Methylenetetrahydrofolate Reductase Genotypes and Early-Onset Coronary Artery Disease

Aviv Mager, MD; Shadan Lalezari, MD; Tamar Shohat, MD; Yochai Birnbaum, MD; Yehuda Adler, MD; Nutrit Magal, PhD; Mordechai Shohat, MD

Methods and Results—Patients (n=169) with documented myocardial infarction or angiographically documented CAD who were aged ≤55 years at onset of CAD symptoms and DNA samples from control subjects (n=313) were studied. The prevalence of homozygosity among patients with early CAD onset (aged ≤45 years) was 28%, which was significantly higher than that in patients with later onset (13%) and in control subjects (14%) (odds ratio 2.4, 95% CI 1.24 to 4.69, P=0.006, and odds ratio 2.7, 95% CI 1.15 to 6.42, P=0.01, respectively). Plasma folate was lower in TT homozygotes who had early CAD onset than in those with later onset (P<0.005). Among patients with plasma folate in the lowest quintile (≤12.6 μmol/L), 31% were homozygotes, as were 45% of those with low plasma folate and early CAD onset. There was no difference in the prevalence of traditional risk factors among genotypes. The frequency of homozygosity in patients with ≤1 risk factor was higher than in those with ≥2 risk factors (30% versus 12%, P<0.05). In multiple regression analysis, TT homozygosity and plasma folate were independently associated with CAD, but the impact of folate was small.

Conclusions—Homozygosity for the 677C→T mutation of MTHFR is common and is associated with an increased risk of premature CAD in this population. (Circulation. 1999;100:2406-2410.)

Key Words: genetics ■ coronary disease ■ risk factors ■ myocardial infarction

Hyperhomocysteinemia confers an increased risk of coronary artery disease (CAD),1-3 stroke,4 and deep vein thrombosis5 and is a strong predictor of mortality among patients with ischemic heart disease.6 Methylenetetrahydrofolate reductase (MTHFR) reduces 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the main circulatory form of folate and the methyl donor for homocysteine remethylation, thus regulating the fasting plasma homocysteine level. A common point mutation (677C→T) in the MTHFR gene renders the enzyme thermolabile and less active.7-9 Homozygosity for this mutation is associated with mild hyperhomocysteinemia,7,10-14 particularly among those with low plasma folate levels.10,13,15,16 However, there is uncertainty as to the association between this mutation and CAD.8,10-14,16-25 The present study, undertaken in Israel, examined the association between homozygosity for this mutation and age at onset of CAD.

Methods

We studied 169 Israeli Jewish patients (26 women and 143 men) from our Post Myocardial Infarction Clinic who were aged ≤55 years when they first developed symptoms of CAD and who had angiographically documented CAD (>50% stenosis of at least one epicardial coronary artery) or had suffered a myocardial infarction diagnosed by clinical, ECG, and enzymatic criteria. All the patients were interviewed, and data involving smoking habits, body weight, premature CAD in first-degree relatives, use of medications including vitamins, age at onset of CAD symptoms, anginal status, previous myocardial infarction, hypertension, diabetes mellitus, and dyslipidemia were recorded. After a 6-hour fast, blood was drawn from participants for determination of MTHFR genotype, lipid profile, creatinine, and plasma levels of folate and vitamin B12. All participants underwent coronary angiography for purposes unrelated to the present study. Each angiogram was reviewed for determination of the number of lesions with >50% stenosis and the number of involved coronary arteries. Patients were allocated to 2 groups according to their age at onset of CAD symptoms: early onset, ≤45 years; later onset, 46 to 55 years. For control, DNA samples were collected from 313 random Jewish males and females who underwent prenatal screening testing in the genetic clinic at our center. All were aged <45 years and had no history of CAD.

Coronary risk factors included diabetes mellitus, hypertension, dyslipidemia, smoking, obesity, and family history of premature CAD. The following definitions were used: hypertension, blood pressure >140/90 mm Hg or antihypertensive treatment; dyslipidemia, LDL >130 mg%, HDL <35 mg%, triglycerides >300 mg%.

