Thermolabile Defect of Methylenetetrahydrofolate Reductase in Coronary Artery Disease

Soo-Sang Kang, MD, PhD; Edward L. Passen, MD; Neal Ruggie, MD; Paul W.K. Wong, MD, MSc; Hyunchoo Sora, MS

Background. To determine whether or not a moderate genetic defect of homocysteine metabolism is associated with the development of coronary artery disease, we studied the prevalence of thermolabile methylenetetrahydrofolate reductase, which is probably the most common genetic defect of homocysteine metabolism.

Methods and Results. Three hundred thirty-nine subjects who underwent coronary angiography were classified into three groups: (1) patients with severe coronary artery stenosis (≥70% occlusion in one or more coronary arteries or ≥50% occlusion in the left main coronary artery), (2) patients with mild to moderate coronary artery stenosis (<70% occlusion in one or more coronary arteries or <50% occlusion in the left main coronary artery), and (3) patients with non–coronary heart disease or noncardiac chest pain (nonstenotic coronary arteries). The thermolability of methylenetetrahydrofolate reductase was prospectively determined in all subjects. Plasma homocyst(e)ine levels were then measured in those with thermolabile methylenetetrahydrofolate reductase. The traditional risk factors for coronary artery disease were thereafter ascertained by chart review of all subjects. The prevalence of thermolabile methylenetetrahydrofolate reductase was 18.1% in group 1, 13.4% in group 2, and 7.9% in group 3. There was a significant difference between the prevalence of thermolabile methylenetetrahydrofolate reductase in groups 1 and 3 (P<.04). All individuals with thermolabile methylenetetrahydrofolate reductase irrespective of their clinical grouping had higher plasma homocyst(e)ine levels than normal (group 1, 14.86±5.85; group 2, 15.36±5.70; group 3, 13.39±3.80; normal, 8.50±2.8 nmol/mL). Nonetheless, there was no statistically significant difference in the plasma homocyst(e)ine concentrations of these patients with or without coronary artery stenosis. Using discriminant function analysis, thermolabile methylenetetrahydrofolate reductase was predictive of angiographically proven coronary artery stenosis. The traditional risk factors—age, sex, diabetes, smoking, hypercholesterolemia, and hypertension—were not significantly associated with the presence of thermolabile methylenetetrahydrofolate reductase.

Conclusions. Thermolabile methylenetetrahydrofolate reductase is a risk factor for coronary artery disease and is unrelated to other risk factors. (Circulation. 1993;88[part 1]:1463-1469.)

Key Words • methylenetetrahydrofolate reductase • coronary artery disease • genetics • risk factors

During the past 15 years, many studies have substantiated the positive correlation between moderate defects of homocysteine metabolism and the occurrence of occlusive vascular disease. These studies have implicated hyperhomocyst(e)inemia* as a risk factor for arteriosclerotic disease, including coronary artery disease.1-15 Since plasma lipoprotein abnormalities and other well-recognized risk factors do not account for all the cases of occlusive vascular disease,16 plasma homocyst(e)ine levels may be an independent atherogenic factor in some patients. Nevertheless, determination of plasma homocyst(e)ine alone does not reliably reveal moderate defects of homocysteine metabolism because hyperhomocyst(e)inemia is induced or masked by various genetic, physiological, pathological, nutritional, and iatrogenic factors.17-30 Although reduced cystathionine β-synthase activity was reported in cases of moderate hyperhomocyst(e)inemia,4,13 neither determination of plasma homocyst(e)ine nor cystathionine β-synthase activity is able to reliably define heterozygosity for cystathionine β-synthase deficiency.3,31-33

