age and ethnicity to evaluate if the polymorphism could predict the risk of obesity and increased overall adiposity. Analysis of covariance using the univariate General Linear Model with adjustment for covariates (age and ethnicity) was performed for anthropometric measurements and blood pressures. A p-value of less than 0.05 was considered as statistically significant.

3. Results

Table 1 shows the demographic and anthropometric characteristics of the subjects. More than half of the subjects were females, where the distributions of subjects under different BMI and WHR classes were not significantly different among genders. However, there were more females from all the ethnicities, age groups and TBF classes ($p<0.05$).

The distributions of all *UCP2* 45-bp I/D genotypes and alleles according to ethnicities and genders are as shown in table 2, which did not deviate from the Hardy-Weinberg equilibrium. The overall minor allele frequency (MAF) for *UCP2* 45-bp I/D was 0.14, while according to Malay/Chinese/Indian and non-obese/obese categories, their MAFs were 0.17/0.12/0.21 and 0.13/0.17, respectively. The allele distribution of *UCP2* 45-bp I/D was significantly associated with ethnicity ($p<0.001$) – MAF of Indians being significantly higher. The genotype and allele distribution was also significantly associated with obesity status and overall adiposity (TBF) status, but not central adiposity (WHR) status. The allele distribution of the polymorphism was also not significantly different among gender ($p=0.35$).

Table 1. Demographic and anthropometric characteristics of the subjects according to gender

Variables	Male (n =416)	Female (n =510)
Ethnicity		
Malay	34 (8.2)	68 (13.3)
Chinese	320 (76.9)	352 (69.0)
Indian	62 (14.9)	90 (17.6)
Age group		
≤30	339 (81.5)	371 (72.7)
31-45	8 (1.9)	35 (6.9)
46-60	33 (7.9)	71 (13.9)
>60	36 (8.7)	33 (6.5)
BMI Class		
Non-obese	285 (68.5)	376 (73.7)
Obese	131 (31.5)	134 (26.3)
WHR Class		
Normal	208 (50.0)	254 (49.8)
High	208 (50.0)	256 (50.2)
TBF Class		
Normal	231 (55.5)	316 (62.0)
High	185 (45.5)	194 (38.0)

Parentheses indicate percentage within the same gender.

Due to the high heterogeneity of the sampled subjects, their allele frequencies were also analysed separately by Pearson's χ^2 test based on gender and ethnicity (Table 3). Within males, the genotype and allele distribution was not associated with ethnicity and anthropometric classes. However, within females, significantly more Indians, obese and those with high TBF (all $p<0.001$) carried the I allele, indicating that the *UCP2* 45-bp I/D polymorphism was significantly associated with ethnicity, BMI and TBF. In ethnically-stratified analysis, only TBF class was significantly associated with the *UCP2* allele among Malays ($p=0.03$), and with the *UCP2* genotype among Chinese ($p=0.02$). Meanwhile, a further stratified analysis based on both gender and ethnicity (male – Malay, male – Chinese, female – Malay, etc.) revealed that only TBF was significantly associated with *UCP2* allele distribution among female Chinese subjects only ($\chi^2=7.57$, $p=0.01$; data not shown). These results indicate that the female gender had the main effect in associating obesity and adiposity with the *UCP2* 45-bp I/D polymorphism and hence, analyses involving only females were conducted (tables 4 and 5).

Table 4 shows that females with ID genotype and I allele were 2.10 times and 1.87 times, respectively, more likely to be obese; and 2.34 times and 2.19 times, respectively, more likely to have high TBF/overall adiposity. However, when adjusted for age and ethnicity, this association of *UCP2* 45-bp I/D polymorphism with overall obesity was abolished ($p=0.10$) but not so for high adiposity ($p=0.01$). Indeed, covariate analysis of variance after controlling for age and ethnicity, also showed similar result, where females carrying ID genotype or I allele had significantly higher TBF than the rest (both $p=0.006$) (table 5). Interestingly, SBP, DBP and PR were also significantly different among the genotypes (all $p<0.001$), with DD, ID and II having the highest SBP, DBP and PR, respectively (table 5).

