The TT genotype of the methylenetetrahydrofolate reductase C677T gene polymorphism is associated with the extent of coronary atherosclerosis in patients at high risk for coronary artery disease

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Background There are conflicting results on the relationship of N5,N10-methylenetetrahydrofolate reductase C677T gene variation in coronary artery disease and myocardial infarction.

Methods and Results We analysed this gene variation in 2453 male Caucasians whose coronary anatomy was defined by coronary angiography. In the total sample, the C677T gene polymorphism was not associated with the presence or the extent of coronary artery disease (defined by the degree of vessel disease or by the coronary heart disease score according to Gensini). However, after excluding individuals with low risk profiles, an association between the C677T TT genotype and the Gensini score was found. This observation applies only to individuals (i) with high glucose levels, (ii) with low apolipoprotein Al/apolipoprotein B ratios, (iii) with low apolipoprotein Al/apolipoprotein B ratios and high lipoprotein (a) levels and (iv) with low apolipoprotein Al/apolipoprotein B ratios and high glucose concentrations. In patients with high glucose levels, the paraoxonase 191 A/B gene variation presupposed whether differences in Gensini scores between C677TC allele carriers and TT homozygotes became apparent, since only in paraoxonase 191 AA homozygotes, but not in paraoxonase 191 B allele carriers, did C677 T TT homozygotes have clearly higher Gensini scores than C allele carriers (two-way interaction; \(P=0.013\)). The MTHFR C677T gene polymorphism was not associated with non-fatal myocardial infarction.

Conclusion The present study extends previous observations by the finding that carriers of the N5,N10-methylenetetrahydrofolate reductase C677T TT genotype with various coronary high risk profiles had clearly higher coronary heart disease scores than individuals with at least one C677T C allele.

Key Words: Homocysteine metabolism, coronary artery disease, myocardial infarction, coronary risk factors.

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Introduction

Homocysteine is increasingly recognized as an independent risk factor for coronary artery disease (for review see[1–3]). Experimental evidence shows that homocysteine-induced atherosclerosis is characterized by endothelial dysfunction and injury followed by platelet activation and thrombus formation[4–6]. In blood, homocysteine is rapidly auto-oxidized to form homocystine, mixed disulfides, and homocysteine thiolactone[7,8]. The potent reactive superoxide and hydrogen peroxides, which are produced during this process, are mainly responsible for the vascular toxicity of homocysteine[9]. It has been proposed that endothelial cell injury, caused by homocysteine and mediated by reactive oxygen species, might expose the underlying matrix and promote activation of platelets and leukocytes[4]. Homocysteine also alters the normal antithrombotic phenotype of endothelial cells (i) by enhancing the activities of factor V[10] and factor XII[11], (ii) by...
depressing the activation of protein C\(^{[12]}\), and (iii) by inhibiting the expression of thrombomodulin\(^{[13]}\) and of endothelial heparan sulfate\(^{[14]}\). In addition, homocysteine (i) is a potent mitogen for vascular smooth muscle cells\(^{[13]}\), (ii) has been postulated to decrease the bioavailability of nitric oxide by impairing its synthesis\(^{[5,16]}\) and (iii) is able to increase the formation of highly atherogenic oxysterols, the peroxidation of lipids and the oxidation of low-density lipoprotein in vitro\(^{[17,18]}\).

The sulphur-containing amino acid homocysteine is formed during the metabolism of methionine. It is metabolized by either remethylation or transsulfuration. In the remethylation cycle, homocysteine is salvaged by the acquisition of a methyl group in a reaction catalyzed by methionine synthase\(^{[19]}\). Vitamin B\(_{12}\) is an essential cofactor for methionine synthase, N\(^5\) -methyltetrahydrofolate is the methyl donor in this reaction, and N\(^5\),N\(^10\)-methylene tetrahydrofolate reductase (MTHFR) functions as a catalyst in the remethylation process\(^{[19]}\). Increases in homocysteine plasma levels are caused by nutritional deficiencies in vitamin cofactors, or by genetic defects in the enzymes which are involved in the metabolism of this amino acid (for review see\(^{[1–3]}\)). Cystathionine \(\beta\)-synthase deficiency is the most common genetic cause of severe hyperhomocystinaemia\(^{[2]}\).

