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The Association of MTHFR C677T Gene Variants and Lipid Profiles or Body Mass Index in Patients With Diabetic and Nondiabetic Coronary Heart Disease

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Background: The aim of this study is to investigate whether methylenetetrahydrofolate reductase (MTHFR) C677T mutation is associated with the development of hyperlipoproteinemia and obesity in coronary heart disease (CHD). *Methods:* This study was carried out in 82 diabetic and 112 nondiabetic patients with CHD and in 138 CHD-free healthy controls. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and agarose gel electrophoresis techniques were used to determine the MTHFR C677T. *Results:* Distributions of *MTHFR* genotypes (C677T dbSNP: rs1801133) were similar in our study groups ($P > 0.05$). There was no statistical association between biochemical parameters and genotype distribution in nondiabetic CHD patients, while diabetic CC genotype carriers have elevated levels of body mass index (BMI) independently from lipid profiles ($P = 0.002$). In diabetic CHD patients, while evaluating the clinical parameters according to gender, it was found that gender had an impact on BMI ($P = 0.013$). Due to this gender effect, a multivariate analysis was conducted on the diabetic CHD patient group. The multivariate logistic regression analysis confirmed that the MTHFR-CC genotype was associated with elevated BMI levels in diabetic CHD patients (odds ratio [OR] = 5.42, $P = 0.003$). *Conclusion:* The results of the present study demonstrated that possessing T allele of *MTHFR C677T* mutation indicates a protective association on BMI independently from other risk factors. J. Clin. Lab. Anal. 27:427–434, 2013.

Key words: coronary heart disease; lipid profiles; BMI; MTHFR; polymorphism

INTRODUCTION

Coronary heart disease (CHD) is a complex disease in which numerous genetic and environmental factors contribute to have a high morbidity and mortality prevalence worldwide (1). Traditionally, hypertension, gender, age, family history, hyperlipidemia, dyslipidemia, hyperglycemia, diabetes, obesity, and smoking are the main risk factors for the development of CHD and these factors may differ in each race and ethnic group. In past 20 years, extensive studies were done on lipid profiles and possible genes that coordinate the cholesterol levels (1–6). CHD is frequently characterized by dysregulation of fatty acid metabolism likewise lipid profiles particularly in patients with metabolic disease (5, 6).

Genetic polymorphisms that alter the activity of biotransformation enzymes have been reported to be associated with the biological processes (7, 8). Although the previous reports revealed the critical role of methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms

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in lipid profiles and body mass index (BMI), their potential impact on diabetic or nondiabetic CHD has not been well studied (9–11).

MTHFR is a key enzyme (EC 1.5.1.20) in folate metabolism and irreversibly catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which is essential in remethylation of homocysteine (Hcy) into methionine. Methionine is then activated to a universal methyl donor called S-adenosylmethionine (SAM) functioning in numerous trans-methylation reactions, including methylation of DNA, proteins, lipids, and synthesis of polyamines. Indeed this conversion is critical in controlling intracellular levels of Hcy and maintaining adequate SAM levels (8–11).

Genetic deficiencies in MTHFR gene is one cause of elevated Hcy levels (9, 12) and several studies have shown that the fluctuations in plasma Hcy levels were strongly associated with the development of CHD (13,14). In addition, raised concentrations of Hcy (hyperhomocyteinemia), in parallel with decreased methionine levels (hypomethionemia), suggested as an independent risk factor (15, 16) by causing endothelial injury; smooth muscle cell proliferation; hydrogen peroxide generation; and protein and lipid, especially low-density lipoprotein, oxidation (17–19). However, all reports did not show such association (20).

MTHFR gene is located on 1p36.3 (cDNA Gen Bank accession number U09806) and several polymorphic sites have been identified on MTHFR gene. A common 677 C→T transition (rs1801133) in the MTHFR gene is a well-identified genetic determinant of hyperhomocysteinemia. The C677T variant lies in exon 4 at the folate binding site of the MTHFR gene and results in the substitution of an alanine by a valine (Ala222Val) residue. This common polymorphism of MTHFR gene causes the reduced activity and thermolability of MTHFR and results in lower levels of 5-methyltetrahydrofolate, an accumulation of 5,10-methylenetetrahydrofolate, and increased plasma homocysteine levels. Recent studies have reported that (MTHFR) gene plays an important role in the pathogenesis of coronary artery disease (7–12,21). However, the results of other studies were controversial (22).

