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ORIGINAL COMMUNICATION

Plasma homocysteine, MTHFR C677T, CBS 844ins68bp, and MTHFD1 G1958A polymorphisms in spontaneous cervical artery dissections

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Abstract Mild hyperhomocysteinemia is a probable risk factor for atherosclerotic diseases and stroke. Recently, associations of elevated plasma homocysteine concentrations in the acute phase and of MTHFR 677 TT genotype with spontaneous cervical artery dissections (sCAD) have been reported. The purpose of this study was to test this hypothesis in the currently largest sample of patients with sCAD, taking into account known factors influencing plasma homocysteine levels. Ninety-five patients with past sCAD were compared with 95 age- and sex-matched healthy individuals. Homocysteine, vitamin B6, B12, folate, and polymorphisms of methylenetetrahydrofolate reductase (MTHFR C677T), cystathionine β-synthase (CBS 844ins68bp) and methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase (MTHFD1 G1958A) were assessed and any associations were analysed using multivariate statistics. The occurrence of sCAD was associated with elevated homocysteine levels with an odds ratio of 1.327 per 20 % percentile. Homocysteine levels were influenced by gender, smoking status, occurrence of hypertension, vitamin B12 and folate levels, and by the MTHFR TT genotype. MTHFR, CBS 844ins68bp, and MTHFD1 G1958A genotype were not independently associated with the occurrence of sCAD. These data suggest that elevated homocysteine is associated with the occurrence of sCAD. The MTHFR C677T polymorphism is associated with the homocysteine level.

Key words cervical artery dissection · stroke · homocysteine · methylenetetrahydrofolate reductase · cystathionine beta-synthase

Introduction

Spontaneous cervical artery dissection (sCAD) is a rare disease with an estimated annual incidence of about 2.6 per 100000 [30]. Nevertheless it accounts for 13–15.5 % of strokes in adults under 45 years [5] and 30–40 % of brainstem and cerebellar infarctions in this population [23]. The pathophysiological basis of sCAD remains poorly understood. Connective tissue disease, preceding

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infections and minor trauma are the major recognized risk factors [5, 12].

Recently the suspicion that mild hyperhomocysteinemia might possibly be a risk factor for sCAD has been raised by association studies with rather small sample sizes, including 25 and 26 patients respectively [15, 28]. These observations are interesting because mild elevation of total plasma homocysteine has frequently been associated with cardiovascular and cerebrovascular diseases, although not all cohort studies confirm this association: while the British Regional Heart Study, the Rotterdam Study, and the Framingham Study found a positive association between hyperhomocysteinemia and vascular risk, this was not corroborated by the US Physicians study, the Caerphilly Study, or a Finnish cohort study [14, 17, 26]. Pathophysiologically, endothelial cell dysfunction, vascular smooth muscle cell growth, and coagulation abnormalities could be the basis of an association with atherosclerotic stroke [8].

A number of enzymes underlying genetic regulation control human homocysteine metabolism. Genetic variations could therefore influence plasma levels of homocysteine and vascular risk [16, 19]. The best known factor contributing to hyperhomocysteinemia is a C to T substitution at nucleotide 677 in the gene for methylenetetrahydrofolate reductase (MTHFR C677T) leading to a thermolabile variant of the enzyme. The homozygous MTHFR-TT form has a reduced specific activity of about 50 % of the wild type. Associations between homozygous MTHFR-TT and vascular disease including stroke have been repeatedly reported [22]. Other genetic polymorphisms influencing homocysteine metabolism are located in the cystathionine β-synthase gene (CBS 844ins68bp) [31] and the trifunctional enzyme methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase (MTHFD1 G1958A) [7].

The genetic background of mild hyperhomocysteinemia in previous studies on sCAD was not consistent [28]: In one cohort, the MTHFR-TT genotype was significantly more frequent in patients with sCAD [15], while in the other cohort no significant association between this genotype and sCAD could be detected [28]. The CBS 844ins68bp mutation was only analysed in one cohort and was not associated with sCAD [15].