4. Discussion

In this study, the MAF of ethnic Chinese was quite similar to that of a previous Chinese study (Liu *et al.* 2012). As for ethnic Indians, their MAF was higher than other ethnic groups at 0.21; in agreement with 0.25 in a study among South Indians of Chennai, Tamil Nadu (Cassell *et al.* 1999). Indeed, majority of Malaysian Indians in this study comprised Tamils and Telugus with ancestries tracing back to the South Indian state (Teo *et al.* 2009). Therefore, the similar MAF found between both ethnic groups in Malaysia and those in China and India reflect the genetic ancestral origins

Table 2. Association of *UCP2* 45-bp I/D polymorphism genotype and allele distribution with demographic and anthropometric classes

Variables	Genotype			Allele	
	DD	ID	II	D	I
Gender					
Male	312 (75.0)	99 (23.8)	5 (1.2)	723 (86.9)	109 (13.1)
Female	374 (73.3)	123 (24.1)	13 (2.5)	871 (85.4)	149 (14.6)
χ^2; *p*	2.24; 0.33			0.87; 0.35	
Ethnicity					
Malay	70 (68.6)	29 (28.4)	3 (2.9)	169 (82.8)	35 (17.2)
Chinese	523 (77.8)	139 (20.7)	10 (1.5)	1185 (88.2)	159 (11.8)
Indian	93 (61.2)	54 (35.5)	5 (3.3)	240 (78.9)	64 (21.1)
χ^2; *p*	NP			19.58; <0.001*	
BMI Class					
Non-obese	505 (76.4)	145 (21.9)	11 (1.7)	1155 (87.4)	167 (12.6)
Obese	181 (68.3)	77 (29.1)	7 (2.6)	439 (82.8)	91 (17.2)
χ^2; *p*	6.60; 0.04*			6.50; 0.01*	
WHR Class					
Normal	353 (76.4)	100 (21.6)	9 (1.9)	806 (87.2)	118 (12.8)
High	333 (71.8)	122 (26.3)	9 (1.9)	788 (84.9)	140 (15.1)
χ^2; *p*	2.76; 0.25			2.07; 0.15	
TBF Class					
Normal	430 (78.6)	110 (20.1)	7 (1.3)	965 (88.2)	129 (11.8)
High	256 (67.5)	112 (29.6)	11 (2.9)	629 (83.0)	129 (17.0)
χ^2; *p*	15.06; 0.001*			10.20; 0.001*	

Parentheses indicate percentage within the same demographic/anthropometric class; NP = Chi-Square Test not performed due presence of cell having the count of less than 5; *p*-values by Pearson's Chi-Square Test; **p*-value significant at <0.05.

based on their migration history. With regard to Malays, observation cannot be made as no published data is available for comparison. The I/I genotype was detectable at the prevalence of 1.9% in this Malaysian population, whereas Tongan (Duarte *et al.* 2003) and French (Otabe *et al.* 1998) populations reported the absence of this genotype.

A recent meta-analysis to assess the effect of the three common *UCP2* polymorphisms – Ala55Val, 45-bp I/D, and -866G/A on the risk of overweight and obesity using in both Asian and European populations found that the *UCP2* I allele was associated with increased BMI in Asians (Brondani *et al*. 2014). In this study, we found that the polymorphism was associated with obesity in the overall Malaysian population. However, stratified analysis revealed that this significance was only found among females but was further abolished after controlling for age and ethnicity. Therefore, *UCP2* I/D polymorphism was not associated with obesity regardless of gender and Malay/Chinese/Indian ethnicity, similar with a previous Chinese study (Liu *et al.* 2012).

