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# Plasma homocysteine levels & 677C→T methylenetetrahydrofolate reductase gene polymorphism in patients with coronary artery disease of different severity

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***Background & objectives*: Numerous studies have identified hyperhomocysteinemia as an independent risk factor for coronary artery disease (CAD). Furthermore, influences of polymorphysim of methylenetetrahydrofolate reductase (MTHFR) on homocysteine levels are documented. However, the relationship between severity of CAD and polymorphism of *MTHFR* has not been systematically evaluated. The present study was undertaken to evaluate this relationship in patients undergoing coronary artery bypass surgery.**

***Methods*: Serum homocysteine and *MTHFR* polymorphism in relation to severity of CAD was examined in 113 male patients, who all underwent coronary artery bypass surgery. The prevalences of 677 C→T transition of the *MTHFR* gene were determined in these patients. Two groups were compared according to GENSINI coronary score : mild atherosclerosis (CAD stenosis < 30) and severe atherosclerosis (CAD stenosis > 30).**

***Results*: Patients with CAD showed a significantly higher serum concentration of homocysteine than control subjects ($P < 0.01$). The serum homocysteine level was significantly higher in patients with increased scores than in patients with mild CAD (Gensini score < 30) both with and without the *MTHFR* polymorphism.**

***Interpretation & conclusions*: The findings of our study showed that hyperhomocysteinemia was significantly related to the severity of CAD independent on *MTHFR* polymorphism.**

**Key words** Coronary artery disease - GENSINI score - homocysteine - methylenetetrahydrofolate reductase polymorphism

Several studies have identified moderate hyperhomocysteinemia as an independent risk factor for atherosclerotic disease[1-4]. Both clinical and experimental investigations suggest that elevated fasting plasma homocysteine concentrations are common in patients with peripheral arterial occlusive disease,

coronary heart disease, carotid artery stenosis, and a history of stroke, myocardial infarction, and venous thrombosis[5-9]. Previous studies have shown that homocysteine may contribute to the pathogenesis of atherosclerosis by adversely affecting endothelial cell function[10-12].

Hyperhomocysteinemia may reflect either genetic defects, vitamin deficiencies, or renal failure. Folate, vitamin B12 (cobalamin) and vitamin B6 (pyridoxal phosphate) are essential coenzymes for homocysteine catabolism[13,14]. The 677 C → T polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene is associated with elevated plasma homocysteine levels[3,8,9].

Kang *et al*[15] first reported a defect of this enzyme, a thermolabile variant of *MTHFR*, which was associated with raised homocysteine levels. Frosst *et al*[16] described a C to T substitution at nucleotide 677 of the *MTHFR* gene that converts an alanine to a valine residue. An increased thermolability of *MTHFR*, connected to 677 C→T methylenetetrahydrofolate reductase homozygous variant, is associated with hyperhomocysteinemia in patients with atherosclerotic disease, especially when plasma folic acid levels are low[5,8, 9,13,17].

However, the relation between *MTHFR* polymorphism and severity of coronary artery disease (CAD) has not been systematically evaluated. The aim of the present study was therefore to evaluate the relationship between the prevalence of this *MTHFR* polymorphism and the severity of coronary artery disease in patients undergoing coronary artery bypass surgery.

## Material & Methods

A total of 113 male patients with CAD (mean age 63 ± 11 yr) of the Clinic of Heart Surgery of the Heart Center Leipzig, University of Leipzig, Germany, scheduled for coronary artery bypass surgery, and 60 male patients of the clinic (age 62 ± 6.5 yr) in whom CAD was excluded by coronary angiography (control subjects) were randomly selected. The present investigation was a case-control study during a period of approximately 2 yr (September 2002-June 2004). The Human Ethics Committee at the Faculty of Medicine, University of Leipzig, Germany, approved the study protocol.

The severity of CAD was calculated using to the GENSINI scoring system[18]. Five millilitre blood were drawn from a peripheral vein of a fasting volunteer and placed into a test tube. The sample was centrifuged at 4000 g at 4 °C for 10 min within 45 min after withdrawal.

