Plasma Homocysteine Predicts Mortality Independently of Traditional Risk Factors and C-Reactive Protein in Patients With Angiographically Defined Coronary Artery Disease

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Background—Plasma homocysteine (tHCY) has been associated with coronary artery disease (CAD). We tested whether tHCY also increases secondary risk, after initial CAD diagnosis, and whether it is independent of traditional risk factors, C-reactive protein (CRP), and methylenetetrahydrofolate reductase (MTHFR) genotype.

Methods and Results—Blood samples were collected from 1412 patients with severe angiographically defined CAD (stenosis ≥70%). Plasma tHCY was measured by fluorescence polarization immunoassay. The study cohort was evaluated for survival after a mean of 3.0 ± 1.0 years of follow-up (minimum 1.5 years, maximum 5.0 years). The average age of the patients was 65 ± 11 years, 77% were males, and 166 died during follow-up. Mortality was greater in patients with tHCY in tertile 3 than in tertiles 1 and 2 (mortality 15.7% versus 9.6%, P = 0.001 [log-rank test], hazard ratio [HR] 1.63). The relative hazard increased 16% for each 5-mmol/L increase in tHCY (P = 0.001). In multivariate Cox regression analysis, controlling for univariate clinical and laboratory predictors, elevated tHCY remained predictive of mortality (HR 1.64, P = 0.009), together with age (HR 1.72 per 10-year increment, P = 0.0001), ejection fraction (HR 0.84 per 10% increment, P = 0.0001), diabetes (HR 1.98, P = 0.001), CRP (HR 1.42 per tertile, P = 0.004), and hyperlipidemia. Homozygosity for the MTHFR variant was weakly predictive of tHCY levels but not mortality.

Conclusions—In patients with angiographically defined CAD, tHCY is a significant predictor of mortality, independent of traditional risk factors, CRP, and MTHFR genotype. These findings increase interest in tHCY as a secondary risk marker and in secondary prevention trials (ie, with folate/B vitamins) to determine whether reduction in tHCY will reduce risk. (Circulation. 2000;102:1227-1232.)

Key Words: coronary disease • amino acids • genes • risk factors • inflammation

One third to one half of the variation in vascular disease occurrence remains unexplained by traditional risk factors.1–2 Epidemiological and experimental evidence suggests that homocysteine may be an additional risk factor.2–4 Homocysteine is produced by catabolism of methionine, an essential amino acid, and is further metabolized by transsulfuration (when methionine is in excess) or by remethylation (when methionine is deficient).2 Approximately 70% of the total plasma pool of homocysteine (tHCY) is protein bound, and 25% circulates as the dimer, homocystine.2,5 Severe elevation of tHCY, caused by homozygous defects of homocysteine metabolism, results in vascular injury and premature atherothrombosis but is rare.2,6,7 In contrast, mild to moderate elevation of tHCY is common, but its causes are less well defined. Genetically determined but milder metabolic deficiencies (eg, the thermolabile methylenetetrahydrofolate [MTHFR] gene polymorphism) merit further evaluation.2,8–10 Deficiencies of folate acid and vitamin B₆ may play a role through their association with increased tHCY.11–13 If mild to moderate elevations of tHCY accelerate the progression of coronary artery disease (CAD), these common nutritional and genetic deficiencies, together with other factors, could have substantial impact on risk at a population level.

Epidemiological studies have associated mild to moderate elevations of tHCY with primary cardiovascular risk and have suggested a graded relationship extending into the "normal" range (< 15 μmol/L).2 However, the fewer data from prospective longitudinal cohort studies, which provide better tests of risk association, are less consistent,2 and only one study has evaluated secondary risk, after CAD diagnosis.14 We endeavored to further evaluate tHCY and MTHFR polymorphism as secondary risk predictors.

Methods

Study Objectives
We prospectively tested whether (1) tHCY predicts all-cause mortality, independent of standard risk factors and C-reactive protein (CRP), in patients with angiographic CAD and (2) homozygosity for...
the MTHFR C677T polymorphism predicts tHCY levels and mortality.\textsuperscript{13,16}

### Study Population

The present study consisted of a cohort of 1412 consecutive patients that underwent coronary angiography from August 1994 through August 1997, had severe CAD, consented for the study (approved by the hospital’s institutional review board), had blood drawn at angiography and tHCY measured, and survived hospitalization. Patient information was entered into a computerized database as previously described.\textsuperscript{17} Subjects from our referral area are primarily of European extraction and are genetically similar to other US white individuals.\textsuperscript{18}

### Angiographic CAD

Severe CAD, defined as \( \geq 1 \) stenosis of \( \geq 70\% \) diameter in \( \geq 1 \) coronary artery, was identified by the patient’s attending cardiologist, who was unaware of tHCY level or MTHFR genotype.

