Original Article

Association of MTHFR genetic polymorphisms with venous thromboembolism in Uyghur population in Xinjiang, China

Zhao Li1, Umesh Yadav1, Ailiman Mahemuti1, Bao-Peng Tang1, Halmurat Upur2

1Heart Center, First Affiliated Hospital of Xinjiang Medical University, Xinjiang, China; 2Basic Medical College, Xinjiang Medical University, Xinjiang, China

Received June 27, 2015; Accepted September 10, 2015; Epub October 15, 2015; Published October 30, 2015

Abstract: Background: The aim of this study was to reveal the association between Methylene tetrahydrofolate reductase (MTHFR) gene mutations (C677T, A1298C and C1317T) and risk of venous thromboembolism (VTE) in Han and Uyghur population in Xinjiang. Material and method: We conducted a case control study composed of 246 cases, including 86 Uyghur and 160 Han ethnic diagnosed VTE were admitted in the First Affiliated Hospital of Xinjiang Medical University between January 2008 to December 2012, and 292 population including 122 Uyghur ethnic and 170 Han ethnic were studied as controls. To detect the polymorphism of MTHFR gene C677T, A1298T, and C1317T, Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was applied. Fluorescence polarization immunoassay was adopted to determine the plasma levels Homocysteine (Hcy), folic acid and vitamin B12 (VitB12). The association of the polymorphism of MTHFR and levels Hcy, folic acid and VitB12 with VTE was analyzed.

Results: The MTHFR gene C677T genotypes distribution in Uyghur VTE patients and control groups were: TT (27.91% vs. 12.29%), CT (41.86% vs. 52.46%) and CC (30.23% vs. 35.25%), respectively; and in Han VTE patients and control groups were: TT (27.49% vs. 14.71%), CT (44.38% vs. 53.53%) and CC (28.13% vs. 31.76%), respectively, and there were significant differences in TT genotype of MTHFR C677T between VTE patients and controls in both Uyghur and Han ethnic (Uyghur: \(x^2=8.070, P=0.005\); Han: \(x^2=8.159, P=0.004\)). However, there were no significant differences in the MTHFR gene A1298T and C1317T genotyping distribution frequency in Uygur and Han ethnic between VTE patients and controls (\(P>0.05\)). Plasma levels of Hcy in MTHFR gene TT genotype were statistically higher than CT and CC genotype (\(P<0.05\)). After adjusting for age, gender, smoking, hypertension, hyperlipidemia, diabetes and MTHFR genotype for plasma Hcy levels, multifactor logistic regression analysis showed (OR=1.025, 95% CI 1.003-1.046, \(P=0.024\)) and obesity (OR=4.660, 95% CI 1.417-15.324, \(P=0.011\)) were independent risk factors for Uygur ethnic with VTE while plasma Hcy levels (OR=1.020, 95% CI 1.006-1.034, \(P=0.004\)) and smoking (OR=2.867, 95% CI 1.062-6.586, \(P=0.024\)) were independent risk factors for Han ethnic with VTE. Conclusions: Our finding supports significant role of MTHFR gene in VTE and evidence of genetically determined HHcy contribute a risk for VTE, and a smoker with tHcy has positive association with a risk of VTE.

Keywords: Venous thromboembolism, uyghur, gene polymorphism, homocysteine, methylene tetrahydrofolate reductase

Introduction

Venous thromboembolism (VTE) is a serious health problem with pathogenic contributions from both genetic and environmental factors [1]. Hyperhomocysteinemia (HHcy) has been identified as an independent risk factor of atherosclerotic and thromboembolic disease, resulting from genetic and nutritional disorder in homocysteine metabolism. Low folate level, Vitamin B12 concentrations associated with elevated plasma total homocysteine (tHcy) are known independent risk factor for cardiovascular disease worldwide [2], but data with genetically evidence have inconclusive results in developing countries. HHcy is associated with increased risk factor for VTE [3, 4]. However there are conflicting data regarding MTHFR gene mutations in Asian patients with venous thromboembolism.

The most frequent genetic causes for mild HHcy is linked to A common C to T transition and A to C transition at nucleotide 677 (C677T) and
1298 (A1298C) respectively of the MTHFR gene coding sequence [5]. Prediction for increased homocysteine levels due to reduced MTHFR enzyme activity are the result of Homozygosity for MTHFR C677T (TT), A1298C (CC), and compound heterozygosity for C677T and A1298C (677CT/1298AC) genotype [6]. Individuals with low plasma folate levels, particularly in Caucasian population are associated with mild HHcy, with evidence of point mutation in the MTHFR gene (C677T) [7]. It was found that widespread of an allele in American Indian populations are far more than Caucasian populations and very less among African Americans [8, 9].

