Abstract

Background/aims—Hyperhomocyst(e)inaemia has been identified as a strong risk factor for stroke, myocardial infarction, and deep vein thrombosis. A point mutation of methylene tetrahydrofolate reductase (MTHFR C677T) has been associated with increased plasma homocyst(e)ine levels. To investigate whether hyperhomocyst(e)inaemia and/or MTHFR C677T mutation are associated with non-arteritic ischaemic optic neuropathy (NAION), a case-control study including 59 consecutive patients with NAION and 59 controls matched for age and sex was performed.

Methods—Fasting plasma homocyst(e)ine levels, MTHFR C677T genotypes, and plasma levels of folate and vitamin B-12 were determined.

Results—Mean plasma homocyst(e)ine levels were significantly higher in patients than in controls (11.8 (SD 5.7) µmol/l vs 9.8 (2.5) µmol/l, p = 0.02). The odds ratio for patients with homocyst(e)ine levels exceeding the 95th percentile of control homocyst(e)ine levels was 5.8 (95% CI 1.5–21.4). Mean plasma folate levels were significantly lower in patients than in controls (4.3 (1.7) ng/ml vs 5.5 (1.9) ng/ml, p = 0.001), whereas plasma vitamin B-12 levels did not differ significantly. Prevalence of the MTHFR C677T mutation was not significantly increased in patients with NAION compared with controls.

Conclusion—These results suggest that hyperhomocyst(e)inaemia, but not MTHFR C677T mutation is associated with NAION. Determination of plasma homocyst(e)ine levels might be of diagnostic value in patients with NAION.

Hyperhomocyst(e)inaemia, but not MTHFR C677T mutation, as a risk factor for non-arteritic ischaemic optic neuropathy

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Hyperhomocyst(e)inaemia is a major risk factor for cardiovascular disease and venous thrombosis. Recent studies suggested that elevated levels of plasma homocyst(e)ine (tHcy) may cause endothelial dysfunction, presumably by generation of increased oxidative stress and impairment of nitric oxide (NO) metabolism. Furthermore, direct toxicity of tHcy on the endothelium has been demonstrated in animal studies. Homocyst(e)ine is a highly reactive sulphur containing amino acid deriving from the intracellular metabolism of methionine by removal of a single methyl group. It is either further metabolised by irreversible transsulphuration to cystathionine and cysteine or remethylated to form methionine again. This important remethylation pathway occurs mainly by means of 5-methyltetrahydrofolate, which is formed by a riboflavin dependent enzyme, 5,10 methylene tetrahydrofolate reductase (MTHFR), with folate as a cosubstrate.

Impairment of homocyst(e)ine remethylation is characterised by elevated levels of fasting plasma homocyst(e)ine. A common qualitative variant of the MTHFR enzyme resulting from a single amino acid substitution (alanine to valine, caused by a 677 C→T nucleotide exchange) was identified in 1988. This genetic defect is defined by its in vitro heat sensitivity and causes an enzyme activity that is approximately 50% of the normal mean.

Prevalence of homozygosity for the MTHFR C677T mutation ranges between 5–15% of subjects of white descent and may be associated with moderately elevated plasma homocyst(e)ine levels. However, reports on an association between the C677T MTHFR variant and vascular disease have been conflicting.

Non-arteritic ischaemic optic neuropathy (NAION) is an infarction of the optic nerve head caused by an insufficient blood supply to the posterior ciliary arteries. It is a common vision threatening disease, primarily affecting patients older than 55 years. Several risk factors for NAION have been identified, including arterial hypertension, diabetes mellitus, hypercholesterolaemia and hyperfibrinogenae mia. However, not all cases can be fully explained by these factors alone and the precise pathomechanism of ischaemic optic neuropathy is still unknown.

Recently, hyperhomocyst(e)inaemia was suggested to be a novel risk factor for NAION. However, the number of patients enrolled in that study was small and MTHFR C677T genotypes were not determined. Salomon et al, who determined MTHFR C677T genotypes, did not find an association with NAION, but did not investigate the role of hyperhomocyst(e)inaemia in those patients.

The aim of our study was therefore to determine the association of both hyperhomocyst(e)inaemia and the MTHFR C677T mutation in a larger cohort of patients with NAION.
We studied 59 consecutive patients with NAION who met the inclusion criteria, and 59 controls matched for age and sex. All participants were seen at our department between January 1996 and April 2000 and gave written informed consent before enrolment. The study was approved by the ethics committee of the Karl-Franzens University, Graz.

Criteria for diagnosis of NAION included sudden visual loss, optic disc oedema followed by optic atrophy, relative afferent pupillary defect and visual field defects consistent with ischaemic optic neuropathy. Exclusion criteria for all subjects were an erythrocyte sedimentation rate above 40 mm in the first hour, a history of jaw claudication, headache of recent onset, scalp tenderness, fever, myalgias, anorexia, and weight loss. Subjects with liver or kidney dysfunction, malignancy, intake of vitamins, and other medication known to influence homocyst(e)ine plasma concentrations such as oestrogens, carbamazepine, phenytoin, antifolates, and tricyclic antidepressants were also excluded.