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TABLE 1. Distribution of MTHFR Genotypes in Patients With Early and Later Onset of CAD and in Control Subjects

<table>
<thead>
<tr>
<th>Genotype, n (%)</th>
<th>Early onset CAD</th>
<th>Later onset CAD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>19 (28)</td>
<td>13 (13)</td>
<td>44 (14)</td>
</tr>
<tr>
<td>CT</td>
<td>32 (48)</td>
<td>53 (52)</td>
<td>139 (44)</td>
</tr>
<tr>
<td>CC</td>
<td>16 (24)</td>
<td>36 (35)</td>
<td>130 (42)</td>
</tr>
<tr>
<td>Total</td>
<td>67 (100)</td>
<td>102 (100)</td>
<td>313 (100)</td>
</tr>
</tbody>
</table>

Genotype, n (%)

TT indicates homozygosity for the mutation; CT, heterozygosity; and CC, normal homozygosity.

Statistical Analysis

The overall frequency of the T (mutant) allele was significantly higher among patients (44.1%) compared with control subjects (36.3%) (OR 1.38, 95% CI 1.05 to 1.83, \( P = 0.02 \)). The distribution of each MTHFR genotype in the 2 groups of patients and in control subjects is shown in Table 1. The prevalence of TT homozygotes was significantly higher among patients with early CAD onset than in control subjects (OR 2.4, 95% CI 1.24 to 4.69, \( P = 0.005 \)) or in patients with later CAD onset (OR 2.7, 95% CI 1.15 to 6.42, \( P = 0.01 \)). There was no difference in the prevalence of TT homozygotes between the group of patients with later CAD onset and control subjects (\( P = 0.87 \)).

Table 2 summarizes the patients’ characteristics according to age at CAD onset. The mean level of plasma folate was significantly lower in patients with early CAD onset than those with later onset (\( P = 0.02 \)). There were no significant differences between the 2 groups in the mean levels of vitamin B\( _12 \), the prevalence of traditional risk factors, the mean number of coronary lesions with \( \geq 50\% \) luminal diameter stenosis, and the mean number of involved vessels.

Table 3 summarizes the patients’ characteristics according to MTHFR genotype. There were no significant differences between TT homozygotes and patients with other genotypes in the mean level of vitamin B\( _12 \), the prevalence of traditional risk factors, the number of coronary lesions with \( \geq 50\% \) luminal diameter stenosis, and the number of involved vessels.

Plasma folate was measured in all participants. Ten patients were current multivitamin users and were excluded from further analysis. The mean plasma level of folate in the 159 multivitamin nonusers was 20.1 \( \pm \) 6.9 nmol/L.

Table 4 indicates the plasma folate levels by genotype for patients with early and later CAD onset. Patients with early CAD onset homozygous for the T allele had lower plasma folate levels than did those with later onset and the TT genotype (\( P = 0.005 \)) and those with early onset and a homozygous normal (CC) genotype (\( P = 0.03 \)). Plasma folate levels did not differ significantly among genotypes in patients with later onset of CAD. The TT genotype was markedly overrepresented among patients with plasma folate levels in the lowest quintile (\( \leq 12.6 \) nmol/L) (Table 5).

The frequency of TT genotype decreased markedly with the increasing number of traditional risk factors. The frequency of homozygosity in patients with 0 to 1 risk factors (30%) was higher than the frequency in patients with 2 risk factors (12%, \( P < 0.05 \)). A family history of premature CAD was reported by 57% of the patients. As shown in Table 6, there were no differences in the prevalence of this or any other specific risk factor among MTHFR genotypes.

Most of our patients (\( n = 151 \)) had myocardial infarction, whereas only 18 patients developed symptoms only. The proportion of patients with symptoms only did not vary by age or by MTHFR genotype. Therefore, the clinical manifestation of CAD could not have had an impact on our findings.

On multiple regression analysis, TT homozygosity and plasma folate were independently associated with early CAD onset compared with later onset, but the association of plasma folate was not as strong.