In order to better define the role of MTHFR and its genetic polymorphisms in the development of VTE in Han and Uyghur population in Xinjiang, the present study was performed. Additionally, exploring the genetic risk factor and total tHcy levels, we analyzed the relationship between VTE and its polymorphism involved in total tHcy remethylation to methionine. The MTHFR polymorphism adenine-to-cytosine (A1298C) and thymine-to-cytosine (T1317C) takes part in folate coenzyme.

Materials and methods

Study population

Two patient groups (Han and Uyghur) with VTE were studied independently (Table 1). One hundred Han patients and one hundred Uyghur...
MTHFR genetic polymorphisms and venous thromboembolism

Figure 2. Nucleotide sequences around the MTHFR C677T polymorphism.

Figure 3. Restriction fragment length polymorphism analyses for determination of MTHFR A1298T genotype. 1 and 1’, 2 and 2’, 3 and 3’, 4 and 4’ are all the same DNA samples; 1-4 are the P1/P2 PCR products; 1’, 2’, 3’, 4’ are the P1/P3 PCR products; 1, 2: AA genotype; 3, 4: AC genotype; 5: Cc genotype.

patient diagnosed with VTE were recruited at the First Affiliated Hospital of Xinjiang Medical University from January 2006 to December 2012. They are grouped as the first VTE group and second VTE groups, respectively. We preferred healthy participants for each VTE patient group matched for sex, ethnicity, and age as the controls. Control subjects were selected from the Physical Examine Centre of Xinjiang Medical University. Approval of this study was confirmed by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China). All the participants take part in written informed consent.

Definition of the cardiovascular risk factor

Hypercholesterolemia was defined as serum cholesterol more than 6.1 mmol/L (235 mg/dl). Hypertension was considered if the mean systolic pressure was above 140 mmHg and/or diastolic pressure was above 90 mmHg, additionally if the patient was taking antihypertensive medications. Smokers were grouped into current smokers or non-smokers, however former smokers were included who had quit smoking for at least six months before the study. Diabetes mellitus was defined if fasting glucose in the serum was 126 mg/dl or higher. Height and Weight were measured. Body mass index (BMI) was measured as total body weight (kg)/height squared (m²).

Blood sampling

For isolation of DNA, measurement of routine chemical variables and lipoprotein and apolipoprotein levels, fasting blood samples were measured. Conventional methods of clinical chemistry were applied for the measurement of total cholesterol, HDL-cholesterol, and triglyceride levels. We used the Friedewald formula to measure LDL-cholesterol levels. To determine the homocysteine levels, fasting blood samples were obtained into EDTA vials with ice packed immediately and were centrifuged within 30 min to avoid false increases in homocysteine due to release from red blood cells. tHcy was obtained with an immunoassay (Axis Biochemicals, Oslo, Norway).

Biochemical determinations

Total Genomic DNA was isolated from peripheral blood leukocytes by the phenol/chloroform extraction procedure (Tiangen Biotech Beijing
Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) (PCR Amplifier: MJ Research Co. USA) was used for the genotype analysis. Briefly, 500 ng genomic DNA was amplified with 7 pmol each of the MTHFR gene C677T forward primer F: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and the reverse primer R: 5'-AGGACGGTGCGGTGAGAGTG-3'. (primer maker: Sangon Biotech Shanghai, China) The following PCR parameters were used: denaturation cycle at 94°C for a 5 min and 38 cycles of the following: 96°C for 1 min, 56°C for 1 min, and 72°C for 1 min, followed by a 10-min extension cycle at 72°C. A 5-μl aliquot of the 198-bp PCR product was digested with 4 μL of 10× MboII buffer and five units of MboII restriction enzyme (Fermentas Company, USA) incubated at 37°C overnight. The PCR Digestion products were silver-stained after electrophoresis separated by 3% agarose gel (Fermentas Company, USA). The MTR PCR product of 198 bp was cut into fragments of 175 and 28 bp in the presence of the mutation (Figures 1 and 2).