In the present study we wanted to test the suspicion raised by previous association studies that hyperhomocysteinemia and/or related polymorphisms are associated with sCAD. We recruited the largest number of patients for an association study in sCAD to date and prospectively selected age- and sex-matched controls with comparable occupational background and identical regional provenance.

Material and methods

Study population

One hundred and thirty-eight patients with a diagnosis of sCAD who were treated in our Department between 1992 and 2002 were contacted for informed consent and subsequent collection of blood samples. The diagnosis of sCAD was based on clinical signs, that is either a local compressive syndrome or cerebral ischemia, and confirmed by at least one neuroradiological investigation (MRI with transversal sections through the neck or/and arterial digital subtraction angiography). Ninety-five patients gave written informed consent and provided blood samples, 12 refused participation, 3 had died, and 28 were not contactable owing to a change of address. At the same time, ninety-five sex- and age-matched control subjects were drawn from the ongoing Prospective Cardiovascular Munster Study (PROCAM), a population-based study on cardiovascular risk factors [2, 4].

Biochemical analysis

For biochemical and genetic analysis, venous blood samples were taken in the early morning after 12 hours overnight fasting. All patients and controls had been lying for more than 10 minutes. Plasma samples for homocysteine measurements were drawn in special tubes with sodium fluoride (Sarstedt, Germany) and set on ice immediately after collection. Centrifugation of these samples was done at 4 °C. For genetic parameters and vitamin B6 we used EDTA blood, the other measurements were carried out in serum. Tubes for vitamin B12 and folate were protected from light until measurement. The median time between occurrence of sCAD and blood test was 833 days, that is 27.3 months (1st quartile 120 days, 3rd quartile 1547 days). Fifteen patients had their blood tests within the 1st month after sCAD, an additional 6 patients between the 1st and 3rd months. Time was considered as a confounding factor in multivariate statistics (see below). Homocysteine fasting levels were measured by an Abbott AxSym fluorescence polarization immunoassay (FPIA) (Abbott, Germany). For our laboratory, the normal homocysteine values are between 4.6 to 8.1 µmol/l for persons aged up to 30 years, 4.5 to 7.9 µmol/l for women and 6.3 to 11.2 µmol/l for men from 31 to 60 years, and 5.8 to 11.9 µmol/l for persons older than 60 years. Vitamin B6 was determined using a commercial kit from Chromsystems (Germany) with isocratic HPLC system with fluorescence detection. Normal values for vitamin B6 are 3.6 to 18.0 ng/ml. Vitamin B12 was measured by an electrochemiluminescence immunoassay (ECLIA) method with the Elecsys-system from Roche Diagnostics (Germany). For our laboratory, normal values for vitamin B12 range from 243 to 894 pg/ml. Folate was measured by a competitive in vitro binding assay on the Elecsys 2010-system from Roche Diagnostics (Germany). Normal values for folate are 4.2–19.9 ng/ml.

Genetic analysis

DNA was extracted from EDTA-anticoagulated blood samples with magnetic beads using the TECAN DNA sample preparation system and frozen until analysis at –20°C. The MTHFR C677T mutation was analysed with the Light Cycler (Roche Diagnostics, Germany) using the following primers and probes from TIB MOLBIOL (Berlin, Germany): MTHFR sense AGGCCAGCCTCTCCTGACTG; MTHFR antisense AGGACGGTGCGGTGAGAGTG; MTHFR probe CGGGAGCCGATTTCATCA-X; reporter LC Red705-CGCAGCTTTTCTTTGAGGCTGACA-p. The 844ins68bp CBS polymorphism was PCR amplified with the oligonucleotide primers CBSinsF 5'-GTTGTTAACGGCGGTATTGG-3' and 5'-GTCTGCTCCGTCTGGTTCAG-3' using standard PCR techniques at an annealing temperature of 62 °C. PCR products were separated on 1.8 % agarose gels to detect the 68 bp insertion. The MTHFD1 MTHFD1 G1958A polymorphism was PCR

amplified with the oligonucleotide primers MTHFD1-R653QF 5'-CCCACTTTGAAGCAGGATTG-3' and MTHFD1-R653QR 5'-CATCCCAATTCCCCTGATG-3' using standard PCR techniques at an annealing temperature of 52°C. The PCR product of 232 bp was digested with the restriction endonuclease *MSP*1 cutting the wild type (G) allele into two fragments of 125 bp and 107 bp. Restriction digests were analysed on 1.8 % agarose gels.

