RESEARCH ARTICLE

Correlations between genetic variance and adiposity measures, and gene × gene interactions for obesity in postmenopausal Vietnamese women

TRAN QUANG BINH[1]*, YUTAKA NAKAHORI[2], VU THI THU HIEN[3], NGUYEN CONG KHAN[4], NGUYEN THI LAM[3], LE BACH MAI[3] and SHIGERU YAMAMOTO[5]

[1]*National Institute of Hygiene and Epidemiology, 1 Yersin, Hanoi 112800, Vietnam*
[2]*Department of Human Genetics and Public Health, Faculty of Medicine, the University of Tokushima, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan*
[3]*National Institute of Nutrition, 48B Tang Bat Ho Street, Hanoi 112807, Vietnam*
[4]*Vietnam Food Administration, Ministry of Health, 138A Giang Vo Street, Hanoi 118401, Vietnam*
[5]*Department of Nutrition and Food Science, Ochanomizu University, 2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan*

Abstract

Although environmental factors are important, there is considerable evidence that genes also have a significant role in the pathogenesis of obesity. We conducted a population-based study to investigate the relationship between candidate genes for obesity (*UCP1, UCP2, ADRA2B, ADRB3, LEPR, VDR* and *ESR1*) and adiposity measures (body mass index, body fat percentage, weight, waist circumference and waist–hip ratio) in terms of individual gene and gene × gene interaction in models unadjusted and adjusted for covariates (age, years since menopause, educational level and total energy intake). Postmenopausal women with TC genotype of *ESR1* gene had higher body fat percentage than those with TT genotype in the models unadjusted and adjusted for the covariates ($P = 0.006$ in adjusted model). In multiple logistic regression analysis, *Bsm*I and *Apa*I SNPs of *VDR* genes were significantly associated with overweight and obesity. The *UCP2–VDR Apa*I interaction to susceptibility of overweight and obesity was first observed from logistic regression analysis, and then confirmed in the multifactor dimensionality reduction method unadjusted and adjusted for the covariates. This interaction had 69.09% prediction accuracy for overweight and obesity ($P = 0.001$, sign test). In conclusion, the study suggests the significant association of *ESR1* and *VDR* genes with adiposity measures and the *UCP2–VDR Apa*I interaction to susceptibility to being overweight and obesity in postmenopausal Vietnamese women.

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Introduction

Obesity, which results from an imbalance between energy intake and expenditure, is an important cause of morbidity and mortality in developed countries, and is also becoming increasingly prevalent in the developing world. In Hanoi and Ho Chi Minh City (Vietnam), the prevalence of overweight and obesity was about 30% in both sexes, and generally increased with age (Walls *et al.* 2009). Obesity and being overweight are also major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases, osteoarthritis, cancer and mental health problems, and these chronic health conditions become more prevalent in postmenopausal women. Thus, obese postmenopausal women stand at a crossroads between living the remainder of their lives in essentially good health or facing the likely onset of chronic diseases that might have been prevented (Comuzzie and Allison 1998).

As a complex disorder, obesity includes both genetic and environmental factors in its pathogenesis. It is estimated that 40%–70% of the variation in body mass index (BMI) is heritable, while cultural and social factors may explain at least 30% of the variation (Hill and Peters 1998; Dennis 2007). Environmental factors, including increased food intake and inactive lifestyle, play important role in the increase of body weight and obesity (Swinburn *et al.* 2009). Genetic factors affecting obesity are categorized by different progresses such as: appetite stimulating group (e.g. neuropeptide Y, leptin receptor, P proopiomelanocortin); energy expenditure group (e.g. uncoupling proteins); metabolism regulating group (e.g.

*For correspondence. E-mail: binhtq@nihe.org.vn.

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beta-2 adrenergic receptor, beta-3 adrenergic receptor); and adipogenesis (e.g., peroxisome proliferator-activated receptor, vitamin D receptor, retinoid X receptor) (Bell *et al.* 2005). However, association studies have given inconsistent results due to population specificity and possible genetic effects masked by different gene × gene and gene × environment interactions (Cooper 2003). Thus, studies in different populations are needed to confirm these relations. In addition, to the best of our knowledge, there is still a dearth of data on the genetic factors of obesity in Vietnam. Therefore, we performed a population-based study to investigate the potential association of common candidate genes with adiposity measures in postmenopausal Vietnamese women.

This is an initial study from Vietnam investigating: (i) the genotype distribution of the candidate genes including uncoupling protein 1 (*UCP1*), uncoupling protein 2 (*UCP2*), alpha-2B adrenergic receptor (*ADRA2B*), beta-3 adrenergic receptor (*ADRB3*), leptin receptor (*LEPR*), vitamin D receptor (*VDR*), and oestrogen receptor 1 (*ESR1*) in the postmenopausal women; (ii) relationship between the single nucleotide polymorphisms (SNPs) of the selected genes and adiposity measures; and (iii) possible gene × gene interaction for being overweight and obesity.

