CLINICAL STUDY

Methylenetetrahydrofolate reductase C677T polymorphism is associated with central adiposity and increased androgenicity in healthy postmenopausal women

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Abstract

Objective: To assess the association of genetic polymorphisms related to cardiovascular disease (CVD) risk with anthropometric parameters and indices of androgenicity in healthy postmenopausal women. **Design:** Cross-sectional study in a University Menopause Clinic.

Methods: The following polymorphisms were assessed in 84 healthy postmenopausal women: glycoprotein IIIa Leu33Pro, apolipoprotein E2/E3/E4, methylenetetrahydrofolate reductase (*MTHFR*) Ala222Val, apolipoprotein B Arg3500Gln, paraoxonase 1 Gln192Arg, plasminogen activator inhibitor 1 4G/5G, cholesterol-7 α -hydroxylase A-204C, and cholesterol ester transfer protein (TaqIB) B1/B2. Hormonal assays included FSH, LH, 17- β -estradiol, testosterone, sex hormone-binding globulin (SHBG), DHEA sulfate, Δ -4-androstenedione (Δ 4A), free androgen index (FAI), free estrogen index (FEI), and homocysteine (Hcy). The anthropometric components were body mass index (BMI) and waist-to-hip ratio (WHR).

Results: *MTHFR* Ala222Val polymorphism was positively associated with testosterone, FAI, and FEI ($P=0.001$, $P=0.0004$, and $P=0.014$ respectively) and negatively with SHBG ($P=0.047$). Furthermore, women bearing this polymorphism had higher BMI and WHR compared with women with the wild-type variant ($P=0.027$ and $P=0.044$ respectively).

Conclusions: *MTHFR* Ala222Val polymorphism is associated with increased androgenicity and elevated BMI and WHR in healthy postmenopausal women. The significance of this association with respect to the CVD risk of postmenopausal women remains to be elucidated in future studies.

European Journal of Endocrinology 159 233–241

Introduction

Menopause-associated estrogen deficiency has metabolic and vascular consequences that increase the risk for cardiovascular disease (CVD). The increased prevalence of CVD after menopause has been partially attributed to the adverse effects of estrogen deficiency on plasma lipid–lipoprotein levels. Menopause associates with an atherogenic profile: increased levels of total and low density lipoprotein (LDL) cholesterol, triglycerides, and Lp(a), and decreased levels of high-density lipoprotein (HDL) cholesterol (1). Of particular importance for the menopausal women are the increased levels of triglycerides, for they associate with the presence of small dense and very low-density particles (1). Furthermore, menopause is related to small increases of both systolic and diastolic blood pressure and increased sympathetic tone. Among the vascular consequences, endothelial dysfunction and vascular inflammation are reported to be augmented (2).

Postmenopause is associated with significant changes in obesity indices. Waist-to-hip ratio (WHR), reflecting abdominal adiposity, increases significantly after menopausal transition (3). High body mass index (BMI) and WHR have been correlated with increased risk for CVD, diabetes mellitus type 2, and other aspects of the metabolic syndrome (4, 5). Furthermore, menopause is associated with reduced glucose tolerance (1). Diet changes, excess weight, central obesity, and sedentary way of life, frequently associating with advancing age, concur to alter glucose tolerance and increase insulin resistance. Impaired glucose tolerance and diabetes mellitus may be found in nearly 20% of women aged 55–65 years. Additionally, estrogen deficiency may lead to decreased pancreatic insulin secretion (2).

High androgenicity in women may be a signal of increased risk for coronary artery disease (6). Elevated androgen levels have been associated with central obesity, insulin resistance, and the development of

type 2 diabetes mellitus (7, 8). Regarding postmenopausal women, data suggest that androgenicity may contribute to the development of insulin resistance independently of obesity (7).

