Polymorphism of the Methionine Synthase Gene
Association With Homocysteine Metabolism and Late-Onset Vascular Diseases in the Japanese Population

Hiroyuki Morita, Hiroki Kurihara, Takao Sugiyama, Chikuma Hamada, Yukiko Kurihara, Takayuki Shindo, Yoshio Oh-hashi, Yoshio Yazaki

Abstract—Methionine synthase and 5,10-methylenetetrahydrofolate reductase (MTHFR) sequentially catalyze the remethylation of homocysteine to methionine. A point mutation in the encoding region of the methionine synthase gene, which results in substitution of an aspartic acid for a glycine residue (D919G), has been identified in patients of the cbIG genetic complementation group; these patients exhibit significantly decreased methionine synthase activity. Nevertheless, the D919G mutation has also been reported to be common in the general population. In this study, we analyzed the distribution of methionine synthase D/G polymorphism in the Japanese population and examined the extent to which it is associated with altered homocysteine metabolism and late-onset vascular diseases. We studied 215 patients with coronary artery disease, 251 patients with histories of ischemic stroke, and 257 control subjects. The methionine synthase genotype was analyzed by polymerase chain reaction followed by HaeIII digestion; allele frequencies for the D919G variant of the enzyme proved to be similar in all 3 subject groups (control subjects, 0.17; coronary artery disease patients, 0.17; and ischemic stroke patients, 0.19). Furthermore, in patients with ischemic stroke, plasma levels of homocyst(e)ine and folate were similar, irrespective of methionine synthase genotype. Thus, the methionine synthase D919G mutation was found to be common in the Japanese general population, and it appears unlikely that this polymorphism has a major effect on homocysteine metabolism and/or the onset of vascular diseases. (Arterioscler Thromb Vasc Biol. 1999;19:298-302.)

Key Words: homocysteine ■ methionine synthase ■ methylenetetrahydrofolate reductase ■ genetics

Recent reports indicate that even modest increases in plasma homocyst(e)ine levels can lead to an increased risk of occlusive vascular disease.1–9 Homocysteine is a sulfur amino acid generated as an intermediate product in methionine metabolism and occurs at the intersection of 2 metabolic pathways, remethylation and transsulfuration. These pathways are known to be regulated by 3 key enzymes: cystathionine β-synthase, 5-methyltetrahydrofolate:homocysteine methyltransferase (methionine synthase), and 5,10-methylenetetrahydrofolate reductase (MTHFR), as well as by the cofactors folate, vitamin B6, and vitamin B12. Many studies,10–14 including ours, have shown that differences in the plasma levels of homocyst(e)ine and folate are associated with variation in the MTHFR genotype, as reported by Frosst et al.15 Moreover, this MTHFR gene polymorphism, which is associated with a predisposition for elevated plasma concentrations of homocyst(e)ine, has been reported to represent a genetic risk factor for occlusive vascular diseases, although it remains controversial.11–14,16–21

Methionine synthase catalyzes the remethylation of homocysteine to methionine in a methylcobalamin-dependent reaction, and a deficiency of methionine synthase activity results in hyperhomocysteinemia. Indeed, homocystinuria, a rare autosomal recessive disease characterized by markedly elevated plasma homocyst(e)ine concentrations, is caused in part by a deficiency in methionine synthase activity.22 Two classes of methionine synthase–associated genetic diseases, cbIE and cbIG, have been proposed on the basis of genetic complementation analysis of fibroblasts isolated from patients.23,24 In both cbIE and cbIG, the capacity to maintain a reduced form of cobalamin on the methionine synthase apoenzyme is impaired; the cbIG group is thought to result from defects in the methionine synthase apoenzyme. Leclerc et al25 determined the cDNA sequences of human methionine synthase and identified a missense mutation, D919G, in patients of the cbIG complementation group. Interestingly, they also showed that this mutation is common in the general population and inferred that it might lead to mild hyperhomocysteinemia with a consequent impact on vascular disease. Thus, analysis of the genetic polymorphism of methionine synthase might provide us with an explanation for elevated homocyst(e)ine levels in those cases that cannot be explained...
by other causes, such as MTHFR genotype. In the present study, we analyzed the distribution of the methionine synthase D/G polymorphism in the Japanese population. We then examined the extent to which this polymorphism is associated with differences in homocysteine metabolism and late-onset cardiovascular and cerebrovascular diseases.

