Methylenetetrahydrofolate Reductase Gene Polymorphism in Korean Patients with Migraine or Ischemic Stroke

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Background: Methylenetetrahydrofolate reductase (MTHFR) gene polymorphism has been implicated in both migraine and ischemic stroke. The homozygous C677T mutation in the MTHFR gene was more frequent in the Japanese and Turkish migraineurs than in the control group. Positive associations have also been found in ischemic stroke. The purpose of this study is to investigate the role of MTHFR C677T polymorphism in Korean patients with migraine or ischemic stroke.

Methods: We analyzed the allele frequencies and genotype of MTHFR C677T polymorphism in 115 patients with migraine, 213 with cerebral infarction, and 73 controls.

Results: There was no significantly increased frequency of homozygosity for the T677 allele in both of the diagnostic groups, compared to the controls.

Conclusions: Our results suggest that MTHFR gene C677T polymorphism is unlikely to play a major role in the pathogenesis of migraine or ischemic stroke in Korean patients.

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Key Words: Migraine, Stroke, Homocysteine, Methylenetetrahydrofolate reductase, Polymorphism

INTRODUCTION

Elevated plasma homocysteine levels have been identified as a risk factor for coronary heart disease¹ and ischemic stroke.² Hyperhomocysteinemia is caused by nutritional deficiencies or genetic defects in one of the enzymes involved in homocysteine metabolism. 5, 10-methylenetetrahydrofolate reductase (MTHFR) is the key rate-limiting enzyme required for the conversion of dietary folate to 5-methyltetrahydrofolate, the methyl group donor required for the remethylation of homocysteine to methionine.

The most common and extensively studied poly–
morphism is the thermolabile C677T MTHFR mutation. Individuals who have a C-to-T substitution at base 677 of MTHFR gene have reduced enzyme activity and hyperhomocysteinemia. This variant has been considered an ideal candidate genetic polymorphism for predisposition to ischemic stroke. However, studies that have examined the risk for ischemic stroke associated with a MTHFR C677T polymorphism have reported conflicting results.3-6

Migraine is a common neurovascular disorder with an unknown pathomechanism. Migraine is believed to be the manifestation of a hereditary abnormal sensitivity of neurovascular reactions to sudden changes in the internal or external environment, or to cyclic changes in the central nervous system.7 Although migraine is also believed to have a strong genetic component, at present the type and the number of genes involved in this diseases are unclear. The relationship between stroke and migraine is one of the most perplexing problems for neurologist. However the clinical experience that migraine may present as stroke like episodes or develop into an ischemic stroke supports a possible relationship between the two disease. Epidemiological studies have shown the presence of a significant comorbidity between migraine and ischemic stroke,8-10 In addition several prothrombotic genetic factors seem to be involved in the migraine.11-13

The homozygous C677T mutation in the MTHFR gene was reported to be more frequent in the Japanese and Turkish migraineurs than in the control group.14-15 However, a true relationship between migraine and MTHFR 677TT genotype is uncertain because elevated blood homocysteine was not observed in patients with migraine16 or primary headache.17

In view of these conflicting results in both migraine and ischemic stroke, we have conducted a study of the C677T MTHFR gene polymorphism.

**MATERIALS and METHODS**

DNA was available from the following groups: migraineurs (n=115), ischemic stroke group (n=213), and controls (n=73). All samples were recruited in the Eulji hospital. All participants were Korean and northeastern in Seoul. An informed consent was obtained from the subjects who involved in the study.