As BMI is just one phenotype of obesity, there is possibility that the *UCP2* I/D polymorphism could have different effects on other phenotypes, such as TBF, WC, WHR, subcutaneous fat and visceral fat. Indeed, the *UCP2* I/D polymorphism was associated with overall adiposity (assessed by TBF via bio-impedence), whether in the overall Malaysian population or in female-stratified analysis (even after controlling for age and ethnicity). Females carrying the ID genotype or I allele had almost two-fold risk of having higher overall adiposity, and I allele carriers had 2.05% higher TBF compared to D allele. However, central obesity (assessed by WHR) was not affected by *UCP2* I/D polymorphism across gender and ethnicities. Consistently, Yanowski *et al.* (2000) also found that TBF (assessed via skinfold thicknesses and dual-energy X-ray absorptiometry) was significantly associated and greater in ID subjects compared to II subjects, while WHR was not significantly different African American, white, and Asian ethnicities (Yanowski *et al.* 2000). Also, no association between WC and waist-to-thigh ratio with this polymorphism was found among Pima Indians (Walder *et al.* 1998).

The lack of association of this polymorphism with BMI but with TBF may be attributed to the low BMI and high TBF paradox among Asians (including Malays, Chinese and

Table 3. Stratified analysis of *UCP2* 45-bp I/D polymorphism genotype and allele distributions based on genders and ethnicities

Variables	Genotype			Allele	
	DD	ID	II	D	I
Males					
Ethnicity					
Malay	23 (67.6)	11 (32.4)	0	57 (83.8)	11 (16.2)
Chinese	247 (77.2)	69 (21.6)	4 (1.2)	563 (88.0)	77 (12.0)
Indian	42 (67.7)	19 (30.6)	1 (1.6)	103 (83.1)	21 (16.9)
χ^2; p	NP			2.81; 0.25	
BMI Class					
Non-obese	214 (75.1)	68 (23.9)	3 (1.1)	496 (87.0)	74 (13.0)
Obese	98 (74.8)	31 (23.7)	2 (1.5)	227 (86.6)	35 (13.4)
χ^2; p	NP			0.02; 0.88	
WHR Class					
Normal	160 (76.9)	46 (22.1)	2 (1.0)	366 (88.0)	50 (12.0)
High	152 (73.1)	53 (25.5)	3 (1.4)	357 (85.8)	59 (14.2)
χ^2; p	NP			0.86; 0.36	
TBF Class					
Normal	172 (74.5)	57 (24.7)	2 (0.9)	401 (86.8)	61 (13.2)
High	140 (75.7)	42 (22.7)	3 (1.6)	322 (87.0)	48 (13.0)
χ^2; p	NP			0.01; 0.92	
Females					
Ethnicity					
Malay	47 (69.1)	18 (26.5)	3 (4.4)	112 (82.4)	24 (17.6)
Chinese	276 (78.4)	70 (19.9)	6 (1.7)	622 (88.4)	82 (11.6)
Indian	51 (56.7)	35 (38.9)	4 (4.4)	137 (76.1)	43 (23.9)
χ^2; p	NP			18.38; <0.001*	
BMI Class					
Non-obese	291 (77.4)	77 (20.5)	8 (2.1)	659 (87.6)	93 (12.4)
Obese	83 (61.9)	46 (34.3)	5 (3.7)	212 (79.1)	56 (20.9)
χ^2; p	12.07; 0.002*			11.52; 0.001*	
WHR Class					
Normal	193 (76.0)	54 (21.3)	7 (2.8)	440 (86.6)	68 (13.4)
High	181 (70.7)	69 (27.0)	6 (2.3)	431 (84.2)	81 (15.8)
χ^2; p	2.28; 0.32			1.21; 0.27	
TBF Class					
Normal	253 (80.1)	58 (18.4)	5 (1.6)	564 (89.2)	68 (10.8)
High	121 (62.4)	65 (33.5)	8 (4.1)	307 (79.1)	81 (20.9)
χ^2; p	19.62; <0.001*			19.73; <0.001*	
Malay					
Gender					
Male	23 (67.6)	11 (32.4)	0	57 (83.8)	11 (16.2)
Female	47 (69.1)	18 (26.5)	3 (2.9)	112 (82.4)	24 (17.6)
χ^2; p	NP			0.07; 0.79	
BMI Class					
Non-obese	27 (79.4)	6 (17.6)	1 (2.9)	60 (88.2)	8 (11.8)
Obese	43 (63.2)	23 (33.8)	2 (2.9)	109 (80.1)	27 (19.9)
χ^2; p	NP			2.09; 0.15	