Statistic analysis

- Baseline demographic characteristics of the study population were tested using chi square test (Fisher's Exact test for $n < 5$) or Wilcoxon signed rank test.
- One-way ANOVA models were used to examine the influences of gender, age, smoking, hypertension, hypercholesterolemia, diabetes mellitus, serum folate, vitamin B6 and B12 serum levels (the latter categorized into quintiles) on log-10-transformed homocysteine levels. The effect of time between dissection and blood withdrawal (in quintiles) on log-10-transformed homocysteine was analysed in patients. The influence of MTHFR C677T, 844ins68bp CBS, and MTHFD1 G1958A polymorphisms on log10-transformed homocysteine levels was examined in the control group.
- Logistic regression was performed with case status (dissection) as the outcome variable and homocysteine levels categorized in quintiles, smoking (yes-no), hypertension (yes-no), diabetes (yes-no), and gender as independent variables. Homocysteine levels were significantly correlated with vitamin B12 (Spearman correlation coefficient –0.28, $p < 0.05$) and folate (–0.32, $p < 0.05$). Intercorrelations were observed between vitamins: Vitamin B12 was correlated with vitamin B6 (0.23, $p < 0.05$) and folate (0.16, $p < 0.05$), and vitamin B6 correlated with folate (0.25, $p < 0.05$). Because of the strong collinearities between vitamins and homocysteine levels we excluded vitamin B12, B6 and folate from the regression analysis.
- After exclusion of collinearities between MTHFR C677T, 844ins68bp CBS, and MTHFD1 G1958A polymorphisms, one-way ANOVA models with dissection as major outcome parameter and polymorphisms as independent variables were tested.
- All statistics were computed using the SPSS for Windows software, release 10.0.

Results

Clinical data

The cohort consisted of 37 female and 58 male patients, with a mean age of 42.6 ± 10.2 years (Table 1). Sixty-three patients could not remember any precipitating event prior to sCAD. Fifteen patients remembered having cervical chiropractic manipulation to alleviate neck pain prior to the diagnosis of sCAD. Seventeen patients reported minor trauma prior to the symptoms of dissection, including working with hyperextension of the neck, rapid neck movements etc. Thirty-eight patients and 28 controls were current smokers or had quit smoking within the last six months (chi square $p = 0.078$). Twenty-eight patients and 15 controls had hypertension with systolic blood pressure > 160 mmHg and diastolic blood pressure > 95 mmHg in two separate measurements (chi square $p = 0.016$). Two patients and 3 controls had diabetes according to the World Health Organization criteria (chi square $p = 0.500$).

Table 1 Baseline characteristics

	Patients with sCAD	Controls
Number	95	95
Gender (female: male)	37:58	37:58
Age in years	42.6 ± 10.2	44.5 ± 8.5
Smoker	38	28
Diabetes mellitus	2	3
Hypertension	28	15
Minor trauma before sCAD	16	not applicable
Chiropractic manipulation before sCAD	14	not applicable
sCAD of carotid: vertebral: basilary artery	71:45:1	not applicable
sCAD in 1:2:3:4 arteries	74:16:1:2	not applicable
Completed strokes	68	not applicable

Baseline analysis of biochemical and genetic factors

Fasting levels of plasma homocysteine were significantly higher in patients than in the age and sex-matched control population (median 11.4 μmol/l and 9.9 μmol/l respectively, $p < 0.05$) (see Table 2). 43.2 % of patients had pathological plasma homocysteine levels while 73.4 % of control subjects had normal values ($\chi^2 = 5.12$, $p = 0.018$). Post hoc stratification showed no difference between sCAD patients with stroke and without stroke (median 11.4 and 11.5 μmol/l respectively). Vitamin B6, vitamin B12, and folate levels of patients and matched controls did not differ significantly. There was also no difference between sCAD patients with and without stroke. Analysis of polymorphisms did not show differences of allele distribution between sCAD patients and matched controls (Table 3).