One hundred fifteen migraineurs (17 males, 98 females; mean age±SD, 33.8±12.2 years), attending the Headache Clinics of Eulji Hospital, were involved in the study. The diagnosis of migraine was made by one neurologist (B Kim) using a structured questionnaire reproducing the International Headache Society (IHS) criteria.18 All individuals were asked to fill out a structured questionnaire. For additional statistical analyses, migraine patients were divided into two groups: migraine without aura (IHS code 1.1; 94 patients; 10 males, 84 females; mean age±SD, 28.5±13.2 years); and migraine with aura (IHS code 1.2; 21 patients; 6 males, 15 females; mean age ±SD, 35.0±11.7 years). Ischemic stroke group included 213 patients (104 males, 109 females; mean age±SD, 62.7 ±8.6 years) who were admitted to the neurological departments due to an acute ischemic stroke. Only patients with a proven infarction on the computerized tomography (CT) or magnetic resonance imaging scan were enrolled as patients with ischemic stroke. Patients who had transient ischemic attacks were not included in this group. Seventy three controls (28 males, 45 females; mean age±SD, 45.3±11.4 years) were screened for the absence of neurological diseases and specific questions were asked concerning the symptoms of migraine and stroke. Table 1 presents clinical and demographic characteristics of the migraine, ischemic stroke and control subjects.

The genomic DNA were prepared from EDTA-treated peripheral blood samples using Puregene blood DNA kit (Gentra Inc.) following manufacturer’s protocol. The genotypes of the patients and control samples were assayed by single base primer extension assay using SNpShot assay kit according to manufacturer’s recommendation (ABI). Briefly, the interested region of the MTHFR was amplified with PCR reaction with 5’ – CGAAGCAAGGCTTGGCCT (Forward) and 5’ – AGGACGGTGCGGTAGAGTG (Reverse) primer pairs. The PCR conditions were : 10 min at 95℃ for 1 cycle, and 30
cycles on 95°C for 30s, 65°C for 1min, 72°C for 1min followed by 1 cycle of 72°C for 7mins. After amplification, the PCR products were treated with 2.0 unit of SAP (shrimp alkaline phosphatase) and 2.0 unit of EXO I (Exonuclease I) at 37°C for 60 minutes and 72°C for 15 minutes. The SNP genotyping primer (5’-AGAAGGTGTCTGCGGGAG) were added to the reaction products with appropriate amount of SNaPShot mix and cycled on PCR machine for 25 cycles of 96°C for 10s, 50°C for 5s, 60°C for 30s, 0.5 unit of SAP was added to the product and incubated for 60 min at 37°C followed by 15 minute at 72°C. The final products were analyzed on ABI 3700 automated sequence analyzer. Gene scan program was used to call genotypes for each sample.

The Pearson chi-square test or Fisher’s exact test were used to compare the frequencies of allele and genotype between cases and controls, if appropriate. Differences in clinical features were compared using the Student’s t-test or the Pearson chi-square test. The level of significance was set at p<0.05.

### RESULTS

Table 2 shows the comparison of genotype and allele frequencies of MTHFR gene in study groups. The allelic frequencies for the MTHFR C677T polymorphism were not significantly different between migraineurs and controls (p=0.18), or when divided into migraine with aura and migraine without aura (p=0.33 and p=0.22, respectively). We also found no differences in the frequency of the TT genotype between migraine subjects and controls (p=0.14), or when divided into migraine with aura and migraine without aura (p=0.37 and p=0.17, respectively). Similarly, there were no differences in the frequencies of allele and TT genotypes between cerebral infarction and controls (p=0.51 and p=0.52, respectively).

The demographic and clinical characteristics of migraineurs according to MTHFR C677T genotypes are shown in Table 3. No significant association was found between the genotype and all the features examined (p>0.05).

### Table 1. Clinical and demographic characteristics of migraine, cerebral infarction and controls

<table>
<thead>
<tr>
<th></th>
<th>Migraine</th>
<th>Cerebral infarction</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA</td>
<td>115</td>
<td>21</td>
<td>213</td>
</tr>
<tr>
<td>MO</td>
<td>34</td>
<td>94</td>
<td>73</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>12-66</td>
<td>12-66</td>
<td>12-66</td>
</tr>
<tr>
<td>Males</td>
<td>17 (14.8)</td>
<td>13-66</td>
<td>104 (48.8)</td>
</tr>
<tr>
<td>Females</td>
<td>98</td>
<td>84</td>
<td>109</td>
</tr>
</tbody>
</table>

MA; migraine with aura, MO; migraine without aura. Figures in parentheses represent percentage values.