According to the exclusion criteria another 24 patients diagnosed with NAION were not enrolled in this study (13 for taking one or more of the above cited medications, five for malignancy, and six for impaired renal function). The control group consisted of 59 age and sex matched consecutive patients, who were referred to our department for other reasons than NAION and retinal vascular diseases.

Blood samples were drawn from the antecubital vein between 7 am and 8 am after an overnight fast of at least 8 hours. Samples for homocyst(e)ine determination were processed immediately, centrifuged at 4°C (3000 g for 10 minutes), and stored at −70°C until analysis. Measurements of plasma homocyst(e)ine in EDTA plasma were performed using high performance liquid chromatography (HPLC) and fluorescence detection according to the method of Araki and Sako25 with modifications by Ubink et al26 and Vester and Rasmussen.27 Because this procedure involved a reducing step, the method did not distinguish between homocysteine and its oxidised analogues. Therefore the measured moiety is referred to as homocyst(e)ine.

Vitamine B-12 and folate were determined with an Abbott Axsym analyser using a micro-particle enzyme immunoassay (vitamin B-12) or an “ion capture” technology (folate).

Genomic DNA was extracted from peripheral blood lymphocytes by standard techniques and the MTHFR mutation analysis was performed by PCR-RFLP according to Frosten et al,28 by a technician unaware of the status of the DNA sample.

### STATISTICAL ANALYSIS

Descriptive statistics were used to calculate frequencies and percentage of discrete variables. Continuous data are given as mean (SD). We performed the Kolmogorov-Smirnov test to assess normal distribution and Levene’s test for homogeneity of variances. Means were compared using independent samples t test, while proportions were compared using chi^2 test statistic or when appropriate Fisher’s exact test. Odds ratios and 95% confidence intervals were calculated as a measure of the association between ischaemic anterior optic neuropathy and homocyst(e)ine levels. Data were analysed using a cut-off level of 95th percentile of homocyst(e)ine level among control values. Allele frequencies were calculated by the gene counting method. All p values are two tailed and all confidence intervals (CIs) were calculated at the 95% level. A p value <0.05 was considered to be significant.

Statistical analysis was performed with the spss statistical package (SPSS version 9.0, Chicago, IL, USA).

### Results

The study group consisted of 59 patients (28 females and 31 males). The mean age of patients was 69.1 (SD 8.2) years (range 53–88 years) and 69.7 (9.1) years (range 52–88 years) in controls, respectively. Baseline parameters and clinical characteristics of both groups are shown in Table 1. In the NAION group arterial hypertension and diabetes mellitus were significantly more frequent than in the control group (p = 0.025).

Mean plasma homocyst(e)ine levels were significantly higher in patients than in controls (Table 2). Hyperhomocyst(e)inaemia defined by the 95th percentile of the homocyst(e)ine levels in the control group was determined as 14.01 µmol/l.

Fourteen patients (23.7%) were therefore classified as hyperhomocyst(e)inaemic compared with three (5%) controls. The odds ratio for these patients was 5.8 (95% CI 1.5–21.4). Five of 13 (38.4%) patients with both eyes affected were hyperhomocyst(e)inaemic compared with none of 46 (19.6%) patients with unilateral involvement (p = 0.27).

### Table 1 Baseline characteristics of patients with NAION and controls

<table>
<thead>
<tr>
<th>NAION (n=59)</th>
<th>Controls (n=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>59</td>
</tr>
<tr>
<td>Female</td>
<td>28 (47.5)</td>
</tr>
<tr>
<td>Male</td>
<td>31 (52.5)</td>
</tr>
<tr>
<td>Mean age (years (SD))</td>
<td>69.1 (8.2)</td>
</tr>
<tr>
<td>Range (years)</td>
<td>53–88</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>18 (30.5)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>18 (30.5)</td>
</tr>
<tr>
<td>Stroke</td>
<td>4 (6.8)</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>9 (15.3)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>17 (28.8)</td>
</tr>
<tr>
<td>Current</td>
<td>7 (11.9)</td>
</tr>
<tr>
<td>Never</td>
<td>35 (59.3)</td>
</tr>
</tbody>
</table>

Numbers are given as n (%); *p<0.05.

### Table 2 Mean plasma levels of homocyst(e)ine, folate, and vitamin B-12 in patients and controls

<table>
<thead>
<tr>
<th>NAION (µmol/l)</th>
<th>Controls (µmol/l)</th>
<th>Significance value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocyst(e)ine</td>
<td>11.8 ± 5.7</td>
<td>9.8 ± 2.5</td>
</tr>
<tr>
<td>Plasma folate</td>
<td>4.3 ± 1.7</td>
<td>5.5 ± 1.9</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td>425.4 ± 207</td>
<td>458.5 ± 277</td>
</tr>
</tbody>
</table>

Results are given as mean (SD).
Hyperhomocyst(e)inaemia, but not MTHFR C677T mutation, as a risk factor for NAION

Numbers are given as n (%); ns = not significant.

