The 677 C/T MTHFR Polymorphism is Associated with Essential Hypertension, Coronary Artery Disease, and Higher Homocysteine Levels

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Background. Essential hypertension (EH) and cardiovascular disease are common, multifactorial disorders likely to be influenced by multiple genes of modest effect. The C677T methylenetetrahydrofolate reductase (MTHFR) gene polymorphism is related to MTHFR enzyme activity and to plasma homocysteine (Hcy) concentration. This study was designed to investigate an association of this polymorphism with coronary artery disease (CAD), EH, and healthy subjects.

Methods. In this study, we measured serum folate, serum vitamin B12, and plasma homocysteine and determined the MTHFR C677T genotype of 78 patients with essential hypertension, 100 patients with coronary artery disease, and 100 healthy subjects. MTHFR genotypes were assessed by real-time polymerase chain reaction.

Results. CC, CT, and TT genotype frequencies were 52, 44.0, and 4.0% in patients with CAD, respectively. In patients with essential hypertension, the CC, CT, and TT genotype frequencies were 46.2, 41.0, and 12.8%, respectively. In control subjects, the CC, CT, and TT genotype frequencies were 72.0, 26.0, and 2.0%, respectively. The C allele was significantly more frequent in controls compared with patients with EH ($p < 0.05$), and CC genotypes were more frequent in controls compared to patients with EH and CAD. Homocysteine level was higher in TT genotypes in CAD patients compared with CC and CT genotypes ($p < 0.01$). MTHFR gene polymorphism is an independent risk factor for EH but not for CAD.

Conclusions. The TT genotype of the 677C/T MTHFR polymorphism is associated with EH and CAD. In addition, TT genotypes had higher plasma Hcy levels in CAD patients compared with CC and CT genotypes. MTHFR gene polymorphism is an independent risk factor for EH but not for CAD.

Key Words: MTHFR gene polymorphism, Essential hypertension, Coronary artery disease.

Introduction

Increased plasma concentrations of homocysteine were previously found in patients with coronary artery disease (CAD) (1) and essential hypertension (EH) (2). It has been suggested that homocysteine is involved in the promotion of platelet activation, hypercoagulability, oxidative stress, endothelial dysfunction, smooth muscle cell proliferation, and oxidation and peroxidation of lipids (1,3,4). The sulfur-containing amino acid homocysteine is formed during the metabolism of methionine. Homocysteine (Hcy) concentration is influenced by vitamin B12 and folate (FA) levels. Methylenetetrahydrofolate reductase (MTHFR), a key enzyme in homocysteine metabolism, seems to play a role in both hypertension and cardiovascular disease (5–8). The T allele of the 677C/T (A222V) MTHFR polymorphism causes a thermolability of the enzyme, reduces its activity, and inhibits the formation of 5-methyltetrahydrofolate, which serves as a methyl donor during the remethylation of homocysteine to methionine. This explains why TT homozygotes exhibit higher plasma homocysteine concentrations than CT heterozygotes and CC homozygotes in
a majority of studies (1,9). However, some studies reported no association between MTHFR genotype and plasma homocysteine levels (10).

Elevated plasma homocysteine has been found in hypertensive patients and showed a positive correlation with blood pressure (11—15). Individuals randomly selected for homocysteine-lowering treatment had a decrease in blood pressure. This provides strong evidence linking homocysteine and blood pressure.

Although the relationship between the 677T variant and CAD risk has not yet been clearly established, the MTHFR polymorphism may play an important role in the pathophysiology of cardiovascular disease because of its influence on plasma Hcy levels. Alternatively, an increase in plasma Hcy levels may occur because of nutritional deficiencies in essential cofactors or enzyme substrates including vitamin B12, folate, and/or vitamin B6. Hyperhomocysteinemia has been identified as an independent risk factor for cerebral, coronary, and peripheral atherosclerosis, although the pathological mechanism of this risk is not fully understood. Thus, the MTHFR C677T causing mild hyperhomocysteinemia is an important genetic risk factor for CVD (16). A large study conducted by Inamoto (17) showed that the TT genotype was associated with hypertension and carotid stenosis in women.

As increased plasma homocysteine levels were observed in diseases constituting coronary artery disease and as the data linking the MTHFR genotype to these common multifactorial diseases are not consistent, we therefore undertook this pilot study to investigate the MTHFR gene in a sample of healthy subjects and patients with CAD and EH in the eastern Anatolia region of Turkey. Knowledge about the genetic background of the Turkish population should enable us to evaluate their susceptibility to CAD and EH.