TABLE 2. Patient Characteristics According to Age at CAD Onset

| Characteristic | Early Onset (\( \leq 45 \) y) | Later Onset (\( 46–56 \) y) | \( P \)
|---------------|-----------------------------|-----------------------------|-----
| No. of lesions | 2.53 \( \pm \) 1.52         | 2.70 \( \pm \) 1.46         | 0.43 |
| No. of involved vessels | 1.90 \( \pm \) 0.90 | 2.03 \( \pm \) 0.90 | 0.37 |
| No. of risk factors | 2.55 \( \pm \) 1.74 | 2.85 \( \pm \) 1.82 | 0.29 |
| Vitamin B\( _12 \), pmol/L | 258 \( \pm \) 102 | 265 \( \pm \) 115 | 0.85 |
| Folate, nmol/L | 18.03 \( \pm \) 8.41         | 21.42 \( \pm \) 8.62         | 0.01 |

Values are mean \( \pm \) SD.

TABLE 3. Patient Characteristics According to MTHFR Genotype

| Genotype | TT | CT + CC | \( P \)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of lesions</td>
<td>2.89 ( \pm ) 1.67</td>
<td>2.57 ( \pm ) 1.44</td>
<td>0.35</td>
</tr>
<tr>
<td>No. of involved vessels</td>
<td>1.96 ( \pm ) 0.94</td>
<td>1.98 ( \pm ) 0.89</td>
<td>0.97</td>
</tr>
<tr>
<td>No. of risk factors</td>
<td>2.62 ( \pm ) 1.59</td>
<td>2.76 ( \pm ) 1.84</td>
<td>0.75</td>
</tr>
<tr>
<td>Vitamin B( _12 ), pmol/L</td>
<td>283 ( \pm ) 140</td>
<td>258 ( \pm ) 101</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Values are mean \( \pm \) SD.

Genetic and Biochemical Analysis

DNA was isolated from peripheral leukocytes with a DNA Isolation Kit for Mammalian Blood (Boehringer-Mannheim). Screening for the 677C \( \rightarrow \) T substitution was performed by polymerase chain reaction of genomic DNA, followed by HindII digestion and agarose gel electrophoresis as described by Frosst et al. \(^7\) Plasma cobalamin (vitamin B\( _12 \)) and folate levels were measured by radioimmunoassay techniques.

The study was approved by the Ethics Committee of the Rabin Medical Center, and informed consent was obtained from each participant.

or lipid-lowering treatment; smoking, current or recent (<1 year before CAD onset); obesity, body mass index >29; and known coagulation disorders (eg, presence of antiphospholipid antibodies) necessitating anticoagulation.

Statistical Analysis

Genotype distributions were examined by \( \chi^2 \) analysis. Comparison of biological parameters between the patients with early CAD onset and later onset was performed by the Wilcoxon rank sum method, and comparison of plasma folate levels according to MTHFR genotype was performed by Kruskal-Wallis ANOVA. Logistic regression analysis was used to calculate the odds ratio (OR) and associated 95% CIs for the frequency of the homozygous genotype (TT) among patients with early and later CAD onset.

Results

The overall frequency of the T (mutant) allele was significantly higher among patients (44.1%) compared with control subjects (36.3%) (OR 1.38, 95% CI 1.05 to 1.83, \( P = 0.02 \)). The distribution of each MTHFR genotype in the 2 groups of patients and in control subjects is shown in Table 1. The prevalence of TT homozygotes was significantly higher among patients with early CAD onset than in control subjects (OR 2.4, 95% CI 1.24 to 4.69, \( P = 0.006 \)) or in patients with later CAD onset (OR 2.7, 95% CI 1.15 to 6.42, \( P = 0.01 \)). There was no difference in the prevalence of TT homozygotes between the group of patients with later CAD onset and control subjects (\( P = 0.87 \)).

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On multiple regression analysis, TT homozygosity and plasma folate were independently associated with early CAD onset compared with later onset, but the association of plasma folate was not as strong.
folate with early CAD onset was much weaker (OR 2.45, 95% CI 0.99 to 6.1, P=0.05, and OR 1.05, 95% CI 1.00 to 1.10, P=0.04, respectively). Plasma folate was modeled as a continuous variable. When plasma folate was not controlled for, the OR for CAD associated with the TT genotype was 2.63 (95% CI 1.15 to 6.04, P=0.02).