Methylenetetrahydrofolate reductase catalyzes the synthesis of 5-methyltetrahydrofolate, which donates its methyl group for the remethylation of homocysteine to methionine. Recently, we found a new variant of methylenetetrahydrofolate reductase that was clearly defined by its in vitro heat sensitivity and was designated as "thermo-
labile methylenetetrahydrofolate reductase.\textsuperscript{34-37} Specific enzyme activity of this thermolabile methylenetetrahydrofolate reductase was 50\% of the normal mean, and it was similar to that found in heterozygotes for severe methylenetetrahydrofolate reductase deficiency.\textsuperscript{34,36,37} Severe hyperhomocyst(e)inemia (homocystinuria) is uncommon and is usually due to homozygosity of cystathionine \(\beta\)-synthase deficiency or methylenetetrahydrofolate reductase deficiency. Thus, homozygosity for these enzyme defects has been proposed as a major cause of moderate hyperhomocyst(e)inemia.\textsuperscript{38} Alternatively, a high proportion of moderate to severe hyperhomocyst(e)inemia may be caused by a high incidence of thermolabile methylenetetrahydrofolate reductase. Approximately 5\% of the population was homozygous for thermolabile methylenetetrahydrofolate reductase.\textsuperscript{36} Thus, in the general population, the frequency of thermolabile methylenetetrahydrofolate reductase is comparable to that of Rh negative blood type in Caucasians and is fivefold to 14-fold greater than that of heterozygotes for cystathionine \(\beta\)-synthase deficiency.\textsuperscript{38}

Our previous studies of the relation between homocysteine metabolism and atherosclerotic coronary artery disease did not have angiographic documentation in all controls and might have included patients with subclinical coronary lesions in the control group.\textsuperscript{36} Including cases with subclinical disease in the control group potentially obscures the relation being investigated. We therefore undertook the present study to evaluate the relation between a moderate genetic defect of homocysteine metabolism and the occurrence of atherosclerotic coronary artery disease in a population with angiographic documentation and grouped according to the extent of coronary occlusion.

**Methods**

Three hundred thirty-nine patients who underwent coronary angiography as part of a comprehensive clinical evaluation were classified angiographically into three groups: (1) patients with severe coronary artery stenosis (at least 70\% stenosis in one or more coronary arteries or at least 50\% stenosis in the left main coronary artery), (2) patients with mild to moderate coronary artery stenosis (less than 70\% stenosis in one or more coronary arteries and less than 50\% stenosis in the left main coronary artery), and (3) patients with nonstenotic coronary arteries. All patients in groups 1 (\(n=155\)) and 2 (\(n=83\)) and 66 patients in group 3 (\(n=101\)) were recruited between 1990 and 1992 without knowledge of the status of their coronary anatomy. However, because of the low prevalence of nonstenotic coronary arteries in patients undergoing clinically indicated coronary angiography, 35 patients in group 3 were the control subjects who underwent coronary angiography in our previous study.\textsuperscript{36} Group 3 included patients with noncoronary heart disease, such as valvular disease, cardiomyopathy, or noncardiac chest pain. Patients were recruited during the study period when cardiac catheterization laboratory and genetics laboratory facilities were mutually prepared for specimen collection and processing. Coronary anatomy, thermolability of methylenetetrahydrofolate reductase, and traditional risk factors were not known to the investigators at the time of recruitment. Patients who were below 30 years of age or had evidence of other vascular diseases such as cerebrovascular and peripheral arterial diseases were excluded from this study. Clinical diagnoses were verified by reviewing medical records.

Coronary angiography was predominantly undertaken to determine whether atherosclerotic coronary artery disease with luminal stenosis was present. The severity of stenosis was determined by visual estimation of the percent diameter stenosis compared with an adjacent angiographically normal portion of the vessel by experienced angiographers also involved in the course of the patient care. Before and during angiographic interpretation, the angiographers were unaware of the results of methylenetetrahydrofolate reductase studies. Information concerning age, sex, history of hypertension and hyperlipidemia, cigarette smoking during or before the time of the present illness, diabetes mellitus, family history of coronary artery disease, and serum total cholesterol values was obtained from the medical records without knowledge of the angiographic results. Most cholesterol values were routinely obtained just before or at admission for coronary angiography. Serum cholesterol values were divided into groups of less than 200, 200 to 239, and 240 mg/dL or higher according to the established guidelines of the National Cholesterol Education Program to represent the levels of risk.\textsuperscript{39}