Materials and methods

Subjects

One hundred and forty healthy postmenopausal women were randomly selected from the rural population of Hai Duong province, Vietnam. Of the 140 subjects in the study, 137 (98%) were farmers and manual workers, and the others were office clerks. All subjects were healthy women who did not smoke or drink alcohol. The mean (± SD) of age, age at menarche and age at menopause of the study group were 55.6 ± 3.8, 16.7 ± 2.1 and 47.7 ± 3.4 years, respectively. The Ethics Committee of the National Institute of Nutrition, Vietnam, and the Ethnical Committee of Tokushima University, Japan, approved the study. All participants provided written informed consent before entering the study.

Measurements

All participants completed a structured questionnaire. Data were collected on current age, age at menarche, age at menopause, ethnicity, educational level, occupation, medical and reproductive history, dietary, smoking and drinking history. Lifelong occupation was defined as the occupation that the subject engaged most frequently in their life. Educational level was categorized in three groups, by number of years of schooling: low level (≤ 5 years), medium level (6–8 years), and high level (≥ 9 years). Dietary intake was measured by previous 24 h dietary recall method on three consecutive weekdays (Witschi 1998).

Anthropometric measurements including weight, height, waist and hip circumference, body fat percentage were collected. Body weight and height were measured in light clothing and without shoes to the nearest 0.1 kg and 0.1 cm respectively. Body mass index (BMI) was calculated as weight per square of height (kg/m^2). Body fat percentage was measured by bioelectrical impedance method by using OMRON scale (HBF-351, Kyoto, Japan). Overweight and obesity were classified by the BMI-value recommendations of the World Health Organization (WHO Expert Consultation 2004) and the International Obesity Task Force for Asian and Pacific Island populations, which corresponds to the BMI cutoffs of 23 and 25 kg/m^2 (International Obesity Task Force 2002).

Genotyping

Peripheral blood samples were obtained from each woman and genomic DNA was extracted from peripheral blood leukocytes, using QIA amp DNA blood kit (Qiagen GmbH, Hilden, Germany). PCR protocols and primers used for genotyping *UCP1* (rs1800592), *UCP2* (rs659366), *ADRA2B* (*12Glu9*), *ADRB3* (rs4994), *LEPR* (rs1137101), *ESR1* (*Pvu*II and *Xba*I), and the common single nucleotide polymorphisms (SNPs) of *VDR* gene (*Fok*I, *Bsm*I, *Apa*I and *Taq*I) were as described previously (Kadowaki *et al.* 1995; Sivenius *et al.* 2001; Nagai *et al.* 2003; Sesti *et al.* 2003; Binh *et al.* 2006; Mitra *et al.* 2006) with some modification (table 1).

Statistical analysis

We coded genotypes as 0, 1, and 2, depending on the number of copies of the risk alleles. Genotype frequencies were compared and tested for Hardy–Weinberg Equilibrium (HWE) by Pearson's χ^2 test or Fisher's exact test, as appropriate. A pairwise |D′| value (the absolute value for the disequilibrium parameter) that ranges from 0 (complete linkage equilibrium status) to 1.0 (complete LD status) among SNPs was measured using the software program SNPstats (Sole *et al.* 2006). Haplotype frequencies for multiple loci were estimated by the expectation-maximization method, by use of the same software program SNPstats.

Quantitative variables were checked for normal distribution and compared using one-way ANOVA or independent-sample *t*-test. We used a general linear model (GLM) for one variate to evaluate the relationships between candidate genes and adiposity measures. The raw variables were adjusted by regression for covariates of age, years since menopause, educational level and total energy intake.

Gene × gene interactions were examined using both the logistic regression model and the multifactor dimensionality reduction analysis, with and without controlling for the covariates. In the logistic regression, interactions were

Table 1. PCR protocols and primers for typing the candidate genes.

