Prevalence of familial mild hyperhomocysteinemia

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Abstract

Previous studies have shown that elevated basal homocysteine levels are correlated among family members of patients with coronary vascular disease and juvenile venous thrombosis. This suggests the possibility of the presence of inherited basal mild hyperhomocysteinemia (mHH). We studied homocysteine levels, fasting as well as after methionine load, among 96 family members of 21 post-load hyperhomocysteinemic vascular index patients, i.e. 6 parents, 27 offspring, 38 siblings, 19 uncles and aunts and 6 cousins. In 15 out of 21 screened families post-load mHH was established in at least one family member. Fasting and post-load mHH was observed in 19 out of 89 (21%) screened family members (fasting homocysteine levels not measured in seven family members), and 31 out of 96 screened family members (32%), respectively. In 40% of all family members, post-load mHH was not accompanied by fasting mHH. We conclude that both fasting and post-load mHH seems to be inherited in the majority of hyperhomocysteinemic vascular patients.

Keywords: Hyperhomocysteinemia; Familial; Vascular disease

1. Introduction

Mild hyperhomocysteinemia (mHH), a risk factor for premature arteriosclerosis, is detected in a frequency of 9.47% in patients with premature cerebral, peripheral or coronary stenotic arterial disease or with thromboembolism [1–11]. In the general population mHH is present in up to 8% [1,2]. mHH can be caused by enzymatic defects such as heterozygosity for cystathionine synthase (CS) deficiency, or homozygosity for thermolabile 5-methylentetrahydrofolate reductase (MTHFR) deficiency, indicating a genetic cause in at least a subset of the detected patients [3,4,12–15]. Environmental influences such as vitamin B12 and folic acid deficiency, and renal or liver disease can also induce mHH [1,16,17].

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Homocysteine blood concentrations were found to be correlated in monozygotic and dizygotic healthy twins [18,19] and were strongly correlated among family members of patients with coronary vascular disease [20–22]. These studies in family members of arterial occlusive diseased patients were performed on the base of fasting homocysteine concentration measurements only. Falcon et al. [8] reported high plasma homocysteine levels in 5 out of 8 families of patients with juvenile venous thrombosis.

In the present study, we have performed methionine loading tests in 96 family members of 21 hyperhomocysteinemic vascular patients. In these patients as well as their family members vitamin deficiencies or liver or renal failure which could lead to secondary mHH had been excluded. The prevalence of mHH, fasting as well as after methionine loading, among the family members was assessed to examine the possibility of the presence of an inherited cause of mHH in the vascular patients.

2. Materials and methods

2.1. Methionine loading test and determination of hyperhomocysteinemia

In 21 vascular disease patients hyperhomocysteinemia was established on the basis of their homocysteine level after methionine loading exceeding the mean + 2 S.D.s post-load homocysteine level in 95 controls. Methionine loading tests (0.1 g L-methionine/kg body weight) were performed in the family members according to a protocol reported previously [23]. Plasma samples in the fasting state and 6 h after methionine load were collected and centrifuged instantly. The total homocysteine concentration (free plus protein-bound) was measured by high-performance liquid chromatography (HPLC) [24]. At the time of screening, the index patient of family 10, his parents and sibships, and one cousin (fasting and post-load homocysteine levels of 11 and 46 μmol/l, respectively) presented with decreased vitamin B12 levels. Hypercholesterolemia was detected in the mother of family 4 (8.3 mmol/l; reference value ≤ 6.5 mmol/l), in the index patient of family 13 (7.7 mmol/l), hypertriglyceridemia was detected in the index patient of family 8 (5.46 mmol/l; reference value ≤ 2.00 mmol/l), and diabetes in the index patient of family 3 (fasting glucose: 9.4 mmol/l; reference value ≥ 7.8 mmol/l). All other index patients and family members had vitamin B6, vitamin B12, folic acid, glucose, cholesterol, triglycerides levels within the normal ranges, were without liver or renal disease or hypertension of non-reno-vascular origin. From all patients and the family members information of intake of vitamin supplements and medical history was obtained. None of the 21 families and the studied parents were related to one other within the second generation.

2.2. Index vascular patients

Of the 21 hyperhomocysteinemic index patients with documented vascular disease, 10 patients had suffered from cerebral occlusive arterial disease, 3 from peripheral occlusive arterial disease (2 intermittent claudication, and 1 subclavian arterial occlusion), 2 from anterior spinal arterial syndrome, 2 from coronary occlusive arterial disease, 3 from venous thrombosis (1 deep crural thrombosis and 2 cerebral thrombosis), and 1 from concomitant coronary occlusive arterial disease and deep crural thrombosis. One family (Fig. 1, family number 13) was also known with inherited protein C-deficiency [25]. The vascular disease became symptomatic in the index patients between their 16th and 50th year of age (38 ± 8 years; mean ± S.D.), the mean age ± S.D. at their screening was 39 ± 7 years.

