Association Between High Homocyst(e)ine and Ischemic Stroke due to Large- and Small-Artery Disease but Not Other Etiologic Subtypes of Ischemic Stroke

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Background and Purpose—Elevated plasma homocyst(e)ine may be a causal and modifiable risk factor for ischemic stroke, but the results of previous studies have been conflicting. One possible explanation is that homocyst(e)ine may only be associated with certain pathophysiological subtypes of ischemic stroke.

Methods—We conducted a case-control study of 219 hospital cases with a first-ever ischemic stroke and 205 randomly selected community control subjects stratified by age, sex, and postal code. With the use of established criteria, cases of stroke were classified by etiologic subtype in a blinded fashion. The prevalence of conventional vascular risk factors, fasting plasma homocyst(e)ine levels, vitamin levels, and nucleotide 677 methylene tetrahydrofolate reductase (MTHFR) genotypes were determined in cases and controls.

Results—Increasing homocyst(e)ine was a strong and independent risk factor for ischemic stroke (adjusted OR 2.7, 95% CI 1.4 to 5.1 for a 5-μmol/L increase in fasting plasma homocyst(e)ine from 10 to 15 μmol/L). Compared with the lowest quartile, the highest quartile of homocyst(e)ine was associated with an adjusted OR of ischemic stroke of 2.2 (95% CI 1.1 to 4.2). Mean plasma homocyst(e)ine was significantly higher in cases of ischemic stroke due to large-artery disease (14.1 μmol/L, 95% CI 12.5 to 15.9, P<0.001) and small-artery disease (12.7 μmol/L, 95% CI 11.4 to 14.1, P=0.004) compared with control subjects (10.5 μmol/L, 95% CI 10.0 to 11.0) but not in cardioembolic or other etiologic subtypes of ischemic stroke. Compared with the lowest quartile of homocyst(e)ine, the upper 3 quartiles were associated with an adjusted OR of ischemic stroke due to large-artery disease of 3.0 (95% CI 0.8 to 10.8) for the second quartile, 5.6 (95% CI 1.6 to 20) for the third quartile, and 8.7 (95% CI 2.4 to 32) for the fourth quartile (P for trend=0.0005). However, despite a clear association between the TT MTHFR genotype and elevated fasting plasma homocyst(e)ine, there was no association between MTHFR genotype and ischemic stroke or subtype of ischemic stroke.

Conclusions—There is a strong, graded association between increasing plasma homocyst(e)ine and ischemic stroke caused by large-artery atherosclerosis and, to a much lesser extent, small-artery disease, but not cardioembolic or other etiologic subtypes of ischemic stroke. Our results are consistent with the hypothesis that the deleterious effect of high homocyst(e)ine is mediated primarily via a proatherogenic effect. (Stroke. 2000;31:1069-1075.)

Key Words: arterial occlusive disease ■ homocyst(e)ine ■ ischemic risk ■ stroke

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troke is the second most common cause of death in the world,1 and it remains a major cause of long-term disability.2 The most promising strategy to reduce the worldwide burden of stroke is effective stroke prevention. However, the success of this strategy depends on the recognition and control of all important causal and modifiable risk factors.3 At present, only about two thirds of all strokes can be attributed to known causal risk factors.4 A previously unrecognized risk factor for stroke, which is prevalent and modifiable and may be causal, is elevated plasma homocyst(e)ine.5