<table>
<thead>
<tr>
<th></th>
<th>Allele frequency (%)</th>
<th>Genotype frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migraine</td>
<td>C (60.4) T (39.6)</td>
<td>CC (57.9) CT (42.1) TT*</td>
</tr>
<tr>
<td>MA</td>
<td>26 (61.9) T (38.1)</td>
<td>35 (35.1) 70 (33.9) 17 (14.8)</td>
</tr>
<tr>
<td>MO</td>
<td>113 (60.1) T (39.9)</td>
<td>33 (35.1) 70 (33.9) 17 (14.8)</td>
</tr>
<tr>
<td>Cerebral infarction</td>
<td>241 (56.6) T (43.4)</td>
<td>70 (33.9) 101 (47.4) 42 (19.7)</td>
</tr>
<tr>
<td>Controls</td>
<td>78 (53.4) T (46.6)</td>
<td>22 (30.1) 34 (46.6) 17 (23.3)</td>
</tr>
</tbody>
</table>

*No differences in the frequency of TT genotype between migraine and control (p=0.14), and between cerebral infarction and control (p=0.52).

MA; migraine with aura, MO; migraine without aura.
Table 3. Clinical features of migrainers according to MTHFR C677T genotype

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>41</td>
<td>57</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Mean age (years)*</td>
<td>34.8±13.6</td>
<td>33.6±12.0</td>
<td>31.8±9.3</td>
<td></td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>8:33</td>
<td>6:51</td>
<td>3:14</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Mean age at onset (years)*</td>
<td>28.3±14.1</td>
<td>26.4±8.5</td>
<td>22.8±10.7</td>
<td></td>
</tr>
<tr>
<td>Mean disease duration (years)*</td>
<td>6.5±11.9</td>
<td>7.2±15.4</td>
<td>9.0±8.2</td>
<td></td>
</tr>
<tr>
<td>Family history of migraine (%)</td>
<td>32 (68.10)</td>
<td>43 (75.4)</td>
<td>13 (76.5)</td>
<td></td>
</tr>
</tbody>
</table>

*Mean±SD.
† No significant association was found between the genotype and all the features examined (p>0.1).

DISCUSSION

We found no allelic or genotypic association between MTHFR C677T polymorphism and migraine or ischemic stroke. In addition, the MTHFR genotype did not significantly influence the migraine subtype or clinical features that we have examined.

Although the precise mechanism of migraine-related stroke is not known, risk factors for hypercoagulability have been found in migrainers.\(^\text{11-13}\) From this point of view, several studies have investigated the relationship between migraine and hyperhomocysteinemia\(^\text{14,17}\) or MTHFR gene polymorphism.\(^\text{14,15}\) Contrarily to our results, previous studies revealed positive association between MTHFR C677T gene polymorphism and migraine. That our findings differ from previous results can be accounted for in several ways. It may be partly due to the ethnic difference. Since there is no biological marker for migraine, diagnosis of migraine is entirely made by combination of history and neurological examination. Heterogeneity of study group due to diagnostic vagueness may explain these conflicting results. However, the fact that hyperhomocysteinemia was not observed in patients with migraine or primary headache suggests no association between MTHFR gene and migraine.

Most studies to date have indicated that hyperhomocysteinemia is independently associated with ischemic stroke,\(^\text{2,5,6}\) and that TT genotype is associated with hyperhomocysteinemia.\(^\text{19}\) However, studies that have examined the risk for ischemic stroke associated with a MTHFR C677T polymorphism have reported conflicting results.\(^\text{2-6}\) It may be due to diversity of risk factors for stroke and heterogeneity of pathomechanism according to the stroke subtypes.\(^\text{5}\) This can be overcome by larger sample size with well classified subtypes and risk factors for stroke. In conclusion, C677T polymorphism is unlikely to play a major role in the pathogenesis of migraine or ischemic stroke in Korean patients.

REFERENCES