Gene	dbSNP[a]	Primers	Tm	Restriction enzyme	Allele size (bp)
UCP1	rs1800592	5′-cttgggtagtgacaaagtat-3′	52°C	*Bcl*I	A: 470 bp
	A/G	5′-ccaaagggtcagatttctac-3′			G: 310 bp+160 bp
UCP2	rs659366	5′-cacgctgcttctgccaggac-3′	68°C	*Mlu*I	A: 360 bp
	G/A	5′-aggcgtcaggagatggaccg-3′			G: 290 bp+70 bp
ADRA2B	12Glu9	5′agggtgtttgtggggcatctcc-3′	63°C	–	Glu^{12}:112 bp
		5′-caagctgaggccggagacactg-3′			Glu^{9}:103 bp
ADRB3	rs4994	5′-cgcccaataccgccaacac-3′	61°C	*Bst*NI	C:161 bp;
	T/C	5′-ccaccaggagtcccatcacc-3′			T: 99 bp+62 bp
LEPR	rs1137101	5′-acccttaagctgggtgtcccaaatag-3′	57°C	*Msp*I	A: 421 bp
	A/G	5′-agctagcaaatatttttgtaagcaatt-3′			G: 294 bp+127 bp
ESR1	rs2234693	5′-gatatccagggttatgtggca-3′	60°C	*Pvu*II	C(P): 346 bp
	T/C	5′-aggtgttgcctattatattaaccttga-3′			T(p): 241 bp+105 bp
	rs9340799	5′-gatatccagggttatgtggca-3′	60°C	*Xba*I	G(X): 346 bp
	A/G	5′-aggtgttgcctattatattaaccttga-3′			A(x):196 bp+150 bp
VDR	rs2228570	5′-agctggccctggcactgactctgctct-3′	60°C	*Fok*I	C(F): 265 bp
	C/T	5′-atggaaacaccttgcttcttctccct-3′			T(f): 196 bp+69 bp
	rs1544410	5′-caacaaagactacaagtaccgcgtcagtga-3′	54°C	*Bsm*I	A(B): 822 bp
	G/A	5′-aaccagcggaagaggtcaaggg-3′			G(b): 646 bp+176 bp
	rs7975232	5′-cagagcatggacagggagcaag-3′	62°C	*Apa*I	A(A): 746 bp
	C/A	5′-gcaactcctcatggctgaggtctca-3′			C(a): 532 bp+214 bp
	rs731236	5′-cagagcatggacagggagcaag-3′	62°C	*Taq*I	T(T): 746 bp
	T/C	5′-gcaactcctcatggctgaggtctca-3′			C(t): 497 bp+249 bp

[a]Accession number of each polymorphism to dbSNP at http://www.ncbi.nlm.nih.gov/. Alleles underlined were proposed to be the risk alleles for obesity identified by previous studies.

assessed using a likelihood-ratio test. Here, data are presented as odds ratios with 95 per cent confidence intervals (CI). The above statistical procedures were performed using SPSS version 16.0 (SPSS, Chicago, USA). Multifactor dimensionality reduction (MDR) analysis was used to detect gene × gene interactions to obesity and overweight. A detailed explanation on MDR has been provided elsewhere (Hahn *et al.* 2003; Moore *et al.* 2006; Lou *et al.* 2007). Briefly, MDR is a genetically model-free and nonparametric alternative to logistic regression. MDR acts by reducing a set of multilocus genotypes to one dimension with two groups: a high-risk and a low-risk set of genotypes. A particular multilocus genotype can be declared to be high-risk if the ratio of number of cases to controls exceeds the proportion of cases in the total sample. By grouping the high-risk multilocus genotypes together and the low-risk genotypes together, the model is reduced to one dimension, i.e., essentially one variable with two possible values: high or low risk 2-locus genotypes. Models are evaluated on the testing balanced accuracy statistic (TBA), the cross-validation consistency (CVC), and the statistical significance of the model. The TBA measures how often individuals are correctly classified with respect to their case/control status and the CVC evaluate the consistency with which individuals are classified. We used 10000 permutations to determine the statistical significance of the best models. These data were analysed using an extension of the MDR algorithm that includes adjustment for covariates, the generalized multifactor dimensionality reduction (GMDR, v. 0.7) software package (Lou *et al.* 2007).

Results

Genotype distribution of the candidate genes

Genotype frequency of the selected SNPs in the postmenopausal women is shown in table 2. All SNPs were in Hardy–Weinberg equilibrium ($P > 0.3$), except for *ADRB3*, which was marginally significant ($P = 0.076$). In the *UCP* gene, SNP rs1800592 (*UCP1*) and SNP rs659366 (*UCP2*) were in different LD block ($D' = 0.21$, $P = 0.001$). From four SNPs of the *VDR* gene, the polymorphisms at the 3′end of the gene (*Bsm*I, *Apa*I and *Taq*I) exhibited a strong linkage disequilibrium (D′ ranging from 0.91 to 0.99, $P < 0.001$), forming three frequent haplotypes: baT (66.6%), bAT (24.5%), Bat (5.7%), which jointly represent nearly 98% of all haplotypes. SNP rs2228570 (*Fok*I) was separated from the others in *VDR* gene by over 33 kb, with D′ ranging from 0.014 to 0.096. In *ESR1* gene, the *Pvu*II (rs2234693) and *Xba*I (rs9340799) polymorphisms were 45 bp apart and located approximately 400-bp upstream of exon 2, and the two polymorphisms were in the same LD block ($D' = 0.99$, $P < 0.001$).

Correlations between genetic variance and adiposity measures

Table 2 shows the comparison of adiposity measures among genotypes of each candidate genes. No significant association was observed between polymorphisms of the selected SNPs and weight, BMI, body fat percentage, waist

Table 2. Comparison of adiposity measures among genotypes of each candidate gene.

