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Association of Methylenetetrahydrofolate Reductase Gene 677C>T Polymorphism with Hypertension in Older Women in a Population of Buenos Aires City

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Abstract

We examined the relationship between the 677C >T polymorphism in the MTHFR gene and tHcy in normotensive (NT) and hypertensive (HT) subjects and the influence of sex and age in a cross-sectional study. Smoking habits, history of vascular disease, diabetes, and tHcy were significantly associated with T allele as hypertension risk factors. The T allele was significantly related with higher tHcy in (i) men versus women ($P < .01$), (ii) men and women older than 47 years versus the younger ones ($P < .05$ and $P < .001$, respectively), (iii) HT women versus NT women ($P < .01$), and (iv) older HT women versus older NT women ($P < .01$). We found an association between the 677C>T MTHFR polymorphism and tHcy with hypertension that in women is manifested with age.

Keywords: age, gender, hyperhomocisteinemia, hypertension, MTHFR

INTRODUCTION

Several experimental, clinical, and epidemiological studies have reported that moderate hyperhomocysteinemia (HHcy) plays a role as an independent risk factor for coronary and cerebrovascular atherosclerosis, carotid artery stenosis, and venous thrombosis (1–4) independent of conventional risk factors such as diabetes mellitus, smoking, and hyperlipidemia (5,6). Hyperhomocysteinemia has been also linked to hypertension (7,8), and it was proposed that the interaction between homocysteine (Hcy) and endothelium in hypertensive (HT) patients may promote thrombogenesis and atherogenesis, leading to adverse cardiovascular events (5,8,9). Homocysteine is synthesized through *S*-adenosyl-L-methionine-dependent methylation reactions and is catabolized by remethylation and transsulfuration reactions that form methionine and cystathionine, respectively (10). Methionine synthase together with betaine–homocysteine methyltransferase are the two enzymes involved in the remethylation pathway (11). Methylenetetrahydrofolate reductase (MTHFR) reduces methylenetetrahydrofolate to methyltetrahydrofolate, which then donates its methyl group by methionine synthase to Hcy to form methionine (11). A reduction in the activity of this enzyme is very common, and can be the result of both genetic and nongenetic factors (12). Until now, four single nucleotide polymorphisms (SNPs) (203G>A, 677C>T, 1286A>C, and 1793G>A) in the MTHFR gene have been studied in relation to different pathologies (13). Within this four SNPs, 677C>T results in the replacement of alanine for valine that produced a thermolabile enzyme with decreased MTHFR-specific activity. As a result, homozygous 677TT has 30% of residual activity and heterozygous 677CT has 70% of residual activity when compared with 677CC variant (14–16). This reduced specific activity results in the enhanced level of tHcy and low folate level on plasma (15,17). The frequency of 677C>T polymorphism varies according to the ethnic group analyzed (18). To date, despite the high prevalence of hypertension, very few studies have been carried out on Argentinians with regard to the polymorphisms in Hcy-associated genes such as the MTHFR gene and hypertension, in order to address the role of these polymorphisms and their contribution to premature atherosclerosis. The objective of this cross-sectional study was to evaluate the relationship between tHcy concentrations

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in a sample of people of both sexes living in Buenos Aires city, and the prevalence of the 677C>T MTHFR polymorphism taking into account age and gender.

MATERIALS AND METHODS

We carried out a cross-sectional study comprising 225 subjects aged 18–87 years, randomly selected in three health centers from Buenos Aires city, belonging to the University Hospital of Universidad Abierta Interamericana. All were physically active. The exclusion criteria were pregnancy, use of any type of (multi-) vitamin tablet in the preceding year, and younger than 18 years. Written informed consent was obtained from all participants before the study began. The hospital local ethics committee approved the study protocol in accordance with the Declaration of Helsinki as revised in 1983. A questionnaire with clinical data was completed by all subjects on the day of enrollment. The medical history also included family history in the first-degree relatives. Participants were classified as HT if they were taking antihypertensive medication. The risk factors of vascular disease recorded in this study were hypertension ($\geq$140/90 mm Hg), diabetes (glysemia $\geq$6.96 mmol/L), renal failure (creatinemia >132.5 μmol/L), hyperlipidemia (high-density lipoprotein [HDL] <1.04 mmol/L for men and <1.30 for women, low-density lipoprotein [LDL] >3.36 mmol/L, triglyceride >1.50 g/L, and total cholesterol >5.20 mmol/L) (19). History of smoking and antihypertensive treatment were also noted. Body mass index (BMI) was calculated as weight (kg)/height (m^2)