Many case-control and cohort studies have identified a strong, independent and dose-related association between moderately elevated homocyst(e)ine and atherosclerotic vascular disease, including stroke.5 However, not all reports have been consistent; several prospective cohort studies have failed to demonstrate a positive association between elevated homocyst(e)ine and stroke,6,7 and studies examining the association between the common C677T methylene tetrahydrofolate reductase (MTHFR) mutation and stroke have also been conflicting,8,9 despite a strong association between this mutation and elevated homocyst(e)ine.
One potential explanation for these inconsistent findings is that epidemiological studies have correlated homocyst(e)ine and the MTHFR mutation with all stroke but not etiologic subtypes of stroke. It is well recognized that stroke is pathologically and etiologically heterogeneous and that risk factors for one etiologic subtype may not be risk factors for other subtypes of stroke. We hypothesized that increased levels of homocyst(e)ine with atherosclerotic disease that elevated homocyst(e)ine would only be a risk factor for the subtypes of ischemic stroke in which atherosclerosis plays a primary pathogenic role (ie, large-artery disease and possibly small-artery disease) and that any association with other pathogenic subtypes of ischemic stroke would be less strong (eg, embolism from the heart) or absent (eg, dissection, hypoperfusion). To explore this hypothesis, we undertook a prospective case-control study of consecutive patients hospitalized with a first-ever ischemic stroke and examined specifically whether there may be an association between homocyst(e)ine, the C677T MTHFR mutation, vitamin status, and the specific etiologic subtypes of ischemic stroke caused by atherosclerosis (large-artery disease and possibly small-artery disease).

Subjects and Methods
The study was approved by the Institutional Review Board of Royal Perth Hospital, and informed consent was provided by all study participants.

Cases
Consecutive patients presenting to a university teaching hospital in Western Australia between March 1996 and June 1998 with first-ever ischemic stroke were approached for consent to participate in our study. Stroke was defined as a clinical syndrome characterized by rapidly developing clinical symptoms and/or signs of focal and at times global loss of brain function, with symptoms lasting >24 hours or leading to earlier death, and with no apparent cause other than that of vascular origin. Ischemic stroke was defined as a stroke with either a normal CT brain scan or evidence of a recent infarct in the clinically relevant area of the brain on a CT or MRI brain scan performed within 3 weeks of the event or at autopsy. Patients with cerebral hemorrhage or cerebral venous thrombosis were not included. Baseline demographic data (age, sex), history of conventional vascular risk factors (hypertension, diabetes, hypercholesterolemia, current smoker), and history of previous vascular events (myocardial infarction, angina, claudication, amputation) was obtained. All patients underwent a CT brain scan. Echocardiography and extracranial duplex ultrasound were performed at the discretion of the clinician. Within 7 days of the acute stroke event, an overnight fasting blood sample was obtained for biochemical and genetic analyses.

On the basis of clinical evaluation and results of imaging studies, the study neurologist (G.J.H.) (who remained blinded to the results of homocyst(e)ine and vitamin assays and MTHFR genotype) classified all strokes into 4 major etiologic subtypes according to the following predefined criteria: (1) large-artery disease: ischemic stroke with (a) evidence of extracranial or intracranial occlusive large-artery disease (eg, Doppler, angiographic) and (b) no major cardioembolic source (atretic cerebral artery, carotid, vertebral, basilar, and posterior cerebral arteries; 2) small-artery disease: ischemic stroke with (a) consciousness and higher cerebral function maintained plus (b) 1 of the classic lacunar syndromes (ie, pure motor hemiparesis, pure hemisensory loss, pure hemisensormotor loss, ataxic hemiparesis) or nonlacunar small-artery clinical syndromes (eg, basal branch artery syndromes); (3) cardioembolic disease: ischemic stroke with (a) a major cardioembolic source plus (b) no definite evidence of occlusive large artery disease, and (c) clinical opinion that the most likely cause of brain infarction was embolism from the heart; (4) other: ischemic stroke that did not meet the criteria for 1 of the categories outlined above (eg, periprocedural, hypoperfusion, dissection, procoagulant state) or where there was more than 1 likely explanation (eg, concurrent large-artery occlusive disease and major cardioembolic source).