Gene	Genotype	N (%)	Adiposity measures				
			Weight (kg)	BMI (kg/cm^2)	Body fat (%)	Waist circumference (cm)	Waist–hip ratio
UCP1	AA	26 (18.6)	46.5 ± 7.7	20.9 ± 2.4	31.5 ± 8.7	72.2 ± 8.3	0.83 ± 0.07
	AG	76 (54.3)	45.4 ± 6.5	20.5 ± 2.7	29.5 ± 4.1	70.1 ± 7.3	0.82 ± 0.05
	GG	38 (27.1)	45.0 ± 6.0	20.0 ± 2.5	28.9 ± 4.1	69.9 ± 7.0	0.82 ± 0.06
UCP2	AA	25 (17.9)	45.5 ± 6.6	20.6 ± 2.6	29.8 ± 4.2	71.4 ± 7.4	0.84 ± 0.06
	AG	69 (49.3)	45.7 ± 7.1	20.6 ± 2.7	29.6 ± 4.2	70.7 ± 7.9	0.82 ± 0.06
	GG	46 (32.9)	45.2 ± 5.9	20.0 ± 2.4	29.1 ± 4.2	69.6 ± 6.7	0.81 ± 0.06
ADRA2B	Glu^{12}/Glu^{12}	50 (35.7)	46.0 ± 6.6	20.4 ± 2.5	29.8 ± 3.7	71.4 ± 7.3	0.82 ± 0.06
	Glu^{12}/Glu^{9}	65 (46.4)	44.8 ± 6.5	20.2 ± 2.5	28.8 ± 4.5	69.6 ± 7.2	0.82 ± 0.05
	Glu^{9}/Glu^{9}	25 (17.9)	46.4 ± 6.8	21.0 ± 2.7	30.8 ± 4.2	70.9 ± 8.3	0.81 ± 0.07
ADRB3	TT	102 (72.9)	45.3 ± 6.7	20.4 ± 2.6	29.5 ± 4.0	70.2 ± 7.4	0.82 ± 0.06
	TC	38 (27.1)	45.9 ± 6.4	20.4 ± 2.4	29.4 ± 4.7	71.0 ± 7.5	0.82 ± 0.06
LEPR	AA	3 (2.1)	48.2 ± 3.4	21.8 ± 1.5	33.3 ± 1.9	75.6 ± 3.0	0.84 ± 0.02
	AA	28 (20)	43.9 ± 5.4	19.9 ± 2.5	29.2 ± 3.8	69.3 ± 6.6	0.81 ± 0.06
	GG	109 (77.9)	45.8 ± 6.9	20.5 ± 2.6	29.4 ± 4.3	70.6 ± 7.7	0.82 ± 0.06
ESR1	TT (pp)	52 (37.1)	44.3 ± 5.8	19.9 ± 2.2	28.3 ± 3.9	68.8 ± 6.7	0.81 ± 0.06
PvuII	TC (Pp)	66 (47.1)	46.3 ± 6.7	20.7 ± 2.8	30.3 ± 4.2*	71.4 ± 7.9	0.82 ± 0.06
	CC (PP)	22 (15.7)	46.1 ± 7.7	20.9 ± 2.6	29.9 ± 4.2	71.6 ± 7.1	0.83 ± 0.05
ESR1	AA (xx)	90 (64.3)	45.0 ± 6.4	20.2 ± 2.5	29.1 ± 4.2	70.2 ± 7.8	0.82 ± 0.06
XbaI	AG (Xx)	45 (32.1)	45.9 ± 6.7	20.6 ± 2.6	29.9 ± 4.1	70.5 ± 6.6	0.82 ± 0.05
	GG (XX)	5 (3.6)	50.5 ± 6.8	22.5 ± 3.0	32.3 ± 5.4	75.2 ± 6.1	0.83 ± 0.03
VDR	CC (FF)	39 (27.9)	45.3 ± 7.2	20.5 ± 2.5	29.7 ± 4.0	71.5 ± 8.1	0.83 ± 0.06
FokI	CT (Ff)	72 (51.4)	46.0 ± 6.5	20.6 ± 2.6	29.5 ± 4.3	70.3 ± 7.5	0.82 ± 0.06
	TT (ff)	29 (20.7)	44.6 ± 6.1	20.0 ± 2.6	29.3 ± 4.2	69.3 ± 6.2	0.81 ± 0.04
VDR	AA (BB)	1 (0.7)	39.4	17.6	29.4	64.2	0.78
BsmI	AG (Bb)	23 (16.4)	46.5 ± 8.3	20.7 ± 2.8	29.5 ± 4.7	69.5 ± 7.3	0.81 ± 0.05
	GG (bb)	116 (82.9)	45.4 ± 6.2	20.4 ± 2.5	29.5 ± 4.1	70.7 ± 7.5	0.82 ± 0.06
VDR	AA (AA)	12 (8.6)	45.2 ± 4.6	20.6 ± 1.5	30.1 ± 2.4	70.7 ± 7.0	0.83 ± 0.07
ApaI	AC (Aa)	68 (48.6)	45.3 ± 7.2	20.1 ± 2.6	29.0 ± 4.5	69.8 ± 7.6	0.82 ± 0.06
	CC (aa)	60 (42.8)	45.8 ± 6.3	20.8 ± 2.7	29.9 ± 4.1	71.2 ± 7.4	0.82 ± 0.05
VDR	TT (TT)	124 (88.6)	45.7 ± 6.5	20.5 ± 2.6	29.6 ± 4.2	70.6 ± 7.4	0.82 ± 0.06
TaqI	TC (Tt)	16 (11.4)	44.2 ± 7.3	19.6 ± 2.5	29.0 ± 4.5	69.2 ± 8.0	0.81 ± 0.05