2.3. Family members

The methionine loading tests in the studied family members were performed on their own request after detection of the index patient, except for 2 families (Fig. 1, family number 10 and 13) which were asked to volunteer in the screening of mHH. The index patients of these 2 families were exceptionally young, i.e. 16 years of age at the time of their vascular incident. Only those families have been included in this study, in whom at least
one complete family tree, i.e. both parents, or all children, or all brothers and sisters, had been screened for hyperhomocysteinemia. The 21 index patients had, in total, 131 first degree family members, i.e. 42 non-consanguineous parents, 30 offspring and 59 siblings. Homocysteine concentrations, fasting and post-load, were determined in 72 out of these 131 family members (29 male
Fig. 1. (a and b) Twenty-one family pedigrees of vascular patients with post-load mild hyperhomocysteinemia. At the left side of the symbols, the total homocysteine concentration before (above) and after (below) methionine loading are shown. CVD indicates cerebral vascular disease; PAD, peripheral arterial disease; MI, myocardial infarction; VT, venous thrombosis; ASAS, anterior spinal artery syndrome; CRAO, central retinal artery occlusion; CRVO, central retinal venous occlusion; and Neo, neoplasm.
and 43 female); i.e. 6 parents, 27 offspring and 39 siblings. In family number 10 and 13 (Fig. 1) 10 uncles and 9 aunts were also tested. These second degree family members were siblings of the fathers of both index cases in whom post-load mHH was revealed. In family number 10 another 6 third degree relatives (offspring of a post-load hyperhomocysteinemic second degree family member) were screened with the methionine loading test. At the time of screening the mean age ± S.D. of the 97 family members was 33 ± 9 years (range 12–68 years). One sister used vitamins and was excluded from the study (Fig. 1, family number 7). Four family members suffered from vascular complications at the time of screening (Fig. 1, family number 4, 7, 13, 15). Their age at onset of vascular complications ranged from 37 to 59 years of age (mean ± S.D. 44 ± 8 years).

2.4. Control subjects

Because of previously observed differences in mean fasting and post-load homocysteine levels between male, pre-menopausal and post-menopausal female control subjects the studied index patients and family members were categorized accordingly [3,26]. The fasting and post-load homocysteine concentration (2.5–97.5 percentile) respectively was in control men 8–18 and 25–54 μmol/l (n = 23), in control pre-menopausal women 6–15 and 18–51 μmol/l (n = 46) and in control post-menopausal women 6–19 and 25–69 μmol/l (n = 26) [27].

3. Results

3.1. Post-load mild hyperhomocysteinemia (Fig. 2a, and Table 1)

In 15 out of the 21 screened families post-load mHH was established in at least one family member. In 31 out of 96 screened family members post-load mHH was observed (32%). Post-load mHH was detected in 50% of the parents (3 out of 6 parents), 26% of the offspring (7 out of 27 children) and in 29% of the siblings (11 out of 38 siblings among 16 index vascular patients). Seven out of 19 screened second degree and 3 out of 7 screened third degree family members revealed post-load mHH. By definition, the post-load homocysteine concentrations of vascular index patients, and post-load hyperhomocysteinemic family members were significantly higher than in controls (Table 1). Also the total group of studied family members showed significantly higher levels than controls. Plasma folic acid concentrations of vascular index patients, family members as one group and as subgroups with and without post-load hyperhomocysteinemia, were statistically significantly lower compared to controls. Plasma vitamin B₆ was significantly lower in post-load hyperhomocysteinemic family members (Table 1). There was no evident sex difference among the hyperhomocysteinemic family members (17 of the 42 male, and 14 of the 54 female family members; χ² P > 0.05).

3.2. Fasting mild hyperhomocysteinemia (Fig. 2b, and Table 1)

Fasting mHH was present in 19 out of 89 family members (21%), including 17 among 29 post-load hyperhomocysteinemic family members, and 2 among 60 post-load normohomocysteinemic family members (fasting homocysteine levels not measured in 7 family members). Thus, in the included family members, in 40% post-load hyperhomocysteinemia was not accompanied by fasting elevated levels.

Fasting homocysteine levels were within the normal range in 6 out of 20 index patients (Fig. 1, family number 3, 4, 17, 18, 19, 20; fasting homocysteine level not measured in 1 index patient). In 5 out of the 6 families of these 6 patients, one more post-load hyperhomocysteinemic family member was detected, and also in all these 5 family members the fasting homocysteine level was within the normal range. In the 14 families, in whom the index case showed elevated fasting homocysteine level, only 17 out of 24 post-load hyperhomocysteinemic family members showed also elevated fasting homocysteine levels (71%).