Besides other genetic defects, a thermolabile variant of MTHFR has been described which is caused by a point mutation (C\(_{677}\)T) in the coding region for the MTHFR binding site, leading to the substitution of valine for alanine\(^{[20–21]}\). It has been shown that this gene variation was associated with increased levels of homocysteine; carriers of the C\(_{677}\)T TT genotype had clearly higher plasma levels than CT heterozygotes or CC homozygotes\(^{[21–23]}\). In several studies, the potential link between this gene polymorphism and ischaemic heart disease has been investigated. Whereas some investigators identified the TT genotype as a risk factor of cardiovascular disease\(^{[22,24–27]}\), other scientists failed to identify a link between the MTHFR C\(_{677}\)T genotype and this disease\(^{[28–39]}\). These discrepancies might be due to differences in the study design. It is also conceivable that gene variation other than MTHFR C\(_{677}\)T gene polymorphism, which have also been postulated to be associated with coronary heart disease, might contribute to the discrepant results\(^{[22,24–39]}\), by interfering with the link between the MTHFR C\(_{677}\)T gene variation and the risk of coronary artery disease and myocardial infarction. Potential candidates are polymorphisms of the renin–angiotensin system (angiotensin I-converting enzyme insertion/deletion polymorphism\(^{[40]}\), angiotensinogen T174M\(^{[41]}\) and M235T gene variations\(^{[42]}\), angiotensin II type 1 receptor A1166C gene variation\(^{[43]}\) and gene polymorphisms of the serum enzyme paraoxonase (54 L/M\(^{[44]}\), 191 A/B\(^{[45]}\), which is entirely bound to high density lipoprotein and is considered to play an important role in limiting the accumulation of lipid oxidation products in low density lipoprotein\(^{[46,47]}\). In the present study population, the angiotensin I-

\[\text{converting enzyme insertion/deletion polymorphism}^{[48]}\], the angiotensinogen T174M and M235T gene variations (unpublished observation) and the paraoxonase 54 L/M gene polymorphism (unpublished observation), but not the angiotensin II type 1 receptor A1166C gene variation\(^{[49]}\) and the paraoxonase 191 A/B gene polymorphism (unpublished observation) were associated with the extent of coronary artery disease. It was the aim of the present study to investigate in an overall group of 2453 male Caucasians, whose coronary anatomy was defined by means of coronary angiography, whether the MTHFR C\(_{677}\)T gene polymorphism was related to the presence and extent of coronary artery disease and to myocardial infarction in the total population and among subjects who were at lower or higher risk of these diseases. In addition, we analysed potential interactions between the MTHFR C\(_{677}\)T polymorphism and gene variations of the renin–angiotensin system, and of the serum enzyme paraoxonase, on the risk of coronary heart disease.

**Methods**

*Detection of coronary artery disease and myocardial infarction*

The MTHFR C\(_{677}\)T gene polymorphism was analysed in 2453 male Caucasian individuals. These were consecutive patients who underwent coronary angiography for diagnostic purposes. About 80% of the participants underwent coronary angiography on account of coronary heart disease as a verified illness or presumptive diagnosis. In the remainder of the group consisted almost completely of patients who underwent coronary angiography for clarification of restricted left ventricular function. In 90% of these patients, coronary artery disease was the reason for this dysfunction. Only in 10% of this subpopulation (=2% of the total sample), was restricted left ventricular function caused by dilated cardiomyopathy or longstanding arterial hypertension. All patients who agreed to participate in the study were evaluated with a detailed questionnaire which provided information about coronary risk factors such as smoking, diabetes mellitus and hypertension.

The retrospective study lasted 2.5 years and ended in July 1997. Patients were initially recruited from the departments of cardiovascular surgery and from the department of cardiology of the university hospital in Giessen, and — during the last year — from the department of cardiology of the Kerkhoff-Klinik in Bad Nauheim. (W. H. who carried out or supervised the majority of the coronary angiographies, moved from Giessen to Bad Nauheim in July 1996.)

