Pathogenicity of Thermolabile Methylenetetrahydrofolate Reductase for Vascular Dementia

Jun-Hyun Yoo, Gyu-Dong Choi, Soo-Sang Kang

Abstract—Although the major biochemical abnormality due to methylenetetrahydrofolate reductase (MTHFR) deficiency is hyperhomocyst(e)inemia, its pathogenicity appears to involve more than homocysteine toxicity. In patients with severe MTHFR deficiency, a metabolite(s) other than hyperhomocyst(e)inemia also appears to be associated with its clinical manifestation in cerebrovascular disease. To elucidate the specific role of the TT genotype of MTHFR in the development of cerebral infarction with and without cognitive impairment, we determined the prevalence of hyperhomocyst(e)inemia and the C677T genotypes of MTHFR in 143 patients with vascular dementia, 122 patients with cerebral infarction, and 217 healthy subjects matched for age and sex. Prevalence of hyperhomocyst(e)inemia [homocyst(e)ine ≥15 μmol/L] was higher in cerebrovascular patients with or without dementia than in normal control subjects (42.6%, 20.5%, and 10.1%, respectively; \( P<0.001 \)). In contrast, a higher frequency of MTHFR TT genotype was found only in demented patients compared with nondemented patients and healthy controls (25.2%, 9.8%, and 12.0%, respectively; \( P<0.01 \)). When the study subjects were divided into normohomocyst(e)inemic and hyperhomocyst(e)inemic groups, the TT genotype was significantly associated with the risk for vascular dementia in the hyperhomocyst(e)inemic group (odds ratio 4.13, 95% CI 2.18 to 7.85; \( P<0.03 \)) but not in the normohomocyst(e)inemic group. Demented patients with multiple infarcts had a higher frequency of TT genotype (odds ratio 3.13, 95% CI 2.23 to 4.39; \( P<0.0007 \)), whereas those with a single infarct did not (odds ratio 2.03, \( P<0.15 \)). In contrast, there was no significant association of the TT genotype with multiple infarcts in hyperhomocyst(e)inemic stroke patients. Taken together, these findings indicate a possible role of MTHFR TT genotype combined with hyperhomocyst(e)inemia in the pathogenesis of vascular dementia. Similar to the relationship between homocystinuria due to severe MTHFR deficiency and severe cystathionine β-synthase deficiency, the TT genotype of MTHFR in hyperhomocyst(e)inemic subjects is differentiated from the cases of the TT genotype without hyperhomocyst(e)inemia or hyperhomocyst(e)inemia without the TT genotype in the development of cerebrovascular disease. (Arterioscler Thromb Vasc Biol. 2000;20:1921-1925.)

Key Words: methylenetetrahydrofolate reductase ■ genes ■ cerebral infarction ■ hyperhomocyst(e)inemia ■ vascular dementia

Determination of plasma homocyst(e)ine (the total of free and protein-bound forms of homocysteine and its derivatives) became an important method in evaluating the risk of cardiovascular and cerebrovascular diseases. It is understood that the severity and duration of hyperhomocyst(e)inemia is closely related to the extent and progress of occlusive vascular disease. Although severe hyperhomocyst(e)inemia (known as homocystinuria) causes thromboembolism and vascular damage in children and young adults, moderate and intermediate hyperhomocyst(e)inemia is believed to be associated with occlusive vascular disease in adults.1,2 Moderate hyperhomocyst(e)inemia is caused by genetic or environmental factors or a combination of both factors.1,2 The most common genetic defect is thermolabile methylenetetrahydrofolate reductase (MTHFR), such as the homozygous C677T mutation.3,4 This mutation causes an increased susceptibility to produce hyperhomocyst(e)inemia. However, the TT genotype of MTHFR requires an additional genetic or environmental factor for persistent hyperhomocyst(e)inemia.2 This might explain the inconsistent results found when the association between the TT mutation of MTHFR and cerebrovascular disease was evaluated.5-7

In severe hyperhomocyst(e)inemia, neurological and vascular manifestations are more pronounced despite less severe hyperhomocyst(e)inemia in patients with severe MTHFR deficiency compared with patients with severe cystathionine β-synthase deficiency.5,9 This suggests that the pathogenic feature of hyperhomocyst(e)inemia with MTHFR deficiency...
may be different from that of hyperhomocyst(e)inemia alone. Demyelination of the brain in patients with severe MTHFR deficiency appears to be associated with hypomethioninemia and reduced S-adenosylmethionine accumulation or depletion of other metabolites in addition to hyperhomocyst(e)inemia. However, except for hyperhomocyst(e)inemia, determination of other biochemical abnormalities in mild MTHFR deficiency seems beyond the range of detection. Nonetheless, there is evidence to support the unique aspect of thermolabile MTHFR and the TT genotype in nonvascular diseases. It has been reported that mild MTHFR deficiency is positively associated with various nonvascular diseases, such as nonvascular cardiac disease, neural tube defects, colorectal cancer, and schizophrenia/depression.