Control Subjects
Control subjects were randomly selected from the Western Australian electoral roll, stratified by 5-year age group, sex, and postal code. A letter of invitation to participate, together with a stamped and self-addressed envelope, was sent to potential control subjects. Nonresponders were contacted by telephone. Control subjects who agreed to participate in the study were required to fast for a minimum of 8 hours before their appointment and were given the option of attending the hospital outpatient clinic or being visited at home by the study nurse. Baseline demographic data (age, sex), history of conventional vascular risk factors, and history of previous vascular events were obtained for each control subject. A fasting blood sample was obtained for biochemical and genetic analyses.

Laboratory Analysis
All samples were collected and processed with the use of a standardized protocol and were analyzed in the central core laboratory. Fasting plasma homocyst(e)ine was measured with high-performance liquid chromatography. Serum and red cell folate and serum cobalamin were measured with an automated microparticle enzyme immunoassay (Abbott Laboratories), and serum pyridoxine was measured with a microbiological assay (Lactobacillus casei). Genomic DNA was isolated from nucleated blood cells by use of a Triton X-100 salt-precipitation method, and the prevalence of the C677T MTHFR mutation was determined by polymerase chain reaction and HinfI restriction enzyme digestion as described by Frooss et al.

Statistical Methods
Baseline differences between cases and controls were examined by means of the ch² test for categorical data and an unpaired Student t test for continuous data. Logarithmic transformations were used for variables showing a marked positive skew (homocyst(e)ine, serum folate, red cell folate, pyridoxine, cobalamin). The associations between homocyst(e)ine, vitamin status, and MTHFR genotype (independent variables) and stroke (dependent variable) were examined by means of a logistic regression model, with adjustment for age, sex, conventional vascular risk factors, and history of previous vascular events. Potential interactions between homocyst(e)ine, conventional vascular risk factors, and history of previous vascular events were examined by use of multiplicative interaction terms in the logistic regression model. Results were expressed as OR together with their 95% CI.

Determinants of homocyst(e)ine were assessed by stepwise linear regression. ANOVA was used to compare mean homocyst(e)ine levels between etiologic subtypes of stroke and MTHFR genotypes. If overall significance was confirmed after adjustment for other determinants of homocyst(e)ine, pairwise comparisons were performed with adjustment for multiple comparisons by means of Tukey's test. Statistical significance was taken as a 2-sided P<0.05. Separate logistic regression models were used to examine the association between homocyst(e)ine and large-artery disease and between homocyst(e)ine and small-artery disease, adjusting for age, sex, conventional vascular risk factors, and history of previous vascular events. Mean levels of vitamins were compared in subjects with a homocyst(e)ine greater than or less than the median level with...
the use of an unpaired Student t test. The SPSS for windows (version 8.0) statistical package was used for all analyses.

**Results**

Two hundred nineteen consecutive patients with ischemic stroke (142 men and 77 women; mean age 66.1 years [SD 12.4]) and 205 control subjects (131 men, 74 women, mean age 67.0 years [SD 11.8]) were studied.

**Case-Control Differences**

There was a significantly higher prevalence of all conventional vascular risk factors, with the exception of hypercholesterolemia, among the cases (Table 1). Mean fasting plasma homocyst(e)ine was significantly higher in cases (12.4 μmol/L; 95% CI 11.7 to 13.2) compared with that in control subjects (10.5 μmol/L; 95% CI 10.0 to 11.0, P<0.001). Serum folate (15.1 nmol/L; 95% CI 14.1 to 16.0 versus 16.8 nmol/L; 95% CI 15.8 to 17.6, P=0.015), red cell folate (592.5 nmol/L; 95% CI 555.1 to 632.4 versus 674.8 nmol/L; 95% CI 635.9 to 716.0, P=0.004), and pyridoxine (28.0 nmol/L; 95% CI 25.7 to 30.5 vs 31.7 nmol/L; 95% CI 29.1 to 34.5, P=0.049) levels were significantly lower in cases compared with that in controls. There was no difference in serum cobalamin levels (280.7 pmol/L; 95% CI 256.0 to 307.7, versus 270.8 pmol/L; 95% CI 252.1 to 290.9, P=0.54) or in the distribution of the TT MTHFR genotype (12% versus 11%; χ²=0.02, 1 df, P=0.89) between cases and controls (overall P=0.34) (Table 2).