Materials and Methods

A total of 278 subjects, aged 57.2 ± 10.4 years (mean ± SD), 180 males and 98 females, were enrolled in an association study. Hospitalized patients or patients attending outpatient clinics of cooperating centers or those affiliated with several general polyclinics and with diagnoses of CAD and EH were included in the study as well as subjects without the above-mentioned diagnoses.

Patient Sources

All CAD and EH patients (n = 178) were subjects referred to the Department of Cardiology of Firat University, Firat Medical Center with angina pectoris or a history of myocardial infarction. Disease status of each CAD patient was defined according to the angiography of the coronary vessels. Inclusion criteria were a ≥50% reduction in the diameter of the left main stem or a ≥70% reduction in the diameter of one of the major coronary arteries or their branches. Patients with stenosis <50% were not included in our study. CAD in the non-CAD group was excluded by an absence of symptoms and clinical signs of CAD. Participants who met the following criteria were recruited as essential hypertensive patients: (1) systolic blood pressure (SBP) was between 140 and 200 mmHg, or diastolic blood pressure (DBP) was between 90 and 120 mmHg, diagnosed in the previous 12 months and confirmed during two visits at the outpatient clinic; (2) no history or clinical evidence of congestive heart failure, myocardial infarction, cardiac valve disease, coronary bypass surgery or angioplasty, diabetes mellitus and renal insufficiency, pregnancy, hypercalcemia and no treatment with urate-lowering medication (allopurinol and probenecid); (3) any condition preventing technically adequate ambulatory BP monitoring (ABPM) (e.g., atrial fibrillation or other major arrhythmias); and (4) secondary hypertension. A total of 100 normotensive (NT) age-matched healthy individuals as control subjects. None of the control subjects had a family history of hypertension, all had systolic BP <130 mmHg and diastolic BP <85 mmHg and an absence of any symptoms and clinical signs of CAD. The structure of the total sample is summarized in Table 1. All patients who agreed to participate in the study were evaluated with a detailed questionnaire that provided information about coronary risk factors such as arterial hypertension, smoking history and hyperlipidemia (total cholesterol >240 mg/dL and triglycerides ≥250 mg/dL, above borderline high values according to the European Atherosclerosis Society; in most cases values from medical records).

Genotyping Protocols

DNA was extracted from EDTA anti-coagulated whole blood from all subjects (controls and patients) and was prepared from DNA isolation kit (High Pure PCR Template Preparation kit; Roche Diagnostic, Mannheim, Germany). Patients were screened for the C677T gene polymorphism of MTHFR using PCR amplification with specific primers and LightCycler apparatus (Roche Diagnostic).

Homocysteine, B12, Folic Acid, and Biochemical Analysis

Blood was obtained from an antecubital vein after a 12-h fast and blood samples without anticoagulant were immediately centrifuged at 2500 rpm for 10 min; the separated serum was aliquoted and stored at —70°C until homocysteine and lipid measurement. TC, HDL, LDL, and triglycerides were determined using enzymatic assay on an autoanalyzer AU600 (Hitachi, Tokyo, Japan). Plasma homocysteine level was measured by HPLC (High Performance Liquid Chromatography, Shimadzu 10 AVP, Tokyo, Japan) with Chromsystems kits (cat. #45000; Chromsystems, Instruments & Chemicals, Munich, Germany). Serum folate and B12 levels were measured by chemiluminescent enzyme
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Patients with CAD</th>
<th>Patients with EH</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.30 ± 9.9</td>
<td>57.15 ± 10.0</td>
<td>57.47 ± 11.1</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>78/22</td>
<td>28/50</td>
<td>74/26</td>
</tr>
<tr>
<td>Smoking (n)</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134.48 ± 31.51</td>
<td>151.02 ± 14.8</td>
<td>1200.20 ± 6.24</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84.07 ± 16.9</td>
<td>85.00 ± 9.9</td>
<td>76.67 ± 7.7</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>221.48 ± 45.00</td>
<td>208.64 ± 44.28</td>
<td>198.41 ± 32.85</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>159.66 ± 79.79</td>
<td>177.51 ± 89.35</td>
<td>145.22 ± 56.07</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>44.04 ± 9.78</td>
<td>47.35 ± 8.66</td>
<td>47.58 ± 12.00</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>153.40 ± 37.79</td>
<td>137.15 ± 39.76</td>
<td>135.14 ± 26.78</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>15.30 ± 6.27</td>
<td>17.65 ± 17.14</td>
<td>13.79 ± 6.61</td>
</tr>
<tr>
<td>B12 (pg/mL)</td>
<td>387.59 ± 152.71</td>
<td>422.08 ± 161.42</td>
<td>463.03 ± 188.07</td>
</tr>
<tr>
<td>Folic acid (ng/mL)</td>
<td>6.26 ± 2.36</td>
<td>5.52 ± 2.05</td>
<td>9.11 ± 3.56</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease; EH, essential hypertension.