Blood Collection

Blood samples were collected after an overnight fast (>10 h) by venipuncture into an ethylenediaminetetraacetic acid (EDTA)-containing tube. A separated aliquot was kept for DNA extraction. Plasma samples were obtained by double centrifugation at room temperature for 15 minutes at 2000 × *g*. The plasma aliquots were immediately frozen at −80°C until use.

Laboratory Measurements

Biochemical measurements including cholesterol, triglycerides, HDL, LDL, and creatinine were carried out using Metrolab 2300 auto-analyzer (Metrolab SA, Buenos Aires, Argentina). Plasma tHcy was determined by chemiluminescence using ADVIA Centaur apparatus (Siemens Diagnostics Inc., Tarrytown, NY, USA).

MTHFR Mutation Analysis

DNA Extraction

Genomic DNA was isolated from nucleated blood cells using FlexiGene DNA Kit (QIAGEN GmbH, Hilden, Germany) following the manual instruction. The DNA samples were kept at −80°C until analyzed.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Analysis

The 677C>T *MTHFR* gene mutation was detected using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method according to Frosst et al. (15). Briefly, about 50–80 ng DNA samples were amplified in a final volume of 25 μL containing 1 × PCR buffer with 2.0 mmol/L $MgCl_2$, 0.75 unit GoTaq DNA polymerase (Promega, Madison, WI, USA) 200 μmol/L dNTP, and 0.5 μmol/L of each primer (5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and 5′-AGGACGGTGCGGTGAGAGTG-3′). Polymerase chain reaction was performed in a Mastercycler personal thermocycler (Eppendorf, Hamburg, Germany), and the profile consisted of an initial melting step of 5 minutes at 94°C, followed by 45 cycles of 40 seconds at 94°C, 50 seconds at 62°C, and 40 seconds at 72°C, and a final elongation step of 7 minutes at 72°C. The restriction enzyme *Hinf* I (Promega) was used to distinguish the 677C>T polymorphism, and the gain of a *Hinf* I restriction site occurs in the polymorphic allele. The wild genotype (677CC) has a single band representing the entire 198-bp fragment, and the heterozygous genotype (677CT) results in three fragments of 198, 175, and 23 bp, whereas the homozygous genotype for the MTHFR mutation (677TT) results in two fragments of 175 and 23 bp. Finally, the products of the *Hinf* I digestion were electrophoresed on a 3% agarose. To ensure quality control, genotyping was performed blindly, and random samples were tested twice by different persons. The participants were categorized as homozygous wild type (CC), heterozygous for wild type and thermolabile (CT), or homozygous for the thermolabile (TT) variant.

Statistical Analysis

Continuous variables were expressed as mean ± SEM. Differences between the examined quantitative parameters were compared using the Mann–Whitney *U*-test. The frequency of genotype was determined by direct counting. Deviation of the genotype distribution from the Hardy–Weinberg equilibrium was determined using χ^2 test/Fisher's exact test (two tailed). The adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated to estimate the association of different MTHFR genotypes with risk factors for HT. The software package SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used for the analysis. *P* value <.05 was considered statistically significant.

RESULTS

The population sample was composed of 59 men and 166 women aged between 18 and 87 years. Family history of hypertension, coronary artery disease (CAD), stroke, peripheral vascular disease, and dementia or cognitive impairment in first-degree relatives and the subjects themselves were manifested in 108 (48%) persons. Hypertension was present in 75 (33.3%) subjects and

Table 1. Demographic and cardiovascular risk variables of subjects and genotype frequencies for MTHFR C677T polymorphism

