Thermolabile Methylenetetrahydrofolate Reductase in Coronary Artery Disease

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Background  Hyperhomocysteinemia, an independent and graded risk factor for coronary artery disease (CAD), may result from both environmental and hereditary factors. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of methylenetetrahydrofolate to methyltetrahydrofolate, the methyl donor in the remethylation of homocysteine to methionine. A 677C——T mutation in the MTHFR gene has been associated with elevated homocysteine concentrations in homozous (+/+ ) individuals.

Methods and Results  We assessed the frequency of this common mutation in 735 CAD patients from the Regression Growth Evaluation Statin Study (REGRESS), a lipid-lowering coronary-regression trial, and in 1250 population-based control subjects. Furthermore, the association between the mutation and serum homocysteine concentrations was studied. The frequency of the homozygous (+/+ ) mutation was 9.5% among patients versus 8.5% among control subjects, resulting in an odds ratio of 1.21 (95% confidence interval [CI], 0.87 to 1.68), relative to the (—/—) genotype. Homocysteine concentrations were significantly elevated in both (+/+ ) and (+/-) individuals compared with (—/—) individuals (median homocysteine levels, 15.4, 13.4, and 12.6 mmol/L, for (+/+), (+/-), and (—/—) individuals, respectively). For a summary estimation of the risk of the (+/+ ) genotype for CAD, we performed a meta-analysis on 8 different case-control studies on thermolabile MTHFR in CAD. In the meta-analysis, the homozygous (+/+ ) genotype was present in 299 of 2476 patients (12.1%) and in 257 (10.4%) of 2481 control subjects, resulting in a significant odds ratio of 1.22 (95% CI, 1.01 to 1.47) relative to the (—/—) genotype.

Conclusions  Both the homozygous (+/+ ) and heterozygous (+/-) genotype result in elevated homocysteine concentrations. From our meta-analysis, we conclude that the homozygous (+/+ ) genotype is a modest but significant risk factor for CAD. (Circulation. 1997;96:2573-2577.)

Key Words  • hyperhomocysteinemia • methylenetetrahydrofolate reductase • coronary disease • meta-analysis • risk factors
Recently, Frost and coworkers\textsuperscript{17} were able to identify a relatively common 677C\textrightarrow{T} mutation in the MTHFR gene, which substituted a conserved alanine by a valine residue. Individuals who are homozygous for this mutation often have elevated homocysteine concentrations,\textsuperscript{17,18} especially in combination with a low folate status.\textsuperscript{19,20}

In the present study, we investigated the prevalence of the 677C\textrightarrow{T} mutation in a well-defined population of 735 male CAD patients and in 1250 population-based control subjects and assessed the association of this mutation to serum homocysteine concentration. Several studies have investigated the homozygous 677C\textrightarrow{T} mutation as a risk factor for CAD but with conflicting results,\textsuperscript{21-26} probably because of the relatively small numbers of individuals included in each study separately. We therefore performed a meta-analysis of eight case-control studies reporting data on the MTHFR genotype distribution to estimate the relative risk of the homozygous (+/+) genotype for CAD.

Methods

Study Population

We studied 735 male patients with angiographically assessed CAD enrolled in REGRESS, which was conducted under auspices of the Interuniversity Cardiology Institute The Netherlands. REGRESS is a double-blind, placebo-controlled, multicenter trial designed to assess the effects of lipid-lowering therapy with pravastatin on progression and regression of CAD in 885 male patients with normal to moderately elevated serum cholesterol levels (4 to 8 mmol/L). The overall study design and inclusion criteria of patients have been described extensively.\textsuperscript{27}

A large control group was constructed, consisting of individuals recruited from several published\textsuperscript{28-29} and unpublished Dutch case-control studies (L.A.J.K., H.J.B., Van der Put, Den Heijer, and Rosendaal, unpublished results), which resulted in a control group consisting of 1250 unrelated population-based control subjects. All 677C\textrightarrow{T} mutation analyses were performed in our laboratory.

For a summary quantitative risk assessment of the 677C\textrightarrow{T} mutation in CAD, we evaluated eight international case-control studies,\textsuperscript{21-26} including the present one. In this analysis, we confined ourselves to case-control studies in which MTHFR genotype distributions among both CAD patients and control subjects either were given or could be calculated from their data. We calculated the MTHFR genotype distribution and odds ratios of the (+/+) genotype for CAD in each study separately and for all studies combined.

MTHFR Genotype Analysis

Genomic DNA was extracted from peripheral blood lymphocytes by standard procedure, and mutation analysis was performed essentially as described by Frost et al.\textsuperscript{17} Electrophoresis in a 4% agarose gel followed by ethidium bromide staining and UV illumination allowed detection of mutated alleles.