The serum was separated and stored at -80 °C. Total serum homocysteine was measured by HPLC (high-pressure liquid chromatography) with fluorescence detection according to the method of Araki and Sako[19]. For genetic analysis, DNA was isolated from whole blood by using puregene-DNA isolation kit (Puregene-Genetic Analysis Products, Minneapolis, USA). The 677 missense mutation in the *MTHFR* gene was analyzed after polymerase chain reaction (PCR) and digestion with HinfI. A 198 bp fragment of the *MTHFR* gene was amplified by PCR using primers described by Frosst *et al*[16]. In the case of mutation an additional restriction site for HinfI was introduced. The restriction pattern was analyzed after agarose gel electrophoresis and staining with ethidiumbromide. Total serum cholesterol and triglyceride concentrations were measured using enzymatic test kits from Roche Diagnostics GmbH, Mannheim, Germany. For the quality controls, Precinorm L (Roche Diagnostics GmbH) was used. To determine serum high-density lipoprotein (HDL) cholesterol, we used the polyethylene glycol 20000 precipitation method (Quantolip, Immuno GmbH, Heidelberg, Germany). Low-density lipoprotein (LDL) cholesterol was measured with the help of precipitation reagent polyvinylsulfate from Roche Diagnostics GmbH, Mannheim, Germany. Serum creatinine concentration was determined using enzymatics assay (Roche Diagnostic GmbH). Serum folate and vitamin B12 were measured with a chemiluminescense immunoassay (Bayer, Leverkusen, Germany) on an ACS Centaur (Bayer). Control sera were obtained from the same company (between-day CVs, 9% for serum folate and 2.7% for vitamin B12).

*Statistical analysis*: Continuous data were shown as median with quartiles as appropriated. Comparisons were performed using the Mann-Whitney U- test and $\chi^2$-test. Logistic regression model was fitted to evaluate the independent impact of homocysteine on severity of CAD adjusted for *MTHFR* genotype. For the analysis the software package SPSS 15.0 (Statistical Package for the Social Science, SPSS, Inc., Chicago, Illinois) was used.

## Results

The demographic characteristics, cardiovascular risk factors and the prevalence of the MTHFR 677 C→T polymorphism for the CAD patients and controls are shown in Table I. The prevalences of diabetes mellitus, hypertension, and current smokers were higher in patients compared with control subjects. Serum LDL

cholesterol and triglyceride concentration were elevated in CAD patients in comparison with the control subjects ($P< 0.05$). The average HDL cholesterol levels were significantly lower in patients than in controls ($P< 0.05$). The average homocysteine level was significantly higher in CAD patients than in controls (median = 13.8 μmol/l and median 8.50 μmol/l, respectively; $P< 0.01$). There were no significant differences between patients and controls regarding serum folate and vitamin B12 levels. Creatinine concentration in patients with CAD was significantly higher than in controls (median 88.0 μmol/l and 80.0 ± 12.0 μmol/l, respectively; $P< 0.01$).

In CAD patients as well as in control subjects, a polymorphism in the gene encoding methylenetetrahydrofolate reductase (MTHFR) 677 C→T was identified. The prevalence of wildtype (C/C) in CAD patients (41.1%) was significantly lower ($P< 0.01$) than in the control group (52.5%). The prevalence of heterozygous and homozygous variants (C/T genotype, T/T genotype) in CAD patients was also higher ($P< 0.05$) than in controls (Table I).

Compared with CAD patients with mild coronary stenosis (GENSINI score < 30) the serum homocysteine concentration of patients with severe stenosis (GENSINI score > 30) was significantly higher (median=13.1 μmol/l vs. median=15.0 μmol/l, $P=$ 0.022). The distribution of *MTHFR* genotype was not significantly different in the two observed CAD groups (GENSINI score < 30 and GENSINI score > 30) (Table III).

To assess the independent impact of serum homocysteine on severity of CAD according GENSINI-score a multiple logistic regression model was built. The

**Table I.** Demographic and cardiovascular risk variables of CAD patients and control subjects (median, 1. quartile - 3. quartile)

| Characteristic | Control subjects (n = 60) | CAD patients (n = 113) |
|---|---|---|
| Age (yr) | 61.0 (55.0 - 65.0) | 65.0 (57.0 - 71.5) |
| Diabetes mellitus (%) | 2 | 35 |
| Hypertension (%) | 20 | 71 |
| Current smokers (%) | 29 | 61 |
| Previous myocardial infarction (%) | - | 61 |
| Cholesterol (mmol/l) | 5.45 (4.76 - 6.03) | 5.70 (5.05 - 6.35) |
| LDL cholesterol (mmol/l) | 3.29 (2.59 - 4.04) | 4.04 (3.43 - 4.71)* |
| HDL cholesterol (mmol/l) | 1.63 (1.36 - 2.00) | 1.10 (0.92 - 1.29)* |
| Triglycerides (mmol/l) | 1.17 (0.95 - 1.78) | 1.67 (1.25 - 2.40)* |
| Creatinine (μmol/l) | 80.0 (79.9 - 97.6) | 88.0 (71.0 - 99.5)** |
| Homocysteine (μmol/l) | 8.50 (7.00 - 9.80) | 13.8 (11.0 - 17.2)** |
| Folate (ng/ml) | 8.65 (7.52 - 10.4) | 11.0 (7.35 - 18.0) |
| Vitamin B12 (pg/ml) | 334 (260 - 502) | 242 (177 - 321) |
| MTHFR 677C → T genotypes | | |
| Homozygons C/C (%) (Wildtype) | 52.5 | 41.0** |
| Heterozygons C/T (%) | 35.5 | 42.0** |
| Homozygons T/T (%) | 12.0 | 17.0* |