Factors possibly influencing homocysteine levels

Male patients, smoker, subjects with hypertension, subjects with low vitamin B12 or folate levels had higher mean homocysteine levels. ANOVA showed that the effects of gender, smoking status, occurrence of hypertension, vitamin B12 levels and folate levels on log-10-transformed homocysteine levels were significant ($p < 0.001$, $p = 0.004$, $p = 0.041$, $p = 0.014$, and $p = 0.011$ respectively), while the presence of diabetes mellitus or hypercholesterolemia, age, and vitamin B6 levels had no significant effect ($p = 0.214$, $p = 0.533$, $p = 0.630$, $p = 0.467$ respectively). The time interval between sCAD and blood withdrawal had no significant effect on homocysteine levels (ANOVA $p = 0.889$) and there was no correlation either (Spearman $p = 0.71$). A comparison of sCAD patients who had blood withdrawal within the first month versus later showed no differences in homocysteine, vitamin B6, vitamin B12, and folate serum lev-

Table 2 Fasting homocysteine, vitamin B6, vitamin B12, and folate levels

	Patients: median, mean, and standard deviation	Controls: median, mean, and standard deviation	Wilcoxon-test
Homocysteine in µmol/l	11.4 12.2 ± 4.0	9.9 10.7 ± 3.7	p = 0.005*
Vitamin B 6 in ng/ml	10.5 15.1 ± 14.3	12.8 16.4 ± 11.8	p = 0.386
Vitamin B12 in pg/ml	418 459 ± 223	448 477 ± 165	p = 0.124
Folate in ng/ml	7.70 8.52 ± 3.49	6.65 7.31 ± 3.25	p = 0.325

* significant $p < 0.05$

Table 3 MTHFR C677T, CBS 844ins68bp, and MTHFD1 G1958A

	Mutation	Patients	Controls	Chi square test
MTHFR C677T	CC	48.3%	51.7%	$\chi^2 = 0.43$
	CT	43.8%	39.1%	p = 0.806
	TT	7.9%	9.2%	
844ins68bp CBS	No insertion	84.4%	86.7%	$\chi^2 = 0.19$
	Heterozygous insertion	14.4%	12.2.%	p = 0.908
	Homozygous insertion	1.1%	1.1%	
MTHFD1 G1958A	AA	22.5%	16.7%	$\chi^2 = 1.90$
	AG	50.6%	47.8%	p = 0.387
	GG	27%	35.6%	

els ($p = 0.884$, $p = 0.283$, $p = 0.445$, $p = 0.293$ respectively). In controls, 844ins68bp CBS and MTHFD1 G1958A polymorphisms had no significant effect on fasting homocysteine levels ($p = 0.843$ and $p = 0.591$ respectively). In contrast, the MTHFR C677T polymorphism had a significant effect on fasting homocysteine levels ($p = 0.021$). Mean homocysteine levels for CC, CT and TT genotypes were 10.5 ± 3.6 µmol/l, 10.1 ± 3.7 µmol/l, and 14.5 ± 5.3 µmol/l respectively. Post hoc analysis revealed a significant effect of the TT genotype compared with CC and CT genotypes (Scheffé $p = 0.048$ and $p = 0.022$ respectively), but no significant difference between CC and CT genotype ($p = 0.814$).

Risk associated with homocysteine levels

Binary logistic regression was performed with homocysteine as major covariate. Homocysteine was categorized into quintiles, with the cutoff for 20, 40, 60 and 80 % – percentiles at 8.20, 9.66, 11.5, and 13.26 µmol/l respectively. Regression analysis adjusted for smoking status, diabetes mellitus, hypertension, and gender revealed a significant effect of homocysteine levels on the occurrence of sCAD (Table 4). With a regression coefficient of 0.283, the crude odds ratio was 1.327 (95 % – confidence interval from 1.035 to 1.701).