### Assessment of Outcomes

Subjects were followed until death or August 1999 (mean cohort follow-up 3.0 \( \pm \) 1.0 years, range 1.5 to 5.0 years). Study subject status was determined by a telephone survey and by a search of a national Social Security database, which was completed on all study subjects by August 20, 1999.

### Laboratory Testing

At the time of angiography, blood samples were collected in EDTA and refrigerated at 4\(^\circ\)C. Within 24 hours, samples were centrifuged, and plasma and DNA were separated and stored cryogenically. Plasma tHCY was measured by a fluorescence polarization immunoassay (Abbott Diagnostics). In a subgroup of 526 patients, genotyping for the MTHFR point polymorphism C677T was performed by polymerase chain reaction amplification of a 198-bp segment that brackets nucleotide 677.\textsuperscript{9} The amplicon was digested with the Hinfl restriction enzyme, and genotypes were determined after electrophoresis in 1.5% agarose.

CRP was initially assayed by a fluorescence polarization immunoassay (Abbott Diagnostics) with use of a high-sensitivity (0.5- to 26-mg/dL) protocol; 95% of healthy individuals have CRP levels \( <0.5 \) mg/dL, and 98% have levels \( <1.0 \) mg/dL.\textsuperscript{20,21}

Lipid panel assays were performed on a Vitros 950 instrument (Johnson & Johnson Clinical Diagnostics). Total cholesterol, triglyceride, and HDL cholesterol concentrations were determined by enzymatic methods. LDL cholesterol was calculated by an indirect method.

### Risk Factors and Definitions

Ten clinical factors were assessed: age, sex, diabetes, history of hypertension, history of hyperlipidemia, family history of CAD, smoking status (current or \( >10 \) pack years), disease presentation at index hospitalization, index treatment, and renal failure. Age was analyzed as a continuous variable. Diabetes was defined as fasting blood sugar \( >126 \) mg/dL, glycosylated hemoglobin \( >7.5\% \), or antidiabetic therapy. Hypertension was defined as a history of systolic blood pressure \( >160 \) mm Hg, diastolic blood pressure \( >90 \) mm Hg, or antihypertensive therapy. Family history was positive if clinical CAD appeared in a first-order relative before the age of 65 years. At index hospitalization, clinical presentation was categorized as stable angina, unstable angina, or myocardial infarction, and clinical treatment was categorized as medical therapy (only), percutaneous coronary intervention, or CABG.

Eight laboratory risk factors were analyzed in addition to tHCY and MTHFR genotype: left ventricular ejection fraction (contrast method or another, if unavailable), systolic and diastolic blood pressures, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and CRP.

### Statistical Considerations

Highest versus lower tertile tHCY groups were compared at baseline by Pearson \( \chi^2 \) (categorical variables) or Student t test (continuous variables), and survival was assessed by the Kaplan-Meier method (log-rank statistic) and Cox regression. Other univariate and multivariate survival analyses were performed by using Cox regression (SPSS, version 9.0); a backward stepwise logistic regression approach was taken for the multivariate analyses, with \( P=0.10 \) as the critical value for entering and excluding variables in the model. Hazard ratios (HRs) and 95% CIs are reported with 2-tailed probability values; \( P<0.05 \) was taken to be nominally significant.

### Results

#### Patient Characteristics

Table 1 shows patient characteristics. The average age of patients was 65 \( \pm \) 11 years, 77% were males, and 166 (11.8\%) died after a mean of 1.4 years (range 0 to 4.8 years). Those with higher tHCY levels (tertile 3) were similar in age, sex, and most other risk factors. They had a less-frequent history of hyperlipidemia, and some differences were noted in lipid fractions; however, total cholesterol levels were similar.

#### Mortality by Baseline tHCY Level

Mortality over follow-up was significantly greater in patients with tHCY in the highest versus the lower 2 tertiles (mortality 15.7\% versus 9.6\%), as shown in the Figure (log-rank statistic 10.1, \( P=0.0014 \)) and Table 2 (HR 1.63, 95% CI 1.20 to 2.22). Absolute tHCY concentration increments also strongly predicted risk (eg, HR 1.16 per 5-\( \mu \)mol/L increment, \( P=0.0007 \)) (Table 2).

#### Other Univariate Predictors

CRP was strongly associated with mortality (HR 1.46 per tertile, \( P=0.0001 \)). Other univariate predictors were age, ejection fraction, diabetes, and number of diseased vessels and lesions (Table 2). A diagnosis of hyperlipidemia paradoxically predicted lower risk after CAD diagnosis, in part because of its correlation with prescription of a statin and younger age. Initial treatment and diastolic blood pressure were marginal predictors. HDL cholesterol (which correlated with female sex and older age) weakly predicted increased risk; total cholesterol (\( P=0.38 \)), LDL cholesterol (\( P=0.48 \)), and triglycerides (\( P=0.14 \)) were not predictors of secondary risk.