Strong determinants of plasma homocyst(e)ine concentrations.29

Mean plasma folate levels were significantly lower in patients than in controls, whereas mean plasma vitamin B-12 levels did not differ significantly between patients and controls (Table 2).

Distribution of the MTHFR C677T mutation is shown in Table 3. The prevalence of the homozygous variant of thermolabile MTHFR (TT genotype) did not differ significantly between the two groups, and MTHFR genotypes were not significantly associated with different plasma homocyst(e)ine levels among patients or controls.

Discussion

The main finding of our study is that plasma homocyst(e)ine levels are significantly higher in patients with NAION than in controls. The prevalence of homozygosity for the MTHFR C677T mutation, however, does not significantly differ between the two groups.

Hyperhomocyst(e)inaemia is an independent risk factor for cardiovascular diseases comparable with hypercholesterolaemia and smoking.2–7 Plasma homocyst(e)ine levels may be influenced by various factors including nutritional deficiencies, diseases like renal insufficiency and malignancies, medications, and constitutional causes.28 Above all, folate and vitamin B-12 deficiencies are known to be associated with higher homocyst(e)ine levels.28,29 Furthermore, homozygosity (TT genotype) for the MTHFR C677T mutation was reported to be associated with higher homocyst(e)ine plasma levels, especially in the presence of suboptimal folate intake.27 28 30 31 Nevertheless, the distribution of the TT genotype of the MTHFR mutation in our cohort did not significantly differ between the two groups. Moreover, plasma homocyst(e)ine levels in patients with the TT genotype were not significantly higher than in patients with other genotypes. We therefore assume that homozygosity for the MTHFR C677T mutation does not have a role in NAION, which confirms the findings of previous studies.24 32

In our study patients suffering from NAION had significantly higher tHcy levels than controls. When calculating the 95th percentile of control homocyst(e)ine levels, 14 patients (23.7%) exceeded this level. These patients had an approximately fivefold higher risk of suffering from NAION. Results from previous large scale studies suggest that the overall risk increase for vascular disease associated with homocyst(e)ine is dose dependent without threshold level.32 A meta-analysis, including 27 studies relating homocyst(e)ine to atherosclerotic vascular disease, the odds ratio was estimated to increase 1.6 for every 5 µmol/l of elevated plasma homocyst(e)ine. Moreover, a recent study in 12 patients suggested an association between increased plasma homocyst(e)ine levels and NAION in patients under 50 years of age.33 Plasma folate levels are known to be a strong determinant of homocyst(e)ine levels and were significantly lower in our patients compared with controls. Therefore, the increased plasma homocyst(e)ine levels may reflect the low plasma folate levels. In contrast, plasma vitamin B-12 levels did not differ significantly between the two groups.

Hyperhomocyst(e)inaemia was shown to cause endothelial dysfunction by impairing synthesis and bioavailability of nitric oxide (NO).34 Because a balance between vasodilatation, partly mediated by NO and vasoconstriction, has been suggested to have an important role in the physiological blood flow of the optic nerve head, endothelial dysfunction caused by hyperhomocyst(e)inaemia could lead to insufficient perfusion and ischaemia of the optic nerve head.

Treatment of hyperhomocyst(e)inaemia is simple, safe, and effective through vitamin supplementation. The administration of 250 µg/day of folic acid has been shown to reduce plasma homocyst(e)ine levels by approximately 25%.35 Moreover, recent studies have shown a beneficial effect of folic acid supplementation on plasma homocyst(e)ine induced endothelial dysfunction in both healthy probands and in patients with vascular disease.36 37 Therapies designed to lower plasma homocyst(e)ine levels may therefore directly attenuate arterial endothelial injury and dysfunction.

The following limitations of our study have to be considered: firstly, the present study shares the general limitations of retrospective studies. Secondly, a methionine loading test, which is a provocation test and detects up to 27% of otherwise undiagnosed patients with hyperhomocyst(e)inaemia, was not performed.38 It may be assumed that applying the methionine loading test in our patients would have resulted in an even stronger association between NAION and hyperhomocyst(e)inaemia.

In conclusion, our study demonstrates that mild hyperhomocyst(e)inaemia, but not the MTHFR C677T mutation, is a risk factor for non-arteritic ischaemic optic neuropathy.

Clearly, large scale prospective studies are needed to further determine the role of homocyst(e)ine in NAION and to investigate the potential benefit of homocyst(e)ine lowering treatment on the development of NAION.

Table 3  Prevalence of MTHFR genotypes in patients with NAION and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>NAION (n=59)</th>
<th>Controls (n=59)</th>
<th>Significance p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>31 (52.5)</td>
<td>28 (47.5)</td>
<td>ns</td>
</tr>
<tr>
<td>CT</td>
<td>21 (35.6)</td>
<td>23 (38.9)</td>
<td>ns</td>
</tr>
<tr>
<td>TT</td>
<td>7 (11.9)</td>
<td>8 (13.6)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Numbers are given as n (%); ns = not significant.

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37 Brattström L. Vitamins as homocysteine-lowering agents. J Nutr 1996;126:1276S–80S.


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