Correlation between the 677C>T polymorphism in the methylene tetrahydrofolate reductase gene and serum homocysteine levels in coronary heart disease

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ABSTRACT. The aim of the current study was to explore the correlation between serum homocysteine (HCY) levels and the methylene tetrahydrofolate reductase (MTHFR) gene 677C/T polymorphism and coronary heart disease (CHD). We consecutively enrolled 208 patients with CHD confirmed by CTA or coronary angiography from our hospital. An additional 200 healthy volunteers were enrolled as the control group. Serum HCY levels, MTHFR C677T genotype, and other related indicators were evaluated for the two groups. Compared to those in the control group, the serum HCY levels in the CHD patients were significantly higher (P < 0.05). The proportion of individuals with the heterozygous MTHFR CT genotype and homozygous mutant TT genotype among CHD
patients was significantly higher than that in the control group (P < 0.05). In the acute coronary syndrome (ACS) subgroup, the proportion of those with the CT and TT genotypes was significantly higher than that of the stable CHD subgroup (P < 0.05). In summary, serum HCY levels were elevated in CHD patients, and the frequency of the CT and TT genotypes were also significantly increased, especially among the ACS subgroup. Taken together, this suggests that serum HCY levels and \textit{MTHFR}\textsubscript{C677T} genotypes are correlated with CHD.

\textbf{Key words:} Coronary heart disease; Serum homocysteine levels; Methylene tetrahydrofolate reductase; Gene polymorphism

\section*{INTRODUCTION}

Methylene tetrahydrofolate reductase (MTHFR) is the key enzyme in the metabolism of homocysteine (HCY), and is closely related to serum HCY levels. MTHFR mainly catalyzes the 5,10-methylene tetrahydrofolate reaction, and provides methyl to HCY for methyl transfer metabolism. If the activity of MTHFR is decreased, which affects the most important HCY metabolism pathway, i.e., remethylation, thus impeding the transformation of HCY to methionine, then hyperhomocysteinemia will be induced (Botto and Yang, 2000). The MTHFR\textsubscript{C677T} genotype and HCY are closely related with hypertension, congenital heart disease, coronary heart disease (CHD), stroke, and depression among other diseases. Moreover, hyperhomocysteinemia is an independent risk factor for CHD coronary heart disease among the Chinese population. Serum folic acid levels and the C677T homozygous mutant of the \textit{MTHFR} gene are independently associated with plasma HCY levels, demonstrating that both folic acid and the \textit{MTHFR} gene influence HCY. It has previously been reported that when low folic acid levels and the TT genotype exist simultaneously HCY reaches its highest levels (Frosst et al., 1995). The aim of the current study was to investigate the correlation between changes in HCY serum levels and the \textit{MTHFR}\textsubscript{C677T} polymorphism in patients with CHD to provide a theoretical basis for better prevention and treatment of this deadly disease.

\section*{MATERIAL AND METHODS}

\textbf{Subjects}

From June 2012 to March 2015, 208 CHD patients (118 males, 90 females) with ages ranging from 32 to 85 years were continuously enrolled in this study from both inpatient and outpatient departments at Shenzhen Second People’s Hospital Affiliated to Anhui Medical University. Those with high blood pressure, congenital heart disease, pregnancy and/or lactation, stroke, or liver and/or kidney dysfunction were excluded. Two hundred healthy volunteers (114 males, 86 females) from the Physical Examination Division were enrolled as the control group, and those with high blood pressure, congenital heart disease, pregnancy and/or lactation, stroke, or liver and/or kidney dysfunction were excluded.

\textbf{Diagnostic criteria of CHD}

CHD was confirmed by coronary computed tomography angiogram or coronary
angiography, and the diagnosis of acute myocardial infarction was made based on electrocardiogram and troponin I levels.

**Measurement indices**

Blood lipids, serum HCY, and other biochemical markers were assessed.

**MTHFR C677T genotyping**

Venous blood samples were collected from fasting participants, and genomic DNA was isolated from white blood cells using the QiAamp Blood Kit (standard salting). The \textit{MTHFR} C677T genotype was obtained from the extracted DNA using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. All experimental procedures were performed in the Central Laboratory in Shenzhen Second People’s Hospital Affiliated to Anhui Medical University.