Table 3 (continued)

Variables	Genotype			Allele	
	DD	ID	II	D	I
WHR Class					
Normal	29 (74.4)	10 (25.6)	0	68 (87.2)	10 (12.8)
High	41 (65.1)	19 (30.2)	3 (4.8)	101 (80.2)	25 (19.8)
χ^2; p	NP			1.67; 0.20	
TBF Class					
Normal	17 (73.9)	6 (26.1)	0	29 (96.7)	1 (3.3)
High	53 (67.1)	23 (29.1)	3 (3.8)	140 (80.5)	34 (19.5)
χ^2; p	NP			4.73; 0.03*	
Chinese					
Gender					
Male	247 (77.2)	69 (21.6)	4 (1.2)	563 (88.0)	77 (12.0)
Female	276 (78.4)	70 (19.9)	6 (1.7)	622 (88.4)	82 (11.6)
χ^2; p	NP			0.05; 0.83	
BMI Class					
Non-obese	416 (78.2)	109 (20.5)	7 (1.3)	941 (88.4)	123 (11.6)
Obese	107 (76.4)	30 (21.4)	3 (2.1)	244 (87.1)	36 (12.9)
χ^2; p	NP			0.36; 0.55	
WHR Class					
Normal	290 (79.0)	70 (19.1)	7 (1.9)	650 (88.6)	84 (11.4)
High	233 (76.4)	69 (22.6)	3 (1.0)	535 (87.7)	75 (12.3)
χ^2; p	NP			0.23; 0.63	
TBF Class					
Normal	369 (80.9)	82 (18.0)	5 (1.1)	829 (88.8)	105 (11.2)
High	154 (71.3)	57 (26.4)	5 (2.3)	356 (86.8)	54 (13.2)
χ^2; p	8.21; 0.02*			1.02; 0.31	
Indian					
Gender					
Male	42 (67.7)	19 (30.6)	1 (1.6)	103 (83.1)	21 (16.9)
Female	51 (56.7)	35 (38.9)	4 (4.4)	137 (76.1)	43 (23.9)
χ^2; p	NP			2.14; 0.14	
BMI Class					
Non-obese	62 (65.3)	30 (31.6)	3 (3.2)	154 (81.1)	36 (18.9)
Obese	31 (54.4)	24 (42.1)	2 (3.5)	86 (75.4)	28 (24.6)
χ^2; p	NP			1.35; 0.25	
WHR Class					
Normal	34 (60.7)	20 (35.7)	2 (3.6)	88 (78.6)	24 (21.4)
High	59 (61.5)	34 (35.4)	3 (3.1)	152 (79.2)	40 (20.8)
χ^2; p	NP			0.02; 0.90	
TBF Class					
Normal	45 (67.2)	21 (31.3)	1 (1.5)	107 (82.3)	23 (17.7)
High	47 (56.0)	33 (39.3)	4 (4.8)	133 (76.4)	43 (23.6)
χ^2; p	NP			1.54; 0.21	

Parentheses indicate percentage within the same demographic/anthropometric class; NP – Chi-Square Test not performed due presence of cell having the count of less than 5; p-values by Pearson's Chi-Square Test; *p-value significant at <0.05.