*Coronary artery disease*

Coronary angiography was performed by the Judkins method. Coronary vessels with at least 50% stenosis were defined as diseased. The severity of coronary heart disease was also estimated by calculating the Gensini
score[50]; this score is subsequently designated the ‘CHD score’. By means of coronary angiography, the study population was divided into subjects without any angiographically detectable coronary artery disease or with coronary arterial stenoses less than 50% (no vessel disease) and individuals with single-, double- or triple-vessel disease.

**Myocardial infarction**
Angina pectoris and acute myocardial infarction were diagnosed according to criteria established by the World Health Organisation. Sixteen percent of patients without vessel disease, 48% of individuals with single-vessel disease, 55% of patients with double-vessel disease and 61% of patients with triple-vessel disease had suffered a myocardial infarction before recruitment in the study.

**Measurements of substrates, definition of variables and detection of the MTHFR C677T gene polymorphism**
Triglycerides, total cholesterol, apolipoprotein B, apolipoprotein AI, lipoprotein (a), glucose and fibrinogen were measured by conventional methods of clinical chemistry[48]. Diabetes mellitus was defined as a binary variable and not divided into subcategories. Hypertension (binary variable in the present study) was defined by either treatment or diastolic blood pressure greater than 95 mmHg on two consecutive visits for those untreated. Cigarette consumption was given as pack years (1 pack year: e.g. 20 cigarettes per day for one year). The MTHFR C677T gene polymorphism was analysed in all study patients according to Nishinaga et al.[14]. Genotypes for angiotensin I-converting enzyme insertion/deletion polymorphism[51,52], angiotensinogen T174M[53] and M235T gene variations[54], angiotensin II type 1 receptor A1166C gene variation[55], paraoxonase 54 L/M[44] and paraoxonase 191 A/B gene polymorphisms[56] were determined in all study patients as described.

**Definition of low and high risk subpopulations**
The mean values of coronary risk factors from the whole study population were used as cut points for the definition of low and high risk populations. With respect to hypertension and diabetes, low and high risk groups were defined by the absence or presence of these diseases. With respect to the plasma glucose level and the apolipoprotein AI/apolipoprotein B ratio not only the mean value but also the 10th, 25th, 50th, 75th and 90th percentiles were used to define low and high risk populations.

**Statistical analysis**
Statistical analyses were performed using the SPSS for Windows 95 software (Version 7.52). In order to compare established risk factors between MTHFR C677T genotypes, the relationship of the MTHFR C677T gene polymorphism to continuous variables was tested by Kruskal–Wallis one-way ANOVA; the relationship of the MTHFR C677T gene polymorphism to diabetes and hypertension was checked by chi-square analysis. Established risk factors of coronary artery disease and myocardial infarction were identified by multiple regression analysis (extent of coronary artery disease, CHD score) or multiple logistic regression (absence/presence of coronary artery disease or myocardial infarction). The chi-square test was used to test for deviation of genotype distribution from Hardy–Weinberg equilibrium. The relationship of the MTHFR C677T gene polymorphism to the extent of coronary artery disease (coronary artery disease, CHD score) was determined by multiple regression analysis; coronary risk factors (age, cholesterol, apolipoprotein AI, apolipoprotein B, lipoprotein (a), fibrinogen, body mass index, smoking habit, diabetes, hypertension) were introduced into the calculation. The relationship of the MTHFR C677T gene polymorphism to the presence of coronary artery disease and to myocardial infarction was determined by multiple logistic regression with adjustment for the above-mentioned coronary risk factors. The interactions between the paraoxonase 191 A/B gene variation and the MTHFR C677T gene polymorphism on the CHD score were tested by a two-factor ANOVA procedure. A two-sided probability value of less than 0.05 was considered to indicate statistical significance.

