Abstract—An inborn error of metabolism, homocystinuria due to cystathionine β-synthase deficiency, results in markedly elevated levels of circulating homocysteine. Premature vascular events are the main life-threatening complication. Half of all untreated patients have a vascular event by 30 years of age. We performed a multicenter observational study to assess the effectiveness of long-term homocysteine-lowering treatment in reducing long-term vascular risk in 158 patients. Vascular outcomes were analyzed and effectiveness of treatment in reducing vascular risk was evaluated by comparison of actual to predicted number of vascular events, with the use of historical controls from a landmark study of 629 untreated patients with cystathionine β-synthase deficiency. The 158 patients had a mean (range) age of 29.4 (4.5 to 70) years; 57 (36%) were more than 30 years old, and 10 (6%) were older than 50 years. There were 2822 patient-years of treatment, with an average of 17.9 years per patient. Plasma homocysteine levels were markedly reduced from pretreatment levels but usually remained moderately elevated. There were 17 vascular events in 12 patients at a mean (range) age of 42.5 (18 to 67) years: pulmonary embolism (n=3), myocardial infarction (n=2), deep venous thrombosis (n=5), cerebrovascular accident (n=3), transient ischemic attack (n=1), sagittal sinus thrombosis (n=1), and abdominal aortic aneurysm (n=2). Without treatment, 112 vascular events would have been expected, for a relative risk of 0.09 (95% CI 0.036 to 0.228; P<0.0001). Treatment regimens designed to lower plasma homocysteine significantly reduce cardiovascular risk in cystathionine β-synthase deficiency despite imperfect biochemical control. These findings may be relevant to the significance of mild hyperhomocysteinemia that is commonly found in patients with vascular disease.

Key Words: homocysteine ■ cardiovascular disease ■ cystathionine beta-synthase deficiency ■ risk factors

The association between modest elevations of circulating homocysteine and coronary artery disease, first shown in 1976 and subsequently confirmed for vascular disease generally in many studies, arose from observations in patients with the inborn error of homocystinuria due to cystathionine β-synthase deficiency (CBS). This is a recessively inherited disorder of the transsulfuration pathway of methionine metabolism. Homocystinuria was first recognized in 1962 and the enzyme defect identified 2 years later. The worldwide birth prevalence based on newborn screening has been reported at 1 in 344 000, a minimal incidence because cases are known to be missed by such screening. There is a much higher birth prevalence in Ireland, estimated at 1 in 65 000, derived from both newborn screening and clinically detected cases, and in New South Wales, Australia. CBS deficiency is characterized biochemically by severe hyperhomocysteinemia, hypermethioninemia, and hypocysteinemia. The pathological sequelae include ectopia lentis, osteoporosis, mental retardation, and most importantly, a greatly increased risk of vascular events.

Approximately 50% of patients worldwide are biochemically responsive to pharmacological doses of pyridoxine. Since 1967, many patients have been treated by dietary methionine restriction, pyridoxine (vitamin B6) in combination with folate, and sometimes vitamin B12. Since the 1980s, betaine has been used to remethylate homocysteine back to methionine. Early anecdotal reports showed that these regimens result in some biochemical control and might prevent or delay clinical complications.

In 1985, an international study by Mudd and colleagues documented the natural history of CBS deficiency in 629 patients by time-to-event analyses for patients before treat-
TABLE 1. Demographics of Patients With Cj/S Deficiency Treated in Sydney, Nijmegen, Dublin, Manchester, and London

