Hyperhomocysteinemia
A Risk Factor for Ischemic Stroke in Children

Ingrid M. van Beynum, MD; Jan A.M. Smeitink, MD, PhD; Martin den Heijer, MD, PhD; Maria T.W.B. te Poel Pothoff; Henk J. Blom, PhD

Background—Moderate hyperhomocysteinemia is a risk factor for arterial vascular disease and venous thrombosis in adults. We performed a case-control study to assess a possible relation between moderate hyperhomocysteinemia and ischemic stroke in Dutch children (age range, 0 to 18 years).

Methods and Results—We measured plasma total homocysteine levels (tHcy) in 45 patients with ischemic stroke and in 234 controls. Hyperhomocysteinemia was defined as a tHcy above the 95th percentile regression line for the respective age of the controls. Hyperhomocysteinemia was present in 8 (18%) of the 45 patients with ischemic stroke. The odds ratio was 4.4 (95% CI, 1.7 to 11.6).

Conclusions—We conclude that moderate hyperhomocysteinemia is a risk factor for ischemic stroke in children.

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Key Words: hyperhomocysteinemia cerebral infarction stroke pediatrics

Cerebrovascular disease in childhood is relatively rare. The incidence for ischemic and hemorrhagic strokes together is 2.7 cases per 100 000 population per year.1 Despite extensive evaluation, an etiologic factor or associated conditions remain undetermined in from 20% to 50% of all stroke patients.2-3 Patients with classic homocystinuria, a rare inborn error due to cystathionine β-synthase deficiency, suffer from premature cardiovascular disease and venous thrombosis at a very young age.4 Over the last 2 decades, it has become evident that moderate hyperhomocysteinemia is an independent risk factor for arteriosclerosis, including stroke,5,6 as well as for venous thrombosis.7 These studies have all been performed in adults. Whether moderate hyperhomocysteinemia is a risk factor for arterial vascular disease or venous thrombosis in children is unknown.

We performed a case-control study to assess a possible association between moderate hyperhomocysteinemia and ischemic stroke in Dutch children.

Methods
In children with cerebrovascular disease, we analyzed blood samples for total homocysteine (tHcy) from January 1989 through December 1997. These samples were collected primarily for tHcy measurement. We included 45 patients with objectively confirmed episodes of ischemic stroke (30 boys and 15 girls). The diagnosis of stroke was radiologically confirmed by CT scan or MRI. If required, MR angiography or single photon emission computerized tomography (SPECT) was also performed. Hemiparesis was the most common presenting symptom (29 [64%] of 45 children). The medial cerebral artery (26 patients) and basal ganglia (14 patients) were predominantly affected. The exclusion criteria were overt liver and renal dysfunction, hormonal therapy, neoplastic disease, and closure defects such as cleft lip and spina bifida. Overall, we excluded 2 patients with neoplastic disease and 1 with overt renal dysfunction. Classic risk factors for vascular disease, such as smoking, diabetes, and hypertension, were not present in the patients. Cholesterol was not routinely examined.

The control group, recruited in 1997, consisted of 234 subjects (115 boys and 119 girls). Children of secondary school age served as healthy volunteers, whereas for ethical reasons, the younger children were recruited in a hospital setting. Information about medical history was obtained from medical records or by questionnaire. The same exclusion criteria were applied for the control group as for the patients. Blood was drawn by venipuncture, and in the very young, blood obtained from capillaries was used. tHcy levels were determined in EDTA plasma by an automated high-performance liquid chromatography method with reverse-phase separation and fluorescent detection.8

Because of a sharp increase in tHcy with age, we calculated a 95th percentile regression line of tHcy against age. Hyperhomocysteinemia was defined as a concentration that exceeded this 95th percentile regression line for the respective age. ORs and 95% CIs were calculated to estimate the relative risk of hyperhomocysteinemia. ORs were calculated with a logistic regression model. Analyses were performed with the statistical package SPSS.

The protocol was approved by the local ethics committee.

Results
tHcy values plotted by age of individual patients and controls are shown in the Figure. tHcy increased sharply with age for both sexes in patients and the control group. There was no apparent difference between girls and boys except that in adolescence, tHcy tended toward higher levels in boys than in girls.

Patients with stroke had a median age at the time of blood sampling of 1.8 years (range, 0 to 15.7 years), and controls...
were 8.6 years of age (range, 0 to 19.3 years). Therefore, all data had to be corrected for age. The highest incidence of cerebrovascular disease in childhood is predominantly in the very young, but cerebrovascular disease can occur at any age, as previously published.2 The median time between onset of symptoms and homocysteine determination was 0.1 year (range, 0 to 11.9 years). The median tHcy level was 8.5 mmol/L (range, 5.0 to 77 mmol/L) for the patients and 9.1 mmol/L (range, 4.3 to 20.0 mmol/L) for the controls. Among the patients with ischemic stroke, 8 (18%) of 45 versus 11 (5%) of 234 in the control group had a tHcy level above the age-corrected cutoff points of homocysteine concentration. The OR for ischemic stroke was 4.4 (95% CI, 1.7 to 11.6). ORs for different age-corrected cutoff points of tHcy are shown in Table 1 and demonstrate gradually increasing ORs at higher cutoff points in the highest quartile. A continuous dose-response relation seemed to be present above a threshold tHcy value of ≈70th percentile of controls. To evaluate a potential change in risk with increasing age of the child, we calculated ORs for 3 different age groups, balanced for number of cases (Table 2). A trend toward an increase in the risk of ischemic stroke associated with hyperhomocysteinemia in increasingly older age groups is demonstrated.