**Methods**

**Study Population**

Patients with coronary artery disease (>50% narrowing of 1 or more major coronary arteries) were consecutively enrolled in the study at the time of their diagnostic cardiac catheterization at the Sakakibara Heart Institute. Coronary angiograms were interpreted by 2 or more independent, experienced cardiologists. After exclusion of patients with renal dysfunction, we enrolled 215 coronary artery disease patients in this study group (age of onset, 41 to 90 years; mean, 62.8±9.0 years). At the time they entered the study, 137 of the patients exhibited clinical evidence of acute or previous myocardial infarction, 20 patients had crescendo-type unstable angina, and 58 had stable angina.

Patients with ischemic stroke were enrolled at the Kitamura Neurosurgery Clinic. The diagnosis of ischemic stroke was made when neurological deficits were accompanied by corresponding abnormal findings on CT of the brain; neurological and CT findings were interpreted by 2 or more independent, experienced neurologists. After exclusion of patients with renal dysfunction, we enrolled 257 coronary artery disease patients in this study group (age of onset, 41 to 90 years; mean, 62.8±9.0 years). At the time they entered the study, 137 of the patients exhibited clinical evidence of acute or previous myocardial infarction, 20 patients had crescendo-type unstable angina, and 58 had stable angina.

Patients with cardiovascular and cerebrovascular diseases.

**TABLE 1. Clinical Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Patients With Coronary Artery Disease (n=215)</th>
<th>Patients With Ischemic Stroke (n=257)</th>
<th>Controls (n=257)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of men (%)</td>
<td>190 (88)*</td>
<td>122 (49)</td>
<td>140 (54)</td>
</tr>
<tr>
<td>Age, y</td>
<td>62.8±9.0</td>
<td>70.3±8.6†</td>
<td>67.3±8.5</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>107 (50)*</td>
<td>152 (61)‡</td>
<td>63 (25)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>76 (35)*</td>
<td>52 (21)†</td>
<td>34 (13)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>195.9±31.9</td>
<td>213.5±35.5</td>
<td>193.3±37.3</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>46.6±13.6‡</td>
<td>56.6±16.4</td>
<td>58.9±15.6</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>147 (68)*</td>
<td>97 (39)§</td>
<td>66 (26)</td>
</tr>
</tbody>
</table>

*P<0.01 patients with coronary artery disease vs control.
†P<0.05 patients with ischemic stroke vs control.
‡P<0.01 patients with ischemic stroke vs control.
§P<0.05 patients with coronary artery disease vs control.

**Results**

Two hundred fifty-seven volunteers with no history of cardiovascular or cerebrovascular disease and no present neurological or electrocardiographic abnormalities (age, 41 to 88 years old; mean, 67.3±8.5 years) were recruited as control subjects at their annual health examination at the Institute for Adult Diseases, Asahi Life Foundation, which is in the same area of the Tokyo megalopolis as the Sakakibara Heart Institute and the Kitamura Neurosurgery Clinic. The criteria of exclusion from the control groups were the same as those used for the patient groups. In addition, to assess allele frequencies of the methionine synthase gene, another panel of volunteers consisting of 262 healthy men from the Nikon clinic (age, 34 to 59 years old; mean, 43.4±5.3 years) were also genotyped.

At the time of subject enrollment, relevant data on past medical history, current smoking habits, and alcohol consumption were obtained from all study participants (Table 1). Hypertension and diabetes mellitus were diagnosed according to the respective World Health Organization criteria for each disease. Fasting venous blood samples were drawn for estimation of biochemical measurements. All subjects were of Japanese ancestry and were non-first- or second-degree relatives. All female participants were postmenopausal. Informed consent was obtained from every subject after a full explanation of the study, which was approved by the Ethics Committee of University of Tokyo.

**Genetic Analysis**

Venous blood samples were collected in tubes containing disodium EDTA and applied to genomic DNA extracting columns (QIAamp blood kit, Qiagen) according to the manufacturer’s protocol. Polymerase chain reaction (PCR) amplification of genomic DNA samples was performed using specific oligonucleotide primers in a GeneAmp PCR kit (Perkin Elmer Cetus). Thirty-five cycles (95°C for 60 seconds, 60°C for 90 seconds, and 72°C for 60 seconds) were used to amplify 189-bp products. The amplified fragments were then cut with HaeIII. HaeIII-treated PCR fragments were separated by electrophoresis in 9.6% polyacrylamide gels and stained with ethidium bromide. The methionine synthase genotype was classified as DD (aspartic acid, aspartic acid), DG (aspartic acid, glycine), and GG (glycine, glycine). We also examined the MTHFR A/V genotype as previously described.