**Statistical analysis**

SPSS17.0 (SPSS Inc., Chicago, IL, USA) software was used for statistical processing. Quantitative data between the two groups were compared using \(t\)-tests, and classification data were compared using \(\chi^2\) tests; a level of significance of 0.05 was set as the two-tailed test standard.

**RESULTS**

The comparison of characteristics between CHD patients and the control group revealed that the age difference was statistically significant, but no significant differences were found in the other indices (\(P > 0.05\); Table 1).

<table>
<thead>
<tr>
<th>Table 1. Characteristics of coronary heart disease patients and the control group.</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>LDL-C</td>
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<tr>
<td>Blood Sugar</td>
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</table>

The serum HCY level of CHD patients was 17.13 ± 3.01 \(\mu\)M compared to 10.19 ± 2.73 \(\mu\)M in the control group, which was significantly different (\(P < 0.001\)).

The percentage of those with the CT and TT genotypes of the \textit{MTHFR} C677T polymorphism was 55.77\% in CHD patients compared to 19.11\% in the control group, and this difference was statistically significant (\(P < 0.05\); Table 2).

The acute coronary syndrome (ACS) subgroup had 126 patients in total, among which the percentage of those with the CT and TT genotype was 62.70\%. A subgroup of 82 patients had stable coronary artery disease, among which the percentage of those with the CT and TT genotype was 45.12\%. The former percentage was significantly higher than the latter (\(P < 0.05\); Table 3).
Table 2. *MTHFR* C677T genotype comparison between coronary heart disease (CHD) patients and the control group.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CHD patients [N (%)]</th>
<th>Control group [N (%)]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT and TT</td>
<td>116 (55.77%)</td>
<td>38 (19.11%)</td>
<td>$\chi^2 = 58.66$ $P &lt; 0.001$</td>
</tr>
<tr>
<td>CC</td>
<td>92 (44.23%)</td>
<td>162 (80.89%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>208</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. *MTHFR* C677T genotype comparison between the ACS and stable coronary artery disease subgroups.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ACS [N (%)]</th>
<th>Stable CHD [N (%)]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT and TT</td>
<td>79 (62.70%)</td>
<td>37 (45.12%)</td>
<td>$\chi^2 = 42.36$ $P &lt; 0.001$</td>
</tr>
<tr>
<td>CC</td>
<td>47 (37.30%)</td>
<td>45 (54.88%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>126</td>
<td>82</td>
<td></td>
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</table>