Results

Study Group Characteristics and Distribution of MTHFR 677 C/T Polymorphism

Characteristics of the study patients are shown in Table 1. Eighteen CAD patients had hypertension. Hypertensive patients showed higher systolic-diastolic blood pressure (p < 0.0001) and CAD patients had higher total cholesterol and LDL cholesterol than control subjects (p < 0.05). No difference in total homocysteine level was detected between groups. Mean serum homocysteine level was higher in hypertensive patients (17.6 ± 17.1 µmol/L) than in CAD patients (15.33 ± 6.27) and controls (13.79 ± 6.6), but this was not statistically significant. Mean serum folic acid and B12 level was lower in CAD patients, similar changes compared to control subjects (p < 0.0001, p < 0.05, respectively).

Genotypic and allelic frequencies for the study groups are shown in Table 2. There was no deviation from Hardy-Weinberg equilibrium for the polymorphisms considered. CC genotype frequency was higher in control subjects than in patients with EH and patients with CAD (72.0% vs. 46.2% vs. 52.0%, respectively), and the difference was statistically significant (p < 0.05). The C allele was more frequent in healthy subjects (85.0%) compared to patients with EH (66.7%) (p < 0.05).

Comparison of Serum B12, Folic Acid, and Homocysteine Levels Related to MTHFR Genotypes

Levels of serum B12, folic acid and homocysteine in patients related to their MTHFR genotypes are shown in Table 3. In CAD patients, CC and CT genotypes had lower serum homocysteine levels compared with TT genotypes (p < 0.01), and in hypertensive patients B12 level was lower in CC genotypes compared with CT and TT genotypes (p < 0.05). In hypertensive patients, B12 level was higher in T allele compared with C allele (p < 0.05).
The aim of the study was to investigate the association of the 677C/T MTHFR polymorphism with CAD and EH; therefore, multiple logistic regression was performed to analyze the relevance of selected parameters (Hcy, FA, B12, and MTHFR genotypes) to these clinical outcomes (Table 4). MTHFR proved to be significantly linked with only EH patients ($p < 0.05$).

**Correlation Coefficients between Mean tHcy and B12, FA, BMI in Patients with CAD, EH, and Total Subjects**

Correlation coefficients between tHcy and B12, FA, BMI in all subjects or with CAD and EH patients appear in Table 5. In correlation analysis of tHcy to B12 and FA, no significant correlations were detected, but significant positive correlation was observed between tHcy and BMI in patients with CAD and EH or among all subjects ($r = 0.378$, $p = 0.009$; $r = 0.268$, $p = 0.09$; $r = 0.281$, $p = 0.001$, respectively).

**Discussion**

The absence of information on the reported genetic risk factors in the Turkish population, which is considered to be genetically very heterogeneous, led us to design the present study. In our study of a cohort of the eastern Anatolia regions in Turkey, we report that the presence of the TT genotypes confers increased risk for CAD and EH. The MTHFR gene is found to be associated with increased cardiovascular risk in several populations including Lebanese, Japanese and French Canadians (9,18–20), whereas no association between MTHFR C677T mutation and CAD could be demonstrated in other studies (21–25). In the present study, hypertensive subjects present a significant increase in MTHFR allele T frequency, demonstrating an enzymatic variant with minor activity. This observation may be related to endothelial cell lesions associated with hypertension because the 677T mutation decreased nucleotide synthesis, affecting DNA reparation mechanism and cellular division.

Tokgozoglu et al. (26) reported that TT was not an independent predictor factor of CAD in the Turkish population, but it was an important predictor of the extent of the disease and plasma homocysteine, especially in the presence of plasma folate values below the median of the population. The frequency of T allele (15.0%) observed in this study could be demonstrated in other studies (21). Nevertheless, the frequency of CC genotype (52.0%) in CAD patients in our study is consistent with the 50.6% frequency reported by Yilmaz et al. (28). The frequencies of T allele for different ethnic populations were found to vary between 9.7% and 53% (29,30).