Beyond metabolic factors, the genetic background may affect the risk of CVD in postmenopausal women. Polymorphisms in genes involved in homocysteine (Hcy) and lipid metabolism, oxidative stress as well as the hemostasis cascade have been reported to influence the susceptibility to ischemic heart disease, stroke, and venous thromboembolism in men. However, the relevant data for postmenopausal women are sparse. Glycoprotein IIIa Leu33Pro polymorphism is correlated with a sixfold increase in the risk of myocardial infarction in homozygous carriers of the unfavorable *PLA2* (pro/pro) allele, compared with *PLA1* homo- or heterozygotes (9). Apolipoprotein E2/E3/E4 polymorphism is associated with increased blood cholesterol concentrations as well as with increased risk of myocardial infarction (10). Methylenetetrahydrofolate reductase (*MTHFR*) Ala222-Val polymorphism may lead to elevated Hcy levels that are associated with increased risk of thromboembolism, atherosclerosis, and myocardial infarction (11). Apolipoprotein B Arg3500Gln polymorphism is associated with increased LDL cholesterol and elevated risk of ischemic heart disease (12). Paraoxonase 1 Gln192Arg polymorphism is related to augmented exposure of HDL to oxidation (13). Plasminogen activator inhibitor 1 4G/5G polymorphism is responsible for increased levels of plasminogen activator inhibitor (PAI)-1, leading to an elevated risk for obesity (BMI), hypertension, dyslipidemia, insulin resistance, metabolic syndrome, and type 2 diabetes mellitus (14). Cholesterol-7 α -hydroxylase A-204C polymorphism increases the expression of circulating cholesterol levels (15). Finally, cholesterol ester transfer protein (*TaqIB*) B1/B2 polymorphism is correlated with reduced levels of HDL cholesterol and increased risk of myocardial infarction in men (16). In order to globally assess the diptych CVD-androgenicity, the whole spectrum of the aforementioned polymorphisms has been included in this study.

The preventive management of the postmenopausal women with respect to their personal CVD risk bears a high significance in clinical practice. Given the paucity of data in postmenopausal women, the present study aimed to investigate the association of genetic polymorphisms known to affect CVD risk in men with anthropometric and hormonal factors in healthy postmenopausal women.

Subjects and methods

Subjects

Subjects were recruited from the Menopause Clinic of the Second Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital. They

consisted of 84 women aged 49–69 years who had been menopausal for at least 1 year. Women who were past users of HT, tibolone, or raloxifene were not included in the study unless they had been off-therapy for at least 6 months.

Before recruitment, patients had a gynecological and biochemical evaluation that included: bimanual examination, Papanicolaou (Pap) smear and transvaginal sonography, breast examination and mammography, thyroid–liver–renal function, as well as blood coagulation tests and bone densitometry. Criteria for inclusion were the absence of climacteric complaints, an endometrial thickness <5 mm, the absence of a history of gynecological malignancy, ischemic heart disease, thromboembolism, diabetes mellitus, non-treated thyroid dysfunction, and the intake of lipid-lowering or antihypertensive medication. Patients were instructed to fast and not to smoke for 12 h before blood collection. Samples were centrifuged immediately and stored at -80°C , until assayed.

Anthropometric components were assessed in the morning in light clothing, in order to estimate BMI and WHR. Height was measured by using a stadiometer in the upright position and weight was measured on an electronic scale. BMI was calculated using the following equation: $\text{BMI} = \text{body weight (kg)} / \text{height}^2 (\text{m})$. Waist circumference was measured at the midpoint between the bottom of the rib cage and the top of the lateral border of the iliac crest during minimal respiration. Hip circumference was measured approximately 10 cm below the lateral border of the iliac crest. WHR was estimated using the values resulting from the above measurements. Written informed consent was obtained by all participants. The local Institutional Review Board has approved the present study.

Hormonal assays

Follicle-stimulating hormone (FSH) and luteinizing hormone were measured with the Microparticle Enzyme Immunoassay kit: Abbott AxSYM measured on the AxSYM analyzer (Abbott Laboratories). The total coefficient of variation (CV) ranged from 5.3 to 8.5%. 17- β -Estradiol (E_2) was measured with a commercial enzyme immunoassay kit: DSK-10-4300 (Diagnostic Systems Laboratories Inc., Webster, TX, USA). The total CV ranged from 4.3 to 6.1% and the analytical sensitivity was 8 pg/ml. Δ -4-Androstenedione (Δ 4A) was measured with an IBL 'Androstenedione ELISA' kit (IBL GmbH, Hamburg, Germany). The inter-assay CV ranged from 6.5 to 8.1%. Total testosterone and DHEA- SO_4 were measured with DPC 'Total Testosterone' and 'DHEA- SO_4 ' Immulite analyzer kits (Diagnostic Products Corporation, Los Angeles, CA, USA). The total CV ranged from 8.0 to 16.0% and from 8.1 to 15% respectively. Sex hormone-binding globulin (SHBG) concentrations were measured with a DPC Immulite SHBG chemiluminescent enzyme