#### Multivariate Predictive Models

The 13 clinical and laboratory variables that predicted \( P<0.10 \) univariate risk were entered into multivariate Cox regression analyses. Third-tertile tHCY (HR 1.64, \( P=0.009 \)) was selected as an independent predictor of mortality, together with age (HR 1.72 per 10-year increment, \( P<0.0001 \)), ejection fraction (HR 0.84 per 10% increment, \( P=0.001 \)), diabetes (HR 1.98, \( P=0.001 \)), CRP (HR 1.42 per tertile, \( P=0.004 \)), history of hyperlipidemia (protective), and, marginally, less invasive treatment (Table 3). As a continuous variable, tHCY also was a strong independent predictor (HR 1.16 per 5-\( \mu \)mol/L increment, \( P<0.005 \)) (Table 4).

### tHCY and Mortality by MTHFR Genotype

Neither the presence of an MTHFR mutant allele (HR 1.01, 95% CI 0.65 to 1.57, \( P=0.96 \)) nor C677T homozygosity
(mortality 11.7% for homozygous genotype versus 15.9% for wild-type or heterozygous genotype, HR 0.74, 95% CI 0.34 to 1.62) was associated with increased mortality (Table 5). However, homozygosity was associated weakly with tHCY (Pearson correlation coefficient 0.145, \( P < 0.001 \); mean 18.5 \( \mu \text{mol/L} \), 95% CI 15.7 to 21.3, and median 14.5 \( \mu \text{mol/L} \), for homozygous genotype versus mean 14.9 \( \mu \text{mol/L} \), 95% CI 14.2 to 15.5, and median 12.9 \( \mu \text{mol/L} \), for wild-type or heterozygous genotype; \( P = 0.015 \) \( t \) test). MTHFR homozygosity was weakly predicted by absolute tHCY levels (odds ratio [OR] 1.25 per 5-\( \mu \text{mol/L} \) increment, \( P = 0.0013 \) but less so by third-tertile tHCY (OR 1.69, \( P = 0.059 \) [logistic regression]).

Entry of the MTHFR genotype into Cox bivariate regression analysis did not diminish the predictive value of elevated (top tertile) tHCY (HR 1.84, 95% CI 1.19 to 2.85; \( P = 0.006 \); \( n = 526 \)). Also, the MTHFR genotype was not retained when forced into multivariate risk analyses.

tHCY and Lipids
Patients in tHCY tertile 3 had somewhat lower triglyceride levels (Table 1), carried a diagnosis of hyperlipidemia less frequently (45% versus 54%, Table 1), and tended to be treated less often with lipid-lowering medication (9.5% versus 13.6%) in contrast, they had somewhat higher LDL and slightly lower HDL levels (Table 1). These differences did not affect the independent predictive value of tHCY (Tables 3 and 4).

Discussion
Summary of Study Findings
In this large prospective study of patients with angiographically documented CAD, tHCY was strongly predictive of all-cause mortality. The predictive value of tHCY was independent of traditional risk factors, CRP, and MTHFR genotype and was robust, being present consistently in all primary and supplemental analyses and undiminished when adjusted for multiple other predictive variables. The magnitude of tHCY risk (HR 1.64 per third tertile) approached that of a 10-year increase in age or of diabetes. In contrast, the MTHFR genotype was not associated with mortality, although mutant homozygosity was weakly related to increased tHCY.

Comparison With Previous Studies
An overview of cross-sectional and case-control observational studies (\( n = 4000+ \) subjects, 27 studies) suggested an
association between elevated (>90th percentile) plasma tHcy levels and occurrence of coronary events (OR 1.7), carotid or cerebrovascular atherosclerosis (OR 2.5), peripheral vascular disease (OR 6.8), and aortic atherosclerosis. In contrast, prospective cohort studies have been fewer, and results have been more variable. Eight reports found significant positive associations of tHcy and vascular risk, whereas 6 did not. Most observations were in subjects without evident vascular disease at entry. Because of lack of consistency in the database and measurement standardization, tHcy is not yet recommended for routine risk assessment in primary prevention.