	Overall sample ($n = 225$)	Male ($n = 59$)	Female ($n = 166$)	P value	NT ($n = 150$)	HT ($n = 75$)	P value
Age (y)	45.9 ± 1.1	49.7 ± 1.9	44.5 ± 1.3	<.05	41.00 ± 1.25	56.29 ± 1.66	<.001
BMI (kg/m^2)	26.8 ± 0.4	27.5 ± 0.7	26.5 ± 0.5	NS	26.14 ± 0.49	28.13 ± 0.71	<.05
Diabetes mellitus, n (%)	16 (7.1)	8 (13.6)	8 (4.8)	<.05	5 (3.4)	11 (15.3)	<.01
Current smokers, n (%)	82 (36.4)	22 (37.3)	60 (36.1)	NS	59 (40.1)	21 (29.2)	NS
History of vascular diseases in at least themselves or one or more in the first degree relatives, n (%)	108 (48.0)	27 (45.8)	81 (48.8)	NS	64 (42.7)	44 (58.7)	NS
Previous vascular disease in themselves, n (%)	38 (19.9)	7 (11.9)	31 (18.7)	NS	20 (13.6)	18 (25.0)	NS
Cholesterol (mmol/L)	5.38 ± 0.08	5.49 ± 0.16	5.33 ± 0.10	NS	5.29 ± 0.10	5.66 ± 0.15	<.05
LDL cholesterol (mmol/L)	3.17 ± 1.14	3.15 ± 0.13	3.17 ± 0.09	NS	3.11 ± 0.09	3.40 ± 0.15	NS
HDL cholesterol (mmol/L)	1.41 ± 0.02	1.34 ± 0.04	1.44 ± 0.03	<.05	1.42 ± 0.03	1.36 ± 0.04	NS
Triglycerides (mmol/L)	1.75 ± 0.11	2.15 ± 0.24	1.59 ± 0.12	<.01	1.63 ± 0.13	2.11 ± 0.24	<.05
Creatinine (μmol/L)	73.04 ± 0.88	77.44 ± 1.76	71.28 ± 0.88	<.05	71.3 ± 0.88	75.69 ± 1.76	<.05
Hcy (μmol/L)	8.02 ± 0.19	8.60 ± 0.32	7.78 ± 0.23	<.05	7.58 ± 0.20	8.95 ± 0.40	<.001
MTHFR C677T genotypes							
CC, n (%)	100 (44.4)	28 (47.5)	72 (43.4)		71 (47.3)	29 (38.7)	
CT, n (%)	104 (46.2)	28 (47.5)	76 (45.8)		64 (42.7)	40 (53.3)	
TT, n (%)	21 (9.3)	3 (5.1)	18 (10.8)		15 (10.0)	6 (8.0)	

Abbreviations: BMI – body mass index; HDL – high-density lipoprotein; HT – hypertensive; LDL – low-density lipoprotein; NS – nonsignificant; NT – normotensive.

The results are given in mean ± SEM. Comparisons of male versus female and HT versus NT were performed using the Mann–Whitney U-test. The genotype distribution did not significantly deviate from the Hardy–Weinberg equilibrium ($\chi^2 = 0.67$, df = 1, $P = 0.72$).

vascular diseases in 38 (16.9%) subjects (Table 1). The prevalence of genotypes determined in the overall sample population of this study was CC 44.4%, CT 46.2%, and TT 9.3%. The frequencies of C and T alleles were 0.68 and 0.32, respectively, and this polymorphism was in Hardy–Weinberg equilibrium ($\chi^2 = 0.67$, df = 1, $P = .72$). These frequencies are consistent with other studies of this variant (20,21). Demographic characteristics, cardiovascular risk factors, and genotype frequencies for MTHFR 677C>T polymorphism for the overall sample, male and female and normotensive (NT) and HT are shown in Table 1. Average age, prevalence of diabetes mellitus, and serum concentration of creatinine, tHcy ($P < .05$), and triglycerides ($P < .01$) were lower in female than in male, whereas serum HDL cholesterol was higher in female ($P < .05$). The parameters associated with hypertension were average age ($P < .001$), prevalence of diabetes mellitus ($P < .01$), BMI > 30, total serum cholesterol, tryglicerides, creatinine ($P < .05$), and total plasma tHcy ($P < .001$). There were no significant differences between male and female with regard to BMI > 30, current smokers, previous vascular disease, total serum cholesterol, and LDL cholesterol levels. Finally, between NT and HT there were no significant differences with regard to current smokers, previous vascular disease, and serum LDL cholesterol and HDL cholesterol.