The other variables adjusted for in the model were sex (OR 2.31, 95% CI 0.84 to 6.36, P=0.10) and number of coronary risk factors (OR 1.05, 95% CI 0.87 to 1.28, P=0.59). Because of the low prevalence of coronary risk factors and the small size of some patient groups, we could not adequately control for each of these factors. Confounding was addressed, however, by including a term in the model for the number of risk factors. Although there were no differences in any of these factors among genotypes or age groups, it is possible that the control of confounding in this model was not optimal.

### Discussion

We found a significantly higher frequency of homozygosity for the 677C→T mutation of MTHFR in patients with early CAD onset (aged ≤45 years) than in patients with later onset or control subjects. The OR for CAD in this age group was 2.4. Other epidemiological studies that estimated the risk of CAD associated with the TT genotype showed conflicting results. Although some investigators found this mutation to be a significant risk factor for coronary heart disease (OR of 3.1 to 2.1), others found only a mild increase in the risk of CAD or even no increase at all. Summarizing the results of 12 studies, Verhoeft et al reported that 8 of them did not find an increased risk of cardiovascular disease for the TT genotype despite the fact that homozygosity for this mutation is associated with elevated fasting homocysteine levels. However, critical analysis of previous reports highlight methodological differences that could have limited their ability to demonstrate an association between this mutation and the risk of CAD. For example, age was not restricted in most of the studies that found no association between MTHFR polymorphism and CAD, and most of these studies did not include a separate analysis of patients with very early onset of coronary heart disease. Interestingly, only 2 of studies of 4 studies that included mostly younger patients found no association between the TT genotype and CAD. One of them compared the frequency of homozygosity in CAD patients aged <50 years with control subjects and did not find a significant difference in TT distribution between the 2 groups. Another study investigated the risk of myocardial infarction specifically in young women. This patient population was relatively small, and there were significant differences in the prevalence of coronary risk factors between the patients and control subjects. To the best of our knowledge, ours is the first study to assess the relation between MTHFR polymorphism and CAD in patients aged <45 years at the time of CAD onset. Our results are consistent with those of Gallagher et al and Kluitmans et al in Irish and Dutch populations, respectively, and show that the TT mutation is a significant risk factor for very early onset of CAD in the Israeli population as well. Therefore, it is possible that the TT genotype will emerge as a risk factor for premature cardiovascular disease in other populations with a specific genetic background (eg, non-Anglo Saxon) and in those with low folate intake, such as the Dutch, Japanese, and Irish populations as well as ours, where folate supplementation or fortification is not common. Our findings also suggest that an investigation of the association between the TT mutation and coronary heart disease specifically in patients aged ≤45 years is warranted.

We found lower plasma folate levels in patients homozygous for the 677C→T mutation than in those without this genotype. This is consistent with previous reports of low plasma folate in those with homozygosity for this mutation. Additionally, we compared the prevalence of homozygosity between patients with lower and higher plasma folate levels and found a significantly higher prevalence among those with plasma folate in the lowest quintile compared with those with higher levels. There was no difference in the prevalence of homozygosity between patients with plasma folate below versus above the median. van Boxmeer et al also reported a lack of difference in the prevalence of homozygosity between patients with plasma folate below versus above the normal median. However, analysis of the distribution of MTHFR genotypes in other folate strata was not performed in the study of van Boxmeer et al, and folate status was not addressed in any of the studies.

### Table 4. Plasma Folate Levels According to MTHFR Genotype and Age at Onset

<table>
<thead>
<tr>
<th>Plasma Folate Levels, nmol/L</th>
<th>TT (n=31)</th>
<th>CT (n=50)</th>
<th>CC (n=16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early onset CAD</td>
<td>15.0±6.0</td>
<td>19.5±7.0</td>
<td>19.6±6.0</td>
<td>0.04</td>
</tr>
<tr>
<td>Later onset CAD</td>
<td>21.7±5.8</td>
<td>20.3±6.9</td>
<td>22.9±7.3</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Values are mean±SD.