Briefly, 500 ng genomic DNA was amplified with 7 pmol each of the MTHFR gene A1298T forward primer F: 5'-TCTTTG TTCTTGGAAGCGG-3' and the reverse primer R1: 5'-CGAAGACTCTAAAAGACACTTG-3', R2: 5'-CGAAGACTCTAAGACACTTT-3'. (primer maker: Sangon Biotech Shanghai, China) PCR thermal cycling conditions were as: denaturation cycle at 95°C for 2 min and 35 cycles of the following: 94°C for 40 s, 50°C for 40 s, and 72°C for 1 min. This was followed by a 10-min extension cycle at 72°C. HaeIII restriction digestion using 1 μl of 10× HaeIII buffer (Fermentas Company, USA) and five units of HaeIII restriction endonuclease (Fermentas Company, USA) added to 8.5 μl of PCR product incubated at 37°C overnight. Digestion products were silver-stained after electrophoresis separated by 3% agarose gel (Fermentas Company, USA). The MTR PCR product of 189 bp was cut into fragments of 159 and 30 bp in the presence of the mutation. Sequencing reactions were undertaken by Biomed (Beijing Biomed Co.) (Figures 3 and 4).

Briefly, 500 ng genomic DNA was amplified with 7 pmol each of the MTHFR gene C1317T forward primer F: 5'-ACAGGATGGGGAAGTCACAG-3' and the reverse primer R: 5'-AGGAGGAGCTGTAAGATG-3' (primer designer: Sangon Biotech Shanghai, China). The following PCR parameters were used: denaturation cycle at 95°C for a 5 min and 30 cycles of the following: 94°C for 1
MTHFR genetic polymorphisms and venous thromboembolism

Table 2. Allele frequencies of the MTHFR polymorphisms among the Uyghur patients with VTE and control individuals

<table>
<thead>
<tr>
<th>Gene polymorphism</th>
<th>Uyghur</th>
<th></th>
<th></th>
<th></th>
<th>Han</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-/-</td>
<td>26 (30.23)</td>
<td>43 (35.25)</td>
<td>56 (65.12)</td>
<td>76 (62.30)</td>
<td>86</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>36 (41.86)</td>
<td>64 (52.46)</td>
<td>23 (26.74)</td>
<td>39 (31.97)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>24 (27.91)</td>
<td>15 (12.29)</td>
<td>7 (9.14)</td>
<td>7 (5.73)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.017</td>
<td>0.620</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Comparison of MTHFR C677T in Hcy between Uyghur and Han population

<table>
<thead>
<tr>
<th>Hcy (mmol/L)</th>
<th>VTE</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uyghur CC</td>
<td>22.43±12.21</td>
<td>12.32±5.61</td>
</tr>
<tr>
<td>CT</td>
<td>25.13±11.71</td>
<td>12.62±7.65</td>
</tr>
<tr>
<td>TT</td>
<td>26.11±13.02</td>
<td>15.62±6.32</td>
</tr>
<tr>
<td>Han CC</td>
<td>23.31±12.51</td>
<td>14.21±5.51</td>
</tr>
<tr>
<td>CT</td>
<td>22.9±11.01</td>
<td>14.41±8.12</td>
</tr>
<tr>
<td>TT</td>
<td>26.52±12.31</td>
<td>15.42±6.53</td>
</tr>
</tbody>
</table>

Note: compared with CT in the same ethnic group,
^P<0.05; compared with CC in the same ethnic group,
^P<0.05.

Results

Characteristics of the participants

Table 1 Clinical characteristics and blood parameters measured in the study subjects of Uyghur and Han population with case status. Patients were older and more likely to be male with their VTE risk profile unfavorable compared to control subjects. For example, they had a higher blood pressure and total cholesterol level. In addition, they were more likely to be smokers with their lower HDL cholesterol level. The mean (±SD) homocysteine concentration in VTE patients (27.87±9.51 μmol/L) was significantly higher than the mean concentration in controls (21.87±8.67 μmol/L) in both Uyghur and Han ethnic (P<0.001). According to clinical and laboratory parameters, the estimation of the relative risk (RR) of VTE examined in this study are given in Table 1.

Genotype and allele distribution in cases and control subjects

The frequencies of the alleles in case and controls were 36.7% (95% CI 32.0-41.5), 15.7% (95% CI 12.3-19.0) and 11.4% (95% CI 12.3-19.0) for the C677T, A1298C, and C1317T allele in the MTHFR resembles to those previously reported in other populations. Table 2 shows Allele frequencies of the MTHFR poly-
MTHFR genetic polymorphisms and venous thromboembolism

Table 4. Logistic regression for the risk factors of VTE for Uyghur and Han population