**Measurement of Plasma Levels of Homocyst(e)ine and Folate**

Plasma homocyst(e)ine and folate levels were measured in 143 randomly selected, ischemic stroke patients. Fasting venous blood samples were collected in tubes containing disodium EDTA. After collection, samples were promptly centrifuged and stored at −20°C. Plasma homocyst(e)ine levels were determined as total homocysteine by high-performance liquid chromatography with fluorescence detection as previously described. Plasma folate levels were measured using commercially available radioimmunoassay kits.

**Statistical Analysis**

Means (±SDs) and proportions for baseline risk factors were computed for patients and control subjects. Alleles and genotype frequencies among the patients and control subjects were compared by χ² tests with Hardy-Weinberg predictions. Confounding influences of other risk factors were assessed in a multiple logistic regression model. The gene–gene interaction between methionine synthase and MTHFR were analyzed using χ² tests and logistic regression analysis. Plasma homocyst(e)ine and folate levels were analyzed by univariate analysis with the Mann-Whitney rank sum test, and multiple linear regression analysis was used to examine the determinants of plasma homocyst(e)ine levels. A 2-tailed value of P<0.05 was considered significant. Statistical analysis was done with SAS (Statistical Analysis System).

**Results**

The baseline characteristics of the patients and the control subjects are shown in Table 1. The patients with coronary artery disease had a significantly higher prevalence of men, hypertension, diabetes, and smoking and lower value of HDL.
cholesterol than the control subjects. There was a significant difference in age, hypertension, diabetes, and smoking between patients with ischemic stroke and controls.

In these subjects, the 3 methionine synthase genotypes for the aspartic acid to glycine mutation (DD, DG, and GG) were diagnosed by digestion of the 189-bp PCR products by HaeIII, as shown in the previous report by Leclerc et al. The data summarized in Table 2 demonstrate that the D919G methionine synthase variant was present in a substantial fraction of the control (0.17) and healthy reference (0.19) groups, which is consistent with previous findings in Caucasians (0.15), and similar allele frequencies were found among coronary artery disease and ischemic stroke patients (0.17 and 0.19, respectively). The genotype distributions of all groups were consistent with Hardy-Weinberg equilibrium. In addition, in the patients with ischemic stroke, plasma homocyst(e)ine and folate levels were similar irrespective of methionine synthase genotype (Table 3). Thus, no association was observed between the methionine synthase D919G variant and late-onset vascular diseases; the absence of association was independent of all other risk factors examined (data not shown). The remethylation pathway of homocysteine is regulated by methionine synthase and MTHFR. Therefore, we assessed the extent to which the association between late-onset vascular disease and the AV polymorphism was not found to be a determinant of plasma levels of homocyst(e)ine (Table 6).

**Discussion**

In the present report, we describe 3 major findings: first, the methionine synthase D919G mutation initially described in a white population by Leclerc et al. is also common in the Japanese general population; second, this mutation is not a determinant of plasma levels of homocyst(e)ine and folate; and third, there are no significant differences in the mutant allele frequencies between patients with late-onset vascular diseases and controls.

Previously, Kang et al. reported that thermolabile MTHFR, which corresponds to the C677T mutation in the MTHFR gene, may be an inherited risk factor for coronary artery disease. Such a genetic factor likely contributes to the risk of late-onset vascular disease and the AV polymorphism of MTHFR was not influenced by the methionine synthase D/G polymorphism.

Multiple linear regression analysis was used to examine the determinants of plasma homocyst(e)ine levels. Age, sex, plasma folate concentration, and the MTHFR VV genotype were found to be independent factors significantly associated with plasma homocyst(e)ine levels. In contrast, the methionine synthase D/G polymorphism was not found to be a determinant of plasma levels of homocyst(e)ine (Table 6).