**Results**

**Distribution of MTHFR C677T genotypes within the study population and effect of MTHFR C677T gene polymorphism on variables of clinical chemistry and clinics**
The distributions of the MTHFR C677T genotypes in controls (subjects without coronary artery disease and myocardial infarction) were in Hardy–Weinberg’s equilibrium. Age, total cholesterol, triglycerides, apolipoprotein AI, apolipoprotein B, apolipoprotein AI/apolipoprotein B ratio, lipoprotein (a), glucose, fibrinogen, prevalence of diabetes and arterial hypertension, body mass index and cigarette consumption were almost identical between MTHFR C677T genotypes (data not shown). The mean delay between the occurrence of myocardial infarction and recruitment in this study (5.38±0.3 years for the total population) did not differ between MTHFR C677T genotypes (data not shown).
### Table 1 Risk factors of coronary artery disease and myocardial infarction

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>− CAD (n=560)</th>
<th>+CAD (n=1893)</th>
<th>2P</th>
<th>− MI (n=1301)</th>
<th>+MI (n=1152)</th>
<th>2P</th>
<th>Mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>58±3 ± 10·7</td>
<td>62±6 ± 9·3</td>
<td>&lt;0·0001</td>
<td>61±1 ± 10·1</td>
<td>62±2 ± 9·5</td>
<td>&lt;0·01</td>
<td>61±6 ± 9·8</td>
</tr>
<tr>
<td><strong>BMI (kg·m⁻²)</strong></td>
<td>26±9 ± 3·8</td>
<td>26±9 ± 3·3</td>
<td>ns</td>
<td>26±9 ± 3·5</td>
<td>26±9 ± 3·3</td>
<td>ns</td>
<td>26±9 ± 3·4</td>
</tr>
<tr>
<td><strong>Smoking habit (pack years)</strong></td>
<td>19±6 ± 24</td>
<td>23±5 ± 27</td>
<td>&lt;0·05</td>
<td>20±1 ± 25</td>
<td>25±4 ± 27</td>
<td>&lt;0·0001</td>
<td>22±6 ± 26</td>
</tr>
<tr>
<td><strong>% Diabetes</strong></td>
<td>11 ± 20</td>
<td>&lt;0·01</td>
<td></td>
<td>17</td>
<td>19</td>
<td>ns</td>
<td>18</td>
</tr>
<tr>
<td><strong>% Hypertension</strong></td>
<td>54 ± 66</td>
<td>&lt;0·002</td>
<td></td>
<td>64</td>
<td>62</td>
<td>ns</td>
<td>63</td>
</tr>
<tr>
<td><strong>% treated hyperchol.</strong></td>
<td>109±40</td>
<td>109±49</td>
<td>ns</td>
<td>110±43</td>
<td>114±53</td>
<td>&lt;0·05</td>
<td>112±48</td>
</tr>
<tr>
<td><strong>Glucose (mg·dl⁻¹)</strong></td>
<td>206±43</td>
<td>211±43</td>
<td>ns</td>
<td>210±42</td>
<td>209±43</td>
<td>ns</td>
<td>210±43</td>
</tr>
<tr>
<td><strong>Triglycerides (mg·dl⁻²)</strong></td>
<td>144±94</td>
<td>156±94</td>
<td>ns</td>
<td>153±98</td>
<td>155±89</td>
<td>ns</td>
<td>154±94</td>
</tr>
<tr>
<td><strong>ApoAI (g·l⁻¹)</strong></td>
<td>1·47±0·30</td>
<td>1·42±0·29</td>
<td>&lt;0·005</td>
<td>1·45±0·29</td>
<td>1·40±0·29</td>
<td>&lt;0·005</td>
<td>1·43±0·29</td>
</tr>
<tr>
<td><strong>ApoB (g·l⁻¹)</strong></td>
<td>1·21±0·32</td>
<td>1·30±0·35</td>
<td>&lt;0·0001</td>
<td>1·26±0·34</td>
<td>1·29±0·35</td>
<td>&lt;0·0001</td>
<td>1·28±0·34</td>
</tr>
<tr>
<td><strong>ApoAI/ApoB ratio</strong></td>
<td>0·44±0·14</td>
<td>0·44±0·14</td>
<td>ns</td>
<td>0·45±0·14</td>
<td>0·45±0·14</td>
<td>ns</td>
<td>0·45±0·14</td>
</tr>
<tr>
<td><strong>Lp(a) (mg·dl⁻¹)</strong></td>
<td>21±30</td>
<td>30±39</td>
<td>&lt;0·0001</td>
<td>26±9±37</td>
<td>29±1±37</td>
<td>&lt;0·05</td>
<td>28±2±37</td>
</tr>
<tr>
<td><strong>Fibrinogen</strong></td>
<td>3·42±1·61</td>
<td>3·51±1·22</td>
<td>ns</td>
<td>3·44±1·31</td>
<td>3·60±1·11</td>
<td>&lt;0·05</td>
<td>3·51±1·22</td>
</tr>
</tbody>
</table>