<table>
<thead>
<tr>
<th></th>
<th>Sydney</th>
<th>Nijmegen</th>
<th>Dublin</th>
<th>Manchester</th>
<th>London</th>
<th>Overall Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of Cj/S-deficient patients</td>
<td>40</td>
<td>30</td>
<td>28</td>
<td>31</td>
<td>41</td>
<td>170</td>
</tr>
<tr>
<td>No. of pedigrees</td>
<td>25</td>
<td>21</td>
<td>22</td>
<td>27</td>
<td>34</td>
<td>129</td>
</tr>
<tr>
<td>Deaths before treatment</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Deaths during treatment</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Vascular events before/off treatment</td>
<td>12</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>Total No. followed up with treatment</td>
<td>32</td>
<td>28</td>
<td>27</td>
<td>30</td>
<td>41</td>
<td>158</td>
</tr>
<tr>
<td>B6 responders</td>
<td>17</td>
<td>19</td>
<td>1</td>
<td>8</td>
<td>25</td>
<td>70</td>
</tr>
<tr>
<td>B6 nonresponders</td>
<td>15</td>
<td>9</td>
<td>26</td>
<td>22</td>
<td>16</td>
<td>88</td>
</tr>
<tr>
<td>Mean period of treatment, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B6 responders</td>
<td>18.5</td>
<td>13.2</td>
<td>13.7</td>
<td>22.9</td>
<td>19.3</td>
<td>17.8</td>
</tr>
<tr>
<td>B6 nonresponders</td>
<td>19.2</td>
<td>18.8</td>
<td>17.1</td>
<td>17.5</td>
<td>18.2</td>
<td>17.9</td>
</tr>
<tr>
<td>Mean (range) age, y at start of treatment</td>
<td>12.9 (1–41)</td>
<td>22.8 (3–54)</td>
<td>0.8 (0–11)</td>
<td>6.7 (0–29.7)</td>
<td>11.4 (0–57)</td>
<td>11 (0–57)</td>
</tr>
<tr>
<td>Current (1998) mean age, y</td>
<td>32</td>
<td>38.5</td>
<td>18.1</td>
<td>26.5</td>
<td>30.7</td>
<td>29.4</td>
</tr>
<tr>
<td>Range of ages, n</td>
<td>1–68</td>
<td>18–70</td>
<td>4.5–33.8</td>
<td>4.8–50.7</td>
<td>12–69</td>
<td>4.5–70</td>
</tr>
<tr>
<td>&lt;10 years old</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>5 (3%)</td>
</tr>
<tr>
<td>&gt;30 years old</td>
<td>11</td>
<td>18</td>
<td>1</td>
<td>9</td>
<td>18</td>
<td>57 (36%)</td>
</tr>
<tr>
<td>&gt;50 years old</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>10 (6.3%)</td>
</tr>
</tbody>
</table>

Of the 7 deaths that occurred during treatment, each of the Australian and Irish groups have 1 death (accidental poisoning and drowning) unrelated to homocystinuria. The remaining 5 deaths were vascular deaths (pulmonary embolism, n=3; myocardial infarction, n=1; sagittal sinus thrombosis, n=1).

The Irish national newborn screening program for homocystinuria was started in 1971 and accounts for the relatively younger group with comparable mean periods of treatment. Newborn screening program for homocystinuria in Manchester was started in 1969.

Biochemical Markers

Methods for the measurement of plasma amino acids in each center have been reported.14–19 Homocysteine exists in several forms in plasma, with a protein-bound and a non–protein-bound (free) fraction. Total homocysteine (tHcy) includes all homocysteine species, both protein bound and free. Free total homocysteine (tHcy) is the non–protein-bound fraction, which is calculated as twice the concentration of free homocysteine (the homocysteine-homocystine disulfide) plus the concentration of homocysteine-cysteine mixed disulfide. Homocysteine (Hcy-Hcy) is the non–protein-bound (free) homocysteine, the disulfide. To monitor biochemical control, the centers in Sydney and Nijmegen have used tHcy.15,18 Dublin, Manchester, and London have used homocystine (Hcy-Hcy) is not detectable.
TABLE 2. Treatment Regimens, Biochemical Criteria for B₆ Responsiveness, Biochemical Markers Used in Monitoring, and the Frequency of Monitoring in Each Respective Center Treating Patients With CJS Deficiency

<table>
<thead>
<tr>
<th>Treatment regimens used</th>
<th>Sydney</th>
<th>Nijmegen</th>
<th>Dublin</th>
<th>Manchester</th>
<th>London</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary methionine restriction, mg/d</td>
<td>General advice</td>
<td>600</td>
<td>200–625</td>
<td>160–900</td>
<td>400–1375</td>
</tr>
<tr>
<td>Pyridoxine (B₆), mg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>100–200</td>
<td>750</td>
<td>100–800</td>
<td>50–500</td>
<td>20–500</td>
</tr>
<tr>
<td>Child</td>
<td>200–500</td>
<td>150 (Neonate)</td>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5–10</td>
</tr>
<tr>
<td>Vitamin B₆ (intramuscular or oral)</td>
<td>Routine to all</td>
<td>Given, if deficient</td>
<td>Given, if deficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betaine, g/d</td>
<td>6–9</td>
<td>6</td>
<td>3–6</td>
<td>4.5–15</td>
<td>2–6</td>
</tr>
<tr>
<td>Biochemical markers monitored</td>
<td>tfHcy</td>
<td>tfHcy; tHcy (from 1990)</td>
<td>Hcy-Hcy and tHcy (from 1997)*</td>
<td>Hcy-Hcy</td>
<td>Hcy-Hcy</td>
</tr>
<tr>
<td>Frequency of biochemical monitoring, per year</td>
<td>1–4</td>
<td>1–2</td>
<td>≥8–10</td>
<td>1–4</td>
<td>2–4</td>
</tr>
<tr>
<td>Criteria for B₆ responsiveness, μmol/L</td>
<td>tfHcy &lt;20</td>
<td>tfHcy &lt;20 or tHcy &lt;50</td>
<td>Hcy-Hcy &lt;5</td>
<td>Hcy-Hcy &lt;10</td>
<td>Hcy-Hcy &lt;10</td>
</tr>
</tbody>
</table>