Bjorck et al. found that serum levels of Hcy were positively associated with serum insulin levels as well as with insulin resistance independently from age and sex (23). Moreover, it was suggested that insulin resistance and hyperinsulinemia were associated with increased plasma Hcy levels, independently from body weight (24). However, epidemiological studies found that plasma homocysteine was correlated with BMI (25). Jacques et al. reported that the BMI showed a positive association with tHcy ($p = 0.03$). Koehler et al. (26) also reported a positive relation between BMI and tHcy concentrations. Due to the relationship between MTHFR gene polymorphism and hyperhomocysteinemia, we suggested that this mutation could be a risk factor for elevated BMI. Thus, the aim of this study is to investigate the association between MTHFR C677T mutation and the risk of CHD whether with presence or absence of type 2 diabetes with respect to lipid profiles and BMI in Turkish population.

METHODS

Study Participants and Clinical Investigation

The study groups were composed of 82 diabetic and 112 nondiabetic CHD patients diagnosed by Department of Cardiology, Istanbul Faculty of Medicine in Istanbul University who volunteered for the study. Patients with severe coronary vascular disease were documented by angiography. The presence of CHD was estimated according to previous medical history, present symptoms of angina pectoris, and ECG changes. Angiographic inclusion criteria were $\geq$50% stenosis of at least one major coronary vessel because of atherosclerosis, or a vascular event, defined as myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery by-pass grafting. A total of 138 CHD-free healthy volunteers were included as controls. Control subjects lacking any symptoms of CHD were selected for the control group. Coronary angiography was not performed on these individuals, and therefore the presence of atherosclerotic coronary arteries could not be excluded. However, none of these individuals had a history of a vascular event.

The study protocol was approved by both the Ethical Committee of the Istanbul Faculty of Medicine and the Research Fund of Istanbul University. All participants in the study signed an informed consent form in accordance with ethics guidelines regarding the study.

Lipid Measurement

Blood samples were drawn into plain tubes after the participants had fasted overnight. The samples were centrifuged for 10 min at 15,00 $\times$ g at room temperature and the serum was immediately removed and frozen at -20°C. Total cholesterol (TC) levels were measured by cholesterol oxidase-peroxidaseaminoantipyrine (CHOD-PAP) enzymatic calorimetric method. Serum high-density lipoprotein cholesterol (HDL-C) levels were measured by CHOD-PAP test, following precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic acid and magnesium ions. The glycerol phosphate oxidase-peroxidaseaminoantipyrine (GPO-PAP) enzymatic calorimetric test was used to measure serum triglyceride levels. Serum low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald formula.

Polymerase Chain Reaction (PCR)-based Detection of MTHFR C677T Mutation

Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole blood with a salting-out procedure (27).

PCR, restriction fragment length polymorphism (RFLP), and agarose gel electrophoresis techniques were used to determine MTHFR C677T. The DNA samples were analyzed for the C677T missense mutation by PCR with locus-specific primers and subsequent analysis of an RFLP created by the mutation as reported in Frosst et al. (9). The primers for PCR amplification of the region spanning the 677 locus were 677F (5′-TGAAGGAGAAGGTGTCTGCGGGA-3′) and 677R (5′-AGGACGGTGCGGTGAGAGTG-3′). The 677 C→T substitution creates a HinfI recognition sequence, therefore, after amplification of the isolated DNA with PCR, the MTHFR C677T mutation was detected by cutting the PCR product with the restriction endonuclease HinfI (MBI Fermentas, ON, Canada) (10, 11). The digested DNAs were separated on 3% agarose gel in 1XTris borate EDTA buffer followed by staining with ethidium bromide solution. The genotypes were typed by visualization under ultraviolet light. All the results were confirmed with double replicates.