The fasting homocysteine levels were statistically significantly higher in vascular index patients, in family members as one group, in
Fig. 2. (a and b) Frequency distribution in percentages of post-load (Fig. 2a) and fasting (Fig. 2b) plasma homocysteine concentrations in controls (n = 95), index patients (n = 21) and family members of the index patients (n = 96). Post-load homocysteine concentrations above 54 μmol/l in men, 51 μmol/l in premenopausal women and 69 μmol/l in post-menopausal women are defined as hyperhomocysteinemia.

Post-load hyperhomocysteinemic family members, but also, albeit less strongly significantly, in post-load normohomocysteinemic family members compared to the control group (Table 1).

3.3. Vascular complications in family members

Four screened family members were known with vascular manifestations at the time of the methionine loading test. Three of them revealed post-load mHH (Fig. 1, family number 4, 7 and 13). The fourth vascular affected family member (Fig. 1, family number 15) showed a high but normal post-load homocysteine concentration (fasting homocysteine level was not measured).

4. Discussion

In the present study, 71% of the families of a patient with various forms of arteriosclerosis had at least one other family member with post-load mHH. In 6 out of the 21 studied families no other relative had post-load mHH, but only 15 of the 40 first degree relatives were studied, and therefore, hyperhomocysteinemic family members could have been missed. Mildly elevated homocysteine levels were present in family members in 21% on the base of fasting and in 32% on the base of
Table 1

Mean ± SD of laboratory characteristics of 21 vascular index patients with post-load mild hyperhomocysteinemia, 96 of their family members (FM), and 95 controls

<table>
<thead>
<tr>
<th>Vascular index patient (n = 21)</th>
<th>FM (n = 96)</th>
<th>FM with elevated post-load (n = 31)</th>
<th>FM with normal post-load (n = 65)</th>
<th>Controls (n = 95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting 25 ± 16 (n = 20)</td>
<td>17 ± 14 (n = 89)</td>
<td>27 ± 21 (n = 29)</td>
<td>12 ± 3 (n = 60)</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Post-load 85 ± 28 (n = 20)</td>
<td>47 ± 20 (n = 29)</td>
<td>69 ± 20 (n = 29)</td>
<td>37 ± 8</td>
<td>37 ± 13</td>
</tr>
<tr>
<td>Post-load 47 ± 20 (n = 29)</td>
<td>69 ± 20 (n = 29)</td>
<td>37 ± 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA 9.3 ± 2.3 (n = 20)</td>
<td>11.3 ± 3.4 (n = 20)</td>
<td>12.3 ± 3.5 (n = 64)</td>
<td>14.4 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>B6 52 ± 16 (n = 20)</td>
<td>48 ± 14</td>
<td>50 ± 14</td>
<td>52 ± 19</td>
<td></td>
</tr>
<tr>
<td>B12 280 ± 136 (n = 20)</td>
<td>251 ± 85</td>
<td>258 ± 77 (n = 64)</td>
<td>265 ± 101</td>
<td></td>
</tr>
<tr>
<td>Age 38 ± 10 (n = 20)</td>
<td>33 ± 12</td>
<td>33 ± 14</td>
<td>33 ± 12</td>
<td>41 ± 9</td>
</tr>
<tr>
<td>Sex (m/f) 4/17</td>
<td>42/54</td>
<td>25/40</td>
<td>23/72</td>
<td></td>
</tr>
</tbody>
</table>

The family members are divided into 31 family members with post-load mild hyperhomocysteinemia, and 65 family members with normal post-load homocysteine concentration.

FA, plasma folic acid; B6, blood vitamin B6; B12, blood vitamin B12.

The age is given in years; M, male; F, female.

* < 0.05; ** < 0.01; *** < 0.0001; **** < 0.00001 (Wilcoxon rank sum W-test).