*Hcy-Hcy indicates free homocysteine, the disulfide.

Published data on biochemical control in growing children with homocystinuria have used Hcy-Hcy, and there are currently no reported data using tfHcy in growing children. B₆ responsiveness is determined by serial homocysteine assessments in relation to B₆ administration, and the patient is classified as responsive when the homocysteine levels meet the criteria set by each respective center.

In the normal population. The mean±SD value for tfHcy in the normal Australian population is 3.7±1 μmol/L. The mean±SD value for tfHcy in the normal Dutch population is 3.3±1.3 μmol/L, whereas the upper limit of normal for tHcy is 19 μmol/L. The upper limit of tfHcy for the normal Irish and New South Wales populations is 15 μmol/L.

TABLE 3. Patient-Years of Treatment, Predicted and Actual Number of Vascular Events, and the Biochemical Control of CJS-Deficient Patients Treated in the Respective Centers

<table>
<thead>
<tr>
<th>Study Procedure</th>
</tr>
</thead>
</table>

Patient records were analyzed for age at diagnosis, mode of diagnosis, B₆ responsiveness, treatment regimen, period of treatment for each regimen, and frequency of biochemical monitoring. For vascular events, the frequency, age of occurrence, and type were identi-

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PE indicates pulmonary embolism; MI, myocardial infarction; AAA, abdominal aortic aneurysm; CVA, cerebrovascular accident; SST, sagittal sinus thrombosis; DVT, deep vein thrombosis; TIA, transient ischemic attack; and Hcy-Hcy, homocysteine (disulfide).
fied. All vascular events were diagnosed by appropriate contemporary diagnostic methods used by the respective teaching hospitals at the time of the events. The study period was until the end of 1998, and the current age of each patient was taken as of December 31, 1998. The period of treatment was calculated and expressed as patient-years of treatment. For biochemical control, the homocysteine level was expressed as mean±SD for patient groups attending each of the centers and was compared with the mean±SD for the respective normal population. The total number of expected vascular events if the study population had remained untreated was derived from the data of Mudd and colleagues. Data were analyzed for each individual center with a final calculation from the pooled data.

Statistical Analysis

All statistical analyses were performed in collaboration with a medical statistician using SAS version 6.12 data analysis software. Two-tailed significance tests and the conventional level of significance (P<0.05) were used. The number of actual events related to the number of patient-years of treatment was compared with the expected number of events from Mudd and colleagues based on the same number of patient-years. The odds ratios from each of the 2×2 contingency tables for the different countries were combined by the Mantel-Haenszel procedure to give an estimate of the common ratio. The probability values quoted are based on χ² values from analysis of the individual contingency tables. The Breslow-Day test for homogeneity of the odds ratios was also performed. This statistical approach tests the hypothesis that odds ratios for the various centers are not different.

Results

Demographics of the Study Population

Until the end of 1998, a total of 158 patients with C/B₆ deficiency (45% B₆ responsive) at the 5 centers had been treated and followed up for a mean of 17.9 years. The mean age was 29.4 years, with a range of 4.5 to 70 years. Only 3% were younger than 10 years of age, with 36% older than 30 years and 6.3% older than 50 years. The Irish group was younger, with a comparable mean period of treatment, because of Ireland’s national newborn screening program for homocystinuria (n=21). Approximately one third of the patients (n=11) attending Manchester were also detected through newborn screening. (See Table 1.)

