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

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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) $\mu\text{mol/l}$ v 9.8 (2.5) $\mu\text{mol/l}$, $p = 0.02$). 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 v 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.

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Hyperhomocyst(e)inaemia is a major risk factor for cardiovascular disease and venous thrombosis.^{1–5} 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.^{6–9} Furthermore, direct toxicity of tHcy on the endothelium has been demonstrated in animal studies.^{10 11}

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.¹² 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.¹³ 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 1988.¹⁴ This genetic defect is defined by its in vitro heat sensitivity and causes an enzyme activity that is approximately 50% of the normal mean.¹⁵

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.^{16 17} However, reports on an association between the C677T MTHFR variant and vascular disease have been conflicting.^{18 19}

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.²⁰ Several risk factors for NAION have been identified, including arterial hypertension, diabetes mellitus, hypercholesterolaemia and hyperfibrinogenemia.^{21 22} 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.²³ 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.²⁴

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.

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Material and methods

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 Ubbink *et al*²⁶ 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.

Vitamins 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 Frosst *et al*,¹⁶ 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 χ^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. 1998, 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 $\mu\text{mol/l}$.

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 nine of 46 (19.6%) patients with unilateral involvement (*p* = 0.27).

Table 1 Baseline characteristics of patients with NAION and controls

	NAION (<i>n</i> =59)	Controls (<i>n</i> =59)
Number	59	59
Female	28 (47.5)	28 (47.5)
Male	31 (52.5)	31 (52.5)
Mean age (years (SD))	69.1 (8.2)	69.7 (9.1)
Range (years)	53–88	52–88
Risk factors		
Arterial hypertension	30 (50.8)*	18 (30.5)
Diabetes mellitus	18 (30.5)*	8 (13.5)
Stroke	4 (6.8)	1 (1.7)
Coronary heart disease	9 (15.3)	6 (10.2)
Smoking status		
Past	17 (28.8)	15 (25.4)
Current	7 (11.9)	7 (11.9)
Never	35 (59.3)	37 (62.7)

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

	NAION (<i>n</i> =59)	Controls (<i>n</i> =59)	Significance <i>p</i> value
Homocyst(e)ine ($\mu\text{mol/l}$)	11.8 $\pm$ 5.7	9.8 $\pm$ 2.5	0.020
Plasma folate (ng/ml)	4.3 $\pm$ 1.7	5.5 $\pm$ 1.9	0.001
Vitamin B-12 (pg/ml)	425.4 $\pm$ 207	458.5 $\pm$ 277	0.468

Results are given as mean (SD).

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

Genotype	NAION (n=59)	Controls (n=59)	Significance p value
CC	31 (52.5)	28 (47.5)	ns
CT	21 (35.6)	23 (38.9)	ns
TT	7 (11.9)	8 (13.6)	ns

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

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.^{2,3} 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 strong determinants of plasma homocyst(e)ine concentrations.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.^{17,18,30,31}

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.³³ In 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.²

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.³⁴

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).³⁵ 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,³⁶ 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%.³⁷ 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.^{38,39} 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.⁴⁰ 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.

1 Clarke R, Daly L, Robinson K, *et al.* Hyperhomocysteinaemia: an independent risk factor for vascular disease. *N Engl J Med* 1991;324:1149–55.

2 Boushey CJ, Beresford SA, Omenn GS, *et al.* A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intake. *JAMA* 1995;274:1049–57.

3 Graham IM, Daly LE, Refsum HM, *et al.* Plasma homocysteine as a risk factor for vascular disease: the European Concerted Action Project. *JAMA* 1997;277:1775–81.