Data are presented as mean ± SD unless otherwise indicated. P value was derived from one-way ANOVA or independent t-test, as appropriate. *$P = 0.036$ TC versus TT, the other P values > 0.05.

circumference and waist–hip ratio, except for the *Pvu*II polymorphism of *ESR1* gene (women with TC genotype had higher body fat percentage than those with TT genotype, $P = 0.036$). Moreover, the significant association between the *ESR1 Pvu*II polymorphism and body fat percentage was consistently found after adjustment for age, years since menopause, educational level and total energy intake ($P = 0.006$, TC versus TT) (table 3). Significant associations were observed in the *Bsm*I (rs1544410) and *Taq*I polymorphisms (rs731236) of *VDR* gene for both weight and BMI, and in the *ESR1 Xba*I polymorphism for BMI (table 3) in the ANCOVA analysis controlling for covariates. Further analysis on relationship between *ESR1 Pvu*II–*Xba*I haplotype and body fat percentage indicated that carries with 1 px haplotype had consistently higher body fat percentage than those with 2 px haplotype in models unadjusted and adjusted for age, years since menopause, educational level and total energy intake ($P = 0.036$ in unadjusted model and $P = 0.014$ in adjusted model, data not shown). A similar analysis showed no association between body fat percentage and number copies of the Px or PX haplotypes.

Association between candidate genes and obesity

To identify potential risk variables for obesity and being overweight, we used binary logistic regression in the models, including the SNPs selected, age, years since menopause, educational level, and total energy intake. As presented in table 4, we found no association between obesity and the tested SNPs in the models with and without adjustment for covariates. However, the *Bsm*I and *Apa*I polymorphisms of *VDR* gene were statistically significantly associated with being overweight and obesity in both unadjusted and adjusted models, taking into account the effect of the other SNPs.

Possible gene × gene interaction in predisposition of overweight and obesity

Table 5 summarizes the potential pairwise gene × gene interactions for overweight and obesity by logistic regression analysis. The *UCP2–VDR Apa*I interplay was the most significant interaction among all possible pairwise gene ×

Table 3. Analysis of covariates of adiposity measures among candidate genes.

Gene	Genotype	Adiposity measures				
		Weight (kg)	BMI (kg/cm^2)	Body fat (%)	Waist circumference (cm)	Waist–hip ratio
UCP1	AA	47.4 ± 1.9	21.3 ± 0.7	31.9 ± 1.2	73.2 ± 2.2	0.82 ± 0.02
	AG	47.1 ± 1.6	21.2 ± 0.6	31.0 ± 1.0	72.0 ± 1.9	0.82 ± 0.02
	GG	45.7 ± 1.8	20.3 ± 0.7	30.0 ± 1.1	71.2 ± 2.1	0.82 ± 0.02
UCP2	AA	46.8 ± 2.0	21.1 ± 0.8	31.3 ± 1.3	73.0 ± 2.3	0.83 ± 0.02
	AG	46.7 ± 1.6	21.1 ± 0.6	30.9 ± 1.0	72.2 ± 1.8	0.82 ± 0.01
	GG	46.6 ± 1.7	20.7 ± 0.7	30.6 ± 1.1	71.2 ± 2.0	0.81 ± 0.02
ADRB3	TT	46.2 ± 1.5	20.9 ± 0.6	30.9 ± 1.0	71.7 ± 1.8	0.82 ± 0.01
	TC	47.2 ± 1.8	21.1 ± 0.7	31.1 ± 1.1	72.5 ± 2.0	0.82 ± 0.02
LEPR	AA+AG	46.1 ± 1.9	20.9 ± 0.7	31.0 ± 1.2	72.0 ± 2.1	0.81 ± 0.02
	GG	47.3 ± 1.5	21.1 ± 0.6	30.9 ± 0.9	72.3 ± 1.7	0.82 ± 0.01
ADRA2B	Glu^{12}/Glu^{12}	46.8 ± 1.7	20.6 ± 0.7	30.8 ± 1.1	73.0 ± 2.0	0.83 ± 0.02
	Glu^{12}/Glu^{9}	45.3 ± 1.6	20.5 ± 0.6	29.9 ± 1.1	70.7 ± 1.9	0.82 ± 0.02
	Glu^{9}/Glu^{9}	48.1 ± 1.9	21.7 ± 0.7	32.2 ± 1.2	72.7 ± 2.2	0.81 ± 0.02
ESR1	TT	46.2 ± 1.9	20.6 ± 0.7	30.1 ± 1.2	70.5 ± 2.2	0.80 ± 0.02
*Pvu*II	TC	48.4 ± 1.8	21.7 ± 0.7	32.4 ± 1.1[f]	73.8 ± 2.0	0.82 ± 0.02
	CC	45.5 ± 1.9	20.6 ± 0.7	30.3 ± 1.2	72.1 ± 2.2	0.83 ± 0.02
*ESR*1	AA	44.0 ± 1.4	19.8 ± 0.5	29.3 ± 0.9	70.4 ± 1.6	0.82 ± 0.01
XbaI	AG	43.9 ± 1.6	19.8 ± 0.6	29.6 ± 1.0	69.2 ± 1.9	0.81 ± 0.02
	GG	52.2 ± 3.5	23.3 ± 1.3[c]	34.0 ± 2.2	76.8 ± 4.0	0.82 ± 0.03
VDR	CC	47.2 ± 1.8	21.3 ± 0.7	31.3 ± 1.1	73.4 ± 2.1	0.83 ± 0.02
FokI	CT	47.3 ± 1.8	21.1 ± 0.7	31.1 ± 1.2	72.6 ± 2.1	0.82 ± 0.02
	TT	45.7 ± 1.8	20.4 ± 0.7	30.4 ± 1.1	70.5 ± 2.0	0.81 ± 0.02
VDR	AA + AG	49.5 ± 2.0[a]	22.2 ± 0.8[d]	31.7 ± 1.3	71.7 ± 2.3	0.81 ± 0.02
BsmI	GG	43.9 ± 2.0	19.7 ± 0.8	30.2 ± 1.3	72.6 ± 2.3	0.83 ± 0.02
VDR	AA	46.7 ± 2.3	21.0 ± 0.9	31.2 ± 1.5	72.6 ± 2.7	0.82 ± 0.02
ApaI	AC	46.5 ± 1.5	20.6 ± 0.6	30.5 ± 1.0	71.7 ± 1.8	0.81 ± 0.01
	CC	47.0 ± 1.6	21.2 ± 0.6	31.2 ± 1.1	72.2 ± 1.9	0.82 ± 0.02
VDR	TT	50.2 ± 1.9[b]	22.6 ± 0.7[e]	31.9 ± 1.2	72.5 ± 2.2	0.81 ± 0.02
TaqI	TC	43.2 ± 2.4	19.3 ± 0.9	30.0 ± 1.5	71.8 ± 2.7	0.82 ± 0.02