Treatment Regimens and Biochemical Monitoring

All 5 centers used recognized treatment regimens with minor modifications, as outlined in Table 2. Biochemical markers used for monitoring differed slightly among centers (Table 2). The frequencies of monitoring were similar except that the Irish group was monitored more often to avoid nutritional deficiency while trying to achieve good biochemical control in a group of patients who were predominantly in a growth period. Pretreatment plasma free homocystine levels were between 11 and 187 μmol/L (Dublin, London, and Manchester), and plasma tHcy levels were between 42 and 266 μmol/L (Sydney and Nijmegen), which equates to tHcy levels well in excess of 150 μmol/L. tHcy measurements were not available at the time.

Patient-Years of Treatment, Vascular Risk, and Biochemical Control

The 158 patients studied had a total of 2821.6 patient-years of treatment (Table 3). There were 1243.8 patient-years of treatment in the B₆ responders and a further 1577.8 patient-years of treatment in the B₆ nonresponders. As 1 vascular event per 25 years was expected, in 2821.6 patient-years at least 112 vascular events would have occurred if these patients had remained untreated. Instead, only 17 vascular events occurred in 12 patients who were undergoing treatment. This difference is highly significant (relative risk 0.09, 95% CI 0.036 to 0.228; P<0.0001). Statistical analyses were repeated that used only the first vascular event in each patient, and the results were not different from that including all vascular events (relative risk 0.09, 95% CI 0.036 to 0.228; P<0.0001); indeed, it was more significant for the London center (relative risk 0.1243, 95% CI 0.043 to 0.353; P<0.0001). These events occurred at a mean (range) age of 42.5 (18 to 67) years. There was also no evidence of nonhomogeneity in the vascular outcomes of each center (P=0.156) despite the slightly different combinations of treatment regimens used by the 5 centers.

The documented vascular events were pulmonary embolism (n=3), myocardial infarction (n=2), abdominal aortic aneurysm (n=2), transient ischemic attack (n=1), sagittal sinus thrombosis (n=1), deep venous thrombosis (n=5), and cerebrovascular accident (n=3). One B₆ nonresponsive patient had 2 episodes of deep vein thrombosis at 21 and 24 years, respectively. Another patient, who was B₆ responsive, had 3 cerebrovascular accidents and 2 episodes of deep vein thrombosis in her 60s. Of the 17 vascular events, 12 occurred in 8 B₆ responders at a mean (range) age of 51.6 (25 to 67) years and another 5 in 4 B₆ nonresponders at a younger mean (range) age of 20.6 (18 to 24) years. There were 5 vascular deaths resulting from the above events, 2 in B₆ responders at ages 30 (pulmonary embolism, Sydney) and 43 (myocardial infarction, Nijmegen) years. The remaining 3 were among B₆ nonresponders at ages 18 and 21 (pulmonary embolisms, Manchester) and 19 years (sagittal sinus thrombosis, Manchester). Postmortem examinations were performed on these deaths where the diagnoses were not defined clinically before death.

Biochemical control was expressed as the mean (±SD) level of plasma homocysteine measured during long-term treatment. These levels were several times higher than the mean for the respective normal population of each center, despite the aim of achieving normal levels.

The patient-years of treatment were calculated separately for B₆ responders and nonresponders. In the total of 825 patient-years of betaine treatment, there were no reports of significant side effects. The longest period of betaine treatment was 17 years.

Discussion

The immediate aim of treatment in C/B₆ deficiency is to control biochemically and, if possible, normalize the severe hyperhomocysteinemia characteristically associated with the condition, with the hope that the development of recognized complications of untreated homocystinuria, in particular life-threatening vascular events, can be prevented. The purpose of this study was to determine the effects of long-term treatment on the vascular risk of patients with C/B₆ deficiency. A randomized controlled trial never seemed feasible because of a widespread belief, based on early reports, that lowering homocysteine levels had a beneficial effect. Additionally, the time course of the disease is long and the disease itself is uncommon, so that numbers in any study would be necessarily small. To gather sufficient data, we studied patients from
5 centers treating CBS deficiency, comparing the findings with those in the study on the natural history by Mudd and colleagues.9

Our study provides the first overview of vascular outcome in a large number of patients treated chronically. It confirms and amplifies the findings of an earlier, smaller study.10 The risk reduction we observed is highly significant (P<0.0001) compared with the expected vascular events in untreated patients for a similar period. Despite all 5 centers having slightly different combinations of treatment regimens, there was no evidence of nonhomogeneity in the vascular outcomes of each center. Of the 17 vascular events that occurred, 9 were venous, 5 were arterial (including 2 myocardial infarctions), and cerebrovascular accidents accounted for another 3. This distribution is not dissimilar from that of untreated patients.9 There were only 5 vascular deaths during the treatment period, consistent with a lower mortality rate than that of untreated patients.9 The B6-nonresponsive patients were younger at the time of their vascular event than were the B6-responsive patients, and this may have been because their mean posttreatment homocyst(e)ine levels were higher (Table 3), and consistent compliance with the required regimen was more difficult.