Table 4 Risk for sCAD

	Regression coefficient	Odds ratio	p
Homocysteine	0.283	1.327	0.026*
Gender	0.266	1.305	0.457
Smoking	0.245	1.278	0.483
Diabetes mellitus	–0.240	0.787	0.811
Hypertension	0.721	2.056	0.082

Logistic regression analysis with case status (dissection) as outcome variable and homocysteine levels categorized in quintiles, smoking (yes-no), hypertension (yes-no, diabetes (yes-no), and gender as independent variables. * significant $p < 0.05$

A significant effect for homocysteine remained after patients reporting minor trauma or chiropractic manipulation before arterial dissection were excluded from logistic regression ($p = 0.041$). After exclusion, the regression coefficient was 0.295 and the crude odds ratio 1.343.

A significant effect for homocysteine also remained after exclusion of those patients who had their blood tested within the first month after sCAD ($p = 0.042$, odds ratio 1.322) or within the first three months after sCAD ($p = 0.023$, odds ratio 1.385).

Risk associated with CBS 844ins68bp and MTHFD1 G1958A polymorphisms

MTHFR, CBS 844ins68bp, and MTHFD1 G1958A polymorphisms had no significant effect on the occurrence of sCAD ($p = 0.809$, $p = 0.910$, and $p = 0.392$ respectively).

Discussion

In this case-control study we could show that homocysteine levels were influenced by gender, smoking status, occurrence of hypertension, vitamin B12, and folate levels, and the MTHFR C677T polymorphism, showing significantly higher values with male sex, smoking, hypertension, low vitamin B12 or folate levels, and the homozygous TT genotype. Our main finding was an association of hyperhomocysteinemia with spontaneous cervical artery dissections (sCAD) with an odds ratio of 1.327.

The results of the present study are in accordance with previous studies indicating that gender, smoking habits, blood pressure, folate and vitamin intake are associated with homocysteine levels. Male sex was associated with higher homocysteine levels in population studies from Norway and Singapore [27, 29]. Smoking has repeatedly been linked to high homocysteine levels,

e. g. in the Hoardaland Homocysteine Study [27], the Framingham Offspring Cohort [20], in middle aged Chinese from Singapore [29], and in a population-based Dutch cohort [9, 10]. Arterial hypertension [24, 27, 29] and low vitamin B12 or folate levels [9] have previously been associated with high homocysteine levels as well.

The main finding of the present study, the association of hyperhomocysteinemia with sCAD, is in line with observations suggesting that homocysteine could be an independent risk factor for vascular diseases including arteriosclerosis, coronary heart disease, and stroke. A fair number of studies have confirmed a positive association of mild hyperhomocysteinemia with stroke, although not all cohort studies support this association [14, 17, 26]. The pathophysiological basis of this association is still under debate. A strong graded association of hyperhomocysteinemia with large artery disease seems to point to a primarily atherogenic etiology [13], while other pathophysiological hypotheses emphasize the thrombogenic role of high homocysteine [3].

Pathophysiological concepts of sCAD state that either rupture of the arterial intimal layer allows penetration of blood into the arterial wall, or rupture occurs within the connective tissue of the intramedial layer including vasa vasorum [6]. The deleterious effects of homocysteine could be mediated via endothelial damage and endothelial dysfunction, leading to impaired endothelial-dependent vasoreactivity and decreased endothelium thromboresistance [8]. Further, collagen integrity might be impaired through substitution of cysteine-cysteine with cysteine-homocysteine bonds. Whether these mechanisms could trigger sCAD is unknown. So far, a role of homocysteine in sCAD has been claimed by two Italian association studies that suffer from a number of methodological limitations [15, 28]: Both studies reported on sample sizes of 25 and 26 patients, which is too small to allow for a generalization of the observations. Further, both studies relied on highly selected control populations including outpatients from a Headache Center [15] and local general practitioners [28].