Data are presented as mean ± SD. *P*-value was derived from ANCOVA test with adjustment for age, years since menopause, educational level, and total energy intake. [a]$P = 0.033$, AA + AG vs. GG; [b]$P = 0.022$, TT vs. TC; [c]$P = 0.043$, GG vs. AG and AA; [d]$P = 0.013$ AA + AG vs. GG; [e]$P = 0.006$, TT vs. TC; [f]$P = 0.047$, TC vs. TT and CC.

gene interactions in the models unadjusted and adjusted for covariates (table 5).

Table 6 presents the potential gene × gene interaction in predisposition for obesity and being overweight among 11 polymorphisms, using GMDR analysis. From all possible pairwise interactions, the most significant gene × gene interplay was the *UCP2–VDR Apa*I interaction, which had 69.09% prediction accuracy for both overweight and

Table 4. Associations between SNPs in seven candidate genes with overweight and obesity.

Gene	Obesity				Overweight and obesity			
	OR (95% CI)	*P* value[a]	OR (95% CI)	*P* value[b]	OR (95% CI)	*P* value[a]	OR (95% CI)	*P* value[b]
UCP1	0.80 (0.23-2.75)	0.722	0.77 (0.22-2.75)	0.686	0.85 (0.39-1.87)	0.688	0.86 (0.39-1.93)	0.718
UCP2	0.45 (0.12-1.67)	0.232	0.42 (0.10-1.72)	0.226	0.58 (0.28-1.20)	0.142	0.60 (0.28-1.30)	0.192
ADRA2B	0.95 (0.28-3.20)	0.936	0.96 (0.29-3.22)	0.953	1.78 (0.85-3.37)	0.126	1.80 (0.83-3.90)	0.140
ADRB3	0.35 (0.03-3.74)	0.383	0.37 (0.03-4.13)	0.417	0.69 (0.22-2.19)	0.530	0.56 (0.17-1.85)	0.339
LEPR	1.99 (0.28-14.1)	0.493	1.88 (0.25-14.1)	0.536	0.57 (0.14-2.39)	0.445	0.60 (0.14-2.67)	0.505
ESR1 PvuII	0.69 (0.18-2.73)	0.600	0.70 (0.17-2.78)	0.608	0.82 (0.36-1.88)	0.635	0.78 (0.32-1.90)	0.585
ESR1 XbaI	1.92 (0.42-8.77)	0.403	2.00 (0.41-9.85)	0.393	2.16 (0.79-5.86)	0.132	2.07 (0.71-5.99)	0.181
VDR FokI	0.91 (0.28-2.98)	0.881	0.83 (0.24-2.86)	0.763	1.55 (0.73-3.30)	0.256	1.80 (0.80-4.04)	0.153
VDR BsmI	0.15 (0.01-3.74)	0.247	0.13 (0.01-3.67)	0.233	0.15 (0.02-0.93)	**0.042**	0.13 (0.02-0.91)	**0.039**
VDR ApaI	6.01 (0.70-51.9)	0.103	6.30 (0.71-56.0)	0.099	3.03 (1.10-8.34)	**0.032**	3.00 (1.08-8.36)	**0.036**
VDR TaqI	–	0.998	–	0.998	6.44 (0.65-63.7)	0.111	6.50 (0.61-69.1)	0.121