Although our study shows that patients with CBS deficiency treated with various combinations of low-methionine diet, pyridoxine, folic acid, vitamin B12, and betaine have much improved vascular outcome, it is not clear whether this results entirely from lowering the extremely high pretreatment levels of homocysteine or from some other aspect of the treatment. The biochemical control achieved by treatment did not result in normal levels of circulating homocysteine in most patients; levels usually remained moderately elevated. In B6-nonresponsive patients, this was some 3 to 5 times the upper limit of normal for the populations. It is possible that some of the treatment modalities that lowered homocysteine levels may have had other as-yet-unidentified protective effects related to vascular events. In addition, some patients in the study were taking other treatment than that specifically designed to lower homocysteine. Some older patients (n = 23) were taking aspirin therapy, and 1 was undergoing lipid-lowering therapy. This may have been different from the experience of the control group collected in 1985. However, before the diagnosis of CBS deficiency, 9 of the older Dutch patients (>30 years) had had recurrent thromboembolic events despite anticoagulation with warfarin. All but 1 had no further episodes of thrombosis after the commencement of homocysteine-lowering therapy. This experience supports the earlier report by Schulman and colleagues28 that some CBS-deficient patients with persistent severe hyperhomocysteinemia may continue to have serious vascular complications despite treatment with dipyridamole and aspirin.

The use of historical controls was unavoidable, as already discussed, but could introduce biases because of changes in the background rate of vascular disease. However, it is unlikely that lifestyle changes could account for the uniformly good results obtained in the widely separated centers of the study. Indeed, reductions in the incidence of cardiovascular disease in the general population, presumably due to lifestyle changes, have only occurred in the United Kingdom and Ireland very recently. Based on the prevalence of a CBS mutation among newborns in the Danish population, Gaustadnes and colleagues9 estimated the incidence of homocystinuria to be 1 in 20,500, albeit with a wide confidence interval. This incidence is much higher than any reported frequency based on newborn screening,6 but newborn screening for homocystinuria is recognized to have false-negative results.30 Despite the presence of characteristic clinical signs and complications, the diagnosis of CBS deficiency is often missed. Cruyssen et al31 reported a mean delay of 11 (0 to 43) years between the first major sign of the condition at mean age 13 (1 to 40) years and the ultimate diagnosis of CBS deficiency in 34 patients. Early diagnosis and prompt treatment of CBS deficiency appears crucial in the prevention of complications.9,14

Mild hyperhomocysteinemia is regarded as a risk factor for vascular disease. An increase in plasma homocysteine to 1 SD above the mean in the normal population has been shown to be associated with a 60% increase in relative risk for coronary disease.32 Despite that, vascular events in our study population were uncommon, although the posttreatment mean homocysteine levels were still several times higher than those in the respective normal populations. This finding could have relevance to the current concept of mild hyperhomocysteinemia and its association with cardiovascular disease, in which the causal role of a mildly elevated homocysteine level is not yet definitively proven.33,35 The results of the several large ongoing trials of the effect of folic acid on vascular outcome and plasma homocysteine levels are awaited with interest.2

We conclude that long-term treatment to lower the markedly elevated plasma levels of homocysteine seen in homocystinuria due to CBS deficiency is effective in reducing the potentially life-threatening vascular risk, despite giving imperfect biochemical control. It is as yet uncertain whether lowering plasma homocysteine levels in the general population of patients with vascular disease will reduce cardiovascular risk.

References

Vascular Outcome in Patients With Homocystinuria due to Cystathionine β-Synthase Deficiency Treated Chronically: A Multicenter Observational Study
Sufin Yap, Godfried H.J. Boers, Bridget Wilcken, David E.L. Wilcken, David P. Brenton, Philip J. Lee, John H. Walter, Pamela M. Howard and Eileen R. Naughten

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