Values are means ± SD or % of a group. The relations of the coronary risk factors to the presence of coronary artery disease and to myocardial infarction were analysed by multiple logistic regression. BMI=body mass index; hyperchol.=hypercholesterolaemia; ApoAI=apolipoprotein AI; ApoB=apolipoprotein B; Lp(a)=lipoprotein (a); ns=not significant.

### Table 2 Distribution of the MTHFR C₆₇₇T genotypes in patients with or without coronary artery disease and with or without myocardial infarction

<table>
<thead>
<tr>
<th>Controls/Cases</th>
<th>Mean age (± SD)</th>
<th>n</th>
<th>MTHFR C₆₇₇T genotype</th>
<th>C/T Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>− CAD CHD score=0 (no MI)</td>
<td>53±9 ± 11·2</td>
<td>185</td>
<td>n (CC)</td>
<td>n (CT)</td>
</tr>
<tr>
<td>No vessel disease</td>
<td>58±3 ± 10·7</td>
<td>560</td>
<td>242</td>
<td>254</td>
</tr>
<tr>
<td>Single-vessel disease</td>
<td>61±0 ± 9·7</td>
<td>487</td>
<td>220</td>
<td>211</td>
</tr>
<tr>
<td>Double-vessel disease</td>
<td>62±4 ± 9·8</td>
<td>539</td>
<td>256</td>
<td>237</td>
</tr>
<tr>
<td>Triple-vessel disease</td>
<td>63±6 ± 8·6</td>
<td>867</td>
<td>415</td>
<td>357</td>
</tr>
<tr>
<td>Single, double or triple vessel disease</td>
<td>62±6 ± 9·3</td>
<td>1893</td>
<td>891</td>
<td>805</td>
</tr>
<tr>
<td>− MI No MI</td>
<td>61±1 ± 9·7</td>
<td>1301</td>
<td>606</td>
<td>544</td>
</tr>
<tr>
<td>At least 1 MI</td>
<td>62±2 ± 9·6</td>
<td>1152</td>
<td>527</td>
<td>515</td>
</tr>
</tbody>
</table>

CAD=coronary artery disease; MI=myocardial infarction; CHD=coronary heart disease.

### Relation of established coronary risk factors and of the MTHFR C₆₇₇T genotypes to coronary artery disease

**Established coronary risk factors**

- Apolipoprotein B (P<0·0001), hypercholesterolaemia (P<0·001), lipoprotein (a) (P<0·0001), diabetes mellitus (P<0·01), hypertension (P<0·002), smoking habit (P<0·05) and age (P<0·0001) could be demonstrated as risk factors for coronary artery disease, and apolipoprotein AI (P<0·005) and consequently high apolipoprotein AI/apolipoprotein B ratios (P<0·0001) as protective factors against coronary artery disease (Table 1). The mean values of coronary risk factors (Table 1) were used as cut points for definition of low and high risk populations.