Methodologically, a strength of the present study is the sample size of 95 patients with sCAD which is large in relation to the low annual incidence of about 2.6 per 100000 of this disease [30]. (Theoretically the number of sCAD investigated here corresponds to the annual incidence of sCAD in 3.65 million inhabitants.)

Another strength is the simultaneous random collection of an age- and sex-matched occupational control population from an ongoing prospective population-based study carried out in the same region in Germany [2, 4]. While previous studies of homocysteine in sCAD obtained blood samples within the first 3 or 7 days after sCAD [15, 28], the present investigation focuses on homocysteine levels beyond the acute phase. This procedure is preferable as homocysteine levels might be affected by the vascular event during the first days after stroke [18, 25]. In addition, time between sCAD and blood withdrawal was included as confounding factor in the statistic analysis as recommended by Howard et al. [18] and did not significantly interact with homocysteine levels.

Limitations to our report include the fact that, like any other association study so far, it is not feasible to determine homocysteine levels before sCAD. Therefore we cannot exclude that changes in homocysteine levels might reflect a consequence rather than a cause of sCAD.

In addition to the investigations on the role of homocysteine in sCAD, several genetic analyses were carried out to better understand the association of homocysteine-related genes with homocysteine levels and sCAD. MTHFD1 G1958A is a trifunctional nicotinamide adenine dinucleotide phosphate-dependent cytoplasmatic enzyme catalyzing the conversion of tetrahydrofolate to precursors of 5-methylene tetrahydrofolate that acts as a methyl group donor in the homocysteine to methionine metabolism. Therefore we considered MTHFD1 G1958A as a putative candidate to screen for associations with hyperhomocysteinemia and sCAD. In line with a previous study that did not find an association with pediatric stroke [1], we could not detect an association of MTHFD1 G1958A with homocysteine levels or occurrence of sCAD. If MTHFD1 does have an effect at all on homocysteine it could be so modest that we could not detect it in the present setting (type 2 error). In addition we examined the association of homocysteine and sCAD with the 844ins68bp CBS polymorphism, coding for an enzyme involved in the transsulfuration of homocysteine to cystathione. Some forms of CBS deficiency may cause severe hyperhomocysteinemia with homocysteinuria [21]. The risk of venous or arterial occlusive disease may increase with combined CBS 844ins68 and MTHFR C677T mutations [11]. However, the CBS 844ins68 mutation has rather been associated with elevated CBS enzyme activity and decreased homocysteine levels [16, 31]. Therefore it was hypothesized that CBS 844ins68 might have a protective effect against vascular thromboembolic disease [32]. Here we did not find any association of CBS 844ins68 with homocysteine levels or sCAD, which does not exclude minor effects that could not be detected it in the present setting (type 2 error). Concerning sCAD, our results are in line with a previous study [28], confirming that CBS 844ins68 does not play a role in the occurrence of sCAD.

Concerning the MTHFR C677T polymorphism, our results are in line with the current literature. Moderate influences of the MTHFR C677T polymorphism on total homocysteine levels have been observed in a number of studies [16, 19]. In a large epidemiological study, the MTHFR C677T polymorphism accounted for 6 % of the phenotypic variation in total homocysteine [19]. In another study, individuals with homozygous TT-alleles had a 2.3 μmol/l higher homocysteine level with low fo-

late [16]. The relation of MTHFR C677T polymorphism with stroke is more ambiguous. In a meta-analysis, the TT-genotype was associated with an odds ratio > 1 in 11 out of 20 studies with a pooled risk estimation of 1.23 across all studies [22]. For sCAD, evidence so far has been conflicting. With 7 TT-genotypes, 15 CT and 5 CC genotypes, Gallai et al. found no significant differences [15], while logistic regression showed a significant effect of MTHFR C677T polymorphism in the study of Pezzini and coworkers [28]. Our study confirms the influence of the MTHFR C677T polymorphism on homocysteine. However, we did not find evidence for the hypothesis that it could be an independent risk factor for sCAD.