**Determinants of Homocyst(e)ine**

Independent determinants of homocyst(e)ine selected by stepwise linear regression were serum folate, red cell folate, and vitamin B6 (Table 2).

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**TABLE 1. Baseline Demographics, Conventional Vascular Risk Factors, and History of Previous Vascular Events in Cases and Control Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=219)</th>
<th>Control Subjects (n=205)</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y (SD)</td>
<td>66.1 (12.4)</td>
<td>67.0 (11.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>140 (64%)</td>
<td>131 (60%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>118 (54%)</td>
<td>68 (33%)</td>
<td>2.4</td>
<td>1.6–3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>55 (25%)</td>
<td>22 (11%)</td>
<td>2.8</td>
<td>1.6–4.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>52 (24%)</td>
<td>45 (22%)</td>
<td>1.1</td>
<td>0.7–1.7</td>
<td>0.66</td>
</tr>
<tr>
<td>Current smoker</td>
<td>72 (33%)</td>
<td>36 (18%)</td>
<td>2.3</td>
<td>1.5–3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Previous vascular event</td>
<td>59 (27%)</td>
<td>26 (13%)</td>
<td>2.5</td>
<td>1.5–4.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*χ² for categorical data, unpaired Student t test for continuous data.

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**TABLE 2. Fasting Plasma Homocyst(e)ine, Vitamin Status, and Nucleotide 677 MTHFR Genotype in Cases and Controls***

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=219)</th>
<th>Control Subjects (n=205)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocyst(e)ine, μmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>12.3 (3.6–93.3)</td>
<td>10.2 (3.3–38.0)</td>
<td></td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>12.4 (11.7–13.2)</td>
<td>10.5 (10.0–11.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum folate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>15.5 (4.6–77.6)</td>
<td>17.4 (5.0–33.9)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>15.1 (14.1–16.0)</td>
<td>16.8 (15.8–17.6)</td>
<td>0.015</td>
</tr>
<tr>
<td>Red cell folate, nmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>616.6 (162.2–1584.9)</td>
<td>660.7 (154.9–2344.2)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>592.5 (555.1–632.4)</td>
<td>674.8 (635.9–716.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>Pyridoxine, nmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>25.7 (7.9–120.2)</td>
<td>28.8 (7.9–120.2)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>28.0 (25.7–30.5)</td>
<td>31.7 (29.1–34.5)</td>
<td>0.049</td>
</tr>
<tr>
<td>Cobalamin, pmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>269.2 (28.8–1479.1)</td>
<td>269.2 (75.9–1479.1)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>280.7 (256.0–307.7)</td>
<td>270.8 (252.1–290.9)</td>
<td>0.54</td>
</tr>
<tr>
<td>rMTHFR genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tt (%)</td>
<td>102 (47%)</td>
<td>84 (41%)</td>
<td></td>
</tr>
<tr>
<td>Tt (%)</td>
<td>88 (41%)</td>
<td>98 (48%)</td>
<td></td>
</tr>
<tr>
<td>TT (%)</td>
<td>25 (12%)</td>
<td>23 (11%)</td>
<td>0.34†</td>
</tr>
</tbody>
</table>

*Unpaired Student t test.

†χ²=2.13, df=2.
cobalamin, creatinine, MTHFR genotype, and age (P<0.001 for each), which together explained approximately 50% of the total variance in homocyst(e)ine levels (R^2=0.51, P<0.001).

