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The influence of age, sex, vitamin B_{12}, folate levels and methylenetetrahydrofolate reductase C677T genetic mutations on plasma homocysteine in the Chinese population

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ABSTRACT

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 B_{12} 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 B_{12} could affect the $\log_{Hcy}$ 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.**

Key words: folic acid level, methylenetetrahydrofolate reductase gene mutation, plasma homocysteine level, thromboembolic disease, vitamin B_{12} level

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Thromboembolic diseases (TED) remain a major cause of morbidity and mortality in most countries. In 1993, factor V Leiden and PT20210 A allele were found to be major causes of TED in Western countries. However, the Chinese people are less prone to TED.[1,2] While our recent studies found neither factor V Leiden nor PT20210 A allele in the Chinese,[3-5] another of our previous studies showed that the Chinese carry the same mutation of the methylenetetrahydrofolate reductase (MTHFR) gene described in Western populations,[5] with a C→T substitution at nucleotide 677 being another possible cause of thrombosis. Whether this mutation affects homocysteine (Hcy) levels and could, thus, be a risk factor for TED in the Chinese deserves investigation. The present study was, therefore, conducted to determine the influences of age, sex, the MTHFR gene mutation and B vitamins on plasma Hcy levels in the Chinese.

Design and Methods

A total of 445 subjects were consecutively enrolled into the present study. These subjects presented for a physical check-up or were patients who attended the hematology outpatient clinic for a blood examination but had no past history of diabetes or TED and had not taken any medication in the preceding 2 weeks. Their mean age was 63.7 years (SD 14.5, range 19-90 years). Blood samples were taken from all subjects between 9 and 10 a.m. after fasting for 12 hours to test for MTHFR gene mutations and plasma Hcy levels. Plasma vitamin B_{12} and folate levels were also measured in a random sample of 258 of the 445 subjects. All subjects signed informed consent to the study.

DNA analysis for MTHFR 677C→T mutation was performed using the methods described by Frosst *et al.*[6] DNA was extracted from the buffy coat of the collected peripheral blood as previously described[7] and approximately 0.5 µg was used for polymerase chain reaction (PCR) amplification. The DNA samples were stored at 4°C

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. *Hin*fI 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 B_{12} and folate levels were measured by a radioimmunoassay method (Radioassay Kit Vitamin B_{12} [^{57}Co]/ folate [^{126}I] (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 log_{Hcy} 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 and 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 B_{12}, folate levels and MTHFR mutations on plasma homocysteine levels in a subgroup of 258 subjects

In addition to Hcy, plasma vitamin B_{12} and folic 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).

	Homozygous Mean ±SD	*Heterozygous Mean ±SD*	*Normal Mean ±SD*	*Total Mean ±SD*	*p value°*
Males					
Age (years)	69.2±10.0 (52)*	66.6±13.7 (113)	67.3±12.4 (139)	67.4±12.5 (304)	0.47
Hcy (μmol/L)	11.0±7.7	9.7±5.2	9.6±5.0	9.9±5.6	0.27
Females					
Age (years)	62.5±10.9 (17)	56.6±13.4 (51)	53.3±17.0 (73)	55.6±15.4 (141)	0.07
p value§	0.002	<0.0005	<0.0005	<0.0005	
Hcy (μmol/L)	6.0±2.6	7.5±3.6	7.7±4.6	7.4±4.1	0.29
p value§	<0.0005	0.008	0.006	<0.0005	
Males + Females					
Age (years)	67.6±10.6 (69)	63.5±14.3 (164)	62.5±15.6 (212)	63.7±14.5 (445)	0.04#
Hcy (μmol/L)	9.8±7.1	9.0±4.9	9.0±4.9	9.1±5.3	0.51

**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: homocysyteine.*

445 subjects. There was no significant difference in age, vitamin B_{12}, or folate levels among these 3 groups of subjects with different MTHFR mutations (p = 0.681, 0.072 and 0.694, respectively, Kruskal-Wallis ANOVA). Hcy concentration (also checked with log_{Hcy} and the square root of Hcy) was significantly higher in the homozygous MTHFR group than in the heterozygous MTHFR group or the normal controls (p < 0.0005, Kruskal-Wallis ANOVA and multiple comparisons). Univariate analysis showed that MTHFR affected plasma Hcy levels (p < 0.0005, enter method, Table 2), and that age, MTHFR and vitamin B_{12} level affected log_{Hcy} level (p = 0.020, < 0.0005 and 0.022, respectively, Enter method). Multivariate analysis, using the stepwise method, showed that MTHFR affected the plasma Hcy levels (p < 0.0005, Table 2), whereas age, MTHFR and B_{12} levels affected log_{Hcy} level (p = 0.005, < 0.0005, and 0.022, respectively).

Discussion

TED occurs less frequently in Chinese than in Westerners.[1,2,8] During the past few years, much effort has been made to understanding better the causes of idiopathic venous thrombosis, and indeed factor V Leiden, PT20210A allele and MTHFR gene mutation have been discovered.[6,9–15] Though factor V Leiden and PT20210A allele have been found to be the most frequent causes of TED in Western countries, they are absent in the Chinese.[3-5,16] However, the MTHFR C677T mutation is found in the Chinese.[5] The mutation change of MTHFR is not in itself a risk factor for TED, but high plasma levels of the amino acid Hcy are.[17] One of the main functions of MTHFR is remethylation of Hcy to methionine.[18] A common MTHFR mutation, an alanine-to-valine substitution, renders the enzyme thermolabile to elevated plasma levels of the amino acid Hcy,[19] which increases the risk of occlusive vascular disease.[20] However, in addition to genetic factors, plasma Hcy levels can also be influenced by environmental factors such as folate, vitamin B_{12} and B_6 levels.[21-23] 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 B_{12} 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).

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

	Hcy (p value)		*logHcy (p value)*	
	Univariate	*Multivariate*	*Univariate*	*Multivariate*
Age	0.275	0.204	0.020	0.005
Sex	0.796	0.808	0.520	0.960
MTHFR mutation	<0.0005	<0.0005	<0.0005	<0.0005
Vitamin B_{12}	0.053	0.088	0.022	0.013
Folate	0.274	0.351	0.101	0.181

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 B_{12} 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 log_{Hcy} level as a variant, both univariate and multivariate analyses showed that age, MTHFR mutation and vitamin B_{12} affected log_{Hcy} levels. In addition, both univariate and multivariate analysis showed that MTHFR mutation could affect both Hcy and log_{Hcy} levels, whereas univariate and multivariate analysis showed that age, MTHFR mutation, and vitamin B_{12} affected log_{Hcy} levels. As previously reported, plasma Hcy levels can be affected by both hereditary and environmental factors.[21-23] The Chinese, who have been reported to have higher folate levels,[24-26] and a much lower folate deficiency rate than European populations[27] are, thus, probably protected from hyperhomocysteinemia. On the other hand, the MTHFR mutation, and probably age and vitamin B_{12} 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.

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Potential implications for clinical practice

- Population studies on the prevalence of genetic and acquired thrombophilic factors[28-30] may help to reduce the incidence of thrombosis.

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