immunometric assay kit (Diagnostic Products Corporation). The total CV ranged from 4.1 to 9.2%. The free estrogen index (FEI) was calculated using total E₂ and SHBG values by the following equation: $FEI = E_2 \text{ (pg/ml)} \times 0.367 / SHBG \text{ (nmol/l)}$. The free androgen index (FAI) was calculated using total testosterone and SHBG values by the following equation: $FAI = \text{testosterone (ng/ml)} \times 3.47 \times 100 / SHBG \text{ (nmol/l)}$. Fasting blood samples were also used to assess Hcy levels. Total Hcy concentrations were measured by the Abbott commercial kit: IMx Hcy (Abbott Lab). The total CV (%) and the sensitivity was 4.3% and 0.5 µmol/l respectively.

Gene polymorphism assays

Isolation of genomic DNA was made from 200 µl whole blood treated with EDTA by using the blood spin protocol of QIAamp DNA Blood Mini Kit from Qiagen Corporation. Measurement of polymorphisms was made after Multiplex-PCR and Array Tube^R hybridization using the coro/check protocol for solas 2 (Ogham Diagnostics GmbH, Münster, Germany). The following polymorphisms were assessed: glycoprotein IIIa Leu33-Pro, apolipoprotein E2/E3/E4, MTHFR Ala222Val, apolipoprotein B Arg3500Gln, paraoxonase 1 Gln192Arg, plasminogen activator inhibitor 1 4G/5G, cholesterol-7 α-hydroxylase A-204C, and cholesterol ester transfer protein (TaqIB) B1/B2 polymorphism.

Statistical analysis

Statistical analysis was performed with STATA 8.0 statistical software (Stata Corporation, College Station, TX, USA). Polymorphisms have been treated as ordinal, 0–1–2 variables (0, wild-type; 1, heterozygous; 2, homozygous). At the univariate approach, the associations between polymorphisms and hormone variables were evaluated by means of Spearman's rank correlation coefficient.

Subsequently, concerning MTHFR polymorphism, to adjust the univariate *P* for BMI, ordinal logistic regression was performed for all hormone variables in Tables 2 and 3 having exhibited a statistically significant association (except for FEI). More specifically, using the first, second, and third quartiles, a four-level variable was created for each hormone. The four-level variable was treated as the dependent variable in the ordinal logistic model, whereas BMI and MTHFR polymorphism status were included as independent variables (covariates). Regarding the effect of the MTHFR polymorphism status, a linear effect was assumed in the log-odds scale; thus, no dummy variables were created and the polymorphism status was again treated as a 0–1–2 variable. Concerning all models, the proportionality-of-odds assumption was checked by the appropriate likelihood ratio test.

The proportionality-of-odds assumption was valid for all hormone parameters examined, except for FEI. For the latter, linear regression was performed, with BMI and MTHFR status entered as covariates. The normality assumption was verified using the Shapiro–Wilk criterion upon the studentized (jackknifed) residuals.

Results

Table 1 presents the baseline demographic characteristics of the 84 postmenopausal women under investigation. Women were on average 8 years postmenopausal, slightly overweight, and normotensive while 30% of them were smokers.

Tables 2 and 3 present the association of the genetic polymorphisms studied with the hormonal profile of the participants. There is a statistically significant association between MTHFR polymorphism and levels of testosterone, SHBG, FAI, and FEI in postmenopausal women ($P=0.001$, $P=0.047$, $P=0.0004$, and $P=0.014$ respectively) and a trend toward a positive correlation with DHEA sulfate (DHEAS) and Δ4A ($P=0.053$ and $P=0.054$ respectively). On the other hand, an inverse correlation has been observed between SHBG and the presence of MTHFR polymorphisms ($P=0.047$). These associations were independent of smoking status or lipid levels across groups. The association of MTHFR polymorphism with testosterone and FAI persisted after adjustment for BMI ($P=0.007$ and $P=0.005$ respectively). Furthermore, the E2/E3 and E3/E4 genotypes of the apolipoprotein E polymorphism associated with testosterone ($P=0.023$). Finally, there was a statistically significant positive association between glycoprotein IIIa Leu33Pro polymorphism and FSH levels ($P=0.01$).