Blood specimens were obtained during cardiac catheterization for the determination of methylenetetrahydrofolate reductase, its thermolability, plasma homocyst(e)ine, serum folic acid, and cyanocobalamin. The specimens were obtained before coronary angiography without any knowledge of the status of the coronary arteries. Informed consent was obtained from all patients according to a protocol approved by the Human Investigation Committee. After the collection of venous blood in tubes with ethylenediaminetetraacetate, mononuclear leukocytes were separated with Ficoll-Hypaque within 2 hours\textsuperscript{40} and stored at \(-80^\circ\text{C}\) until the preparation of enzyme extracts. The methods to determine methylenetetrahydrofolate reductase activity and thermolability were described previously.\textsuperscript{24-37} Based on our previous study,\textsuperscript{36} patients were classified as having thermolabile methylenetetrahydrofolate reductase when the residual activity was less than 20\% after heat inactivation at \(46^\circ\text{C}\) for 5 minutes. Plasma homocyst(e)ine, serum folic acid, and cyanocobalamin levels were measured in all patients with thermolabile methylenetetrahydrofolate reductase. The methods for the determination of total plasma homocyst(e)ine, serum folic acid, and cyanocobalamin are described elsewhere.\textsuperscript{4,41}

The differences between the three groups (ie, the patients with severe coronary stenosis, the patients with mild to moderate coronary artery stenosis, and the patients with nonstenotic coronary arteries) for each of the traditional risk factors and thermolabile methylenetetrahydrofolate reductase were evaluated by \(\chi^2\) analysis (Statistical Package for Social Sciences) except for age and cholesterol level. Age and cholesterol level were evaluated using a one-way ANOVA. A stepwise linear discriminant function analysis (Statistical Package for Social Sciences) was performed to evaluate the independent predictive value of thermolabile methylenetetrahydrofolate reductase as well as the traditional risk factors between the groups with severe coronary stenosis and nonstenotic coronary arteries. The group
with mild to moderate coronary artery stenosis was excluded from this analysis. The parameters are presented in order of inclusion level, and selection was based on an inclusion criterion of 1, where the F ratio is 1. A χ² analysis was used to determine the independence of methylenetetrahydrofolate reductase thermolability from other traditional risk factors except for age and cholesterol level. One-way ANOVA was used to evaluate age and cholesterol level. In patients with thermolabile methylenetetrahydrofolate reductase and available plasma, homocyst(e)ine, folic acid, and cyanocobalamin levels among the three groups of coronary artery disease were evaluated by ANOVA.

Results

Three hundred thirty-nine patients had coronary angiography and methylenetetrahydrofolate reductase assay. The mean age±SD was 57.6±10.4 years, and 205 (60.5%) were men. Of the total, 155 had severe coronary artery stenosis (group 1) and 83 had mild to moderate coronary stenosis (group 2). One hundred one had angiographically nonstenotic coronary arteries (group 3).

Table 1 presents the distribution of the traditional risk factors for coronary artery disease among the three groups of subjects. Using univariate analysis, all these risk factors except family history were significantly more common in group 1 than in group 3. The values in group 2 for all variables except diabetes were intermediate between those in group 1 and group 3. The statistical significance was reduced when the analysis included group 2.

The frequency of thermolabile methylenetetrahydrofolate reductase in patients with severe coronary artery stenosis, mild to moderate coronary artery stenosis, and angiographically normal coronary arteries was compared (Table 2). There was a significant difference between the incidence of thermolabile methylenetetrahydrofolate reductase in patients with severe coronary artery stenosis (group 1) and that in the controls (group 3) (P<.04). As in the case of the traditional risk factors, the prevalence of thermolabile methylenetetrahydrofolate reductase in patients with mild to moderate coronary artery stenosis (group 2) is intermediate between that in patients with angiographically severe coronary artery stenosis (group 1) and the control patients (group 3).