**MTHFR C₆₇₇T gene polymorphism**

In the total population, the MTHFR C₆₇₇T gene polymorphism was not associated with the presence or the extent of coronary artery disease (defined by the degree of vessel disease) (Table 2). In addition, the mean CHD score was similar between MTHFR C₆₇₇T genotypes in the total sample (Fig. 1). However, exclusion of individuals with 'low risk profiles' resulted in an association of the TT genotype with the CHD score (Fig. 1). This observation applies to restriction of individuals (i) with high glucose levels (>112 mg·dl⁻¹, mean value), (ii) with low apolipoprotein AI/apolipoprotein B ratios (<1·19, mean ratio), (iii) with low apolipoprotein AI/apolipoprotein B ratios (<1·19) and high lipoprotein (a) levels (>27·9 mg·dl⁻¹, mean value) and (iv) with low apolipoprotein AI/apolipoprotein B ratios (<1·19) and

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high glucose plasma concentrations (>112 mg . dl\(^{-1}\)) (Fig. 1). Since the apolipoprotein B ratio, but not the plasma glucose level was normally distributed in our study sample, additional analyses were performed by using the 10th, 25th, 50th, 75th and 90th percentiles of the apolipoprotein AI/apolipoprotein B ratio and of the glucose concentration as cut points for the inclusion of individuals in high risk populations. The results of these analyses support our data, that within subgroups of individuals with low apolipoprotein AI/apolipoprotein B ratios or with high glucose levels, TT homozygotes had clearly higher CHD scores than C allele carriers (data not shown). This observation applies to the 50th (<1·12), 25th (<0·93) and 10th (<0·78) percentile of the apolipoprotein AI/apolipoprotein B ratio and to the 50th (>98 mg . dl\(^{-1}\)), 75th (>111 mg . dl\(^{-1}\)) and 90th (>151 mg . dl\(^{-1}\)) percentile of the plasma glucose level (data not shown). In other high risk and also low risk subpopulations an association of the T allele or of the TT genotype with the presence or extent of coronary artery disease (degree of vessel disease, CHD score) was not detected (data not shown).

**Figure 1** Comparison of mean Gensini scores between MTHFR C\(_{677}T\) TT homozygotes and carriers of at least one C allele. Values are means ± SEM. The relationship of the MTHFR C\(_{677}T\) gene polymorphism (TT vs CT+CC subjects) to mean Gensini scores was checked by multiple regression analysis with adjustment to coronary risk factors. Numbers of subjects are given in parenthesis. ApoAI=apolipoprotein AI; apoB=apolipoprotein B; Lp(a)=lipoprotein (a).

**Interactions between the MTHFR C\(_{677}T\) gene variation and other gene polymorphisms on the risk of coronary artery disease**

We analysed potential interactions between the MTHFR C\(_{677}T\) gene polymorphism and gene variations of the renin–angiotensin system (angiotensin I-converting enzyme insertion/deletion polymorphism\(^{[40]}\), angiotensinogen T174M \(^{[41]}\) and M235T gene variations\(^{[42]}\), angiotensin II type 1 receptor A1166C gene variation\(^{[43]}\) and of the serum enzyme paraoxonase (54 L/M\(^{[44]}\), 191 A/B \(^{[45]}\)) on the risk of coronary artery disease. In the total sample, interactions between the MTHFR gene variation and one of the above-mentioned gene polymorphisms were not detected (data not shown). In low and high risk populations, this observation also applies to potential interactions between the MTHFR C\(_{677}T\) gene variation and one of the polymorphisms of the renin–angiotensin system. In contrast, in individuals with high glucose levels, the paraoxonase 191 A/B gene variation pre-supposed whether differences in CHD scores between MTHFR C\(_{677}T\) C allele carriers and TT homozygotes became apparent; pronounced differences in CHD scores were detected only in paraoxonase 191 AA homozygotes.

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(two-way interaction between both gene variations on CHD score; \( P = 0.013 \)), but not in paraoxonase 191 B allele carriers (Fig. 2). Similar observations were made, for example, in a subpopulation of individuals with high glucose levels and low apolipoprotein AI/apolipoprotein B ratios (data not shown). The paraoxonase 54 L/M gene polymorphism did not influence the association of the MTHFR C677T gene polymorphism with the CHD score (data not shown).