Advanced coronary atherosclerosis is known to be the final event of a multifactorial process involving many interactions among numerous risk factors, whereas other factors such as hyperhomocysteinemia are still a matter of investigation (31–36). Over the past decade, a substantial body of evidence has been accumulated on the importance of the MTHFR C677T polymorphism in thrombotic disorders, and it is certain that MTHFR C677T polymorphism has a pathogenetic role in CAD, especially in the form of advanced coronary atherosclerosis. 

**Table 4. Logistic regression analysis of determinants of clinical outcomes**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CAD</th>
<th>EH</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR (95% CI)</td>
<td>$p$</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>MTHFR</td>
<td>0.092 (0.074–0.257)</td>
<td>0.27</td>
</tr>
<tr>
<td>B12</td>
<td>0.000 (0.001–0.000)</td>
<td>0.14</td>
</tr>
<tr>
<td>FA</td>
<td>0.065 (0.09–0.337)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hcy</td>
<td>0.007 (0.008–0.021)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease; EH, essential hypertension.

**Table 5. Correlation coefficients between mean tHcy and B12, FA, BMI in patients with CAD, EH, and total subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>CAD</th>
<th>EH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with CAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R$</td>
<td>$p$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>$p$</td>
<td></td>
<td></td>
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<tr>
<td>Patients with EH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R$</td>
<td>$p$</td>
<td></td>
<td></td>
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<tr>
<td>$r$</td>
<td>$p$</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R$</td>
<td>$p$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>$p$</td>
<td></td>
<td></td>
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</tbody>
</table>

CAD, coronary artery disease; EH, essential hypertension.
evidence has accumulated implicating the MTHFR C677T mutation as a modest genetic risk factor for cardiovascular disease (16). Although hypertension is a primary risk factor for CVD, few studies have examined the MTHFR C677T variant specifically in relation to EH risk. In addition, this is the first report that examined the relationship between EH and MTHFR gene polymorphism in a Turkish population. A recent large-scale study conducted by Inamoto et al. (17) examined hypertension and carotid atherosclerosis in over 3000 Japanese patients. These researchers reported that the TT genotype of the MTHFR gene was associated with an increased risk of hypertension, conferring a small, but significant, risk of 1.5 (35). A recent study of CVD in an unrelated group from the Czech Republic examined the MTHFR C677T in 193 patients diagnosed with only EH (37). The frequency of the T allele was shown to be over-represented in the Czech group affected with EH compared to the healthy control group in this Czech study (38 vs. 33%). These data also indicated an increased prevalence of the dominant genotype group (CT/TT) compared to controls. It is important to note that the frequency of the MTHFR 677T allele, and indeed EH prevalence, is known to vary substantially among different ethnic populations (38). Frequencies of these differences in gene and disease may partly explain the conflicting association results obtained across independent studies. Our study showed the MTHFR C677T variant for association with EH. The results of this work were consistent with the trends reported by Benes et al. (37), showing that the prevalence of the T allele and its composite genotypes (CT/TT and TT) were increased in the EH group compared to controls.

Our additional observation was that the MTHFR TT genotype represented higher homocysteine levels in CAD patients but not in hypertensive patients. Our study contributes to the assessment of the genotype influence on circulating Hcy levels in the general population, confirming these observations in Turkish CAD patients. In addition, recent studies indicate that the MTHFR TT genotype is associated with higher plasma homocysteine levels only in individuals with low folate status (39,40). However, results of studies investigating associations between the MTHFR genotype and CAD in individuals according to their folate status are not consistent with previous studies (26,40–42). A study investigating vitamin B12 and folate status and plasma homocysteine levels in the Czech population ascertained MHHCy in 17% and 14% of subjects with adequate levels, respectively (43); therefore, the effect of the MTHFR genotype could be more dominant and independent of plasma substrate concentrations.

Because this was an exploratory pilot study, these findings should be interpreted cautiously, bearing in mind the following limitations: sample size was small, and the groups were combined for the primary analysis. Nonetheless, this limits generalization to population-based patients with CAD and EH. Alternatively, the small sample size may have masked the relationship between the MTHFR polymorphism and CAD or EH.

In conclusion, our results indicate that the TT genotype of the 677C/T MTHFR polymorphism is associated with EH and CAD in the eastern region of Turkey. In addition, TT genotypes had higher plasma Hcy levels in CAD patients compared with CC and CT genotypes. We found that the MTHFR gene polymorphism is an independent risk factor for EH but not for CAD. These findings require further investigations in large population-based studies, but our results suggest that EH, like CAD, may be mildly influenced by the MTHFR C677T variant.

References