Table 4. Logistic regression analysis for the association of *UCP2* 45-bp I/D polymorphism with obesity and adiposity status among females

	Unadjusted		Adjusted[§]	
Obesity category	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*
Obesity (based on BMI)				
DD	1.00		1.00	
ID	2.10 (1.35, 3.25)	0.001*	1.67 (0.97, 2.89)	0.07
II	2.19 (0.70, 6.88)	0.18	1.34 (0.34, 5.35)	0.68
D	1.00		1.00	
I	1.87 (1.30, 2.70)	0.001*	1.46 (0.93, 2.30)	0.10
Overall adiposity (based on TBF)				
DD	1.00		1.00	
ID	2.34 (1.55, 3.55)	< 0.001*	2.02 (1.18, 3.45)	0.01*
II	3.35 (1.07, 10.44)	0.04*	2.12 (0.51, 8.87)	0.30
D	1.00		1.00	
I	2.19 (1.54, 3.11)	< 0.001*	1.81 (1.15, 2.84)	0.01*

[§] Adjusted for co-variates: age and ethnicity; Values are by logistic regression enter method; **p*-value significant at <0.05.

Indians) compared to Caucasians; thus, obesity should be regarded as an excess of body fat and not as an excess of weight (increased BMI) (Deurenberg-Yap *et al.* 2000).

At present, there is scarce information about the effect of I/D polymorphism on UCP2 function, although its location in the 3′-untranslated region of *UCP2* exon 8 suggests potential involvement in mRNA processing or in the stability of the transcript. However, it has been reported that this polymorphism had no apparent effect on UCP2 mRNA levels in the skeletal muscles of Pima Indians (Walder *et al.* 1998), or in the adipose tissue of Caucasian, Asians, and African Americans (Wang *et al.* 2004). Furthermore, the ratio of I to D mRNA expression was highly variable in the adipose tissue of subjects heterozygous for 45-bp I/D (Esterbauer *et al.* 2001). The findings suggested an independent role for this polymorphism in mRNA stability, possibly

Table 5. Adjusted means of anthropometric measurements for different genotypes and alleles of *UCP2* 45-bp I/D polymorphism among females

	Genotype			Allele	
Variables	DD	ID	II	D	I
WHR	0.84±0.01	0.85±0.01	0.78±0.03	0.84±0.00	0.84±0.01
p	0.24			0.13	
BMI	23.48±0.65	23.76±1.15	23.64±3.51	23.52±0.43	23.73±1.04
p	0.16			0.09	
TBF	27.00±0.41	29.68±0.72	28.34±2.22	27.37±0.27	29.42±0.66
p	0.006*			0.006*	
SBP	118.80±2.43	117.21±4.25	115.37±13.00	118.57±1.58	116.91±3.84
p	< 0.001*			0.82	
DBP	71.23±0.52	72.85±0.91	70.72±2.79	71.46±0.34	72.47±0.83
p	< 0.001*			0.25	
PR	82.89±0.62	82.11±1.08	85.10±3.31	82.78±0.40	82.64±0.98
p	< 0.001*			0.97	

WHR: Waist-to-hip ratio; BMI: Body mass index; TBF: Total body fat; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; PR: Pulse rate; All values were log transformed before analysis by univariate analysis of variance (General Linear Model), adjusted for co-variates: age and ethnicity; Values are presented as adjusted mean± SEM (estimated marginal means± standard error of the mean); **p*-value significant at <0.05.

by just simply acting as a marker for another genetic variant in this region which affects adipogenesis and energy balance.