We postulate that the pathogenicity of mild MTHFR deficiency is not only confined to hyperhomocyst(e)inemia but is also related to the accumulation or depletion of another metabolite(s). To elucidate the new feature of mild MTHFR deficiency in the development of cerebrovascular disease, we chose to compare the role of the TT genotype of MTHFR with and without hyperhomocyst(e)inemia in the occurrence of 2 different types of cerebrovascular disease, cerebral infarction and vascular dementia.

Methods

Study subjects were all unrelated Korean patients aged 65 to 90 years who were admitted to Incheon Neuropsychiatric Hospital, where patients with dementia had been referred from other hospitals for chronic care. Patients were consecutively recruited from January through October 1998 if they met the following criteria: vascular dementia on the basis of National Institute of Neurological Disorders and Stroke (NINDS)–Association Internationale pour la Recherche et l’Enseignement en Neurologie (AIREN) criteria and a Hachinski Ischemic Score of ≥7. Brain CT or MRI was performed in all patients undergoing the study and assessed by 2 neuroradiologists. ECG, serum multiphasic analysis, and thyroid function tests were examined. Fourteen demented patients with intracranial hemorrhage, cancer, renal dysfunction (serum creatinine ≥132.6 μmol/L), hypothyroidism, alcoholism, folate or vitamin B12 deficiency, or use of diuretics were excluded. One hundred forty-three patients were selected for the dementia group.

During the study period, 196 patients who had undergone brain MRI or CT and were assessed as cerebral infarction subjects were then enrolled for the study. Healthy individuals were eligible for inclusion if they did not have a past history of stroke. Three hundred eighty-two subjects consented to participate in the study. Laboratory data of the cases were examined. Social and medical histories were obtained through interviews by investigators. Subjects with Mini-Mental State Examination scores of ≤24 were excluded. Other exclusion criteria were identical to those of cases, according to which 74 patients with cerebral infarction and 76 healthy subjects were excluded. Of 306 healthy subjects, 89 had evidence of symptomatic coronary artery disease. Matched for sex and age within 4 years, a total of 122 patients with cerebral infarction and 217 healthy subjects free of coronary artery disease and stroke were selected for control group. The study was approved by the local ethics committee. Informed consent was obtained from family members or participants.

As previously described, plasma homocyst(e)ine, folate, and vitamin B12 were determined by use of high-performance liquid chromatography, fluorescence detection, and radioimmunoassay. DNA was amplified by polymerase chain reaction. Polymerase chain reaction primers for amplification of the MTHFR mutation have been described elsewhere.

Amplicated 198-bp fragments were incubated with HindII (Takara) for digestion, because the nucleotide 677 mutation creates a restriction site for HindII. Genotypes were determined through gel electrophoresis and ethidium bromide staining. The mutant allele was designated as T; the wild-type, as C. A χ2 test for categorical variables and a t test for continuous variables were applied for comparison between groups. Because plasma homocyst(e)ine and serum triglyceride levels were not normally distributed, natural logarithmic transformation was used. Plasma homocyst(e)ine and other values among MTHFR genotypes were compared by ANOVA, followed by the Duncan test for multiple comparisons. Multiple logistic regression analysis was used to estimate the adjusted odds ratio (OR) for vascular dementia. SAS statistical software (version 6.12, SAS Institute) was used.

Results

Demographic data, serum lipid profiles, and prevalence of major risk factors in 143 patients with vascular dementia, 122 patients with cerebral infarction, and 217 clinically healthy subjects are shown in Table 1. Average onset of vascular disease was 73.5 years. Mean plasma homocyst(e)ine concentration was higher in the 2 patient groups than in the normal control group. The proportion of moderate hyperhomocyst(e)inemia was higher in patients with vascular dementia than in stroke patients or in control subjects (42.6%, 20%, and 10.1%, respectively; P<0.001). No significant differences of any risk factors were seen in patients with vascular dementia compared with patients with cerebral infarction. Among other risk factors, a statistically significant difference between the 2 groups with cerebrovascular disease and the healthy group was found for the frequency of diabetes mellitus and smoking but not for hypertension, atrial fibrillation, and lipid values.

We found a higher frequency of the TT genotype of MTHFR in patients with vascular dementia than in stroke patients or in control subjects (25.2%, 9.8%, and 12.0%, respectively; P<0.001); data are reported in Table 2. The OR adjusted for hypertension, smoking, diabetes mellitus, atrial fibrillation, age, and sex was 2.56 (95% CI 1.92 to 3.42, P<0.001). Demented patients with multiple infarcts had a 3.1-fold higher frequency of TT genotype (95% CI 2.23 to 4.39, P=0.0007). When patients were subdivided by the severity of stroke lesions, the TT genotype was positively associated with the group with multiple infarcts (OR 3.13, 95% CI 2.23 to 4.39; P=0.0007) but not with the group with single infarcts in patients with vascular dementia. In contrast, neither infract group showed any significant association with the TT genotype in patients with cerebral infarction (Table 3).