Interactions among different polymorphisms of MTHFR gene and gender, age (younger vs. older than 47 y), tHcy dichotomized with cut-off point 11.0 mmol/L, diabetes, current smokers, coffee consumers, exercise frequency, positive history of vascular diseases in at least themselves or one or more in the first degree relatives, and BMI higher and lower than 30 kg/m^2 were summarized with a view to increase the risk of hypertension (Table 2). It was found that tHcy, smoking, coffee consumption, history of vascular diseases in at least themselves or one or more in the first degree relatives, and diabetes were significantly associated with T allele as risk factors of hypertension.

Table 3 shows plasma concentrations of tHcy analyzed by genotypes in the overall sample and in male and female. The higher tHcy concentrations observed in men with respect to women ($P < .05$) were due to the presence of T allele ($P < .01$) because in those with CC genotype there were no significant differences in tHcy concentrations between men and women. Table 4 displays the concentrations of plasma tHcy analyzed by genotypes, again in male and female but stratified by age (younger and older than 47 y). In the overall sample, subjects older than 47 years have higher plasma tHcy concentrations than the younger ones ($P < .05$ for male and $P < .001$ for female). When discriminated for genotype, these differences were expressed with statistical significance only if the T allele was present ($P < .05$ for male and $P < .001$ for female).

The concentrations of tHcy analyzed using genotypes in male and female NT and HT are shown in Table 5. In the overall sample, the higher concentrations of tHcy observed in HT ($P < .001$) were due to women with the T allele. Finally, when tHcy concentrations were analyzed using genotypes in NT and HT and discriminated for gender and age (Table 6) in men both younger and older than 47 years, there were no significant differences between NT and HT. Only in older women the concentration of tHcy was higher in HT than in NT and significantly related to the presence of T allele ($P < .01$).

Table 2. Association of MTHFR genotypes in NT and HT with other risk factors

	CC				CT				TT				CT + TT			
	NT *n*	HT *n*	OR 95%CI	*P**	NT *n*	HT *n*	OR 95%CI	*P**	NT *n*	HT *n*	OR 95%CI	*P**	NT *n*	HT *n*	OR 95%CI	*P**
Sex																
Male	20	8	1.100 0.427–2.816	1.000	14	14	0.480 0.200–1.151	0.114	2	1	0.857 0.089–7.650	1.000	16	15	0.500 0.221–1.132	0.131
Female	50	22			50	24			14	6			64	30		
Age																
<47 y	49	7	6.417 2.497–16.432	**.000**	37	7	6.069 2.363–15.495	**.000**	11	2	5.500 0.862–33.763	.169	48	9	6.000 2.576–13.928	**.000**
>47 y	21	23			27	31			5	5			32	36		
Smoking																
Never	40	15	1.333 0.571–3.115	.520	39	32	0.293 0.110–0.783	**.015**	10	5	0.667 0.114–4.158	1.000	49	37	0.342 0.143–0.818	**.017**
Current smokers	30	15			25	6			6	2			31	8		
Coffee consumption																
Occasional	41	21	0.606 2.219–1.684	.370	33	28	0.380 0.144–0.985	**.037**	10	4	1.250 0.146–10.776	1.000	43	32	0.472 0.200–1.101	.061
≥3 cups/d	29	9			31	10			6	3			37	13		
Exercise																
Rare/never	55	21	1.571 0.536–4.578	.444	39	26	0.720 0.282–1.823	.525	15	5	6.000 0.307–215.98	.209	54	31	0.938 0.397–2.206	1.000
≥1× wk	15	9			25	12			1	2			26	14		
History of vascular diseases in at least themselves or one or more in the first degree relatives																
No	38	13	1.553 0.603–4.021	.385	38	15	2.241 0.914–5.537	.066	9	1	4.167 0.455–45.820	.193	47	16	2.471 1.094–5.620	**.025**
Yes	32	17			26	23			6	5			32	28		
BMI																
<30 kg/m^2	57	22	1.594 0.595–4.294	.424	50	25	1.857 0.769–4.494	.245	15	5	6.000 0.612–55.298	.209	65	30	2.167 0.949–4.952	.082
>30 kg/m^2	13	8			14	13			1	2			15	15		
Coexistence of diabetes																
No	66	25	3.300 0.881–12.340	.123	62	32	5.813 1.252–26.511	**.049**	15	5	6.000 0.616–55.289	.209	77	37	5.550 1.495–20.373	**.008**
Yes	4	5			2	6			1	2			3	8		
tHcy																
<11.0 mmol/L	62	26	1.022 0.191–4.908	1.000	60	30	3.600 0.983–13.744	**.037**	14	4	5.250 0.452–74.291	.142	74	34	3.731 1.224–11.653	**.018**
>11.0 mmol/L	7	3			5	9			2	3			7	12		