*P* *<0.05 **<0.01 compared to control subjects

**Table II.** Serum homocysteine in CAD patients with different severity according GENSINI and methylenetetrahydrofolate reductase (MTHFR) genotypes

| | GENSINI score < 30 (n=43) | GENSINI score > 30 (n= 70) | *P* value |
|---|---|---|---|
| Serum homocysteine (μmol/l) | | | |
| Median (1. quartile - 3. quartile) | 13.1 (9.9 - 16.0) | 15.0 (12.1 - 17.5) | 0.022 |
| Serum homocysteine > 11.0 (μmol/l) | n=29 (67.4%) | n=60 (85.7%) | 0.021 |
| MTHFR - genotype | | | |
| C/C (Wildtype) | n=20 (46.5%) | n=30 (42.9%) | 0.704 |
| C/T and T/T | n=23 (53.5%) | n=40 (57.1%) | |

**Table III.** Impact of serum homocysteine on severity according GENSINI adjusted for methylenetetrahydrofolate reductase (MTHFR) genotypes in CAD patients

| | OR | 95% CI | *P* value |
|---|---|---|---|
| Serum homocysteine | | | |
| < 11.0 (μmol/l) | ref. | | |
| > 11.0 (μmol/l) | 2.9 | 1.2 - 7.4 | 0.023 |
| MTHFR - genotyp | | | |
| C/C (Wildtype) | ref. | | |
| C/T and T/T | 1.2 | 0.5 - 2.6 | 0.651 |

OR, odds ratio; CI, confidence interval

serum homocysteine, dichotomized with cut off point 11 μmol/l, as well *MTHFR* polymorphism to adjust for it were included in the model. The cut off point of 11 μmol/l was chosen by reasons of clinical experiences (Table III).

The risk for severity increased about three-fold (OR=2.9, *P*=0.023) when serum homocysteine was higher than 11 μmol/l related to serum homocysteine lower than or equal 11 μmol/l. This increasing risk was independent of *MTHFR* genotype.

## Discussion

High plasma homocysteine is a risk factor for atherosclerosis and venous thrombosis[1-5,9]. Homocysteine concentrations are influenced by folate, vitamin B6 and vitamin B12 and influence genetic polymorphisms of key enzymes in homocysteine metabolism[13,15,17]. A common genetic variant of the *MTHFR* gene (677 C→T) is known to be associated with thermolability of the *MTHFR* enzyme with a remaining activity of around 50 per cent of the normal molecule and elevated plasma homocysteine levels, especially in those with low folic acid concentrations[3,4,17].

In our study, the average homocysteine level was significantly higher in patients with CAD than in control subjects. This finding was in agreement with the observations of other investigators[1-4,8,9,17,20]. The results of our study showed that the concentrations of serum homocysteine differed significantly between the CAD patients with lower (< 30) and higher (> 30) GENSINI score. Tsai *et al*[21] reported that the homocysteine level correlated with the extent of coronary atherosclerosis only in patients with low cardiovascular risk profiles. Further, Montalescot *et al*[22] demonstrated that the presence of both hypertension and hyperhomocysteinemia was associated with more severe coronary atherosclerosis. Yoo *et al*[23] reported that moderate hyperhomocysteinemia was an independent risk factor for coronary artery disease, and also significantly related to the presence of triple-vessel disease. Other studies found no correlation between homocysteine levels and the extent and severity of CAD[24-26]. According to Gardemann *et al*[27] the TT genotype of *MTHFR* 677 C→T gene polymorphism is associated with the extent of CAD in patients at high risk for CAD. The results of the present study showed that the level of homocysteine in CAD patients with severe lesions was significantly higher than in patients with mild disease. Further, this relationship seemed to be independent on *MTHFR* polymorphism.

In conclusion, our findings showed an association between homocysteine levels and severity of coronary artery disease, and this association appeared to be independent on the genotype of methylenetetrahydrofolate reductase.

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