The risk factors (variables from Tables 1 and 2) were evaluated by a discriminant function analysis (Table 3). Age, sex, hyperlipidemia history, diabetes mellitus, and

### Table 1. Traditional Risk Factors in Patients With Severe, Mild to Moderate, and No Angiographic Coronary Stenosis

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Severe Stenosis, Group 1</th>
<th>Mild to Moderate Stenosis, Group 2</th>
<th>No Stenosis, Group 3</th>
<th>Significance Between Groups 1, 2, and 3 (P)</th>
<th>Significance Between Groups 1 and 3 (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59.9±9.4</td>
<td>59.2±9.5</td>
<td>52.7±10.9</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sex, % men</td>
<td>70.3</td>
<td>56.6</td>
<td>48.5</td>
<td>&lt;.002</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>59.2</td>
<td>56.0</td>
<td>41.7</td>
<td>&lt;.025</td>
<td>&lt;.012</td>
</tr>
<tr>
<td>Cigarette smoking, %</td>
<td>65.2</td>
<td>58.1</td>
<td>52.7</td>
<td>&lt;.046</td>
<td>&lt;.016</td>
</tr>
<tr>
<td>Hyperlipidemia history, %</td>
<td>58.3</td>
<td>49.3</td>
<td>33.3</td>
<td>&lt;.002</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cholesterol level, mg/dL</td>
<td>224.6±51.6</td>
<td>216.3±50.5</td>
<td>204.0±42.6</td>
<td>&lt;.021</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Cholesterol group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;240 mg/dL, %</td>
<td>35.1</td>
<td>32.3</td>
<td>16.5</td>
<td>&lt;.053</td>
<td>&lt;.018</td>
</tr>
<tr>
<td>200-239 mg/dL, %</td>
<td>33.3</td>
<td>29.0</td>
<td>43.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200 mg/dL, %</td>
<td>31.5</td>
<td>38.7</td>
<td>40.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>22.2</td>
<td>7.9</td>
<td>8.2</td>
<td>&lt;.002</td>
<td>&lt;.007</td>
</tr>
<tr>
<td>Family history of CAD, %</td>
<td>49.5</td>
<td>33.9</td>
<td>41.0</td>
<td>&lt;.123</td>
<td>&lt;.298</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease.

### Table 2. Thermolabile Methylenetetrahydrofolate Reductase in Control Patients and Patients With Coronary Artery Stenosis

<table>
<thead>
<tr>
<th></th>
<th>Severe Stenosis, Group 1</th>
<th>Mild to Moderate Stenosis, Group 2</th>
<th>No Stenosis*, Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. with thermolabile methylenetetrahydrofolate reductase</td>
<td>28</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>No. in group</td>
<td>155</td>
<td>83</td>
<td>101</td>
</tr>
<tr>
<td>Percentage with thermolabile methylenetetrahydrofolate reductase</td>
<td>18.1†</td>
<td>13.4†</td>
<td>7.9†</td>
</tr>
</tbody>
</table>

*Control group of patients with noncoronary heart disease such as valvular disease, cardiomyopathy, or noncardiac chest pain.

†P<.07 for the differences among the control patients, mild to moderate, and severe coronary artery stenosis; P<.04 for the difference between the control patients and severe coronary artery stenosis.
thermolabile methylenetetrahydrofolate reductase were included in the model. Thus, thermolabile methylenetetrahydrofolate reductase was associated with angiographically proven coronary artery disease independent of other risk factors. Cigarette smoking, family history of coronary artery disease, and total cholesterol group did not improve classification of the patient in the discriminant function model (Table 3). The relative risk for thermolabile methylenetetrahydrofolate reductase associated with coronary artery disease (calculated from Table 2 data) was 1.35 (95% confidence interval, 1.085 to 1.679). Of additional note, the presence of thermolabile methylenetetrahydrofolate reductase was not significantly related to other traditional risk factors such as age, sex, hypertension, cigarette smoking, hyperlipidemia history, cholesterol level or group, diabetes mellitus, or family history of coronary artery disease (Table 4).