**DISCUSSION**

*MTHFR* is the key enzyme in the metabolism of HCY. The *MTHFR* gene is located on 1p363, and the cDNA has a full length of 22 kb with a total of 11 exons. There have been several types of mutations previously reported in *MTHFR*, and the 677 C>T substitution is the most common site of mutation. The *HinfI* reaction-restriction fragment length polymorphism is at the 677th nucleotide C>T mutation of the cDNA sequence, which produces a *HinfI* reaction-restriction endonuclease recognition sequence. The C677T mutation converts the encoded alanine to valine, and therefore MTHFR activity is decreased (Botto and Yang, 2000), heat resistance is reduced, and the ability of catalyzing the reaction of 5,10-methylenetetrahydrofolate to provide methyl to HCY for methyl transfer metabolism declines. This affects the main metabolic pathway of HCY, which is remethylation, thus impeding the transformation of HCY to methionine, increasing plasma HCY levels, and resulting in hyperhomocysteinemia (Frosst et al., 1995). HCY is cytotoxic, which enables thiol oxidation in the metabolic process to produce free radicals that cause DNA damage and apoptosis. Therefore, HCY damages vascular endothelial cells and accelerates platelet-mediated smooth muscle cell proliferation, which results in arteriosclerosis. As such, HCY is closely associated with different arteriosclerosis diseases. Hyperhomocysteinemia is a risk factor that can trigger atherosclerotic vascular disease independent of hyperlipidemia, hypertension, diabetes, smoking, and age. HCY concentration in the blood is influenced by genetic, environmental, and nutritional factors. Other than external factors, HCY increases caused by metabolic enzyme defect or decreased activity due to mutation may be one of the main causes of CHD. Frosst et al. (1995) found that the heat-labile enzyme produced from the *MTHFR* C677T gene mutation could elevate plasma HCY levels. There have since been a large number of case studies exploring whether this mutation is a genetic risk factor for CHD, and whether there are differences among different ethnic groups. McCully (1969) found that two children with high plasma and urine HCY concentrations and had a wide range of arterial thrombosis and atherosclerosis (AS). Since then, much research has confirmed that an increase in plasma HCY concentration is an independent risk factor of AS. The C677T mutation has a relatively high frequency worldwide, and varies in different races and populations (Morita et al., 1997). It was previously demonstrated that the C677T mutation contributes to decreases in activity and thermolability of MTHFR. Among extracts from TC heterozygous lymphocytes, MTHFR maintains only 65% of normal activity at room temperature, and TT homozygotes maintain only 30%. A similar conclusion can also be drawn from MTHFR expression *in vitro*. Thus, the C677T mutation has been considered as a genetic factor of AS, which
is also related to the occurrence of CHD (Frosst et al., 1995; Morita et al., 1997). Helfenstein et al. (2005) revealed that the MTHFR TT genotype and allele frequencies in patients with myocardial infarction are higher than those in healthy individuals. Multivariate analysis showed that the TT mutation may be an independent risk factor for myocardial infarction. Kang et al. (1988) was the first to report that the homozygous MTHFR mutation was more than three times higher in CHD patients than that of the control group. Subsequently, it was reported that MTHFR of 212 CHD patients in North America became heat labile decreasing its activity by 50%, and of these patients, 17% had coronary artery disease. Strain et al. (2004) believed that the plasma HCY concentration of those carrying MTHFR mutations was significantly higher, and that the risk of cardiovascular disease among those was significantly increased. A study in a Japanese population (Morita et al., 1997) also showed that the MTHFR gene C677T homozygous mutation was significantly higher in CHD patients compared to that in the control group, suggesting that it was closely related to CHD.

In the current study, we found that serum HCY levels in CHD patients were higher than those in the control group, and that the proportion of those with the CT and TT genotypes of MTHFR also increased in CHD patients. Subgroup analysis further revealed that the proportion of those with the CT and TT genotypes in the ACS subgroup was higher than that of the stable CHD subgroup, indicating that serum HCY levels and MTHFR C677T genotypes are closely related to CHD. However, these study results are not fully consistent with other domestic and overseas results, which may be related to the following factors: 1) The sample size was small. 2) There may be geographic and racial differences. For example, Shenzhen is a new immigrant city, and hence the patients may have come from various provinces across the country, which may be different from other studies with a single population source. According to the work of Morita et al. (1997), MTHFR C677T gene mutation occurrence has markedly different frequencies in different ethnic populations, which may affect results of the study. 3) The serum levels of HCY and MTHFR C677T genotypes are related to a variety of diseases, and thus when patients are selected, it is not possible to rule out all confounders that may interfere with the results. Therefore, the CHD patients and the control group were not completely matched, which may have affected the results. 4) In our study, the ACS patients accounted for a higher proportion than in previous research. In the current study, we showed that there was a higher proportion of ACS patients with the CT and TT genotypes compared to that of stable coronary artery disease patients, suggesting that MTHFR C677T genotypes may have a stronger influence in ACS patients. Thus, a relatively high proportion of patients with ACS may further highlight the impact of MTHFR C677T genotypes in CHD. However, in other studies, patients with ACS accounted for a relatively low proportion of the study population or no subgroup analysis was provided, which may dilute the positive results. In the current study, MTHFR gene polymorphisms and HCY levels were measured in order to understand their relationship with CHD, whereas it was previously thought that hyperhomocysteinemia was an independent risk factor for CHD among the Chinese population. In the future, we may be able to predict the risk of CHD by measuring MTHFR gene polymorphisms and HCY levels, which would be useful for the primary prevention of CHD. Additionally, these measures may also be used as accessory indicators for early screening of CHD patients. However, large randomized clinical trials are still required for further investigation.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES


