The influence of age, sex, vitamin B₁₂, folate levels and methylene-tetrahydrofolate reductase C677T genetic mutations on plasma homocysteine in the Chinese population

CHAO-HUNG HO
Division of Hematology, Taipei Veterans General Hospital, & National Yang-Ming University, School of Medicine, Taipei, Taiwan, Republic of China

Background and Objectives. Thromboembolic diseases remain a major cause of morbidity and mortality in most countries. The present study was thus conducted to determine the influences of age, sex, methylenetetrahydrofolate reductase (MTHFR) gene mutation and B vitamins on the plasma homocysteine (Hcy) levels in the Chinese. Our previous study found that Chinese people carry the same mutation of the MTHFR gene described in Western populations, with a 677C→T substitution being another possible cause of thrombosis.

Design and Methods. The study population comprised 445 consecutively enrolled Chinese subjects of different ages and sex. Overall 69 subjects were found to have homozygous 677C→T mutation of the MTHFR gene, and were classified as group I; 164 subjects were found to have heterozygous mutation and classified as group II; 212 had no such mutation and were classified as group III.

Results. The mean plasma Hcy did not differ significantly between these 3 groups. When each group was divided again by gender, we found that both age and plasma Hcy levels were significantly higher in the males than in the females. In addition to Hcy levels, we also measured plasma vitamin B₁₂ and folate levels in 258 randomized subjects. Univariate and multivariate analysis showed MTHFR mutation could affect Hcy level, and univariate and multivariate analysis showed that age, MTHFR mutation and vitamin B₁₂ could affect the logHcy levels.

Interpretation and Conclusions. We demonstrate that some Chinese carry the 677C→T mutation of the methylenetetrahydrofolate reductase gene. This could affect their homocysteine levels and thus be a risk factor for thromboembolic disease. ©2000, Ferrata Storti Foundation

Key words: folic acid level, methylenetetrahydrofolate reductase gene mutation, plasma homocysteine level, thromboembolic disease, vitamin B₁₂ level
for no more than 12 months before being analyzed. For detection of the 677C→T transition in the MTHFR gene, PCR was performed using 5 pmole forward and reverse primer6 in 80 µM dNTPs, 10 mM Tris-HCl, pH 8.8 at 25°C, 1.5 mM MgCl2, 50 mM KCl, 0.1% Triton X-100 and 0.4 U DynaZyme™ II DNA polymerase, Recombinant (Finnzymes Oy) in a total volume of 50 µL. Denaturation was first carried out for 5 min at 94°C, followed by another 35 cycles of denaturation for 30 sec at 94°C, primer annealing for 50 sec at 57°C, and primer extension for 50 sec at 72°C. Finally, extension was performed for 5 min at 72°C, and then at 4°C for 30 min. Hinfl restriction enzyme (New England Biolabs) analysis and subsequent electrophoresis in a 2.5% MetaPhor™ agarose gel (FMC Bioproducts) revealed the mutational status of the subject. All PCR experiments to detect the mutation of MTHFR C677T included known positive and negative controls in order to test the accuracy of the results. Plasma Hcy levels were measured by the enzyme immunoassay method (Axis® Homocysteine EIA, Axis Biochemicals ASA, Oslo, Norway) following the manufacturer’s instructions. The measurement range was from 2.0 to 50.0 µmol/L. Serum vitamin B12 and folate levels were measured by a radioimmunoassay method (Radioassay Kit Vitamin B12 [57Co] / folate [126I] (ICN Pharmaceuticals, New York 10962-1294), again using the procedures recommended by the manufacturer.

Statistics
The Kruskal-Wallis ANOVA test was used to calculate the differences (p value) between the 3 groups. When the p value was <0.05, multiple comparisons were made between 2 groups to reveal the significance. The two-sample t-test was used to compare the significance between two groups. Univariate analysis (Enter method) and multivariate analyse (Stepwise method) were used to detect the influence of different parameters on Hcy and logHcy levels.

Results
Homocysteine levels according to MTHFR 677C→T mutation in all 445 subjects
Table 1 shows the plasma Hcy levels of the 445 subjects divided into groups according to MTHFR 677 mutation status and gender. The mean age of all the subjects was 63.7 years (SD 14.5, range 19-90 years), that of the males 67.4±12.5 years, and that of the females 55.6±15.4 years. Sixty-nine of the 445 subjects (15.5%) had homozygous (TT) MTHFR mutation, 164 (36.9%) had heterozygous (CT) MTHFR mutation, 164 (36.9%) had heterozygous (CT) MTHFR mutation, 212 (47.7%) had no mutation (CC). The plasma Hcy levels in these 3 groups were 9.8±7.1 µmol/L, 9.0±4.9 µmol/L and 9.0±4.9 µmol/L, respectively; these differences are not statistically significant (p = 0.51, ANOVA test). On the other hand, plasma Hcy level was significantly higher in males than in females in each mutation group and in the subjects overall (p < 0.0005, 0.008, 0.006 and <0.0005, respectively), and the males were significantly older than the females (p = 0.002, <0.0005, <0.0005 and <0.0005, respectively). The same results were also obtained considering logHcy or the square root of Hcy instead of Hcy.

The influence of age, sex, vitamin B12, folate levels and MTHFR mutations on plasma homocysteine levels in a subgroup of 258 subjects
In addition to Hcy, plasma vitamin B12 and folate acid were also measured randomly in 258 of the

Table 1. Homocysteine levels in 445 subjects divided into 3 groups according to MTHFR mutation status: homozygous (TT), heterozygous (CT) and normal subjects (CC).

<table>
<thead>
<tr>
<th></th>
<th>Homozygous Mean ±SD</th>
<th>Heterozygous Mean ±SD</th>
<th>Normal Mean ±SD</th>
<th>Total Mean ±SD</th>
<th>p value°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males Age (years)</td>
<td>69.2±10.0 (52)*</td>
<td>66.6±13.7 (113)</td>
<td>67.3±12.4 (139)</td>
<td>67.4±12.5 (304)</td>
<td>0.47</td>
</tr>
<tr>
<td>Hcy (µmol/L)</td>
<td>11.0±7.7</td>
<td>9.7±5.2</td>
<td>9.6±5.0</td>
<td>9.9±5.6</td>
<td>0.27</td>
</tr>
<tr>
<td>Females Age (years)</td>
<td>62.5±10.9 (17)</td>
<td>56.6±13.4 (51)</td>
<td>53.3±7.0 (73)</td>
<td>55.6±15.4 (141)</td>
<td>0.07</td>
</tr>
<tr>
<td>Hcy (µmol/L)</td>
<td>6.9±2.6</td>
<td>7.5±3.6</td>
<td>7.7±4.6</td>
<td>7.4±4.1</td>
<td>0.29</td>
</tr>
<tr>
<td>p value†</td>
<td>&lt;0.0005</td>
<td>0.008</td>
<td>0.006</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>Males + Females Age (years)</td>
<td>67.6±10.6 (69)</td>
<td>63.5±14.3 (164)</td>
<td>62.5±15.6 (212)</td>
<td>63.7±14.5 (445)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Hcy (µmol/L)</td>
<td>9.8±7.1</td>
<td>9.0±4.9</td>
<td>9.0±4.9</td>
<td>9.1±5.3</td>
<td>0.51</td>
</tr>
</tbody>
</table>

* Number of subjects; † comparison among 3 different groups (TT, CT & CC), ANOVA test; § compared with the males in the same groups, two-sample t-test; ANOVA test and multiple comparisons showed significant difference of age between normal and homozygous mutation groups; Hcy: homocysteine.
The present study was, therefore, designed to compare the influence of the genetic and environmental factors on Hcy levels in order to see whether MTHFR mutation could be a risk factor for TED in the Chinese. In our study, 445 consecutive subjects were studied, principally to detect the relationship between MTHFR mutation and plasma Hcy levels. In addition to Hcy levels, we also randomly measured vitamin B₁₂ and folate levels in a subgroup of 258 of the patients. The whole study population of 445 subjects was divided into three groups according to their MTHFR gene mutation status: i.e. CC, CT and TT at position 677. There was no significant difference in Hcy levels between these 3 groups (p = 0.51).

The present study found that Hcy levels were higher in males, who were also older than the females in each of the 3 mutation groups. Thus, gender, age or both appeared to affect the Hcy levels. Meanwhile, in the 258 subjects in whom vitamin B₁₂ and folate levels were also measured, when using Hcy level as a variant, both univariate and multivariate analysis showed that only MTHFR mutation affected the plasma Hcy levels. However, when using logHcy level as a variant, both univariate and multivariate analyses showed that age, MTHFR mutation and vitamin B₁₂ affected logHcy levels. In addition, both univariate and multivariate analysis showed that MTHFR mutation could affect both Hcy and logHcy levels, whereas univariate and multivariate analysis showed that age, MTHFR mutation, and vitamin B₁₂ affected logHcy levels. As previously reported, plasma Hcy levels can be affected by both hereditary and environmental factors. The Chinese, who have been reported to have higher folate levels, and a much lower folate deficiency rate than European populations are, thus, probably protected from hyperhomocysteinemia. On the other hand, the MTHFR mutation, and probably age and vitamin B₁₂ still play important roles in influencing Hcy level.

In conclusion, the MTHFR mutation is found in the Chinese, and might be a risk factor for TED as it affects plasma Hcy levels.

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Disclosures
Conflict of interest: none.
Redundant publications: no substantial overlapping with previous papers.

Table 2. Univariate and multivariate analyses of the factors affecting plasma homocysteine (Hcy) concentration.

<table>
<thead>
<tr>
<th></th>
<th>Hcy (p value)</th>
<th>logHcy (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td>Age</td>
<td>0.275</td>
<td>0.204</td>
</tr>
<tr>
<td>Sex</td>
<td>0.796</td>
<td>0.808</td>
</tr>
<tr>
<td>MTHFR mutation</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>0.053</td>
<td>0.088</td>
</tr>
<tr>
<td>Folate</td>
<td>0.274</td>
<td>0.351</td>
</tr>
</tbody>
</table>
Potential implications for clinical practice

- Population studies on the prevalence of genetic and acquired thrombophilic factors²⁸-³⁰ may help to reduce the incidence of thrombosis.

References


17. Chao-Hung Ho