**Relation of coronary risk factors and of the MTHFR C677T gene polymorphism to myocardial infarction**

**Established coronary risk factors**

Age \( (P < 0.01) \), ApoB \( (P < 0.001) \), hypercholesterolaemia \( (P < 0.005) \), glucose, \( (P < 0.05) \), fibrinogen levels \( (P < 0.05) \) and cigarette consumption \( (P < 0.002) \) were identified as risk factors for myocardial infarction, and apolipoprotein AI \( (P < 0.005) \) and consequently high apolipoprotein AI/apolipoprotein B ratios as protective factors against myocardial infarction (Table 1).

**MTHFR C677T gene polymorphism**

In the total population (Table 2), and in high and low risk subpopulations (data not shown) an association of the MTHFR C677T gene polymorphism with non-fatal myocardial infarction was not detected. This observation applies to a comparison (i) of all three genotypes \( (P = 0.90) \), (ii) of the TT genotype vs CT+TT (odds ratio 0.76 (0.55–1.05); \( P = 0.092) \), (iii) of the T allele (TT+TC) vs CC homozygotes (odds ratio 1.09 (0.90–1.32); \( P = 0.39) \) and (iv) of TT genotypes vs CC homozygotes (odds ratio 0.91 (0.77–1.08); \( P = 0.28) \).

**Interactions between the MTHFR C677T gene variation and other gene polymorphisms on the risk of myocardial infarction**

Neither gene variations of the renin–angiotensin system nor of paraoxonase had any significant influence on the relationship of the MTHFR C677T gene polymorphism to the risk of myocardial infarction (data not shown).

**Discussion**

Elevated levels of the thiol-containing amino acid homocysteine are an independent, graded risk factor for the development of atherosclerotic vascular disease (for review see [1–3]). A thermolabile variant of the enzyme N5, N10-methylenetetrahydrofolate reductase (MTHFR) — involved in the remethylation pathway of homocysteine to methionine — has been described and
is caused by a point mutation (C→T) in the coding region for the MTHFR binding site[20,21]. TT homozygotes of this gene variation have been repeatedly shown to have clearly higher homocysteine plasma values than CT heterozygotes or CC homozygotes[21–23]. However, conflicting results have been obtained with respect to the potential association of the MTHFR C677T gene variation with coronary artery disease and myocardial infarction. Therefore, the present investigation was performed to analyse the relationship of the MTHFR C677T gene polymorphism with coronary heart disease in a large case-control study of 2453 participants. The present findings allow the following conclusions.

**Design of the present study**

Established risk factors of coronary artery disease (age, hypercholesterolaemia, apolipoprotein B, lipoprotein (a), diabetes mellitus, hypertension, cigarette consumption) and of myocardial infarction (age, hypercholesterolaemia, apolipoprotein B, fibrinogen, smoking habit) could be identified in the present study sample. Also, known protective factors against coronary heart disease, such as apolipoprotein AI and high apolipoprotein AI/apolipoprotein B ratios, were found in our investigation. The present study did not show an association between cholesterol or fibrinogen and coronary artery disease and between diabetes, hypertension, cholesterol or lipoprotein (a) and myocardial infarction. However, when the group of patients without myocardial infarction was restricted to individuals without coronary artery disease, lipoprotein (a) (P=0.0001), diabetes (P=0.048) and hypertension (P=0.0006) could be identified as risk factors of myocardial infarction (data not shown). When this group was compared with the subpopulation of patients with myocardial infarction, we obtained essentially the same results for the relationship of the MTHFR C677T gene polymorphism to myocardial infarction (data not shown). Therefore, the present results strengthen the hypothesis that comparisons within our study group would lead to accurate predictions of new coronary risk factors.

**Distributions of the MTHFR C677T genotypes**

The frequencies of the C and T alleles of the present study sample show great similarities to those of previous investigations. For example, C allele frequencies of 0.61–0.70[22–39,57] were calculated in subpopulations of control subjects.