**Association Between Homocyst(e)ine and Ischemic Stroke**

Fasting homocyst(e)ine was a strong and independent risk factor for ischemic stroke (OR 2.7; 95% CI 1.4 to 5.1 for a 5-μmol/L increase in fasting plasma homocyst(e)ine from 10 to 15 μmol/L). The upper quartile of fasting homocyst(e)ine was associated with a crude OR of ischemic stroke of 2.2 (95% CI 1.1 to 4.2) (Figure 1). There was no evidence of an interaction between homocyst(e)ine and any of the conventional vascular risk factors or between homocyst(e)ine and history of previous vascular events. Compared with the lowest quartile, the highest quartile of serum folate (OR 0.52; 95% CI 0.29 to 0.92), red cell folate (OR 0.46; 95% CI 0.25 to 0.82), and serum pyridoxine (OR 0.64; 95% CI 0.29 to 0.92) were associated with a reduced risk of stroke. However, after adjustment for age, sex, conventional vascular risk factors, and history of previous vascular events, these associations between high vitamin levels and low risk of stroke were no longer statistically significant.

**Association Between Homocyst(e)ine and Etiologic Subtypes of Ischemic Stroke**

There was a significant difference in homocyst(e)ine levels among etiologic subtypes of ischemic stroke and control subjects (ANOVA, P=0.003). The mean fasting homocyst(e)ine was significantly higher in large-artery disease (14.1 μmol/L; 95% CI 12.5 to 15.9, P<0.001) and small-artery disease (12.7 μmol/L; 95% CI 11.4 to 14.1, P=0.004) compared with controls (10.5 μmol/L; 95% CI 10.0 to 11.0), and these differences remained statistically significant after adjustment for age, sex, creatinine, MTHFR genotype, and vitamin status (P<0.05) (Table 3). There was no significant difference in homocyst(e)ine between cardioembolic stroke (11.6 μmol/L; 95% CI 10.2 to 13.1, P=0.58) or “other”

### Table 3. Plasma Homocyst(e)ine and Vitamin Status in Etiological Subtypes of Ischemic Stroke

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Mean homocyst(e)ine, μmol/L (95% CI)</th>
<th>Mean red cell folate, nmol/L (95% CI)</th>
<th>Mean S-folate, nmol/L</th>
<th>Mean pyridoxine, nmol/L (95% CI)</th>
<th>Mean cobalamin, pmol/L (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large-Artery (n=63)</td>
<td>14.1 (12.5–15.9)</td>
<td>469.9 (412.1–535.8)</td>
<td>13.6 (11.8–15.7)</td>
<td>28.2 (23.9–33.2)</td>
<td>288.8 (242.7–343.7)</td>
</tr>
<tr>
<td>Small-Artery (n=68)</td>
<td>12.7 (11.4–14.1)</td>
<td>599.0 (535.9–669.4)</td>
<td>15.2 (13.6–16.9)</td>
<td>29.5 (25.3–34.4)</td>
<td>257.6 (213.3–311.0)</td>
</tr>
<tr>
<td>Cardioembolic (n=45)</td>
<td>11.6 (10.2–13.1)</td>
<td>741.7 (650.4–845.9)</td>
<td>16.1 (14.1–18.3)</td>
<td>26.6 (21.1–33.6)</td>
<td>328.3 (265.8–405.5)</td>
</tr>
<tr>
<td>Other (n=43)</td>
<td>10.8 (9.5–12.2)</td>
<td>644.6 (571.3–727.3)</td>
<td>16.1 (14.2–18.2)</td>
<td>27.0 (22.5–32.3)</td>
<td>264.3 (224.1–311.8)</td>
</tr>
<tr>
<td>Control (n=205)</td>
<td>10.5 (10.0–11.0)</td>
<td>674.8 (635.9–716.0)</td>
<td>16.8 (15.8–17.6)</td>
<td>31.7 (29.1–34.5)</td>
<td>270.8 (252.1–290.9)</td>
</tr>
<tr>
<td>Overall Significance</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>0.03‡</td>
<td>0.32</td>
<td>0.30</td>
</tr>
<tr>
<td>Pairwise Comparisons</td>
<td>LA vs control</td>
<td>LA vs all other groups</td>
<td>LA vs control</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*P<0.001 after adjustment for age, sex, creatinine, MTHFR genotype, and vitamin status.  
†P<0.001 after adjustment for age, sex, creatinine, MTHFR genotype, and vitamin status.  
‡P=0.04 after adjustment for age, sex, creatinine, MTHFR genotype, and vitamin status.  
LA indicates large artery; SA: small artery; C: control.
stroke (10.8 μmol/L; 95% CI 9.5 to 12.2, P=0.99) and controls (Table 3). Compared with the lowest quartile of homocyst(e)ine, the upper 3 quartiles were associated with an adjusted OR of ischemic stroke due to large-artery disease of 3.0 (95% CI 0.8 to 10.8) for the second quartile, 5.6 (95% CI 1.6 to 20) for the third quartile, and 8.7 (95% CI 2.4 to 32) for the fourth quartile (P for trend=0.0005) (Figure 2).