- 4 Den Heijer M, Koster T, Blom HJ, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med* 1996;334:759–62.
- 5 Ray JG. Meta-analysis of hyperhomocysteinemia as a risk factor for venous thromboembolic disease. *Arch Intern Med* 1998;158:2101–16.
- 6 Chambers JC, McGregor A, Jean-Marie J, et al. Acute hyperhomocysteinemia and endothelial dysfunction. *Lancet* 1998;351:36–7.
- 7 Tawakol A, Omland T, Gerhard M, et al. Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilatation in humans. *Circulation* 1997;95:1119–21.
- 8 Loscalzo J. The oxidant stress of hyperhomocyst(e)inemia. *J Clin Invest* 1996;98:5–7.
- 9 Upchurch GR, Welch GN, Fabian AJ, et al. Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. *J Biol Chem* 1997;272:17012–7.
- 10 Harker LA, Harlan JM, Ross R. Effect of sulfinpyrazone on homocysteine-induced endothelial injury and arteriosclerosis in baboons. *Circ Res* 1983;53:731–9.
- 11 Lentz SR, Sobey CG, Piegors DJ, et al. Vascular dysfunction in monkeys with diet-induced hyperhomocyst(e)inemia. *J Clin Invest* 1996;98:24–9.
- 12 Refsum H, Ueland PM, Nygard O, et al. Homocysteine and cardiovascular disease. *Annu Rev Med* 1998;49:31–62.
- 13 Miller JW, Nadeau MR, Smith D, et al. Vitamin B-6 deficiency vs folate deficiency: comparison of responses to methionine loading in rats. *Am J Clin Nutr* 1994;59:1033–9.
- 14 Kang SS, Zhou J, Wong PW, et al. Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. *Am J Hum Genet* 1988;43:414–21.
- 15 Engbersen AM, Franken DG, Boers GH, et al. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. *Am J Hum Genet* 1995;56:142–50.
- 16 Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;10:111–3.
- 17 Gemmati D, Prevati M, Serino ML, et al. Low folate levels and thermolabile methylenetetrahydrofolate reductase as a primary determinant of mild hyperhomocysteinemia in normal and thrombotic subjects. *Arterioscler Thromb Vasc Biol* 1999;19:1761–7.
- 18 Verhoef P, Kok FJ, Kluijtmans LA, et al. The 677C→T mutation in the methylenetetrahydrofolate reductase gene: associations with plasma total homocysteine and risk of coronary atherosclerotic disease. *Atherosclerosis* 1997;132:105–13.
- 19 Anderson JL, King GJ, Thomson MJ, et al. A mutation in the methylenetetrahydrofolate reductase gene is not associated with increased risk for coronary artery disease or myocardial infarction. *J Am Coll Cardiol* 1997;30:1206–11.
- 20 Repka MX, Savino PJ, Schatz NJ, et al. Clinical profile and long-term implications of anterior ischemic optic neuropathy. *Am J Ophthalmol* 1983;96:478–83.
- 21 Hayreh SS, Joos KM, Podhajsky PA, et al. Systemic diseases associated with nonarteritic anterior ischemic optic neuropathy. *Am J Ophthalmol* 1994;118:766–80.
- 22 Talks SJ, Chong NH, Gibson JM, et al. Fibrinogen, cholesterol and smoking as risk factors for non-arteritic ischaemic optic neuropathy. *Eye* 1995;9:85–8.
- 23 Pianka P, Almog Y, Man O, et al. Hyperhomocysteinemia in patients with nonarteritic anterior ischemic optic neuropathy, central retinal artery occlusion, and central retinal vein occlusion. *Ophthalmology* 2000;107:1588–92.
- 24 Salomon O, Huna-Baron R, Kurtz S, et al. Analysis of prothrombotic and vascular risk factors in patients with nonarteritic anterior ischemic optic nerve neuropathy. *Ophthalmology* 1999;106:739–42.
- 25 Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 1987;422:43–52.
- 26 Ubbink JB, Hayward-Vermaak WJ, Bissbort S. Rapid high-performance liquid chromatography assay for total homocysteine levels in human serum. *J Chromatogr* 1991;565:441–6.
- 27 Vester B, Rasmussen K. High-performance liquid chromatography for rapid and accurate determination of homocysteine in plasma and serum. *Eur J Clin Chem Clin Biochem* 1991;29:549–54.
- 28 Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. *Lancet* 1999;354:407–13.
- 29 Selhub J, Jacques PF, Wilson PW, et al. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993;270:2693–8.
- 30 Harmon DL, Woodside JV, Yarnell JW, et al. The common thermolabile variant of methylenetetrahydrofolate reductase is a major determinant of mild hyperhomocysteinemia. *Q J Med* 1996;89:571–7.
- 31 Malinow MR, Nieto FJ, Kruger WD, et al. The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. *Arterioscler Thromb Vasc Biol* 1997;17:1157–62.
- 32 Biousse V, Kerrison JB, Newman NJ. Is non-arteritic anterior ischaemic optic neuropathy related to homocysteine? *Br J Ophthalmol* 2000;84:555.
- 33 Ma J, Stampfer MJ, Hennekens CH, et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine and risk of myocardial infarction in US physicians. *Circulation* 1996;94:2410–6.
- 34 Kawasaki A, Purvin VA, Burgett RA. Hyperhomocysteinemia in young patients with non-arteritic anterior ischaemic optic neuropathy. *Br J Ophthalmol* 1999;83:1287–90.
- 35 Stamler JS, Osborne JA, Jaraki O, et al. Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest* 1993;91:308–18.
- 36 Orgul S, Gugleta K, Flammer J. Physiology of perfusion as it relates to the optic nerve head. *Surv Ophthalmol* 1999;43(Suppl 1):S17–26.
- 37 Brattström L. Vitamins as homocysteine-lowering agents. *J Nutr* 1996;126:1276S–80S.
- 38 Title LM, Cummings PM, Giddens K, et al. Effect of folic acid and antioxidant vitamins on endothelial dysfunction in patients with coronary artery disease. *J Am Coll Cardiol* 2000;36:758–65.
- 39 Woo KS, Chook P, Lolin YI, et al. Folic acid improves arterial endothelial dysfunction in adults with hyperhomocysteinemia. *J Am Coll Cardiol* 1999;34:2002–6.
- 40 Bostom AG, Jacques PF, Nadeau MR, et al. Post methionine load hyperhomocysteinemia in persons with normal fasting total plasma homocysteine: initial results from the NHLBI Family Heart Study. *Atherosclerosis* 1995;116:147–51.