Statistical Methods

Statistical analysis was performed by using SPSS software package (revision 11.5 SPSS Inc., Chicago, IL). Clinical laboratory data are expressed as mean ± SD. Mean values were compared between patients and controls by unpaired Student's *t*-test. Differences in the distribution of genotypes and alleles between cases and controls were tested using the chi-square statistic. Allele frequencies were estimated by gene counting methods. Values of $P < 0.05$ were considered statistically significant. Multivariate analysis was performed with binary logistic regression (forward: LR). The odds ratios (OR) and the confidence intervals (CI) were calculated to estimate the relative risk. This analysis was used to identify association of MTHFR C677T mutation among several independent factors. In the logistic regression model, BMI ≥ 27 kg/m^2 as the dependent variable were used. Model included age, gender, and MTHFR C677T mutation as independent variables. The data are shown in Table 6.

RESULTS

Clinical Investigation

Demographic characteristics of the study groups were summarized in Table 1. All the study groups had similar distributions of gender and age.

The nondiabetic patient group had significantly higher levels of BMI (26.45 ± 3.18 vs. 25.26 ± 3.29; $P = 0.006$), systolic blood pressure (SBP) (128.57 ± 30.12 vs. 121.35 ± 10.79; $P = 0.027$), diastolic blood pressure (DBP) (79.69 ± 15.62 vs. 74.34 ± 8.97; $P = 0.003$), TC (5.59 ± 1.39 vs. 4.93 ± 1.26; $P < 0.001$), and LDL-C (1.62 ± 0.74 vs. 1.59 ± 0.95; $P < 0.01$) when compared to the control group. The HDL-C levels (1.03 ± 0.18 vs. 1.11 ± 0.30; $P = 0.015$) were significantly lower and the prevalence of hypertension ($P < 0.001$) and family history ($P = 0.034$) were significantly higher in nondiabetics. However, in diabetic CHD patients, when compared to the control group, BMI, TC, and LDL-C levels were not different ($P > 0.05$), but SBP (133.12 ± 27.39 vs. 121.35 ± 10.79; $P = 0.027$), DBP (81.45 ± 16.95 vs. 74.34 ± 8.97; $P = 0.003$), HDL-C (0.98 ± 0.25 vs. 1.11 ± 0.30; $P = 0.002$) levels, and the prevalence of hypertension ($P < 0.001$) and family history ($P = 0.034$) were the same as nondiabetic CHD group. In all of the study groups, the triglyceride levels had similar distributions ($P > 0.05$). On the other hand, the criteria for obesity (≥ 27 kg/m^2) was significantly different in all the study groups ($P < 0.05$) when compared to controls (Table 1). In addition, the distribution of biochemical characteristics was not significantly altered between the two patient groups, diabetics and nondiabetics, except TC, LDL-C, and hypertension (data not shown).

The frequency of smoking for nondiabetic or diabetic patients versus controls were 70.00%→40.20% ($P < 0.001$) and 30.90%→40.20% ($P > 0.05$), respectively. Due to the high smoking rates among Turkish population, especially in comparison of diabetic patients with controls, healthy subjects resemble heavy consumption of cigarette. However, the prevalence of alcohol consumption was not different between control group and diabetic or nondiabetic CHD patients group ($P > 0.05$).

Distribution of MTHFR C677T Genotypes

The distributions of genotypes and alleles of MTHFR C677T were shown in Table 2. Differences were observed in the distribution of MTHFR C677T genotype or allele frequencies in cases versus controls ($P > 0.05$). MTHFR C677T genotype distributions were consistent with Hardy–Weinberg equilibrium ($P > 0.05$). As seen in Table 2, the homozygous variant allele was extremely rare in the study population. Therefore, the combination of heterozygous CT and homozygous TT variant was taken together for the analysis.

Association of the *MTHFR* C677T Genotypes with Clinical Parameters

In Table 3, the distributions of clinical parameters according to MTHFR C677T genotypes were presented.