**Association Between Homocyst(e)ine and Vitamin Levels**

Among the 424 cases and controls in this study, the median homocyst(e)ine was 11.2 μmol/L. Subjects with a homocyst(e)ine level above the median had a mean lower serum folate (13.9 nmol/L; 95% CI 13.1 to 14.7 versus 18.3 nmol/L; 95% CI 17.3 to 19.3), red cell folate (570.0 nmol/L; 95% CI 535.9 to 606.3 versus 708.3 nmol/L; 95% CI 666.7 to 752.5), serum pyridoxine (26.7 nmol/L; 95% CI 24.7 to 28.9 versus 33.2 nmol/L; 95% CI 30.2 to 36.8), and serum cobalamin (240.2 pmol/L; 95% CI 220.4 to 261.8 versus 312.4 pmol/L; 95% CI 290.6 to 335.8) than those with a homocyst(e)ine level below the median (P<0.001 for each comparison).

**Association Between Homocyst(e)ine and MTHFR Genotype**

There was a significant difference in fasting homocyst(e)ine among MTHFR genotypes (ANOVA, P<0.001). Fasting homocyst(e)ine was significantly higher in TT (12.3 μmol/L; 95% CI 10.6 to 14.2, P=0.05) and Tt (11.9 μmol/L; 95% CI 11.3 to 12.5, P=0.02) genotypes compared with tt genotypes (10.6 μmol/L, 95% CI 10.0 to 11.2). These differences remained statistically significant after adjusting for age, sex, creatinine, and vitamin status (P<0.01 for each pairwise comparison). There was no difference in serum folate (P=0.31), cobalamin (P=0.55), and pyridoxine levels (P=0.75) among MTHFR genotypes. Red cell folate levels were significantly higher in TT (840.2 nmol/L; 95% CI 727.8 to 970.1) compared with Tt (629.9 nmol/L; 95% CI 591.4 to 671.0) and tt (592.4 nmol/L; 95% CI 555.3 to 631.8 nmol/L, P<0.001) genotypes, and these differences remained statistically significant after adjustment for age, sex, and vitamin status (P<0.001). There was no difference in the prevalence of the TT MTHFR genotype in subjects with homocyst(e)ine lower than median levels (12%) compared with subjects with homocyst(e)ine greater than median levels (12% in each group) (χ²=0.01, 1 df, P=0.91).

**Association Between MTHFR Genotype and Etiologic Subtypes of Ischemic Stroke**

There was no difference in the distribution of the TT MTHFR genotype among etiologic subtypes of ischemic stroke (χ²=0.32, 4 df, P=0.99).