Abbreviations: CI – confidence interval; HT – hypertensive; NT – normotensive; OR – odds ratio.
*Calculated by χ^2 analysis comparing genotype distributions among NT and HT subjects. In bold, *P* values <.05.

Table 3. Plasma Hcy concentration (μmol/L) in the overall sample and in male and female

MTHFR C677T genotype	Overall sample	Male	Female	*P* value
All	8.02 ± 0.19 (236)	8.60 ± 0.32 (68)	7.78 ± 0.23 (168)	<.05
CC	8.06 ± 0.8 (105)	8.09 ± 0.35 (33)	8.05 ± 0.43 (72)	NS
CT	7.95 ± 0.60 (109)	9.02 ± 0.56 (31)	7.52 ± 0.27 (78)	<.01
TT	8.18 ± 1.47 (22)	9.58 ± 1.70 (4)	7.87 ± 0.49 (18)	NS
CT + TT	7.98 ± 0.25 (131)	9.08 ± 0.52 (35)	7.59 ± 0.24 (96)	<.01

Abbreviations: Hcy – homocysteine; NS – nonsignificant.
The results are given in mean ± SEM. Comparisons of female versus male were performed using the Mann–Whitney *U*-test. Number of subjects are given in parentheses.

Table 4. Plasma Hcy concentration (μmol/L) in male and female younger and older than 47 y

	Male			Female		
MTHFR C677T genotype	Younger than 47 y	Older than 47 y	*P* value	Younger than 47 y	Older than 47 y	*P* value
All	7.60 ± 0.40 (22)	9.15 ± 0.47 (37)	<.05	7.10 ± 0.25 (91)	8.75 ± 0.40 (75)	<.001
CC	7.60 ± 0.27 (12)	8.76 ± 0.65 (16)	NS	7.33 ± 0.43 (43)	8.44 ± 0.40 (29)	NS
CT	6.96 ± 0.67 (9)	9.45 ± 0.67 (19)	<.05	6.62 ± 0.30 (37)	8.69 ± 0.42 (39)	<.001
TT	14.4 (1)	8.52 ± 0.72 (2)	NS	7.46 ± 0.42 (11)	8.51 ± 0.99 (7)	NS
CT + TT	7.70 ± 0.93 (10)	9.36 ± 0.62 (21)	NS	6.81 ± 0.26 ± (48)	8.67 ± 0.39 (46)	<.001

Abbreviations: Hcy – homocysteine; NS – nonsignificant.
The results are given in mean ± SEM. Comparisons of older versus younger than 47 y in male and female were performed using the Mann–Whitney *U*-test. Number of subjects are given in parentheses.

Table 5. Plasma Hcy concentration (μmol/L) in NT and HT male and female

	Overall sample			Male			Female		
MTHFR C677T genotype	NT	HT	*P* value	NT	HT	*P* value	NT	HT	*P* value
All	7.58 ± 0.20 (150)	8.95 ± 0.42 (75)	<.001	8.47 ± 0.46 (36)	8.63 ± 0.49 (23)	NS	7.28 ± 0.21 (114)	9.11 ± 0.59 (52)	<.001
CC	7.76 ± 0.31 (71)	8.84 ± 0.98 (29)	NS	8.16 ± 0.47 (20)	8.34 ± 0.51 (8)	NS	7.59 ± 0.39 (51)	9.26 ± 1.17 (21)	NS
CT	7.32 ± 0.28 (64)	8.84 ± 0.48 (40)	<.01	8.58 ± 0.85 (14)	8.89 ± 0.70 (14)	NS	7.02 ± 0.25 (50)	8.81 ± 0.66 (26)	<.01
TT	7.76 ± 0.54 (15)	9.45 ± 1.09 (6)	NS	11.97 ± 2.43 (2)	7.50 (1)	NS	7.13 ± 0.30 (13)	9.84 ± 1.24 (5)	<.01
CT + TT	7.41 ± 0.25 (79)	8.93 ± 0.45 (46)	<.001	8.98 ± 0.82 (16)	8.80 ± 0.56 (15)	NS	7.04 ± 0.21 (63)	9.00 ± 0.58 (31)	<.001

Abbreviations: Hcy – homocysteine; HT – hypertensive; NS – nonsignificant; NT – normotensive.
The results are given in mean ± SEM. Comparisons of HT versus NT in the overall sample and male and female were performed using the Mann–Whitney *U*-test. Number of subjects are given in parentheses.