TABLE 1. Demographic and Biochemical Characteristics of the Study Population

	Study groups			*P*-values	
	Control ($n = 138$)	DM (−) CHD ($n = 112$)	DM (+) CHD ($n = 82$)	*1P*	*2P*
Gender (*n*, %)				0.169	0.060
Male	82 (59.42%)	76 (67.86%)	38 (46.34%)		
Female	56 (40.58%)	36 (32.14%)	44 (53.66%)		
Age (years) ($X \pm$ SD)	56.39 ± 11.28	55.54 ± 10.87	58.00 ± 7.87	0.544	0.217
BMI (kg/m^2)	25.26 ± 3.29	26.45 ± 3.18	26.21 ± 4.13	**0.006**	0.124
≥ 27kg/m^2	39 (29.77%)	54 (50.94%)	26 (44.07%)	**0,001**	**0.055**
<27 kg/m^2	92 (70.23%)	52 (49.06%)	33 (55.93%)		
Smoking (*n*, %)				**<0.001**	0.201
Never smoking	76 (59.80%)	33 (30.00%)	47 (69.10%)		
Current smoking	51 (40.20%)	77 (70.00%)	21 (30.90%)		
Alcohol consumption (*n*, %)				0.992	0.167
Consumers	8 (16.00%)	9 (16.10%)	3 (6.80%)		
Nonconsumers	42 (84.00%)	47 (83.90%)	41 (93.20%)		
SBP (mmHg) ($X \pm$ SD)	121.35 ± 10.79	128.57 ± 30.12	133.12 ± 27.39	**0.027**	**0.001**
DBP (mmHg) ($X \pm$ SD)	74.34 ± 8.97	79.69 ± 15.62	81.45 ± 16.95	**0.003**	**0.001**
TC (mmol/l) ($X \pm$ SD)	4.93 ± 1.26	5.59 ± 1.39	5.04 ± 1.36	**<0.001**	0.585
TG (mmol/l) ($X \pm$ SD)	1.59 ± 0.95	1.62 ± 0.74	1.80 ± 1.09	0.771	0.170
LDL-C (mmol/l) ($X \pm$ SD)	3.18 ± 1.12	3.57 ± 1.11	3.08 ± 1.04	**0.010**	0.561
HDL-C (mmol/l) ($X \pm$ SD)	1.11 ± 0.30	1.03 ± 0.18	0.98 ± 0.25	**0.015**	**0.002**
VLDL-C (mmol/l) ($X \pm$ SD)	0.71 ± 0.36	0.73 ± 0.33	0.83 ± 0.55	0.769	0.092
LVH (*n*, %)				NS	NS
Positive	–	30 (30.90%)	10 (31.30%)		
Negative	–	67 (69.10%)	22 (68.70%)		
Hypertension (*n*, %)				**<0.001**	**<0.001**
Positive	–	41 (40.20%)	44 (55.70%)		
Negative	104 (100%)	61 (59.80)	35 (44.30)		
Family story (*n*, %)				**0.034**	**0.012**
Positive	24 (24.74%)	42 (38.53%)	21 (45.65%)		
Negative	73 (75.26%)	67 (61.47%)	25 (54.35%)		

DM(+)CHD, coronary heart disease (CHD) patients with diabetes; DM(−)CHD, CHD patients without diabetes; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; VLDL-C, very low-density lipoprotein-cholesterol; LVH, left ventricular hypertrophy. The results are shown as mean ± SD. *n*, number of individuals; parametric results are shown as mean ± SD; *P*1, control versus DM(−)CHD; *P*2, control versus DM(+) CHD; NS, nonspecific. Bold values of *P* indicate statistical significance.

There was no statistical association between biochemical parameters and genotype distribution in nondiabetic CHD patients.

In diabetic CHD patients, the frequency of obesity (BMI ≥ 27 kg/m^2) was found higher in CC genotype than those carrying T allele (TT + CT genotypes) ($P = 0.002$). In addition, the mean values of BMI according to genotypes were 27.84 ± 4.24 in CC, 24.09 ± 3.38 in TT, and 24.73 ± 3.34 in CT (Table 3). Moreover, according to comparison with TT genotype, it was seen that C allele

TABLE 2. The Distribution of MTHFR 677 C/T Genotypes and Alleles in the Study Groups

	Groups			
MTHFR 677C/T	Control ($n = 138$)	DM(−)CHD ($n = 112$)	DM(+)CHD ($n = 82$)	Total patient ($n = 194$)
Genotypes				
CC	62 (44.93%)	63 (56.25%)	40 (48.78%)	103 (53.09%)
TT	15 (10.87%)	9 (8.04%)	14 (17.08%)	23 (11.86%)
CT	61 (44.20%)	40 (35.71%)	28 (34.14%)	68 (35.05%)
Allelles				
C	185 (67.03%)	116 (74.11%)	108 (65.85%)	274 (70.62%)
T	91 (32.97)	58 (25.89%)	56 (34.15%)	114 (29.38%)

Chi-square test was used to compare genotypes in the study group. DM(−)CHD, diabetic coronary heart disease (CHD) group; DM(+)CHD; nondiabetic CHD group; *n*, number of individuals.