**Risk Factors for Ischemic Stroke Modeled by Logistic Regression**

Logistic regression analysis was carried out by use of a backward selection strategy with continuous variables (age, creatinine, homocyst(e)ine, red cell folate, serum folate, pyridoxine, cobalamin, creatinine) and categorical variables (sex, smoking, history of hypertension, diabetes mellitus, hypercholesterolemia, history of previous vascular events, MTHFR genotype) as independent variables and ischemic stroke as the dependent variable. The most important independent risk factors for ischemic stroke were hypertension (OR 2.2; 95% CI 1.3 to 3.4, P=0.001), homocyst(e)ine (OR 11.7 per 1 μmol/L increase in log homocyst(e)ine; 95% CI 2.4 to 58, P=0.002), history of vascular events (OR 2.1; 95% CI 1.1 to 3.8, P=0.02), smoking (OR 1.9; 95% CI 1.1 to 3.4, P=0.022), and diabetes (OR 2.0; 95% CI 1.1 to 3.8, P=0.031). Vitamin status, creatinine, and MTHFR genotype were not significant predictors in the model (P>0.10). Table 4 shows the adjusted OR for each selected risk factor, controlling for all remaining variables in the model.

**Discussion**

This is the first study to examine the association between homocyst(e)ine, MTHFR genotype, and vitamin status in etiologic subtypes of acute ischemic stroke. It not only confirms the presence of an independent association between increasing homocyst(e)ine and ischemic stroke, which appears to be as strong as all other conventional vascular risk factors, but it also confirms our a priori hypothesis that there is a significant and strong association between increasing homocyst(e)ine and large-artery disease (9-fold increased risk for upper quartile of homocyst(e)ine) and, much less so, with small-artery disease (2-fold increased risk for the upper quartile of homocyst(e)ine), and no association with cardioembolic and other etiologic subtypes of ischemic stroke.

Although previous studies have failed to demonstrate a difference in homocyst(e)ine among subtypes of ischemic stroke, this may be due to methodological limitations such as inadequate statistical power, the use of nonfasting blood samples to measure homocyst(e)ine, delayed measurement of homocyst(e)ine until several months after the acute event, failure to include cases of cardioembolic stroke, retrospective classification of subtypes of ischemic stroke (which may have led to misclassification as a result of missing data in some patients), use of a classification system not based on the underlying cause of stroke, and failure to adjust for potential confounders such as age, vitamin status, and MTHFR genotype.

The strengths of our study are that we prospectively assembled an inception cohort of >200 patients with ischemic stroke and >200 community-based control subjects selected at random from the electoral roll. The diagnosis of
stroke and etiologic subtype of ischemic stroke was made by a single neurologist who specializes in stroke medicine (G.J.H.), on the basis of predefined and established criteria while remaining blinded to the results of blood tests for homocyst(e)ine, vitamin status, and MTHFR genotype. Vitamin status and homocyst(e)ine were measured within a consistent and narrow time frame (7 days) of the acute stroke event and in the fasting state in all subjects. Finally, we adjusted our results for differences in age, sex, conventional vascular risk factors, history of previous vascular events, vitamin status, and MTHFR genotype.

The possible limitations of our study are those inherent in a case-control design. Although cases were classified prospectively and recruited consecutively and controls were randomly selected from the community, potential confounding cannot be entirely eliminated. Without a prospective cohort study, it is impossible to predict the potential impact on risk factors, if any, of preexisting subclinical cerebrovascular disease, changing risk factors among cases, and any treatment for hypertension or diabetes that a patient may have received. However, such factors generally tend to reduce differences between cases and controls. Furthermore, we studied incident cases of ischemic stroke, which is likely to reduce the impact of this potential bias. Meanwhile, the use of relatively restrictive criteria for the subclassification of large- and small-artery stroke may, in some cases, have resulted in their misclassification as “other” strokes. However, the impact of any such misclassification would be to reduce differences among etiologic subtypes of ischemic stroke and thereby bias the study toward the null.

Socioeconomic status is an important cardiovascular risk factor and may have been an important determinant of responder status and may lead to the selection of a “healthy” control group. However, we stratified the selection of control subjects by postal code, which is an established surrogate for socioeconomic status, to overcome this potential source of bias. Control subjects were included in our study, irrespective of whether they had a past history of vascular disease (including stroke), whereas homocyst(e)ine assays were performed in the first 7 days after the acute stroke event, during which time homocyst(e)ine may be lower than during the convalescent phase.19 However, both these factors are likely to reduce the differences between cases and controls and thereby bias the results of the study toward the null.