### Table 3. Comparison of the Plasma Levels of Homocyst(e)ine and Folate Among Methionine Synthase Genotypes in Patients With Ischemic Stroke

<table>
<thead>
<tr>
<th>Homocyst(e)ine, μmol/L</th>
<th>Folate, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD (n=89)</td>
<td>11.9±4.5</td>
</tr>
<tr>
<td>DG (n=48)</td>
<td>11.4±3.5</td>
</tr>
<tr>
<td>GG (n=6)</td>
<td>11.1±4.6</td>
</tr>
</tbody>
</table>

### Table 5. ORs and 95% CIs for Patients With Vascular Diseases to Control Subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD/AA vs DD/VG</td>
<td>1.99</td>
<td>1.35 to 2.93</td>
<td>0.0005</td>
</tr>
<tr>
<td>DD/AA vs G/A</td>
<td>1.19</td>
<td>0.71 to 1.99</td>
<td>0.5194</td>
</tr>
<tr>
<td>DD/AA vs G/V</td>
<td>1.95</td>
<td>1.24 to 3.07</td>
<td>0.0037</td>
</tr>
</tbody>
</table>

**DD** and **G** represent the DD genotype and the DG or GG genotype, respectively, in methionine synthase. AA and V—represent the AA genotype and the AV or VV genotype respectively, in MTHFR.

### Table 4. Distributions of the D/G Polymorphism in Methionine Synthase and the A/V Polymorphism in MTHFR

<table>
<thead>
<tr>
<th>Patients With Vascular Diseases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD (n=306)</td>
<td>DD (n=150)</td>
</tr>
<tr>
<td>DG (n=150)</td>
<td>DG (n=10)</td>
</tr>
<tr>
<td>GG (n=10)</td>
<td>GG (n=4)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>93</td>
</tr>
<tr>
<td>AV</td>
<td>161</td>
</tr>
<tr>
<td>WW</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>MTHFR genotype</td>
<td></td>
</tr>
<tr>
<td>AV (vs AA)</td>
<td>0.022</td>
</tr>
<tr>
<td>VV (vs AA)</td>
<td>2.589</td>
</tr>
<tr>
<td>Smoking, smokers vs nonsmokers</td>
<td>0.732</td>
</tr>
<tr>
<td>Alcohol, drinkers vs nondrinkers</td>
<td>-0.833</td>
</tr>
</tbody>
</table>
vascular diseases by predisposing patients to increased plasma homocyst(e)ine levels. Because methionine synthase catalyzes the remethylation of homocysteine to methionine directly, this enzyme should act in concert with MTHFR and be a key enzyme regulating plasma homocyst(e)ine levels. Leclerc et al found 3 mutations in Canadian patients with deficiencies in methionine synthase activity. In particular, the D919G mutation, which is a missense mutation identified in patients of the cbIG genetic complementation group, was reported to be common. In the context of these earlier findings, we considered it important to assess methionine synthase genotype as a candidate genetic risk factor for late-onset vascular diseases. Our study showed that the D919G mutation is also common in the Japanese population and is apparently unaffected by ethnic differences. However, in contrast to the variation in MTHFR genotype, we found no evidence to suggest an association between this methionine synthase mutation and elevated plasma homocyst(e)ine levels or late-onset vascular diseases.

Our observations are consistent with those of Dudman et al, who reported that the majority of patients with premature vascular disease and impaired homocysteine metabolism have normal levels of methionine synthase. In another report, the significant correlation between homocysteine and 5-methyltetrahydrofolate, a substrate for methionine synthase whose metabolism is regulated by MTHFR, was reported in patients with coronary artery disease. Low levels of 5-methyltetrahydrofolate may lead to elevated homocysteine owing to lack of sufficient substrate for methionine synthase. Taken together with these findings, our results indicate that genetic variation of MTHFR likely has a greater influence on the remethylation pathway than does genetic variation of methionine synthase. Nevertheless, we believe that the D919G mutation should be examined in more detail to confirm its impact on the structure and function of methionine synthase. We could not measure plasma levels of homocyst(e)ine and folate in the participants with coronary artery disease, which is thought to be another limitation of this study.

In conclusion, the methionine synthase D9/G polymorphism is unlikely to have a major effect on homocysteine metabolism in the Japanese population, although the present study does not exclude its involvement in the etiology of late-onset vascular diseases. Further investigations will be needed to clarify how changes in homocysteine metabolism and their effects on vascular diseases are genetically determined.

Acknowledgments
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References
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