Homocysteine Determination

After an overnight fast, blood was drawn from the CAD patients for an assessment of fasting homocysteine concentrations, and serum was stored at \textdegree 70C until analysis. Homocysteine concentrations were determined by high-performance liquid chromatography with use of a 150x4.6-mm Hypersil ODS column in a high-performance liquid chromatography analyzer (Thermo Separation Products) after the thiol groups were bound to a fluorescent label (SBD-F).\textsuperscript{30}

Statistical Analysis

Odds ratios and 95% CIs were calculated as an estimate of the relative risk of the different genotypes in CAD.\textsuperscript{31} Differences in genotype distributions were calculated by \textsuperscript{2} analysis. To assess the relationship between the 677C\textrightarrow{T} transition and homocysteine concentrations, we calculated median homocysteine concentrations in different genotype groups. Differences between homocysteine concentrations in these genotype groups were assessed by one-way ANOVA, followed by pairwise t tests on log-transformed data. All probability values are two-tailed, and a value of \textit{P}<.05 was considered statistically significant.

Results

MTHFR Genotype Analysis

The overall frequency for the (+) allele was 31.8% among patients and 29.5% among control subjects. The numbers of individuals homozygous for the 677C\textrightarrow{T} transition were 70 of 735 CAD patients (9.5%) versus 106 of 1250 controls (8.5%; Table 1). The genotype distributions in both groups of individuals are consistent with those calculated from the Hardy-Weinberg equilibrium. The odds ratios as an estimate of the relative risk of the (+/+) and (+/-) genotypes relative to the risk of the (-/-) genotype for CAD were 1.21 and 1.14, respectively (Table 1). We also assessed the relative risk of the (+/+) genotype in relation to the risk of individuals with both other genotypes. In this model, the risk of the homozygous (+/+) genotype in CAD was 1.14 (95% CI, 0.83 to 1.56).

Association Genotype and Total Homocysteine

Homocysteine concentrations were measured in 515 of 735 CAD patients. The numbers of individuals in the three different MTHFR genotype groups in this subset of individuals were 51 (9.9%), 233 (45.2%), and 231 (44.9%) for the (+/+) (+/-) and (-/-) genotype, respectively, which are not substantially different from the MTHFR genotype distribution observed in the entire patient group (\textsuperscript{2} =0.137, \textit{P}=NS). As homocysteine concentrations in the different genotype groups showed a skewed distribution (data not shown), homocysteine concentrations are expressed in median (range) values. Individuals with the homozygous (+/+) genotype have the highest homocysteine concentrations and the (-/-) individuals have the lowest, whereas heterozygous (+/-) individuals have intermediate homocysteine levels. Both homozygotes (+/+) and heterozygotes (+/-) have significantly elevated homocysteine concentrations.
The frequency of the homozygous (+/+) genotype varies between different populations. The effect of this mutation on homocysteine concentrations depends on study design, inclusion criteria, ethnic background, age, and vitamin intake of the population. Except for the
with the results reported by Harmon et al, who observed elevated homocysteine concentrations in heterozygous (+/-) individuals. The present study supports these observations and indicates again that the homozygous (+/+) genotype is associated with elevated homocysteine concentrations. The effect of the homozygous (+/+) genotype on homocysteine concentrations might therefore differ between separate studies as a result of a different intake of folate. A possible adjustment for plasma folate levels was not determined.

In the present study, we were also able to demonstrate a statistically significant effect of the heterozygous (+/-) genotype on homocysteine concentrations. This is in line with the results reported by Harmon et al, who observed elevated plasma homocysteine concentrations in heterozygous (+/-) individuals in the top 50% of the homocysteine distribution. On the basis of specific and residual MTHFR activities measured in isolated lymphocytes, this observation was not unexpected, because we have shown that heterozygous (+/-) individuals have significantly decreased specific and residual MTHFR activities compared with nonaffected (-/-) individuals. MTHFR-dependent homocysteine remethylation, in which 5-methyltetrahydrofolate (the product of the reaction catalyzed by MTHFR) serves as methyl donor, is present in nearly every cell of the human body. Therefore, any significantly deleterious effect in MTHFR enzyme activity will be reflected in elevation of homocysteine concentration in these cells. Previous studies were unable to detect an effect of the heterozygous (+/-) genotype on homocysteine concentrations, probably because of the relatively limited number of individuals included in those studies.

Kang et al were the first to report on a thermolabile MTHFR variant in two patients with CAD and hyperhomocysteinemia. Subsequent studies by the same group showed an association between this thermolabile MTHFR and (the severity of) CAD. In a large study among CAD patients and healthy control subjects, Kang et al detected thermolabile MTHFR in 36 of 212 cases (17%) versus 10 of 202 control subjects (5%). In the present study, we observed a much lower frequency of the thermolabile (+/+) genotype among Dutch CAD patients and a higher frequency among Dutch population-based control subjects. Several possible explanations for this phenomenon should be considered. In the study by Kang et al, the frequency of thermolabile MTHFR was assessed biochemically and was not based on genotyping of the 677C>T mutation in the MTHFR gene. This study was supported in part by grant 93.176 from the Netherlands Heart Foundation. REGRESS was sponsored by Bristol Myers Squibb, Princeton, NJ.

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References