Table 4 presents the association of the genetic polymorphisms studied with Hcy plasma concentrations (Hcy), BMI, and WHR. MTHFR Ala222Val polymorphism bears a statistically significant association with BMI and WHR ($P=0.027$ and $P=0.044$ respectively). Heterozygosity and homozygosity for this polymorphism were both related to higher mean BMI (28.6 ± 5.3 and 26.7 ± 2.6 respectively) compared with wild-type (24.8 ± 3.3). Similarly, WHR mean values were higher in heterozygous and homozygous carriers (0.88 ± 0.05 and 0.91 ± 0.05 respectively) compared with wild-type (0.85 ± 0.08). Smoking and lipid levels did not affect this association.

Discussion

The present study demonstrates an association of MTHFR Ala222Val polymorphism with central adiposity and indices of androgenicity in healthy postmenopausal women. More specifically, the presence of the MTHFR polymorphism was associated with

Table 1 Study group characteristics.

Demographic and anthropometric variables	Mean	S.D.	Min	Max
Age	57.7	4.9	49	69
Age at menarche	13.0	1.4	10	17
Age at menopause	49.7	4.0	31	55
Systolic arterial pressure	116.1	13.4	80	140
Diastolic arterial pressure	77.6	10.3	60	120
BMI (body mass index)	26.8	4.4	20.2	44.6
WHR (waist-to-hip ratio)	0.87	0.07	0.73	0.99
Categorical variables	Frequency		Percentage	
Smoking				
0 (no)	56/84		66.7%	
1 (occasionally)	24/84		28.6%	
2 (on daily basis)	4/84		4.7%	
Exercise				
0 (no)	20/84		23.8%	
1 (<1 h/week)	14/84		16.7%	
2 (1–4 h/week)	32/84		38.1%	
3 (>3 h/week)	18/84		21.4%	
Diet (calcium intake)				
0 (none or minimum intake)	76/84		21.4%	
1 (medium: 1 glass of milk or 1 cup of yogurt or 25–50 g of cheese per day)	8/84		69.1%	
2 (rich: >2 glasses of milk or two cups of yogurt or 75–100 g of cheese per day)	56/84		9.5%	
Surgical menopause				
No	76/84		90.5%	
Yes	8/84		9.5%	
Serum lipids	Mean	S.D.	Min	Max
Total cholesterol (mg/dl)	234.3	43.5	145	339
LDL (mg/dl)	150.7	39.0	67	227
HDL (mg/dl)	63.6	15.3	40	101
Triglycerides (mg/dl)	100.9	30.5	44	162

higher BMI, WHR, and serum androgens and with lower SHBG. On the contrary, apolipoprotein E2/E3 and E3/E4 polymorphisms are associated with suppressed testosterone levels, acting inversely compared with MTHFR polymorphism.

Hcy is a thiamine acid metabolized via two major pathways. When there is an excess of methionine, Hcy converts, through the trans-sulfuration pathway that requires vitamin B₆ as a co-factor, into cystathionine, and is finally metabolized to cysteine. By contrast, when methionine levels are low, Hcy is metabolized to methionine, through remethylation, a process that is catalyzed by the enzyme MTHFR. The Ala222Val (C677T) polymorphism in the MTHFR gene is characterized by a substitution of alanine for valine leading to the formation of a thermolabile enzyme that maintains only 50% of the normal activity. The wild-type genotype (Ala/Ala) exists in 75%, while heterozygosity (Ala/Val) exists only in 10% of the general population. Homozygosity for the valine variant (Val/Val) is present in about 15% of the general population (Fig. 1).

The Ala222Val polymorphism in the MTHFR gene has been associated with increased risk of ischemic heart disease, stroke, and venous thromboembolism (11, 19, 20). An increase in serum Hcy has been suggested as a possible mechanism (21). In contrast to

this hypothesis, data indicate that lowering of Hcy levels by vitamin B and folate supplementation has not resulted in CVD risk reduction (22). Furthermore, Ala222Val polymorphism, although reported to increase CVD risk, may not be associated with hyperhomocysteinemia (18).