Plasma homocyst(e)ine, serum folic acid, and cyanocobalamin were determined in 44 patients with thermodabile methylenetetrahydrofolate reductase (Table 5). Plasma homocyst(e)ine levels, while higher than normal (normal, 8.50±2.80 nmol/mL), were not significantly different between groups 1, 2, and 3. Neither serum folic acid nor cyanocobalamin values were significantly different between groups 1, 2, and 3.

**Discussion**

Homocystinuria is a rare inherited disorder caused by a severe deficiency of cystathionine β-synthase or methylenetetrahydrofolate reductase and is known to result in the premature development of arteriosclerosis. Based on the fact that heterozygosity for familial hypercholesterolemia is closely associated with the premature development of vascular disease, it has been postulated that heterozygosity for cystathionine β-synthase deficiency has a similar association. Neither plasma homocyst(e)ine level nor cystathionine β-synthase activity provides conclusive evidence for heterozygosity for this defect. In addition, the frequency of heterozygosity

**TABLE 3.** Discriminant Function Analysis of Risk Factors in Patients With Severe Coronary Artery Stenosis or No Angiographic Coronary Artery Stenosis (Groups 1 and 3)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate P Value of Variables in Discriminant Function Analysis</th>
<th>Discriminant Function Coefficient of Variables Included in Model</th>
<th>Significance of Variables Included in Model (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt;.001</td>
<td>0.89</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sex</td>
<td>&lt;.001</td>
<td>0.84</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hyperlipidemia history</td>
<td>&lt;.001</td>
<td>0.77</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>&lt;.001</td>
<td>0.75</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>&lt;.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermolabile methylenetetrahydrofolate reductase</td>
<td>&lt;.023</td>
<td>0.74</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>&lt;.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>&lt;.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol group</td>
<td>&lt;.556</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease.

**TABLE 4.** Traditional Risk Factors in Patients With Thermolabile or Thermostable Methylenetetrahydrofolate Reductase

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Thermolabile MTHFR Mean±SD</th>
<th>Thermostable MTHFR Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59.2±11.0</td>
<td>57.3±10.2</td>
</tr>
<tr>
<td>Sex, % men</td>
<td>59.6</td>
<td>60.6</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>59.5</td>
<td>52.0</td>
</tr>
<tr>
<td>Cigarette smoking, %</td>
<td>57.5</td>
<td>59.9</td>
</tr>
<tr>
<td>Hyperlipidemia history, %</td>
<td>53.8</td>
<td>47.7</td>
</tr>
<tr>
<td>Cholesterol level, mg/dL</td>
<td>214.2±57.3</td>
<td>216.6±47.9</td>
</tr>
<tr>
<td>Cholesterol group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥240 mg/dL, %</td>
<td>25.0</td>
<td>29.2</td>
</tr>
<tr>
<td>200-239 mg/dL, %</td>
<td>38.9</td>
<td>34.7</td>
</tr>
<tr>
<td>&lt;200 mg/dL, %</td>
<td>36.1</td>
<td>36.1</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>11.4</td>
<td>15.0</td>
</tr>
<tr>
<td>Family history of CAD, %</td>
<td>33.3</td>
<td>44.4</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; MTHFR, methylenetetrahydrofolate reductase.