OR, odds ratio; CI, confidence interval. ORs (95% CI) were reported with respect to the risk allele using a log additive model in logistic regression. [a]Unadjusted; [b]adjusted by age, years since menopause, educational level, and total energy intake.

Table 5. The possible pairwise gene × gene interactions for overweight and obesity by logistic regression analysis.

Interacting SNPs	Model	OR (95% CI)	*P* value for interaction	*P* value for model fit (*)
UCP2-VDR ApaI	Unadjusted	0.10 (0.02-0.42)	0.002	0.001
	Adjusted	0.09 (0.02-0.39)	0.001	0.003
ESR1 PvuII-VDR FokI	Unadjusted	3.37 (1.36-8.39)	0.009	0.006
	Adjusted	3.38 (1.32-8.63)	0.011	0.023
ESR1 XbaI-VDR ApaI	Unadjusted	0.14 (0.03-0.66)	0.013	0.004
	Adjusted	0.13 (0.03-0.65)	0.013	0.017
ESR1 XbaI-VDR FokI	Unadjusted	0.37 (0.15-0.96)	0.041	0.027
	Adjusted	0.35 (0.13-0.95)	0.039	0.052
ADRB3-VDR ApaI	Unadjusted	0.14 (0.02-0.83)	0.030	0.046
	Adjusted	0.10 (0.01-0.65)	0.016	0.068

OR, odds ratio; CI, confidence interval. Odds ratios and *P* values were derived from the models unadjusted and adjusted for age, years since menopause, educational level, and total energy intake. (*) Omnibus tests of model coefficients: Model adequately fits the data if *P* value < 0.05.

obesity ($P = 0.001$, sign test), and a maximum prediction accuracy increased up to 75.36% ($P = 0.0054$, on the basis of 10000-fold permutation testing). The CVC (10/10) indicated consistency in the cross validation measures. This interaction remained essentially unchanged in the model adjusted for the covariates (age, years since menopause, educational level and total energy intake). The other possible interactions listed in table 5 were not confirmed in MDR analysis. We could not detect significant gene × gene interactions in models for obesity with and without adjustment for the covariates.

Discussion

As obesity is polygenic disorder, we included in the study the candidate genes from different pathways composed of appetite stimulating gene (*LEPR*), energy expenditure group (*UCP1* and *UCP2*), metabolism regulating group (*ADRA2B* and *ADRB3*), and adipogenesis (*VDR* and *ESR1*). Although the selected SNPs have been widely investigated in developed countries, it is the first report of the relationship between genetic factors and obesity in Vietnam. Among the 11 SNPs studied, we observed the significant association of *ESR*1 and *VDR* with adiposity measures and the *UCP2–VDR Apa*I interaction to susceptibility of overweight and obesity.

With regard to *ESR1* gene, postmenopausal women with TC genotype had higher body fat percentage than those with TT genotype in our study. This significant association also remained essentially unchanged in the models unadjusted and adjusted for the covariates (age, years since menopause, educational level and total energy intake). Although it is not in line with previous reports that the *ESR1 Pvu*II C allele and its associated genotypes and haplotypes were inversely associated with obesity in white postmenopausal women (Goulart *et al.* 2009) and *ESR1 Pvu*II polymorphism was not associated with body fat distribution and obesity in Japanese postmenopausal women (Okura *et al.* 2003), our observation can be explained by the report suggesting that the *ESR1 Pvu*II C allele was associated with increased transcription of *ESR1* when compared with the common allele and thus presumably associated with higher relative levels of functional ESR1 protein (Herrington *et al.* 2002). Further haplotype analysis showed that carries with 1 px haplotype had higher body fat percentage than those with 2 px haplotype in models unadjusted and adjusted for the covariates. This is consistent with the findings that plasma oestradiol level decreased by 1.9 pmol/L per copy number of px haplotype in postmenopausal women (Schuit *et al.* 2005), since the effect of low oestrogen on increased obesity has been linked to *ESR1* gene (Ohlsson *et al.* 2000). Furthermore *ESR1* plays a pivotal role in the regulation of food intake and energy expenditure by oestrogens. The ventromedial nucleus (VMN) of the hypothalamus has a high density of oestrogen-binding sites (Pfaff and Keiner 1973), and neurons in this nucleus express *ESR1* at high levels (Li *et al.* 1993). The VMN lesion or ovariectomy both lead to increased food intake and body weight, suggesting that *ESR1* expressed in VMN neurons is an important player in the central control of body weight by oestrogens. In addition, after *ESR1* silencing in the VMN, the mice displayed a higher increase in food consumption as early as two weeks after surgery, especially suppression of *ESR1* levels in the VMN of adult female animals triggered the development of metabolic syndrome marked by a profound increase in body weight and excess visceral fat (Musatov *et al.* 2007).