**Relation of the MTHFR C677T gene polymorphism with coronary artery disease**

Several studies have dealt with the question whether the MTHFR C677T gene variation might be associated with an increased risk for the presence and/or the extent of coronary artery disease[22,24–26,28,31,32,35,37–39]. In most studies, coronary artery disease has been documented by means of coronary angiography. The majority of investigations failed to detect an association of the MTHFR gene variation with the presence of coronary artery disease[28,31,32,35,37–39]. Only in this study and two others was the relationship of the MTHFR gene polymorphism with the extent of coronary artery disease investigated (defined by the degree of vessel disease)[25,37]. Whereas van Bockxmeer et al.[37] and the present investigation found no association between the gene variation and the extent of coronary artery disease, Morita et al.[25] observed that the frequency of the MTHFR C677T TT genotype was correlated with the severity of coronary artery disease. However, we emphasize that we not only divided our study sample into coronary heart disease-positive and coronary heart disease-negative subjects or patients with single-, double- or triple-vessel disease, but also determined the precise extent of coronary heart disease of all participants by calculating a CHD score[50]. These analyses enabled us to identify the MTHFR C677T TT genotype as an independent risk factor of coronary artery disease in various populations at high risk for coronary artery disease. Since we did not measure homocysteine levels of the 2453 study participants, we do not have direct evidence that the TT genotype was also associated with elevated plasma levels of homocysteine. Nevertheless, it has repeatedly been shown that TT homozygotes have higher homocysteine levels than the other MTHFR C677T genotypes[21–23]. Since Jacques et al.[58] demonstrated that these differences disappeared when individuals had folate levels greater than 15.4 nmol·l−1, they suggested that MTHFR C677T TT homozygotes may require folate supplementation to regulate plasma homocysteine concentrations. Further studies are needed to clarify whether it is justified to perform a PCR test and institute homocysteine-lowering treatment instead of directly determining homocysteine levels to guide homocysteine-lowering therapy.

**Relation of the MTHFR C677T gene polymorphism with myocardial infarction**

In accordance with the majority of investigations[27,29–31,35], but in contrast to one study[24], we failed to detect an association of the gene polymorphism with non-fatal myocardial infarction. It appears inconsistent that in various high risk groups the TT genotype was associated with the CHD score but not with myocardial infarction. Nevertheless, in the present case-control study, it should be noted that survivors of myocardial infarction and not patients with fatal outcome of this disease were included. This bias of selection may explain, at least in part, the discrepancies.
Potential interactions between the MTHFR C<sub>677</sub>T gene polymorphism and other gene variations on the risk of coronary heart disease

Regarding the divergent results obtained in recent studies on the association of the MTHFR C<sub>677</sub>T gene variant with coronary heart disease<sup>[22,24–39]</sup>, we speculated that these discrepancies could, at least in part, be explained by gene variations other than the MTHFR gene polymorphism, which was also postulated to be associated with coronary heart disease<sup>[40–47]</sup> and which might interfere to a different degree with the link between the MTHFR C<sub>677</sub>T gene variation and the risk of coronary heart disease. No interactions could be detected between one of the gene variations of the renin–angiotensin system and the MTHFR gene polymorphism on the risk of coronary artery disease and myocardial infarction. Only an interaction between paraoxonase 191 A/B gene variation, and the MTHFR C<sub>677</sub>T gene variation could be observed in a subpopulation of individuals with high plasma glucose levels. Further investigations are required to examine whether this interaction is also detectable in other populations and to evaluate the clinical significance of this finding.

Conclusion

In the total study sample of 2453 participants, we could not detect an association of the MTHFR C<sub>677</sub>T gene polymorphism with the risk of coronary artery disease and myocardial infarction. These observations are in line with results obtained in the majority of other recent investigations. However, the present study extends previous investigations by the finding that carriers of the MTHFR C<sub>677</sub>T TT genotype with coronary high risk profiles had clearly higher CHD scores than individuals with at least one MTHFR C<sub>677</sub>T C allele.

References

[27] Adams M, Smith , Martin D, Thompson JR, Samani NJ. Genetic analysis of thermolabile methylenetetrahydrofolate