Post-load homocysteine concentration. Our observations are in line with previous reports in which basal homocysteine concentration in healthy twins and in families of coronary vascular patients is reported to show a significant correlation [18-22]. The present study demonstrates that both fasting and post-load mHH, in the absence of vitamin deficiencies and renal or liver insufficiencies, seems to be inherited in the majority of vascular patients. Further enzymatic and molecular studies are needed to reveal the involved genetic enzymatic defects and their inheritance patterns. Reduced activities in the range of obligate carriers for CS-deficiency have been reported in hyperhomocysteinemic vascular patients [3,4]. This finding was difficult to reconcile with the conclusion by Mudd et al. of a normal incidence of heart attacks or strokes in a large group of obligate heterozygotes for CS-deficiency studied by questionnaires [28]. Furthermore, even in populations with the highest prevalence of homozygous CS-deficiency, such as in Ireland, the calculated number of carriers is too low to account for the number of observed hyperhomocysteinemic vascular patients [29]. Indeed, very recently, it was reported that the finding of lowered CS-activity was not reproducible in 96% of studied hyperhomocysteinemic vascular patients [15,30]. Moreover, in the Netherlands where the 833 T → C transition is detectable as the mutation in the CS gene on chromosome 21 in 50% of alleles of homozygotes for CS-deficiency, in 60 cardiovascular patients this mutation could not be identified in any of them [30]. The same holds true for the observation in Ireland where the 919 G → A mutation in the CS gene, determined in 70% of Irish homocystinuric alleles, could not be detected in a group of 100 Irish patients with premature vascular disease [31]. From all these enzymatic and molecular genetic studies summarizing, it can be concluded that there is no evidence, so far, that heterozygosity for CS-deficiency plays a role as the basis of mHH in vascular patients. In a previous study, we observed an incidence of a thermolabile MTHFR enzyme in 28% of hyperhomocysteinemic cardiovascular patients [15]. Such thermolability is consistently associated with the 677 C → T mutation in the MTHFR gene on chromosome 1 [12], and a subject has to be homozygous for such mutation to produce hyperhomocysteinemia [13,14]. Despite a high incidence of this thermolabile MTHFR enzyme among hyperhomocysteinemic patients and controls, i.e. 28% and 5%, respectively [30], the frequency of this defect does not seem sufficient to provide on its own the base of
all familial occurrence of mHH as observed in the present study.

It is still questionable whether, either fasting or post-load homocysteine concentration, is the most sensitive indicator of abnormal homocysteine metabolism. Both fasting and post-load mHH are considered risk factors for cardiovascular disease [1,5,32]. In case we screened for mHH only on the basis of fasting homocysteine levels, no more than 14 out of 20 index patients (70%) and 17 out of 29 family members with post-load mHH (59%) would have been considered hyperhomocysteinemic. Even if family members of only the 14 index patients with fasting mHH had been screened for fasting homocysteine levels alone, 29% (7 out of 24) of the family members who indeed showed post-load mHH, would have been classified as normohomocysteinemic. Post-load elevated homocysteine levels in the absence of fasting hyperhomocysteinemia is probably on the basis of another enzymatic defect than concomitant fasting and post-load hyperhomocysteinemia [33].

In our present study, we have screened family members of vascular index patients with post-load elevated homocysteine levels, in whom only in 70% also abnormal fasting levels were present. None of the 21 index patients were known with fasting elevated and post-load normal homocysteine concentrations. Therefore, a selection in families has been made on the basis of this inclusion criterion. However, studies on fasting homocysteine levels in healthy twins and also in families of cardiovascular patients have been performed already previously, showing a strong correlation [18–22]. We prefer to perform both fasting and post-load homocysteine concentrations in vascular patients and family members to investigate their individual homocysteine status more completely [11,25,34–37].

Plasma folic acid concentrations, were significantly lower in vascular index patients, in family members as one group, and in the subgroups of post-load normo- and hyperhomocysteinemic family members, compared to controls. This finding is in accordance with earlier observations of reduced, though not deficient, folic acid blood levels in cardiovascular patients [4,10,11]. Establishing both in normo- and hyperhomocysteine-mic family members lower folic acid compared to controls, could be an indication of a common habit of low folate intake in these families, leading to hyperhomocysteinemia in some of the members. However, in family members with hyperhomocysteinemia, the decrease in folic acid concentration was much more pronounced than in their normohomocysteinemic family members, comparable with the highly significantly lower levels in the vascular patients in these families. This is much more suggestive of the presence of a hereditary defect in homocysteine metabolism leading to a higher demand for folate in the hyperhomocysteinemic patients and their hyperhomocysteinemic family members.

Among the 96 investigated family members, 4 had known vascular disease. Three of them were hyperhomocysteinemic, and one had high-normal post-load homocysteine concentration. Therefore, screening for mHH of family members suffering from vascular events among families of a hyperhomocysteinemic patient, is recommended. About 90% of the family members with post-load mHH revealed no subjective signs of vascular disease, at least so far. Most screened family members were younger than their respective index case. This suggests that familial mild hyperhomocysteinemia leading to symptomatic vascular disease has a low expression, may be age dependent and that homocysteine in mild excess may require more triggering factors.

mHH in vascular patients can be normalized by vitamin B6 and/or folic acid treatment [11,25,34–37]. In case homocysteine-lowering intervention in hyperhomocysteinemic vascular patients and their hyperhomocysteinemic family members is clinically beneficial in terms of preventing recurrent and first occurrence of arterial disease, respectively, such treatment will be of significant importance in general health care.

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References