TABLE 3. Comparison of Background Characteristics Among the Different Genotypes of the MTHFR 677C/T Polymorphism in the Study Groups

	MTHFR 677 C/T genotypes					
Groups	CC	TT + CT	*P*-value	TT	CC + CT	*P*-value
DM (-) CHD patients						
TC (mmol/L)	5.54 ± 1.59	5.64 ± 1.09	0.714	5.54 ± 1.28	5.59 ± 1.41	0.980
TG (mmol/L)	1.59 ± 0.82	1.68 ± 0.64	0.528	1.57 ± 0.71	1.63 ± 0.75	0.575
HDL-C (mmol/L)	1.03 ± 0.17	1.03 ± 0.21	0.833	0.96 ± 0.28	1.04 ± 0.17	0.828
LDL-C (mmol/L)	3.46 ± 1.22	3.71 ± 0.94	0.237	3.74 ± 1.02	3.56 ± 1.12	0.793
VLDL-C (mmol/L)	0.72 ± 0.36	0.74 ± 0.30	0.827	0.71 ± 0.33	0.73 ± 0.34	0.411
BMI (kg/m^2)	26.20 ± 3.57	26.78 ± 2.60	0.330	26.45 ± 2.16	26.45 ± 3.27	0.166
≥27 kg/m^2	29 53.70%)	25 (46.30%)	0.539	5 (9.30%)	49 (90.70%)	0.772
<27 kg/m^2	31 (59.60%)	21 (40.40%)		4 (7.70%)	48 (92.30%)	
SBP (mmHg)	131.34 ± 35.53	125.43 ± 22.48	0.322	126.25 ± 30.67	128.77 ± 30.23	0.871
DBP (mmHg)	81.53 ± 16.52	77.60 ± 14.44	0.216	77.50 ± 14.88	79.88 ± 15.75	0.726
DM (+) CHD patients						
TC (mmol/L)	5.19 ± 1.40	4.87 ± 1.31	0.301	5.25 ± 1.21	4.99 ± 1.39	0.539
TG (mmol/L)	1.92 ± 0.88	1.67 ± 1.27	0.314	2.08 ± 2.02	1.74 ± 0.79	0.569
HDL-C (mmol/L)	0.96 ± 0.25	1.00 ± 0.25	0.492	1.03 ± 0.18	0.97 ± 0.26	0.462
LDL-C (mmol/L)	3.25 ± 1.08	2.90 ± 0.99	0.151	3.02 ± 1.18	3.09 ± 1.02	0.807
VLDL-C (mmol/L)	0.86 ± 0.35	0.80 ± 0.70	0.614	1.05 ± 1.13	0.78 ± 0.32	0.411
BMI (kg/m^2)	27.84 ± 4.24	24.53 ± 3.31	**0.002**	24.09 ± 3.38	26.59 ± 4.16	0.094
≥ 27 kg/m^2	19 (73.10%)	7 (26.90%)	**0.002**	3 (11.50%)	23 (88.50%)	0.481
< 27 kg/m^2	11 (33.30%)	22 (66.70%)		6 (18.20%)	27 (81.80%)	
SBP (mmHg)	138.55 ± 25.99	127.05 ± 28.01	**0.075**	131.81 ± 23.58	133.36 ± 28.19	0.865
DBP (mmHg)	84.47 ± 16.18	78.08 ± 17.40	0.111	80.45 ± 11.92	81.63 ± 17.78	0.833

DM(+)CHD, patients with diabetes; DM(−) CHD, coronary heart disease (CHD) patients without diabetes; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; VLDL-C, very low-density lipoprotein-cholesterol; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. The results are shown as mean ± SD. Bold values of *P* indicate statistical significance.

(CC + CT genotypes) had an increasing effect on BMI levels, but this positive effect had no statistical significance (24.09 ± 3.38 vs. 26.59 ± 4.16; $P = 0.094$) (Table 3). On the other hand, higher levels of TC (5.19 ± 1.40 vs. 4.87 ± 1.31) and LDL-C (3.25±1.08 vs. 2.90±0.99) were observed in patients with CC genotype, however, this association was not also significantly valued.