<table>
<thead>
<tr>
<th></th>
<th>Han</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Uyghur</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>S.E.</td>
<td>Wald</td>
<td>P</td>
<td>OR</td>
<td>95% CI</td>
<td>B</td>
<td>S.E.</td>
<td>Wald</td>
<td>P</td>
</tr>
<tr>
<td>677TT</td>
<td></td>
<td>-0.568</td>
<td>0.437</td>
<td>1.687</td>
<td>0.194</td>
<td>0.567</td>
<td>0.241-1.225</td>
<td>0.229</td>
<td>0.417</td>
<td>0.301</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td>-0.540</td>
<td>0.466</td>
<td>4.363</td>
<td>0.024</td>
<td>2.867</td>
<td>1.062-6.586</td>
<td>0.625</td>
<td>0.607</td>
<td>6.421</td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td>0.303</td>
<td>0.257</td>
<td>1.386</td>
<td>0.239</td>
<td>1.354</td>
<td>0.815-2.243</td>
<td>1.539</td>
<td>0.607</td>
<td>6.421</td>
</tr>
<tr>
<td>Hhcy</td>
<td></td>
<td>0.020</td>
<td>0.007</td>
<td>8.412</td>
<td>0.004</td>
<td>1.020</td>
<td>1.006-1.034</td>
<td>0.024</td>
<td>0.011</td>
<td>5.096</td>
</tr>
<tr>
<td>Constants</td>
<td></td>
<td>-1.090</td>
<td>0.376</td>
<td>8.405</td>
<td>0.004</td>
<td>0.336</td>
<td></td>
<td>-2.311</td>
<td>0.722</td>
<td>10.252</td>
</tr>
</tbody>
</table>

Discussion

Our study confirmed the presence of MTHFR-677 and MYHFR-1298, and MTHFR-1317 polymorphisms in Han and Uyghur population, and their association with VTE. We found that the presence of the MTHFR gene polymorphisms and Hcy significantly increased in the risk of VTE among the people in Xinjiang.

Elevated plasma thcy assumed to increase vascular disease in a number of ways, perhaps by inducing procoagulant activity of monocytes and promoting endothelial tissue factor expression. Salomon O et al, shows that disequilibrium of the homocysteine remethylation pathway of homocysteine metabolism, MTHFR C677T and MTHFR A1298C, leads to increased homocysteine levels, especially in patients with the inadequacy of folic acid, vitamin B6, or B12 promotes to endothelial dysfunction and therefore may be potential risk factors for VTE [10]. Even though HHCy usually seen in VTE patients, the link between MTHFR genotypes, folate levels and risk of VTE is poorly defined in Asian populations. Several studies have established facts

Association between genotypes and homocysteine levels

To further analyze the potential contribution of the MTHFR C677T, and A1298T polymorphisms to elevated thcy levels, patients and controls were grouped together (n=538). As shown in Table 3, Comparison of MTHFR C677T in Hcy between Uyghur and Han population shows significant differences in TT genotype of MTHFR677T between VTE patients and controls (Uyghur: x²=8. 070, P=0. 004; Han: x²=8. 159, P=0. 004).

Multivariate analysis

Smoking (P<0.001), hypertension (P<0.05), diabetes (P<0.05), HHcy (P<0.001), and MTHFR gene C677T polymorphism (P<0.001) were independent correlates to VTE. Age, gender, smoking, hypertension, diabetes, hypercholesterolemia and MTHFR gene C677T and A1298C polymorphisms were commendable in the study. Multifactor logistic regression analysis confirms that, plasma Hcy levels (OR=1.025, 95% CI 1.003-1.046, P=0.024) and obesity (OR=4. 660, 95% CI 1.417-15.324, P=0.011) were independent risk factors for Uyghur ethnic with VTE while plasma Hcy levels (OR=1.020, 95% CI 1.006-1.034, P=0.004) and smoking (OR=2.867, 95% CI 1.062-6.586, P=0.024) were independent risk factors for Han ethnic with VTE. Furthermore, our results showed that Hcy and smoking habits were related to VTE (P<0.05). Obesity and smoking are independent risk factors for Uyghur ethnic and Han ethnic with VTE, respectively. Analysis shows significant differences in the MTHFR T677T polymorphism genotyping distribution frequency in Uyghur and Han ethnic between controls and between VTE patients (P<0.05). High total plasma levels of Hcy in MTHFR gene TT genotype were statistically higher than CT and CC genotype (P<0.05) as shown in Table 4.
MTHFR genetic polymorphisms and venous thromboembolism