No significant difference (P > .05) in values shown between thermolabile and thermostable MTHFR among groups 1, 2, and 3.
TABLE 5. Homocyst(e)ine, Folic Acid, and Cyanocobalamin Levels in Patients With Thermolabile Methylenetetrahydrofolate Reductase

<table>
<thead>
<tr>
<th>Variable</th>
<th>Severe Stenosis, Group 1</th>
<th>Mild to Moderate Stenosis, Group 2</th>
<th>No Stenosis, Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>27</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Homocyst(e)ine, nmol/mL</td>
<td>14.86±5.85</td>
<td>15.36±5.70</td>
<td>13.39±3.80</td>
</tr>
<tr>
<td>Folic acid, ng/mL</td>
<td>8.02±7.02</td>
<td>8.65±8.17</td>
<td>10.10±8.55</td>
</tr>
<tr>
<td>Cyanocobalamin, pg/mL</td>
<td>433.5±282.9</td>
<td>694.9±521.1</td>
<td>407.0±159.9</td>
</tr>
</tbody>
</table>

No significant differences (P>0.05) among groups 1, 2, and 3.

gotes for cystathionine β-synthase deficiency (0.35% to 1%) is considerably less than that of moderate hyperhomocyst(e)inemia (3% to 5%), implying the participation of a more common genetic defect in homocysteine metabolism or of nongenetic mechanisms to account for the association of hyperhomocyst(e)inemia with atherothrombotic disease.30

In a previous study,30 thermolabile methylenetetrahydrofolate reductase was found in 5% of the normal population and was associated with moderate hyperhomocyst(e)inemia under physiological and normal nutritional conditions, suggesting that the majority of genetic hyperhomocyst(e)inemia was caused by thermolabile methylenetetrahydrofolate reductase. In addition, a statistically significant difference in the incidence of thermolabile methylenetetrahydrofolate reductase was previously demonstrated between patients with angiographically proven coronary artery disease and a normal population without clinical evidence of vascular disease.35,36 However, by documenting the status of the coronary arteries in all the subjects, including the controls, a stronger case may be made to support the positive association between thermolabile methylenetetrahydrofolate reductase and coronary disease.

In the present study, all subjects had coronary angiography to define their coronary anatomy. We demonstrated a positive association between severe coronary artery stenosis and the presence of thermolabile methylenetetrahydrofolate reductase. The association was independent of other risk factors. In addition, the strength of this association was obscured if patients with angiographically mild to moderate coronary artery stenosis were included either in the control group or in the group with severe coronary artery stenosis. When the analysis was limited to angiographically normal controls (group 1) and patients with severe coronary artery stenosis (group 3), thermolabile methylenetetrahydrofolate reductase and all of the known risk factors except family history of coronary artery disease were significantly related to coronary stenosis. Of additional interest is the fact that when thermolabile methylenetetrahydrofolate reductase was evaluated by discriminant function analysis, it was statistically related to coronary artery stenosis, whereas cigarette smoking, family history of coronary artery disease, and the serum cholesterol group were not statistically included in the model. Although the cholesterol level or group is significantly related to coronary stenosis when evaluated by univariate analysis without regard to other factors, the contribution of cholesterol group is no longer significant when other variables including thermolabile methylenetetrahydrofolate reductase also having a significant relation are analyzed by a multivariate technique.

The control subjects in this study were patients with noncoronary heart disease, such as valvular disease, cardiomyopathy, or noncardiac chest pain. In contrast, the majority of control patients in the previous study36 were clinically normal subjects who had no history of vascular disease. Since hyperhomocyst(e)inemia predisposes to cerebrovascular disease, peripheral vascular disease, and coronary artery disease,2,4,7-9 the control group with nonstenotic coronary arteries in this study might have included some patients with subclinical noncoronary vascular disease. This may explain why the frequency of thermolabile methylenetetrahydrofolate reductase in the group with normal coronary arteries is higher than previously reported in the normal control subjects. Nonetheless, the present study has demonstrated a significant difference in the frequency of thermolabile methylenetetrahydrofolate reductase between patients with severe coronary artery stenosis (group 1) and patients with nonstenotic coronary arteries (group 3).