Our observations of a strong association between homocyst(e)ine and ischemic stroke and, in particular, between increasing homocyst(e)ine and ischemic stroke due to large-artery disease, are consistent with the results of studies that have reported an association between homocyst(e)ine and extent of atherosclerotic large-artery disease.20–23 The underlying pathophysiology of ischemic stroke due to small-artery disease is less well understood but appears to involve microatheroma formation as well as lipohyalinosis.24 Elevated homocyst(e)ine has recently been reported in patients with cerebral microangiopathy and vascular dementia due to subcortical vascular encephalopathy,25 but such an association has not been reported in acute ischemic stroke. However, if the putative deleterious effects of elevated homocyst(e)ine are mediated primarily via a proatherogenic effect, it is plausible that homocysteine is not as strong a risk factor for stroke caused by small-artery disease as it is for large-artery disease. Meanwhile, the absence of an association between homocyst(e)ine and other causes of ischemic stroke such as cardiac embolism is consistent with the notion that homocyst(e)ine does not have a strong de novo prothrombotic influence, at least not in the heart.

The absence of an association between MTHFR genotype and stroke in our study is consistent with the majority of reports26 but is nevertheless curious and remains unexplained. Furthermore, we did not demonstrate an association between MTHFR genotype and etiologic stroke subtype despite the fact that the TT genotype was strongly associated with elevated homocyst(e)ine in our study. It has been suggested that the strong modulatory effect of folate on plasma homocysteine in patients with the TT genotype may mask an association with atherosclerotic vascular disease in well-nourished populations with adequate folate stores and that the TT MTHFR mutation may only be a risk factor for ischemic stroke in the setting of low folate levels.8 However, we found no association between serum folate and MTHFR genotype. Instead, we found significantly elevated red cell folate levels in TT homozygotes, as has been previously reported in some27 but not all studies.28 One possible explanation for the finding of elevated red cell folate levels in TT homozygotes is that reduced activity of MTHFR results in an alteration in the distribution of red blood cell folates, with a reduction in methyl folate forms and accumulation of formylated folates,29 and the discordant results of the various studies may be a reflection of differences in analytic laboratory techniques that were used.30

In conclusion, our findings suggest that moderately elevated fasting homocyst(e)ine should be added to the established independent risk factors (hypertension, diabetes, smoking, history of previous vascular disease) for ischemic stroke and, particularly, ischemic stroke caused by large-artery atherosclerotic vascular disease. However, it remains to be established whether elevated homocyst(e)ine is a causal risk factor for atherosclerosis or a marker of another causal risk factor such as low serum folate or pyridoxine.5,31,32 Although we and others have found that homocyst(e)ine is associated with an increased risk of ischemic stroke that is independent of vitamin status, this issue will hopefully be resolved by large trials that are currently in progress to determine whether reducing homocyst(e)ine will result in a reduced risk of stroke.5,33 In the meantime, further laboratory studies are required to more clearly elucidate the possible mechanisms of homocysteine-induced vascular injury and to explain the lack of an association between the MTHFR mutation and vascular disease.

Acknowledgments

This study was supported by grants from the Royal Perth Hospital Medical Research Foundation and the National Heart Foundation of Australia. Dr Eikelboom is the recipient of an Overseas Research Fellowship from the Hematology Society of Australia and the University of Western Australia. Dr Anand is the recipient of a Medical Research Council of Canada Clinician Scientist Award. Elsie Yu and Siobhan Sadler assisted with screening and recruiting.
subjects, Frank van Bockxmeer performed genotyping studies, and Qilong Yi provided statistical support.

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Stroke. 2000;31:1069-1075
doi: 10.1161/01.STR.31.5.1069

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/31/5/1069

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