Limitations of the present study include the small sample size of Malay and Indian subjects, and the lack of other indigenous ethnic groups especially from East Malaysia (Sabah and Sarawak); hence the results from this study may not be fully representative of the general Malaysian population. The case–control design in this study also does not allow for a causality conclusion to be made. Also as only one polymorphism of *UCP2* was evaluated in this study, it is unclear whether other *UCP2* or other *UCP* families (*UCP1*, *UCP3*) polymorphisms in tight linkage disequilibrium with I/D polymorphism instead might have association with obesity. Therefore, population-based studies with a larger and more ethnically-diversed sample size and an investigation on the effects of environmental and lifestyle factors (like physical activity and dietary habits) are necessary in order to clarify the possible gene-environment interaction that causes inconsistent findings in different populations. *UCP2* mRNA and protein amounts in both adipose tissue and skeletal muscle of persons with the three possible genotypes could also be examined to test the direct functional effect of this polymorphism.

Acknowledgements

This project was supported by the Universiti Tunku Abdul Rahman Research Fund (IPSR/RMC/UTARRF/C109/S1, IPSR/RMC/UTARRF/C111/C35). We would like to extend our deepest gratitude to the Kampar District Health Office for granting us permission to carry out this study at the Kampar Health Clinic, the nurses who assisted with the blood sampling, and all the respondents who volunteered to participate in this study.

References

Aquila H, Link TA and Klingenberg M 1985 The uncoupling protein from brown fat mitochondria is related to the mitochondrial ADP/ATP carrier. Analysis of sequence homologies and of folding of the protein in the membrane. *EMBO J.* **4** 2369–2376

Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, Couplan E, Alves-Guerra MC, *et al*. 2000 Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nat. Genet.* **26** 435–439

Brondani LA, Assmann TS, de Souza BM, Bouças AP, Canani LH and Crispim D 2014 Meta-analysis reveals the association of common variants in the *uncoupling protein* (UCP) *1–3* genes with body mass index variability. *PLoS One* **9** e96411

Cassell PG, Neverova M, Janmohamed S, Uwakwe N, Qureshi A, McCarthy MI, Saker PJ, Albon L, *et al*. 1999 An uncoupling protein 2 gene variant is associated with a raised body mass index but not Type II diabetes. *Diabetologia* **42** 688–692

Chan PM, Fan SH and Say YH 2011 No association of Peptide Tyrosine-Tyrosine (PYY) gene variant R72T with obesity in the Kampar health clinic cohort, Malaysia. *Mal. J. Nutr.* **17** 201–212

Dalgaard LT and Pedersen O 2001 Uncoupling proteins: functional characteristics and role in the pathogenesis of obesity and Type II diabetes. *Diabetologica* **44** 946–965

Deurenberg-Yap M, Schmidt G, van Staveren WA and Deurenberg P 2000 The paradox of low body mass index and high body fat percentage among Chinese, Malays and Indians in Singapore. *Int. J. Obes. Relat. Metab. Disord.* **24** 1011–1017

Duarte NL, Colagiuri S, Palu T, Wang XL and Wilcken D 2003 A 45-bp insertion/deletion polymorphism of uncoupling protein 2 in relation to obesity in Tongans. *Obes. Res.* **11** 512–517

Esterbauer H, Schneitler C, Oberkofler H, Ebenbichler C, Paulweber B, Sandhofer F, Ladurner G, Hell E, *et al*. 2001 A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. *Nat. Genet.* **28** 178–183

Evans D, Minouchehr S, Hagemann G, Mann WA, Wendt D, Wolf A, Evans D, Minouchehr S, *et al*. 2000 Frequency of and interaction between polymorphisms in the beta3-adrenergic receptor and in uncoupling proteins 1 and 2 and obesity in Germans. *Int J Obes Relat Metab Disord.* **24** 1239–1245

Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillaud F, Seldin MF, *et al*. 1997 Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* **15** 269–272

Gimeno RE, Dembski M, Weng X, Deng N, Shyjan AW, Gimeno CJ, Iris F, Ellis SJ, *et al*. 1997 Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. *Diabetes* **46** 900–906

Hurt RT, Frazier TH, McClave SA and Kaplan LM 2011 Obesity epidemic: overview, pathophysiology, and the intensive care unit conundrum. *J. Parenter. Enter. Nutr.* **35** 4S–13S