Similar to other studies of moderate hyperhomocyst(e)inemia in patients with vascular disease,6,10-14 the present study also demonstrated no significant association between thermolabile methylenetetrahydrofolate reductase and other risk factors such as diabetes, hypertension, abnormal lipid levels, and smoking. Therefore, it may be concluded that thermolabile methylenetetrahydrofolate reductase is an independent risk factor for coronary artery disease. The association between thermolabile methylenetetrahydrofolate reductase and coronary artery disease is stronger than that between some of the other known risk factors and coronary artery disease. It appears that the inclusion of thermolabile methylenetetrahydrofolate reductase as one of the risk factors may allow a more complete and accurate assessment of risk in patients and may permit the identification of patients with coronary artery stenosis who are currently missed by other assessment, such as hypercholesterolemia.

There is evidence of a positive correlation between hyperhomocyst(e)inemia and the development of coronary artery disease.1,3,5,6,10-15 It has also been established previously that thermolabile methylenetetrahydrofolate reductase is associated with high plasma homocyst(e)ine levels.36 In this study, homocyst(e)ine levels in patients with thermolabile methylenetetrahydrofolate reductase were higher than normal but did not reliably separate groups 1, 2, and 3. Serum folic acid and cyanocobalamin, two of several important factors that may modify plasma homocyst(e)ine concentration, were
similar among these groups. Also, a reliable analysis to
detect a difference may require a larger number of cases
than we collected in this study.

A limitation of the present study is the use of
coronary angiography to define normal vessels. Since
only the arterial lumen is visualized, earlier stages of
atherosclerosis may have been missed in the control
group. However, coronary angiography is the most
widely accepted clinical method available. Visual eval-
uation of coronary artery stenosis might be an associ-
ated limitation. Nevertheless, visual evaluation is widely
used in clinical practice and subject to less intraobserver
and interobserver variability at the extremes of normal
nonstenotic coronary arteries and severe coronary ar-
tery stenosis.\textsuperscript{62} Retrospective collection of the
historical data constitutes another limitation, but the findings
are consistent with those of other prospective studies re-
garding the traditional risk factors. In addition, the
determination of thermolability of methylenetetrahy-
drofale reductase was performed prospectively with-
out the knowledge of angiographic results. Finally, not
all patients had complete lipid analysis, although it is
well known that lipid values other than total cholesterol
may be important. Serum high-density lipoprotein val-
ues probably would have improved the predictive value
of the cholesterol measurement but were not obtained
in a sufficient number of patients for meaningful anal-
ysis. Serum total cholesterol was used in this investiga-
tion because of its longstanding and frequent use as a
risk factor. The fact that a history of hyperlipidemia was
statistically included in the discriminant function anal-
ysis model while cholesterol group was not included may
indicate that a history of hyperlipidemia reflected a prior
cholesterol value that was modified by diet or drug
therapy before the time of angiography. A history of
hyperlipidemia thus may be more closely related to the
risk of coronary artery disease in the present study than
the isolated value of cholesterol obtained near the time
of angiography. This may account for the exclusion of
cholesterol among the variables in the discriminant
analysis model but would not negate the contribution of
thermolabile methylenetetrahydrofale reductase in
coronary artery disease.

Conclusions

This study confirms that there is a positive association
between thermolabile methylenetetrahydrofale reduc-
tase and coronary artery stenosis; that thermolabile meth-
ylenetetrahydrofale reductase is an independent risk
factor of coronary artery stenosis; and that it is more
closely related to coronary artery disease than some other
well-recognized risk factors. Hence, thermolabile methy-
enetetrahydrofale reductase should be added to the
usual studies of risk factors in the assessment of patients
for vascular disease, especially since it is potentially modif-
iable through nutritional means.\textsuperscript{25,30,36}

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