Creatinine concentration is known to be positively correlated with tHcy.9 Creatinine concentrations were determined in 178 controls and 29 patients. The calculated OR after adjustment for creatinine concentration remained virtually unchanged at 5.6 (95% CI, 0.9 to 34.2). The use of anticonvulsant drugs may influence homocysteine levels.9 In the present study, 15 children used anticonvulsant drugs for therapeutic or prophylactic reasons. The OR remained 3.1 (95% CI, 0.9 to 10.5) after exclusion of these 15 subjects.

Discussion

Studies about tHcy in children are not commonly reported. tHcy measurements performed in 41 macrobiotic infants showed evidently higher tHcy in these children, predominantly due to cobalamin deficiency.10 In 12 children with leukemia, tHcy levels varied during different stages of chemotherapy treatment.11 A Norwegian study12 among 8- to 12-year-old children found that a modest elevation in tHcy was related to premature cardiovascular death in their male relatives and may account in part for the contribution of family history to risk of cardiovascular disease. Recently, Vilaseca et al13 screened for hyperhomocysteinemia in selected pediatric patients, including children who had suffered a stroke. They found higher tHcy levels compared with age-matched reference values, which supports our findings. However, no subsequent analysis on this raw data was performed, and both children with ischemic infarct and hemorrhage were included, whereas only an association between occlusive vascular disease and hyperhomocysteinemia has been found.

In the present study, moderate hyperhomocysteinemia was related to a 4-fold increased risk for ischemic cerebrovascular disease in childhood. This OR is comparable to the relative

**Table 1. ORs for Different Age-Corrected Cutoff Points of Homocysteine Concentrations**

<table>
<thead>
<tr>
<th>Age-Corrected Homocysteine Concentration</th>
<th>Cases (n=45)</th>
<th>Controls (n=234)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10th percentile</td>
<td>41</td>
<td>211</td>
<td>1.1 (0.4–3.4)</td>
</tr>
<tr>
<td>20th percentile</td>
<td>36</td>
<td>188</td>
<td>1.0 (0.4–1.8)</td>
</tr>
<tr>
<td>25th percentile</td>
<td>35</td>
<td>176</td>
<td>1.2 (0.5–2.5)</td>
</tr>
<tr>
<td>30th percentile</td>
<td>34</td>
<td>164</td>
<td>1.3 (0.6–2.8)</td>
</tr>
<tr>
<td>40th percentile</td>
<td>32</td>
<td>140</td>
<td>1.6 (0.8–3.3)</td>
</tr>
<tr>
<td>50th percentile</td>
<td>28</td>
<td>117</td>
<td>1.6 (0.8–3.0)</td>
</tr>
<tr>
<td>60th percentile</td>
<td>24</td>
<td>94</td>
<td>1.7 (0.9–3.2)</td>
</tr>
<tr>
<td>70th percentile</td>
<td>22</td>
<td>70</td>
<td>2.2 (1.2–4.3)</td>
</tr>
<tr>
<td>75th percentile</td>
<td>20</td>
<td>58</td>
<td>2.4 (1.3–4.7)</td>
</tr>
<tr>
<td>80th percentile</td>
<td>18</td>
<td>46</td>
<td>2.7 (1.4–5.4)</td>
</tr>
<tr>
<td>90th percentile</td>
<td>12</td>
<td>23</td>
<td>3.3 (1.5–7.3)</td>
</tr>
<tr>
<td>95th percentile</td>
<td>8</td>
<td>11</td>
<td>4.4 (1.7–11.6)</td>
</tr>
<tr>
<td>97.5 percentile</td>
<td>5</td>
<td>5</td>
<td>5.7 (1.6–20.7)</td>
</tr>
</tbody>
</table>

**Table 2. ORs for Ischemic Stroke With Hyperhomocysteinemia (95th Percentile of Age-Corrected Homocysteine Distribution) for Each Age Group**

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Above Cutoff Point</th>
<th>Below Cutoff Point</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
</tr>
<tr>
<td>0.0–1.2</td>
<td>3</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>1.2–4.8</td>
<td>3</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>4.8–19.3</td>
<td>2</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>All ages</td>
<td>8</td>
<td>11</td>
<td>37</td>
</tr>
</tbody>
</table>
risk found for ischemic vascular disease in adults. Although the pathogenesis of vascular disease due to hyperhomocysteinemia is unknown, this same risk for ischemic stroke in early childhood and in adults presumes a dysequilibrium of the coagulation-fibrinolysis status that results in an enhanced coagulation state rather than a prolonged, cumulative effect in arterial vessel wall, as is seen in hypercholesterolemia.

Among the patients, a 16-month-old boy had a very high tHcy level (77 mmol/L). It was suspected that he was homozygous for cystathionine β-synthase deficiency, and this diagnosis was confirmed by very low enzyme activity in cultured fibroblasts and molecular genetic analysis that showed homozygosity for the T833C mutation. After exclusion of this case, the adjusted OR remained virtually unchanged (3.8; 95% CI, 1.4 to 10.5). A vascular accident at this age in cystathionine β-synthase deficiency is rare, but cases have been described. Normalization of tHcy levels in vitamin B6-responsive cystathionine β-synthase–deficient patients with pyridoxine is effective in preventing complications such as arterial and venous thrombosis. Although blood and urine amino acid measurements are recommended in the pediatric textbooks, in our opinion they are still not performed routinely. On the basis of our findings, screening of plasma tHcy levels in children with vascular disease or venous thrombosis should be done at least to exclude rare inborn errors causing severe hyperhomocysteinemia.

Our study contributes to elucidation of idiopathic cases of ischemic cerebrovascular disease, indicating that moderate hyperhomocysteinemia is a common risk factor for ischemic stroke in children.

Acknowledgments
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References
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