In terms of *VDR* gene, no significant association was observed between the polymorphism of individual SNPs and BMI, which is consistent with a previous report in Caucasian postmenopausal women (Tworowska-Bardzińska *et al.* 2008). However, the significant associations with BMI were found for *Bsm*I and *Taq*I polymorphisms after adjustment for the covariates. In addition, the multiple logistic regression analysis indicated that *Bsm*I and *Apa*I polymorphisms were statistically significantly associated with being overweight and obesity in both unadjusted and adjusted model, taking into account the effect of other SNPs. This points the limitation of single SNP-based association study.

Table 6. Gene × gene interaction models for overweight and obesity.

Interacting SNPs	Testing BA	CVC	*P* value
Models for overweight and obesity[a]			
UCP2-VDR ApaI	0.6909	10/10	0.001
UCP1-ADRA2B-ESR1 PvuII	0.4564	3/10	0.623
Models for overweight and obesity[b]			
UCP2-VDR ApaI	0.6965	10/10	0.011
UCP2-VDR ApaI-ESR1 XbaI	0.4783	5/10	0.377

P values are from the sign test. BA, balanced accuracy; CVC, cross validation consistency.
[a]Unadjusted; [b]adjusted by age, years since menopause, educational level, and total energy intake.

One of the major findings of the present study is to detect the most significant *UCP2–VDR Apa*I interaction to susceptibility of overweight and obesity among all possible pairwise gene × gene interactions. This interaction was first observed in logistic regression analysis, and then confirmed in the MDR method unadjusted and adjusted for the covariates (age, years since menopause, educational level and total energy intake). This interesting observation can explain partly the possible genetic effects masked by different gene × gene interaction leading to the controversial results in association studies. Although the molecular mechanisms underlying the interaction between *UCP2* gene and *VDR* gene remain to be explored, a prior study has reported suppression of *UCP2* expression by 1,25(OH)2D3 in human adipose tissues via the nuclear *VDR* (Shi *et al.* 2002) and a recent work has indicated that 1,25(OH)2D3 suppresses *UCP* expressions through direct gene regulation and that basal *UCP* expression is upregulated in the brown fat of *VDR* (–/–) mice. Thus, *VDR* interacts with UCPs in the pathogenesis of obesity, whereas the food intake is not significantly different between *VDR*-null mice and wild-type mice (Wong *et al.* 2009).

Because obesity is known as a lifestyle-related disorder, we took into account the analysis of both genetic factors and environmental factors, including: (i) reproductive status: age and years since menopause; (ii) lifestyle factors: total energy intake, educational level and occupation. As all subjects were healthy women who did not smoke and drink alcohol, and most of them (98%) were farmers and manual workers, we could eliminate the confounding factors of these variables in all analysis models.

In the present study, we used the classification of overweight and obesity according to the recommendations of the World Health Organization (WHO Expert Consultation 2004) and the International Obesity Task Force for Asian and Pacific Island populations, which corresponds to the BMI cutoffs of 23 and 25 kg/m^2 (International Obesity Task Force 2002). These recommendations were based on the presence of excessive body fat and adverse effects on health (mortality and morbidity). Studies in Indonesia, Singapore, Japan and Hong Kong demonstrated that for the same BMI, Asians living in these countries had higher body fat percentage compared to age-matched and sex-matched Caucasians with the same BMI (Guricci *et al.* 1998; Deurenberg-Yap *et al.* 2000; Gallagher *et al.* 2000; Ko *et al.* 2001), while a recent study did not support it (Ho-Pham *et al.* 2010). Although there has been disagreement on this classification, many studies have supported the use of theses cutoff to classification of overweight and obesity for Asian populations (Ko and Tang 2007; He *et al.* 2008; Yamada *et al.* 2008). Further, researches are required to derive a more appropriate BMI threshold for defining obesity and being overweight for Asians.

The present findings must be interpreted in the context of several potential limitations. First, the most important limitation was the small sample size. Although statistically significant genetic associations were indeed identified, the sample lacked the statistical power to detect other potential associations. Next, using the bioelectrical impedance method to measure body fat percentage is not known as a 'gold standard', but rather the dual-energy X-ray absorptiometry method. However, in the context of study in developing countries at the public health level, the bioelectrical impedance method has been proposed as an alternative method for noninvasive assessment of body fat percentage, especially useful for pregnant women, due to its low cost, portable tools and feasibility for a large population study.

In conclusion, the present study showed the association of VDR and ESR1 with adiposity measures and the significant *UCP2–VDR Apa*I interaction to susceptibility of overweight and obesity. For future investigations on the association between adiposity measures and genes, use of larger sample sizes, prospective study designs, and additional markers would enhance the findings.

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