In conclusion, the present study examining a large cohort of patients with sCAD and a carefully selected age- and sex-matched control population confirms an association of elevated homocysteine with the occurrence of sCAD.

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References

1. Akar N, Akar E, Ozel D, Deda G, Sipahi T (2001) Common mutations at the homocysteine metabolism pathway and pediatric stroke. Thromb Res 102: 115–120
2. Assmann G, Schulte H (1988) The Prospective Cardiovascular Munster (PROCAM) study: prevalence of hyperlipidemia in persons with hypertension and/or diabetes mellitus and the relationship to coronary heart disease. Am Heart J 116:1713–1724
3. Ay H, Arsava EM, Tokgozoglu SL, Ozer N, Saribas O (2003) Hyperhomocysteinemia is associated with the presence of left atrial thrombus in stroke patients with nonvalvular atrial fibrillation. Stroke 34:909–912
4. Berger K, Schulte H, Stogbauer F, Assmann G (1998) Incidence and risk factors for stroke in an occupational cohort: the PROCAM Study. Prospective Cardiovascular Muenster Study. Stroke 29:1562–1566
5. Bogousslavsky J, Pierre P (1992) Ischemic stroke in patients under age 45. Neurol Clin 10:113–124
6. Brandt T, Orberk E, Weber R, Werner I, Busse O, Muller BT, Wigger F, Grau A, Grond-Ginsbach C, Hausser I (2001) Pathogenesis of cervical artery dissections: association with connective tissue abnormalities. Neurology 57:24–30
7. Brody LC, Conley M, Cox C, Kirke PN, McKeever MP, Mills JL, Molloy AM, O'Leary VB, Parle-McDermott A, Scott JM, Swanson DA (2002) A polymorphism, R653Q, in the trifunctional enzyme methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase is a maternal genetic risk factor for neural tube defects: report of the Birth Defects Research Group. Am J Hum Genet 71:1207–1215
8. Cook JW, Taylor LM, Orloff SL, Landry GJ, Moneta GL, Porter JM (2002) Homocysteine and arterial disease. Experimental mechanisms. Vascul Pharmacol 38:293–300
9. de Bree A, Verschuren W, Bjorke-Monsen A, van der Put N, Heil S, Trijbels F, Blom H (2001) Association between B vitamin intake and plasma homocysteine concentration in the general Dutch population aged 20–65 y. Am J Clin Nutr 77:687–693
10. de Bree A, Verschuren W, Blom H, Kromhout D (2001) Lifestyle factors and plasma homocysteine concentrations in a general population sample. Am J Epidemiol 154:150–154
11. de Franchis R, Fermo I, Mazzola G, Sebastio G, Di Minno G, Coppola A, Andria G, D'Angelo A (2000) Contribution of the cystathionine beta-synthase gene (844ins68) polymorphism to the risk of early-onset venous and arterial occlusive disease and of fasting hyperhomocysteinemia. Thromb Haemost 84:576–582
12. Dziewas R, Konrad C, Drager B, Evers S, Besselmann M, Ludemann P, Kuhlenbaumer G, Stogbauer F, Ringelstein EB (2003) Cervical artery dissections – clinical features, risk factors, therapy and outcome in 126 patients. J Neurol 250:1179–1184
13. Eikelboom JW, Hankey GJ, Anand SS, Lofthouse E, Staples N, Baker RI (2000) Association between high homocyst(e)ine and ischemic stroke due to large- and small-artery disease but not other etiologic subtypes of ischemic stroke. Stroke 31:1069–1075
14. Ford ES, Smith SJ, Stroup DF, Steinberg KK, Mueller PW, Thacker SB (2002) Homocyst(e)ine and cardiovascular disease: a systematic review of the evidence with special emphasis on case-control studies and nested case-control studies. Int J Epidemiol 31:59–70
15. Gallai V, Caso V, Paciaroni M, Cardaioli G, Arning E, Bottiglieri T, Parnetti L (2001) Mild hyperhomocyst(e)inemia: a possible risk factor for cervical artery dissection. Stroke 32:714–718
16. Geisel J, Zimbelmann I, Schorr H, Knapp JP, Bodis M, Hubner U, Herrmann W (2001) Genetic defects as important factors for moderate hyperhomocysteinemia. Clin Chem Lab Med 39:698–704
17. Homocysteine Studies Collaboration (2002) Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA 288:2015–2022
18. Howard VJ, Sides EG, Newman GC, Cohen SN, Howard G, Malinow MR, Toole JF (2002) Changes in plasma homocyst(e)ine in the acute phase after stroke. Stroke 33:473–478
19. Husemoen LL, Thomsen TF, Fenger M, Jorgensen HL, Jorgensen T (2003) Contribution of thermolabile methylenetetrahydrofolate reductase variant to total plasma homocysteine levels in healthy men and women. Genet Epidemiol 24:322–330
20. Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J (2001) Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr 73:613–621
21. Kelly PJ, Furie KL, Kistler JP, Barron M, Picard EH, Mandell R, Shih VE (2003) Stroke in young patients with hyperhomocysteinemia due to cystathionine beta-synthase deficiency. Neurology 60:275–279
22. Kelly PJ, Rosand J, Kistler JP, Shih VE, Silveira S, Plomaritoglou A, Furie KL (2002) Homocysteine, MTHFR 677C → T polymorphism, and risk of ischemic stroke: results of a meta-analysis. Neurology 59:529–536
23. Kristensen B, Malm J, Carlberg B, Stegmayr B, Backman C, Fagerlund M, Olsson T (1997) Epidemiology and etiology of ischemic stroke in young adults aged 18 to 44 years in northern Sweden. Stroke 28:1702–1709
24. Lim U, Cassano P (2002) Homocysteine and blood pressure in the Third National Health and Nutrition Examination Survey, 1988–1994. Am J Epidemiol 156:1105–1113