We stratified study subjects into normal (<15 μmol/L) and hyperhomocyst(e)inemic (≥15 μmol/L) groups to investigate a possible independent relationship of the MTHFR TT genotype in the development of cerebral infarction with or without dementia. Distribution of the combination of hyperhomocyst(e)inemia and the TT genotype of MTHFR in the 2 groups of patients (those with vascular dementia and those with strokes) and in normal control subjects is shown in Table 4. In the groups with hyperhomocyst(e)inemia, the combination of hyperhomocyst(e)inemia and the TT genotype of MTHFR was significantly associated with the risk of vascular dementia (OR 4.13, 95% CI 2.18 to 7.85; P=0.03) compared with hyperhomocyst(e)inemia combined with the CT/CC genotype. A similar association was not found in patients with cerebral infarction. On the other hand, in the groups with normohomocyst(e)inemia, there was no significant association of the TT genotype with vascular dementia (OR 1.06, 95% CI 0.71 to 1.58; P=0.88). In a multiple logistic regression analysis to assess the effect of hyperhomocyst(e)inemia and the MTHFR TT genotype on vascular demen-
MTHFR and cerebral infarction. If indeed the pathogenic association between the C677T genotype of diabetes mellitus, and hyperhomocyst(e)inemia.

In earlier studies, 2 found a significant association between the TT genotype of MTHFR. Severe hyperhomocyst(e)inemia due to MTHFR deficiency manifests itself in more pronounced clinical features, particularly neurological abnormalities, compared with hyperhomocyst(e)inemia due to cystathionine β-synthase deficiency. To examine whether a pattern similar to that found in severe MTHFR deficiency occurs in moderate MTHFR deficiency, it seems appropriate to compare the role of the TT genotype with hyperhomocyst(e)inemia in patients with cerebrovascular disease with and without cognitive impairment.

The association between the C677T mutation of MTHFR and vascular dementia has been previously evaluated by a number of investigators. Nilsson et al reported a high prevalence of hyperhomocyst(e)inemia in psychogeriatric patients and suggested a possible genetic defect in demented patients with hyperhomocyst(e)inemia irrespective of folic acid depletion. Chapman et al observed the lack of a significant association between the MTHFR genotype and vascular dementia, which was probably due to the insufficient number of the sample. In the present study, we have demonstrated a positive association of hyperhomocyst(e)inemia in cerebrovascular patients with and without vascular dementia. In contrast, we have found a positive association of the TT genotype only with cerebrovascular disease in patients with...
vascular dementia. However, it is noteworthy that a significant association is confined to hyperhomocyst(e)inemic patients but not to normohomocyst(e)inemic patients with vascular dementia. In addition, the severity of infarction is related to the presence of the MTHFR deficiency with hyperhomocyst(e)inemia. When patients were subdivided into 2 groups with single and multiple infarcts, a positive association was found in the group with multiple infarcts but not in the group with single infarcts. The results demonstrated not only the importance of hyperhomocyst(e)inemia but also another feature of MTHFR deficiency in the development of vascular dementia.

In an earlier study, we demonstrated a positive correlation between thermolabile MTHFR and nonvascular heart disease. The TT mutation of C677T is found in the majority (92.6%) of patients with thermolabile MTHFR (S.-S. Kang, M.H. Kim, unpublished data, 1996). A similar positive correlation was also observed in other nonvascular diseases, such as neural tube defects, colon cancer, and schizophrenia/depression. Higher homocyst(e)ine and lower folate levels were found in patients with Alzheimer’s disease than in control subjects, indicating the importance of hyperhomocyst(e)inemia. Because hyperhomocyst(e)inemia is caused by various genetic and nongenetic conditions, it is important to evaluate an associated pathogenicity due to the primary defect. The present study indicated a significant association of these diseases with the mutation of MTHFR when hyperhomocyst(e)inemia was also present. The deficiency of MTHFR causes an accumulation of 5,10-methylenetetrahydrofolate as well as the inhibition of 5-methyltetrahydrofolate synthesis. Reduced synthesis of 5-methyltetrahydrofolate will cause decreased homocysteine remethylation. Hypomethioninemia and reduced S-adenosylmethionine occur frequently in severe MTHFR deficiency. Depletion of cerebrospinal fluid S-adenosylmethionine rather than hyperhomocyst(e)inemia appears to be closely associated with demyelination of the brain. Alternatively, accumulation of 5,10-methylenetetrahydrofolate due to MTHFR deficiency may be involved in the development of cognitive abnormalities. This may inhibit serine hydroxymethyltransferase-directed reaction or enhance trifunctional peptide-directed metabolism of 5,10-methylene- and 10-formyl-tetrahydrofolate synthesis.