<table>
<thead>
<tr>
<th>Plasma Folate Levels, nmol/L</th>
<th>All Patients (n=159)</th>
<th>Early Onset (n=65)</th>
<th>Later Onset (n=94)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest quintile (&lt;12.6 nmol/L)</td>
<td>10/32 (31.2%)</td>
<td>9/20 (45%)</td>
<td>1/12 (8.3%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Upper 4 quintiles (&gt;12.6 nmol/L)</td>
<td>19/127 (15%)</td>
<td>9/45 (20%)</td>
<td>10/82 (12.2%)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Data indicate number of patients with TT genotype per number in quintile group (and the percentage of patients in that group).
in which MTHFR polymorphism was associated with CAD. The marked overrepresentation of homozygosity among our patients with very low plasma folate probably indicates that the association between MTHFR polymorphism and CAD is stronger in those with low plasma folate levels and that sampling of plasma folate is probably warranted in patients with early CAD onset.

Folate intake was not assessed in the present study. Mean plasma folate in the group of patients with late CAD onset was 21.4 nmol/L; the normal range in our laboratory has been 6 to 38 nmol/L. In the US population, for comparison, plasma folate levels of 9.8 to 16.1 nmol/L in control subjects and 9.1 to 12.4 nmol/L in CAD patients have been reported. However, given the marked differences in plasma folate in control subjects even among the studies in the US population, it is not clear whether the absolute values of plasma folate in different studies are comparable. Therefore, although folate levels in our patients were higher than those reported in the presumably well-nourished US population, this may not necessarily imply that folate intake in our population was better. Moreover, the lower levels of folate in the group of patients with early CAD onset and the TT genotype still indicate that folate intake in these patients was insufficient.

We found no difference among the genotypes in the frequency of traditional coronary risk factors. This is consistent with previous observations. However, there was an inverse relation in our patients between the frequency of the TT genotype and the number of risk factors. This strongly suggests that the association of the TT genotype with CAD is independent of and does not enhance the effect of the classic risk factors.

Our findings are best explained by the effects of the mutation on folate and homocysteine metabolism. The product of MTHFR, 5-methyltetrahydrofolate, is the main circulatory form of folate and the methyl donor for homocysteine remethylation. Homozygosity for the 677C→T mutation is typically associated with lower plasma folate levels and with higher plasma homocysteine levels, particularly among those with low plasma folate and with higher plasma homocysteine levels, particularly among those with low plasma folate levels and that the effects on both plasma folate and homocysteine levels may be more marked when folate intake is low. Because there is some variation in MTHFR activity among those homozygous for the 677C→T mutation, the lower plasma folate levels in the group of patients with early CAD onset and the TT genotype probably reflect lower MTHFR activity as well as insufficient folate intake and indicate that plasma homocysteine was increased in these patients. Our findings may imply, therefore, that the TT genotype, together with suboptimal folate nutrition, has a major effect on CAD pathogenesis, because even young people, with few or no risk factors, are affected, whereas among older people this effect presumably is “swamped” by other risk factors.

The present study has some limitations. The control group was younger than the patient group; therefore, it is possible that some control subjects could develop CAD at a later stage. This would decrease the significance of our findings. However, because the occurrence of CAD at this age is low, the impact on our results should not be significant. In addition, folate levels were measured in all our patients after CAD onset and often after the patients had participated in health education programs. It is unknown to us whether this may have affected folate intake or plasma folate levels, and further education programs. It is unknown to us whether this may have affected folate intake or plasma folate levels, and further study is necessary.

### References

### TABLE 6. Prevalence of Risk Factors by Age at Onset and by MTHFR Genotype

<table>
<thead>
<tr>
<th></th>
<th>Early Onset</th>
<th></th>
<th>Later Onset</th>
<th></th>
<th>Onset Groups</th>
<th>Genotypes</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>TT (n=19)</td>
<td>CT+CC (n=48)</td>
<td>TT (n=13)</td>
<td>CT+CC (n=89)</td>
<td>TT (n=67)</td>
<td>CT+CC (n=137)</td>
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<tr>
<td>Smoking</td>
<td>63</td>
<td>71</td>
<td>58</td>
<td>49</td>
<td>83</td>
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<td>Diabetes</td>
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<td>25</td>
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<td>Dyslipidemia</td>
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<td>75</td>
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<td>Hypertension</td>
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<td>Family history</td>
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<td>65</td>
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<tr>
<td>Obesity</td>
<td>20</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>21</td>
<td>27</td>
</tr>
</tbody>
</table>

*Values are for TT vs CT+CC.


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