Insulin resistance has been proposed as a possible mechanism linking defects in Hcy catabolism and CVD risk. Polycystic ovary syndrome (PCOS), characterized by central adiposity, increased androgenicity, and insulin resistance, has been associated with defects in Hcy metabolism. Carlsen *et al.* (23) reported a positive association between Hcy levels and free testosterone index, as well as $\Delta 4$ levels in PCOS women. This association persisted after adjustment for fasting plasma insulin C-peptide. According to the above, there is an independent positive association between androgens and Hcy metabolism, which may be independent of glucose disposal.

Further to the association with androgens, many studies have demonstrated an association of Hcy metabolism with insulin resistance. Insulin levels are associated with increased plasma levels of Hcy in healthy, non-obese subjects (24). Several studies support the hypothesis that insulin resistance is the determinant of Hcy metabolic defect in PCOS women.

Table 2 Association between genetic polymorphisms and follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17- β -estradiol (E₂), and free estrogen index (FEI).

Polymorphisms	FSH (mIU/ml) mean (S.D.)	<i>P</i>	LH (mIU/ml) mean (S.D.)	<i>P</i>	E ₂ (pg/ml) mean (S.D.)	<i>P</i>	FEI mean (S.D.)	<i>P</i>
Methylenetetrahydrofolate reductase Ala222Val		0.936		0.376		0.773		0.014
Wild-type	88.4 (43.4)		46.1 (15.5)		28.4 (8.5)		0.15 (0.04)	
Heterozygous	68.5 (17.3)		35.6 (13.4)		22.8 (4.5)		0.18 (0.10)	
Homozygous	89.9 (30.2)		44.4 (10.4)		36.3 (16.5)		0.27 (0.07)	
Glycoprotein IIIa Leu33Pro		0.010		0.614		0.203		0.920
Wild-type	86.1 (33.6)		42.4 (15.2)		27.8 (10.1)		0.18 (0.08)	
Heterozygous	56.2 (16.8)		36.3 (11.8)		25.4 (10.6)		0.19 (0.12)	
Apolipoprotein E2/E3/E4		0.879		0.633		0.863		0.497
E3/E3	81.2 (32.5)		43.0 (15.4)		27.4 (10.7)		0.19 (0.10)	
E3/E2	76.8 (56.6)		31.4 (18.2)		24.3 (9.3)		0.17 (0.03)	
E3/E4	82.2 (23.2)		40.1 (5.6)		26.2 (7.8)		0.15 (0.08)	
Apolipoprotein B Arg3500Gln		0.542		0.113		0.344		0.231
Wild-type	79.6 (33.5)		40.3 (14.2)		27.4 (10.0)		0.18 (0.09)	
Heterozygous	82.8 (15.0)		55.3 (14.4)		20.0 (15.0)		0.10 (0.14)	
Paraoxonase 1 Gln192Arg		0.875		0.914		0.431		0.548
Wild-type	76.3 (27.2)		39.3 (12.6)		28.2 (9.8)		0.18 (0.09)	
Heterozygous	81.9 (28.6)		45.9 (14.9)		26.9 (11.0)		0.17 (0.07)	
Homozygous	85.5 (67.1)		29.7 (14.3)		24.5 (7.6)		0.24 (0.11)	
Plasminogen activator inhibitor 1 4G/5G		0.297		0.097		0.776		0.930
Wild-type	92.1 (43.4)		40.9 (6.5)		23.1 (4.4)		0.12 (0.06)	
Heterozygous	85.5 (34.2)		47.0 (18.4)		28.6 (12.2)		0.20 (0.06)	
Homozygous	70.0 (23.4)		35.9 (11.4)		26.3 (7.6)		0.18 (0.11)	
Cholesterol-7 α -hydroxylase A-204C		0.911		0.770		0.146		0.244
Wild-type	77.4 (30.5)		42.3 (17.2)		29.8 (13.2)		0.16 (0.08)	
Heterozygous	84.8 (40.0)		40.7 (12.1)		27.1 (8.4)		0.19 (0.09)	
Homozygous	72.1 (9.5)		40.4 (16.6)		22.3 (3.8)		0.20 (0.09)	
Cholesterol ester transfer protein B1/B2		0.880		0.890		0.356		0.236
Homozygous	85.6 (36.0)		42.5 (20.0)		26.1 (6.8)		0.16 (0.03)	
Heterozygous	73.0 (24.7)		41.9 (14.4)		24.1 (7.1)		0.16 (0.08)	
Wild-type	77.9 (25.2)		38.3 (9.7)		31.7 (13.6)		0.23 (0.12)	

Bold indicates statistically significant ($P < 0.05$) associations between the examined variants. Bold and italics indicates the presence of weaker correlations ($0.05 < P < 0.1$) between the variants.