<table>
<thead>
<tr>
<th>Group</th>
<th>MTHFR TT, n (%)</th>
<th>MTHFR CT/CC, n (%)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular dementia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single infarct (n=57)</td>
<td>10 (17.5)</td>
<td>47 (82.5)</td>
<td>2.03 (1.23–3.35)</td>
<td>0.15</td>
</tr>
<tr>
<td>Multiple infarct (n=86)</td>
<td>26 (30.2)</td>
<td>60 (69.8)</td>
<td>3.13 (2.23–4.39)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Cerebral infarction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single infarct (n=111)</td>
<td>10 (9.0)</td>
<td>101 (90.0)</td>
<td>0.83 (0.56–1.21)</td>
<td>0.61</td>
</tr>
<tr>
<td>Multiple infarct (n=11)</td>
<td>2 (18.2)</td>
<td>9 (81.8)</td>
<td>1.65 (0.75–3.75)</td>
<td>0.54</td>
</tr>
<tr>
<td>Healthy control (n=217)</td>
<td>26 (12.0)</td>
<td>191 (88.0)</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4. OR of MTHFR Genotype for Cerebrovascular Diseases According to Plasma Homocyst(e)ine Level and OR of Hyperhomocyst(e)inemia for Cerebrovascular Diseases**

<table>
<thead>
<tr>
<th>Group</th>
<th>MTHFR TT, n (%)</th>
<th>MTHFR CT/CC, n (%)</th>
<th>OR of TT (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocyst(e)ine level (&gt;15.0 \mu mol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular dementia (n=61)</td>
<td>26 (42.6)</td>
<td>35 (57.4)</td>
<td>4.13 (2.18–7.85)</td>
<td>0.03</td>
</tr>
<tr>
<td>Cerebral infarction (n=25)</td>
<td>3 (12.0)</td>
<td>22 (88.0)</td>
<td>0.86 (0.61–1.22)</td>
<td>0.62</td>
</tr>
<tr>
<td>Healthy control (n=22)</td>
<td>3 (13.6)</td>
<td>19 (86.4)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Homocyst(e)ine level (&lt;15.0 \mu mol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular dementia (n=82)</td>
<td>10 (12.2)</td>
<td>72 (87.8)</td>
<td>1.06 (0.71–1.58)</td>
<td>0.88</td>
</tr>
<tr>
<td>Cerebral infarction (n=97)</td>
<td>9 (9.3)</td>
<td>88 (90.7)</td>
<td>0.83 (0.79–1.86)</td>
<td>0.66</td>
</tr>
<tr>
<td>Healthy control (n=195)</td>
<td>22 (11.2)</td>
<td>173 (88.8)</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

**Homocyst(e)ine Level**

<table>
<thead>
<tr>
<th>Total of subjects with homocyst(e)ine (&gt;15.0) and (&lt;15.0 \mu mol/L), n (%)</th>
<th>OR of HHcy (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular dementia (n=143)</td>
<td>61 (42.6)</td>
<td>82 (57.4)</td>
</tr>
<tr>
<td>Cerebral infarction (n=122)</td>
<td>25 (20.5)</td>
<td>97 (79.5)</td>
</tr>
<tr>
<td>Healthy control (n=217)</td>
<td>22 (10.1)</td>
<td>195 (89.9)</td>
</tr>
</tbody>
</table>

HHcy indicates hyperhomocyst(e)inemia.
reactions may cause a different pathogenic effect of MTHFR deficiency unrelated to hyperhomocyst(e)inemia.

In summary, we investigated the nature of the MTHFR TT genotype in the development of cerebrovascular disease. The risk for the TT genotype with and without hyperhomocyst(e)inemia was compared in cerebrovascular patients with and without vascular dementia. The magnitude of cerebral infarction was also included in the evaluation. The present study confirmed a significant correlation between hyperhomocyst(e)inemia and cerebrovascular disease with or without vascular dementia. In contrast, only hyperhomocyst(e)inemic patients who had multiple cerebral infarctions and dementia had a positive association with the TT genotype. Such a unique feature of mild MTHFR deficiency is analogous to that of homocystinuria due to severe MTHFR deficiency, which is different from severe cystathionine β-synthase deficiency. We conclude that the pathogenicity of MTHFR TT genotype involves more than hyperhomocyst(e)inemia. Hence, the presence or absence of the TT genotype appears to be an important condition for understanding the role of hyperhomocyst(e)inemia in patients with cerebrovascular disease.

Acknowledgments
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References
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