25. Lindgren A, Brattstrom L, Norrving B, Hultberg B, Andersson A, Johansson BB (1995) Plasma homocysteine in the acute and convalescent phases after stroke. Stroke 26:795–800
26. Moller J, Nielsen GM, Tvedegaard KC, Andersen NT, Jorgensen PE (2000) A meta-analysis of cerebrovascular disease and hyperhomocysteinaemia. Scand J Clin Lab Invest 60:491–499
27. Nygard O, Refsum H, Ueland PM, Vollset SE (1998) Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. Am J Clin Nutr 67:263–270
28. Pezzini A, Del Zotto E, Archetti S, Negrini R, Bani P, Albertini A, Grassi M, Assanelli D, Gasparotti R, Vignolo LA, Magoni M, Padovani A (2002) Plasma homocysteine concentration, C677T MTHFR genotype, and 844ins68bp CBS genotype in young adults with spontaneous cervical artery dissection and atherothrombotic stroke. Stroke 33:664–669
29. Saw SM, Yuan JM, Ong CN, Arakawa K, Lee HP, Coetzee GA, Yu MC (2001) Genetic, dietary, and other lifestyle determinants of plasma homocysteine concentrations in middle-aged and older Chinese men and women in Singapore. Am J Clin Nutr 73:232–239
30. Schievink WI, Mokri B, Whisnant JP (1993) Internal carotid artery dissection in a community. Rochester, Minnesota, 1987–1992. Stroke 24: 1678–1680
31. Tsai MY, Bignell M, Yang F, Welge BG, Graham KJ, Hanson NQ (2000) Polygenic influence on plasma homocysteine: association of two prevalent mutations, the 844ins68 of cystathionine beta-synthase and A(2756)G of methionine synthase, with lowered plasma homocysteine levels. Atherosclerosis 149:131–137
32. Zhang G, Dai C (2001) Gene polymorphisms of homocysteine metabolism-related enzymes in Chinese patients with occlusive coronary artery or cerebral vascular diseases. Thromb Res 104:187–195