Table 3 Association between genetic polymorphisms and male sex hormones: testosterone (Testo), sex hormone-binding globulin (SHBG), DHEA sulfate (DHEAS), Δ -4-androstenedione (Δ 4A), and free androgen index (FAI).

Polymorphisms	Testosterone (ng/ml) mean (S.D.)	<i>P</i>	SHBG (nmol/l) mean (S.D.)	<i>P</i>	DHEAS (ng/dl) mean (S.D.)	<i>P</i>	Δ 4A (ng/dl) mean (S.D.)	<i>P</i>	FAI mean (S.D.)	<i>P</i>
Methylenetetrahydrofolate reductase Ala222Val		0.001		0.047		0.053		0.054		0.0004
Wild-type	0.23 (0.13)		75.9 (27.3)		598.9 (272.0)		129.8 (63.2)		1.06 (0.56)	
Heterozygous	0.37 (0.12)		60.8 (41.0)		833.3 (527.2)		175.6 (69.7)		2.93 (1.97)	
Homozygous	0.42 (0.15)		60.1 (4.2)		963.4 (358.5)		224.8 (130.3)		2.36 (0.89)	
Glycoprotein IIIa Leu33Pro		0.119		0.461		0.870		0.497		0.175
Wild-type	0.30 (0.15)		68.7 (35.0)		778.2 (473.9)		162.3 (86.5)		1.89 (1.41)	
Heterozygous	0.38 (0.13)		57.8 (29.2)		735.3 (316.1)		179.1 (73.7)		2.97 (2.29)	
Apolipoprotein E2/E3/E4		0.023		0.752		0.149		0.236		0.079
E3/E3	0.36 (0.13)		61.2 (25.4)		838.3 (489.0)		180.7 (86.3)		2.55 (1.82)	
E3/E2	0.17 (0.14)		54.7 (30.7)		590.3 (272.3)		130.0 (77.3)		1.15 (0.98)	
E3/E4	0.26 (0.14)		79.1 (50.5)		562.3 (204.7)		136.5 (74.3)		1.41 (0.78)	
Apolipoprotein B Arg3500Gln		0.517		0.218		0.178		0.506		0.304
Wild-type	0.33 (0.15)		65.2 (34.5)		789.3 (441.9)		168.6 (85.0)		2.20 (1.70)	
Heterozygous	0.26 (0.11)		83.0 (12.9)		435.0 (70.7)		131.5 (0.7)		1.14 (0.65)	
Paraoxonase 1 Gln192Arg		0.589		0.167		0.953		0.579		0.878
Wild-type	0.34 (0.12)		74.5 (41.3)		827.3 (511.1)		178.7 (77.2)		2.21 (1.79)	
Heterozygous	0.31 (0.18)		64.0 (21.0)		638.2 (352.0)		146.3 (95.1)		1.60 (1.12)	
Homozygous	0.31 (0.09)		43.3 (31.2)		998.0 (332.4)		190.3 (51.4)		3.41 (2.25)	
Plasminogen activator inhibitor 1 4G/5G		0.204		0.835		0.370		0.138		0.465
Wild-type	0.31 (0.13)		80.0 (53.0)		610.2 (306.7)		120.8 (54.8)		1.91 (1.38)	
Heterozygous	0.27 (0.16)		53.9 (18.1)		818.4 (572.5)		155.3 (73.4)		1.73 (1.19)	
Homozygous	0.36 (0.13)		68.4 (33.3)		773.2 (369.5)		186.2 (91.7)		2.52 (2.10)	
Cholesterol-7 α -hydroxylase A-204C		0.766		0.119		0.828		0.660		0.299
Wild-type	0.34 (0.18)		82.2 (43.0)		875.9 (578.2)		171.7 (81.1)		1.79 (1.23)	
Heterozygous	0.31 (0.14)		59.9 (25.1)		677.1 (333.6)		168.4 (97.6)		2.12 (1.88)	
Homozygous	0.34 (0.09)		54.3 (26.5)		792.1 (396.3)		153.6 (55.5)		2.78 (1.92)	
Cholesterol ester transfer protein B1/B2		0.112		0.563		0.258		0.959		0.181
Homozygous	0.23 (0.11)		65.5 (25.0)		855.1 (652.1)		155.1 (83.0)		1.42 (1.14)	
Heterozygous	0.34 (0.12)		67.2 (39.3)		812.1 (333.5)		172.0 (84.7)		2.30 (1.54)	
Wild-type	0.36 (0.18)		62.5 (34.7)		559.1 (367.6)		150.4 (78.9)		2.60 (2.42)	

Bold indicates statistically significant ($P < 0.05$) associations between the examined variants. Bold and italics indicates the presence of weaker correlations ($0.05 < P < 0.1$) between the variants.