DISCUSSION

The heterogeneity of the Argentinian population, particularly in the metropolitan area, which results in cultural, socioeconomic, and ethnic diversities, may represent a confounding factor herein. Nevertheless, the few existing studies in the Argentinian population on the MTHFR enzyme and their polymorphisms led us to design this study. It is important to take into account that the frequency of the MTHFR 677T allele, and indeed hypertension prevalence, is known to vary substantially among different ethnic populations (18). Our data on risk factors of atherosclerosis (Table 1) indicated that in the overall sample, both in men with respect to women and in HT with respect to NT, had more age and prevalence of diabetes mellitus, and higher serum triglycerides, creatinine, and plasma tHcy. Men also showed higher values of HDL cholesterol compared with women, whereas HT distinguished by having higher BMI and total cholesterol than NT. The development of hypertension is believed to be largely under genetic control (22). Many case–control studies have addressed, in particular, the putative role of a 677C>T mutation in the *MTHFR* gene. The hypothesis that Hcy may play a role in the pathogenesis of essential hypertension is based on the fact that Hcy induces arteriolar constriction, renal dysfunction, and increased sodium reabsorption, and also increases arterial stiffness (23). Moreover, elevated tHcy concentration is known to increase oxidative stress that causes oxidative injury to the vascular endothelium, diminishes vasodilation by nitric oxide, stimulates the proliferation of vascular smooth muscle cells, and alters the elastic properties of the vascular wall (24). All these are closely associated with a rise in hypertension. Thus, Hcy contributes to blood pressure elevation. However, the results of the genetic association studies on the role of the 677C>T MTHFR polymorphism in hypertension have generated considerable controversy (21,25). We found that current smokers, coffee consumers, diabetics, those with history

Table 6. Plasma Hcy Concentration (μmol/L) in NT and HT, male and female and younger and older than 47 y

MTHFR C677T genotype	Male						Female					
	Younger than 47 y			Older than 47 y			Younger than 47 y			Older than 47 y		
	NT	HT	*P**	NT	HT	*P**	NT	HT	*P**	NT	HT	*P**
All	7.65 ± 0.44 (23)	7.02 ± 0.42 (2)	NS	9.92 ± 0.91 (13)	8.77 ± 0.53 (21)	NS	7.05 ± 0.28 (77)	7.38 ± 0.49 (14)	NS	7.81 ± 0.27 (37)	9.25 ± 0.47 (38)	< 0.05
CC	7.54 ± 0.26 (14)	7.45 (1)	NS	9.61 ± 1.36 (6)	8.61 ± 0.97 (7)	NS	7.37 ± 2.04 (37)	7.80 ± 0.55 (5)	NS	8.27 ± 0.54 (14)	8.62 ± 0.57 (16)	NS
CT	7.01 ± 0.80 (8)	6.60 (1)	NS	10.28 ± 1.56 (6)	8.94 ± 0.69 (13)	NS	6.64 ± 0.34 (31)	6.53 ± 0.76 (7)	NS	7.64 ± 0.33 (19)	9.62 ± 0.77 (19)	< 0.01
TT	14.4 (1)	–	NS	9.54 (1)	7.50 (1)	NS	7.15 ± 0.41 (9)	8.88 ± 1.48 (2)	NS	7.03 ± 0.60 (4)	10.49 ± 1.98 (3)	NS
CT + TT	7.83 ± 1.08 (9)	6.60 (1)	NS	10.18 ± 1.32 (7)	8.84 ± 0.65 (14)	NS	6.75 ± 0.28 (40)	7.12 ± 0.73 (9)	NS	7.53 ± 0.29 (23)	9.75 ± 0.70 (22)	< 0.01