Institute for Public Health Malaysia 2011 *The Fourth National Health and Morbidity Survey (NHMS III) 2011* (Kuala Lumpur: Ministry of Health)

Kopelman PG 2000 Obesity as a medical problem. *Nature* **404** 635–642

Lee YH, Kim W, Yu BC, Park BL, Kim LH and Shin HD 2008 Association of the ins/del polymorphisms of uncoupling protein 2 (UCP2) with BMI in a Korean population. *Biochem. Biophys. Res. Commun.* **371** 767–771

Liu XQ, Zhang B, Liu X, Shen Y, Li J, Zhao N, Ma L, Du Q, *et al*. 2012 A 45-bp insertion/deletion polymorphism in uncoupling protein 2 is not associated with obesity in a Chinese population. *Biochem. Genet.* **50** 784–796

Marti A, Corbalan MS, Forga L, Martinez-Gonzalec MA and Martinez JA 2004 Higher obesity risk associated with the exon-8 insertion of the UCP2 gene in a Spanish case-control study. *J. Nutr.* **20** 498–501

Omron n.d. *Omron KARADA Scan Body Composition and Scale (HBF-362)*, Japan: Omron Healthcare Co. Ltd.

Otabe S, Clement K, Rich N, Warden C, Pecqueur C, Neverova M, Raimbault S, Guy-Grand B, *et al*. 1998 Mutation screening of the human UCP 2 gene in normoglycemic and NIDDM morbidly obese

patients: lack of association between new UCP 2 polymorphisms and obesity in French Caucasians. *Diabetes* **47** 840–842

Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, Pérusse L and Bouchard C 2006 The human obesity gene map: the 2005 update. *Obesity (Silver Spring)* **14** 529–644

Ricquier D and Bouillaud F 2000 The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *J. Biochem.* **345** 161–179

Teo YY, Sim X, Ong RTH, Tan AKS, Chen J, Tantoso E, Small KS, Ku CS, *et al*. 2009 Singapore genome variation project: a haplotype map of three Southeast Asian populations. *Genome Res.* **19** 2154–2162

Walder K, Norman RA, Hanson RL, Schrauwen P, Neverova M, Jenkinson CP, Easlick J, Warden CH, *et al*. 1998 Asoociation between Uncoupling Protein Polymorphisms (UCP2-UCP3) and energy metabolism/obesity in Pima Indians. *Hum. Mol. Genet.* **7** 1431–1435

Wang H, Chu WS, Lu T, Hasstedt SJ, Kern PA and Elbein SC 2004 Uncoupling protein-2 polymorphisms in type 2 diabetes, obesity, and insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* **286** E1–E7

WHO 2011 *Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008* (Geneva: WHO Press)

WHO/IOTF/IASO 2000 *The Asia-Pacific perspective: redefining obesity and its treatment* (Hong Kong: World Health Organization, International Obesity Task Force, International Association for the Study of Obesity)

Yanowski JA, Diament AL, Sovik KN, Nguyen TT, Li H, Sebring NG and Warden CH 2000 Associations between uncoupling protein 2, body composition and resting energy expenditure in lean and obese African American, white and Asian children. *Am. J. Clin. Nutr.* **71** 1405–1412

Yiew SK, Khor LY, Tan ML, Pang CL, Chai VY, Kanachamy SS and Say YH 2010 No association between peroxisome proliferator-activated receptor and uncoupling protein gene polymorphisms and obesity in Malaysian university students. *Obes. Res. Clin. Pract.* **4** e247–e342

Yonezawa T, Kurata R, Hosomichi K, Kono A, Kimura M and Inoko H 2009 Nutritional and hormonal regulation of uncoupling protein 2. *IUBMB Life* **61** 1123–1131

MS received 04 August 2014; accepted 07 October 2014

Corresponding editor: SARAH H ELSEA