Table 4 Association between genetic polymorphisms and homocysteine plasma concentrations (Hcy), body mass index (BMI), and waist-to-hip ratio (WHR).

Polymorphisms	Hcy (mmol/l) mean (s.d.)	P	BMI mean (s.d.)	P	WHR mean (s.d.)	P
Methylenetetrahydrofolate reductase		0.486		0.027		0.044
Ala222Val						
Wild-type	14.0 (8.0)		24.8 (3.3)		0.85 (0.08)	
Heterozygous	10.7 (3.4)		28.6 (5.3)		0.88 (0.05)	
Homozygous	11.5 (3.1)		26.7 (2.6)		0.91 (0.05)	
Glycoprotein IIIa Leu33Pro		0.758		0.317		0.145
Wild-type	11.8 (3.8)		26.1 (3.6)		0.86 (0.07)	
Heterozygous	13.6 (9.9)		28.5 (6.2)		0.90 (0.05)	
Apolipoprotein E2/E3/E4		0.678		0.430		0.585
E3/E3	12.1 (6.6)		27.4 (4.6)		0.88 (0.06)	
E3/E2	13.8 (4.6)		25.2 (4.5)		0.83 (0.04)	
E3/E4	11.0 (3.2)		25.9 (3.4)		0.89 (0.06)	
Apolipoprotein B Arg3500Gln		0.249		0.062		0.033
Wild-type	12.4 (5.9)		27.0 (4.4)		0.88 (0.06)	
Heterozygous	8.5 (0.1)		22.1 (2.1)		0.79 (0.01)	
Paraoxonase 1 Gln192Arg		0.378		0.209		0.365
Wild-type	13.7 (7.6)		26.7 (5.7)		0.88 (0.06)	
Heterozygous	10.8 (3.7)		26.6 (3.5)		0.88 (0.08)	
Homozygous	11.4 (3.9)		27.4 (2.8)		0.85 (0.05)	
Plasminogen activator inhibitor 1 4G/5G		0.664		0.928		0.031
Wild-type	11.1 (4.1)		26.1 (2.7)		0.91 (0.07)	
Heterozygous	12.2 (3.8)		26.9 (4.0)		0.88 (0.06)	
Homozygous	10.6 (3.7)		27.2 (5.7)		0.84 (0.06)	
Cholesterol-7 α -hydroxylase A-204C		0.118		0.485		0.930
Wild-type	14.2 (8.6)		25.8 (3.9)		0.86 (0.07)	
Heterozygous	11.9 (3.5)		27.1 (4.7)		0.89 (0.07)	
Homozygous	8.6 (1.7)		27.4 (4.7)		0.86 (0.06)	
Cholesterol ester transfer protein B1/B2		0.250		0.221		0.945
Homozygous	13.3 (4.2)		24.6 (3.2)		0.87 (0.06)	
Heterozygous	10.4 (3.4)		27.7 (3.1)		0.89 (0.06)	
Wild-type	10.9 (3.4)		27.7 (6.8)		0.87 (0.09)	

Bold indicates statistically significant ($P < 0.05$) associations between the examined variants. Bold and italics indicates the presence of weaker correlations ($0.05 < P < 0.1$) between the variants.

Guzelmeric *et al.* (17) reported a positive association of Hcy levels with BMI, total testosterone, free testosterone, insulin levels, and HOMA-IR in women with PCOS and that BMI is the main factor predicting Hcy levels. According to Bjork *et al.* (27), serum Hcy associated positively with serum insulin as well as with insulin resistance independently of age and sex. By contrast, Schachter *et al.* (26) suggested that insulin resistance and hyperinsulinemia in patients with PCOS is associated with elevated plasma Hcy, independently of body weight.