In total, patients group (diabetic and nondiabetic CHD patients) the allelic distributions according to clinical parameters that were associated with blood pressure levels. T allele carriers have lower levels of DBP ($P = 0.045$; data not shown). On the other hand, higher levels of TC ($P = 0.055$) and LDL-C ($P = 0.039$) were observed in T allele carriers of control group than those having CC genotype (data not shown).

In diabetic CHD patients, while evaluating the clinical parameters according to gender, it was found that gender had an impact on BMI ($P = 0.013$; Table 4). In addition, triglyceride levels tended to be higher ($P = 0.059$) in women, but this was a normal situation in female population. On the other hand, there was no association between other lipid profiles and gender.

Normally, lipids had a positive effect on BMI, however, when the association of BMI and MTHFR C677T genotypes were observed according to gender, it was found that in diabetic females, the wild type of MTHFR C677T genotype, CC, increases BMI independently from lipids ($P = 0.009$; Table 5). But this relation was not seen in males. In addition, any possible relation was detected between MTHFR C677T alleles and lipid profiles according to gender ($P > 0.05$; Table 5). On the other hand, lipid profiles could not be only fractioned by gender; the distribution of lipids could also be altered by life style, diet, occurrence of diabetes, and insulin resistance. Therefore,

TABLE 4. Distribution of Clinical Parameters According to Gender in Diabetic Coronary Heart Disease (CHD) Patients

	Female	Male	*P*-value
TC (mmol/l)	5.27 ± 1.45	4.80 ± 1.23	0.129
TG (mmol/l)	2.03 ± 1.35	1.56 ± 0.64	0.059
HDL-C (mmol/l)	1.03 ± 0.24	0.93 ± 0.25	0.083
LDL-C (mmol/l)	3.20 ± 1.15	2.95 ± 0.92	0.308
VLDL-C (mmol/l)	0.92 ± 0.70	0.73 ± 0.30	0.144
BMI (kg/m^2)	27.51 ± 4.69	24.87 ± 2.97	**0.013**

TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; VLDL-C, very low-density lipoprotein-cholesterol; BMI, body mass index. The results are shown as mean ± SD. Bold values of *P* indicate statistical significance.

TABLE 5. The Effects of MTHFR C677T Allelles on BMI and Lipid Profiles According to Gender

		CC genotype	T allel	*P*-values
TC (mmol/l)	Females	5.43 ± 1.54	5.06 ± 1.33	0.440
	Males	4.90 ± 1.19	4.70 ± 1.29	0.631
TG (mmol/l)	Females	2.09 ± 0.94	1.96 ± 1.78	0.773
	Males	1.71 ± 0.76	1.43 ± 0.50	0.198
HDL (mmol/l)	Females	1.00 ± 0.21	1.06 ± 0.27	0.487
	Males	0.90 ± 0.29	0.95 ± 0.22	0.586
LDL (mmol/l)	Females	3.41 ± 1.17	2.92 ± 1.08	0.192
	Males	3.04 ± 0.94	2.88 ± 0.93	0.624
VLDL (mmol/l)	Females	0.90 ± 0.36	0.94 ± 0.99	0.881
	Males	0.81 ± 0.33	0.67 ± 0.27	0.193
BMI (kg/m^2)	Females	29.39 ± 4.65	25.04 ± 3.58	**0.009**
	Males	25.80 ± 2.60	24.11 ± 3.12	$P > 0.05$

TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; VLDL-C, very low-density lipoprotein-cholesterol; BMI, body mass index. The results are shown as mean ± SD. Bold values of *P* indicate statistical significance.

in order to evaluate the association of lipid profile levels and such factors in diabetic CHD patients another dataset was performed: The association of MTHFR C677T genotypes and BMI or lipid profiles were analyzed according to patients more than 55 years and it was found that patients with CC genotype had higher levels of BMI ($P = 0.009$) versus those having T allele (data not shown). However, to evaluate whether this relation was originated from age or gender, logistic regression analysis was performed. BMI $\geq 27\ kg/m^2$ was included as the dependent variable. The CC genotype of the MTHFR C677T polymorphism, female gender, and age $\geq$55 were included in the model as categorical variables. In the logistic regression analysis, it was confirmed that the effects of age and gender on BMI were removed, while the excessive association of BMI and CC genotype was extended (Table 6).