Abbreviations: Hcy – homocysteine; HT – hypertensive; NS – nonsignificant; NT – normotensive.
The results are given in mean ± SEM. Comparisons of NT versus HT were performed using the Mann–Whitney *U*-test. Number of subjects are given in parentheses. *P**: *P* value.

of vascular diseases in at least themselves or one or more in the first-degree relatives, and hyperhomocysteinemic carrying the T allele present higher risk of being HT than CC homozygous (Table 2). Dietary parameters may be acting as effect modifiers in this genetic association and may cause heterogeneity in the observed genetic effects across studies (26). Male sex and postmenopausal status are two demographic characteristics of HHcy as well (27). In general (28), and confirmed in this study, male subjects have higher plasma tHcy levels than female. We found that the presence of the T allele is determinant for this sex difference (Table 3). After menopause, both the tHcy plasma concentration (29) and the incidence of CAD increase in women (6). Our results showed that this age-related increase of tHcy concentration correlates significantly with the T allele more dramatically in women but also in men (Table 4). Administration of estrogen in men and postmenopausal women is associated with a reduction of tHcy plasma level (30,31), and the incidence of CAD (32). E_2 can reduce reactive oxygen species generation in endothelial cells (33), suggesting a potential protection mechanism in female. Therefore, the gender-dependent vascular response appears to be estrogen-related. There is substantial evidence to suggest that estrogens have a cardioprotective influence in women by improving endothelial function (34,35) and LDL concentrations (36) as well as by decreasing tHcy concentrations (37). There is a large base of literature suggesting that female sex hormones have the ability to alter lipid concentrations (36,38). Most of the literature suggests that oral estrogen appears to decrease LDL and increase HDL in postmenopausal women (36). The T allele of the MTHFR gene also resulted as the determinant for the elevation of tHcy in female HT, and this sex-related tHcy increase is responsible for the higher tHcy concentrations observed in all (male and female) HTs (Table 5). Finally, when NT and HT of both sexes were discriminated by age (younger and older than 47 y), it was observed that only in older women HT presented statistically significant higher tHcy concentration than NT, and it was found that the T allele was responsible for this increase (Table 6). Other studies also provide confirmatory evidence of association between MTHFR 677C>T and hypertension (25,39). In a prospective study consisting of 70 candidate polymorphisms, only the MTHFR 677C>T variant was consistently associated with incident hypertension and may be involved in the pathogenesis of hypertension (40), and a meta-analysis of several small case–control studies suggested a relationship between the T allele of MTHFR and hypertension (41). Contrary to our results, some studies have shown a significant association between the 677C>T MTHFR mutation and HT in men (42,43), but Inamoto et al. (44) have reported that this MTHFR mutation was a risk factor for HT in women only. Other studies have reported that the relationship

between MTHFR polymorphism and HT remains unclear and inconsistent (45,46). We found that the tHcy plasma level was significantly associated with hypertension after age-adjustment in this population. We also found that tHcy plasma levels were significantly higher in HT subjects, and that the estimated risk of HT was also associated with the level of tHcy on plasma. In summary, our findings suggest that 677C>T MTHFR mutation is associated with the prevalence of HT level in postmenopausal women. In conclusion, the main finding in this cross-sectional study is an association between the 677C>T polymorphism of the MTHFR gene and hypertension, particularly in older women. As other causes such as diet and hormonal status could modify the phenotypic expression of this genetic variant, the differences related to sex and age could be due to the endocrine activity. In this study, the following limitations must be considered. First, the relatively small sample size, particularly in men, may limit the power of testing a significant association between MTHFR 677TT and hypertension. 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 as valid and reproducible in larger samples. Indeed, the magnitude of the associations documented herein was such that it overcame the critical *P* value (type I error) of .05 despite the relatively small sample size. Second, our results indicate an association of HHcy and MTHFR 677C>T mutation with hypertension, but they do not prove a causal relationship. MTHFR 677C>T mutation may contribute to hypertension or affect the development of hypertension through HHcy. In the future, large prospective studies are needed to confirm our findings and analyze the possible benefits of prevention and treatment in HT patients. Finally, the assessment of gene–gene and gene–environment interactions was beyond the scope of this study. Further studies are needed to explore these important issues in detail.

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