Several mechanisms may explain the association of Hcy metabolism with insulin resistance. Studies in cell cultures have indicated that elevated insulin levels affect the activity of enzymes participating in Hcy metabolism and this has been most consistently shown for cystathionine β -synthase, which converts Hcy to cysteine. MTHFR and methionine synthase activity were also influenced by insulin levels, although the evidence seems to be controversial. McCarty *et al.* (25) reported that the association between insulin resistance and hyperhomocysteinemia may reflect that hyperinsulinemia suppresses hepatocyte expression of cystathionine β -synthase, as demonstrated in rats. The above alterations caused by increased insulin levels, as in

insulin resistance, lead to tissue Hcy accumulation. Defects in Hcy metabolism, through decreased glutathione levels and the generation of reactive oxygen species (ONOO⁻), may lead to endothelial damage that favors insulin resistance and elevated insulin levels (28, 29). According to the above hypothesis, Hcy

Figure 1 MTHFR allele variant to wild-type ratio of mean sex hormone levels. Wt, wild-type; het, heterozygous; hom, homozygous.

metabolism and insulin resistance are related in terms of a vicious cycle and the atherogenic effects of Hcy may, in part, be manifested through insulin resistance.

In contrast to previous reports, the present study indicates that MTHFR Ala222Val polymorphism does not significantly increase Hcy levels. However, we observed a statistically significant association between MTHFR Ala222Val polymorphism and androgenicity, BMI, and WHR. Insulin resistance bears an independent positive association with total testosterone and FAI (30), while it correlates positively with indices of obesity such as BMI and WHR (31). We propose the following hypothesis: genetic defects in Hcy metabolism may result in impaired Hcy catabolism which, at a cellular level, may lead to endothelial dysfunction thus causing insulin resistance. This metabolic disturbance is related to increased androgenicity, BMI, and WHR.

Apolipoprotein E polymorphism and more specifically E2/E3 and E3/E4 genotypes are associated in the present study with lower levels of testosterone. Apolipoprotein E is a peptide responsible for the transportation and redistribution of lipids, including cholesterol. Cholesterol acts as the main substrate for steroidogenesis in both adrenal and gonads. Although both steroidogenic tissues can synthesize ApoE and cholesterol *de novo*, about 80% of the cholesterol precursor for steroidogenesis is provided by serum lipoproteins. Therefore, ApoE gene is assumed to be the main regulator of cholesterol precursor concentration in both circulation and tissues (32, 33). Data on the association of the ApoE polymorphism and serum androgens in postmenopausal women are sparse and contradicting. According to Zofkova *et al.* (34), postmenopausal women bearing the E4 allele have increased serum androgens compared with women without the E4 allele. On the contrary, Barrett-Connor *et al.* (35) found no association of the E4 allele with serum testosterone. The same group, however, reported lower DHEAS levels in women bearing the E4 allele. These data, therefore, are preliminary and should be interpreted with caution, unless corroborated by larger studies.

An important limitation of this study is the relatively small sample size. As a consequence, the power of the study is limited and type II errors might have prevented the emergence of other weaker associations. However, the associations that have reached statistical significance in this context of limited sample size may be thought of as valid and reproducible in larger samples. Indeed, the magnitude of the herein documented associations was such that it overcame the critical *P* value (type I error) of 0.05 despite the relatively small sample size. An additional limitation is the fact that we did not assess quantitatively insulin resistance.

Commenting further on the limitations of this study, it should be kept in mind that the correlation approach adopted herein does not substitute for biochemical research studies. This study is the first to provide

evidence on the examined associations, but undoubtedly further biochemical studies are needed to elucidate the molecular mechanisms that underlie and explain the present findings. Nevertheless, to the best of our knowledge, this is the first study to indicate a link between genetic defects of Hcy metabolism and indices of androgenicity in healthy postmenopausal women.

In conclusion, the presence of Ala222Val polymorphism in the MTHFR gene is associated with increased central adiposity and serum androgen levels. The significance of this association with respect to the risk of CVD remains to be investigated.

Acknowledgements

We would like to thank the Hormonal and Biochemical Laboratory of the University of Athens in Aretaieio Hospital, who contacted the required biochemical, hormonal assays and genetic analysis, as well as all the colleagues who participated in this protocol.

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Received 13 February 2008

Accepted 16 May 2008