TABLE 6. Association of BMI With Gender, Age, and MTHFR CC Genotype

Dependent variable	Independent variable	Exp(B) (OR)	*P*-value	95%CI
BMI$\geq$27 kg/m^2	Gender (Male = Ref)	→1	NS	
	Age (Age < 55 = Ref)	→1	NS	
	CC genotype (T allele = Ref)	5.42 →1	**0.003**	1.755-16.789

BMI, body mass index; OR, odds ratio; CI, confidence interval; NS, not specified. Bold values of *P* indicate statistical significance.

DISCUSSION

The relation between hyperhomocysteinemia and CHD was not clear yet (20). In addition, many reports declare a positive correlation with elevated plasma homocysteine levels and increased lipid profiles and so the CHD (8,28). The association between MTHFR C677T polymorphism and lipid profiles or CHD has been investigated in several previous studies and different conclusions have been reported. Some studies (9, 10, 29, 30) reported a positive association of MTHFR C677T polymorphism with CHD. The results of these studies regarded that the homozygous mutant form of MTHFR C677T polymorphism resulted in the elevated serum levels of homocysteine in CHD patients, thus, MTHFR C677T polymorphism is suggested as a risk factor for the development of CHD. Conversely, others (20, 31–33) have failed to reveal any significant association. In the present study, TC and LDL-C levels were higher in both total CHD patient group and diabetic patients group with CC genotype. In addition, diabetic CC genotype carriers had elevated levels of BMI independent from lipid profiles.

The conflicting results may be reflective of different ethnicities, environmental influences, dietary folate intake, or other life-style factors. Indeed, it was reported that T allele frequency varied among Australia, Canada, Brazil, United States, Japanese, and Corsican populations as well as Caucasians and Black South Africans (34, 35). On the other hand, it was believed that C677T mutation affects thermolability and decreases activity of the enzyme, thus, remethylation of Hcy was impaired and caused hyperhomocysteinemia in homozygous subjects especially if their plasma folate levels were low (36). Most of the studies reported that adequate ingestion of folate and vitamin B may be sufficient to overcome the adverse effects of MTHFR variants (37, 38). In addition, Huang et al. indicated that the participants with TT genotype who consumed high levels of polyunsaturated fatty acids (PUFAs) had lower plasma Hcy levels when compared with those consumed low levels of PUFA (39).

On the other hand, the result of this study was in accordance with the previous study by Boger et al. (40). Similar to the present study, they reported the lower frequencies of TT genotype and they revealed an association between TT genotype and decreased BMI. Accordingly, in the present study it was found that cases with CC genotype have the higher levels of BMI. Therefore, the results of this study suggested that depending on the plasma folate level alterations in diabetics, MTHFR C677T gene polymorphism affects the plasma cholesterol levels and therefore alters BMI.

However, insulin resistance seems to increase the homocysteine levels (41). Therefore, the observed effect of MTHFR C677T mutation on BMI in diabetic patients

with CHD was caused by high insulin levels. On the other hand, it was shown that with another SNP of MTHFR gene, MTHFR A1298C polymorphism, the plasma homocysteine levels have also been affected (42), thus, it was not clearly well-known which individual or combined SNPs of MTHFR affects homocysteine even at an approximate linkage equilibrium between MTHFR C677T and A1298C loci (40, 42).

Consequently, the present study is a preliminary study to establish the link between MTHFR C677T polymorphism and lipid profiles or BMI in the pathogenesis of CHD in a Turkish population. It was demonstrated that possessing T allele of *MTHFR C677T* polymorphism indicated a protective effect on BMI independently from the other risk factors. However, limitations of this study included two principle domains. First, folate and MTHFR variant analysis was not evaluated because the study was lacking folate intake data. Second, the number of study group was relatively small. Therefore, the adverse effect of C677T polymorphism may not be significant with respect to control group, but still may give us clues on prognosis of the disease. Additional studies with larger sample sizes are needed to define the influence of MTHFR C677T genotyping on clinical outcomes. Finally, possible individual and/or combined effects with environmental factors of MTHFR gene in prognosis of the disease could be more conclusive with further studies including both dietary and intracellular folate intake levels and other polymorphisms of MTHFR.

CONFLICT OF INTEREST

Authors declare that no competing interests exist.

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