that endothelial dysfunction in the long term is caused by serum homocysteine along with inhibition of protein C activation for the further advancement of thrombosis [11] and abnormal methionine metabolism that affects the DNA cell membrane [12]. The prevalence of MTHFR mutations varies between racial and ethnic groups, along with different conflicting data regarding MTHFR gene mutations in Asian patients with VTE [13]. It has been stated that HHcy in Asian Indians has been linked with low folate levels [14], which were also observed in our study. The prevalence of the MTHFR C677T genotype in Asian participants was (3.8%) lower than the predicted 11% reported for Japanese [15] along with Chinese populations [16]. We observe negative correlation between homocysteine and folate levels, implying that hyperhomocysteinemia in our patients can be related to lower folate levels. Nevertheless, we have also observed significant difference in homocysteine levels based on smoking habits in our study along a smoker with tHcy was associated with a significant risk of VTE. After adjusting for age, gender, smoking history, hyperlipidemia, hypertension, diabetes and MTHFR genotype, multifactor logistic regression analysis showed that plasma Hcy levels (OR=1.025, 95% CI 1.003-1.046, P=0.024) and obesity (OR=4.660, 95% CI 1.417-15.324, P=0.011) were independent risk factors for Uyghur ethnic with VTE while plasma Hcy levels (OR=1.020, 95% CI 1.006-1.034, P=0.004) and smoking (OR=2.867, 95% CI 1.062-6.586, P=0.024) were independent risk factors for Han ethnic with VTE. However, there were no significant differences in the SNP genotyping distribution frequency in Uyghur and Han ethnic between controls and between VTE patients (P>0.05). Deficiencies of Vitamin B6 and vitamin B12 are substantial cause of HHcy because both of these play a central role in homocysteine metabolism. Kottkut N, et al established that HHcy as the only risk factor of VTE, which is independent from vitamin B6, vitamin B12, and folate levels [17]. Meta-analyses of two randomized control trials presented that folate supplementation does not reduce vascular events [18, 19] but these data are inconclusive and indicate ethnic differences in genetic polymorphisms that are diet responsive and may be beneficial when examine ethnic variations in chronic disease, developmental anomalies, and folate requirements. Genetic polymorphisms, MTHFR C677T, and A1298C are known to influence the plasma homocysteine concentrations and the increase incidence of cardiovascular diseases [20]. The enzyme MTHFR is found to have an important role in homocysteine metabolism by catalyzing the conversion of 5, 10-methylene tertrahydrofolate to 5-methylene tertrahydrofolate, and the methyl-group donor in the B12-dependent remethylation of homocysteine to methionine. The Study proves that severe deficiency of the MTHFR enzyme advance to homocystinuria which is a rare inborn error of metabolism symbolizes by highly increased blood and urine homocysteine concentrations [21]. Thus the reduction in MTHFR level promotes to hyperhomocysteinemia, represent by increased plasma total homocysteine (Hcy) levels, and is frequently observed in patients with vascular diseases. Even though the MTHFR A1298C polymorphism has been associated with HHcy, its prevalence in Uyghur subjects with VTE has not been studied till date. Heterozygotes for the A1298C polymorphism demonstrate only a 10% reduction in the activity of MTHFR level, at the same time the homozygote’s for this polymorphism show a reduction of 35-45% in the activity of the enzyme and as a result it can possibly elevate the levels of homocysteine. We noticed that there were no significant associations in MTHFR-1298 polymorphisms with the increased risk of VTE in our subjects. We also noticed there were no significant higher plasma homocysteine levels with MTHFR 1298 CC genotype suggest a possible link between MTHFR 1298CC genotype and HHcy in our subjects. We have noticed a similar type of association between MTHFR 1298CC genotype and HHcy in Indian populations [22] along with T1317C was present in 5% of alleles in Canadian individuals which also seems to be extremely common in individuals of African ancestry [23].

In conclusion, this study emphasizes the high prevalence of HHcy in Han and Uyghur patients with VTE. HHcy was significantly associated with increased risk of VTE independent from the other confounding factors. Secondary the C677T mutation is a risk factor for HHcy and significantly associated with VTE in Chinese populations. HHcy is the likely modifiable risk factor that should be deal with screening patients with VTE. Thirdly Assessment of vitamin B12 deficiency that may influence homo-
cysteine levels should be recognized to make an attempt to reverse the conditions.

Acknowledgements

This study has been supported by a grant from State Key Lab Incubation Base of Xinjiang Major Diseases Research (No. SKLIB-XJMDR-2012-7).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ailiman Mahemuti, Department of Cardiology, The First Affiliated Hospital of Xinjiang Medical University, 830054 Liyu Road, Urumqi, Xinjiang, China. Fax: +869914366170; E-mail: Nayisha2006@hotmail.com; 151-9362315@qq.com

References


MTHFR genetic polymorphisms and venous thromboembolism