Much less information is available on tHcy as a risk factor in secondary prevention. Only Nygard et al. have studied mortality in patients with angiographically demonstrated CAD, reporting a graded relation between plasma tHcy levels and mortality (P < 0.02). The mortality ratio was 4.5 for

### TABLE 2. Univariate Predictors of Mortality by Cox Regression Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance (P)</th>
<th>HR</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy/μmol/L (n=1412)</td>
<td>0.0007</td>
<td>1.031</td>
<td>1.013</td>
<td>1.049</td>
</tr>
<tr>
<td>tHcy/5 μmol/L</td>
<td>0.0007</td>
<td>1.16</td>
<td>1.07</td>
<td>1.27</td>
</tr>
<tr>
<td>tHcy/third tertile</td>
<td>0.0014</td>
<td>1.63</td>
<td>1.20</td>
<td>2.22</td>
</tr>
<tr>
<td>Age/10 y (n=1412)</td>
<td>&lt;0.0001</td>
<td>1.90</td>
<td>1.60</td>
<td>2.25</td>
</tr>
<tr>
<td>EF/10% (n=1073)</td>
<td>&lt;0.0001</td>
<td>0.76</td>
<td>0.69</td>
<td>0.84</td>
</tr>
<tr>
<td>Vessels diseased (n=1409)</td>
<td>&lt;0.0001</td>
<td>1.55</td>
<td>1.29</td>
<td>1.86</td>
</tr>
<tr>
<td>Lesions ≥70% (n=1409)</td>
<td>&lt;0.0001</td>
<td>1.13</td>
<td>1.07</td>
<td>1.19</td>
</tr>
<tr>
<td>CRP/tertile (n=1372)</td>
<td>0.0001</td>
<td>1.46</td>
<td>1.20</td>
<td>1.77</td>
</tr>
<tr>
<td>History of hyperlipidemia (n=1412)</td>
<td>0.0001</td>
<td>0.54</td>
<td>0.39</td>
<td>0.74</td>
</tr>
<tr>
<td>Total lesions (n=1409)</td>
<td>0.0004</td>
<td>1.09</td>
<td>1.04</td>
<td>1.14</td>
</tr>
<tr>
<td>Diabetes (n=1412)</td>
<td>0.0007</td>
<td>1.79</td>
<td>1.27</td>
<td>2.52</td>
</tr>
<tr>
<td>DBP/10 mm Hg (n=1412)</td>
<td>0.042</td>
<td>0.87</td>
<td>0.77</td>
<td>1.00</td>
</tr>
<tr>
<td>Treatment (n=1408)</td>
<td>0.048</td>
<td>0.84</td>
<td>0.70</td>
<td>1.00</td>
</tr>
<tr>
<td>HDL/mg/dL (n=992)</td>
<td>0.040</td>
<td>1.012</td>
<td>1.001</td>
<td>1.023</td>
</tr>
<tr>
<td>Renal failure (n=1412)</td>
<td>0.068</td>
<td>1.63</td>
<td>0.96</td>
<td>2.77</td>
</tr>
</tbody>
</table>

**Note:** EF indicates ejection fraction; DBP, diastolic blood pressure; Med, medical therapy; and Interv, percutaneous coronary intervention.

Cox regression was used, with entering of only 1 variable (SPSS, version 9.0). The following clinical variables were tested (yes/no [Y/N] unless stated otherwise): age (per 10-y increment) male sex, history of hyperlipidemia, history of hypertension, diabetes, history of smoking, family history, presentation (stable angina, unstable angina, or myocardial infarction), renal failure, and treatment (CABG vs angioplasty vs medical). The following laboratory variables were entered: number of coronary vessels diseased (>1 lesion, ≥70% diameter stenosis per vessel), number of severe (>70% stenosis) lesions, total (severe + moderate) lesions (perfusion), EF (per 10% increment), systolic blood pressure (per 10-mm Hg increment), DBP (per 10-mm Hg increment), total cholesterol (mg/dL), LDL (mg/dL), HDL (mg/dL), triglycerides (mg/dL), CRP (per tertile, patient mean 2.34 mg/dL), tHcy (per 1- or 5-μmol/L increment or third tertile [Y/N]).

### TABLE 3. Multivariate Cox Predictive Model of Mortality Entering tHcy by Tertile (n=1002 Sets With Complete Data, 118 Events)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance (P)</th>
<th>HR</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/10 y</td>
<td>&lt;0.0001</td>
<td>1.72</td>
<td>1.41</td>
<td>2.10</td>
</tr>
<tr>
<td>EF/10%</td>
<td>0.0012</td>
<td>0.84</td>
<td>0.76</td>
<td>0.93</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.0012</td>
<td>1.98</td>
<td>1.31</td>
<td>2.99</td>
</tr>
<tr>
<td>CRP/tertile</td>
<td>0.0038</td>
<td>1.42</td>
<td>1.12</td>
<td>1.80</td>
</tr>
<tr>
<td>tHcy/third tertile</td>
<td>0.0086</td>
<td>1.64</td>
<td>1.13</td>
<td>2.38</td>
</tr>
<tr>
<td>History of hyperlipidemia</td>
<td>0.015</td>
<td>0.61</td>
<td>0.41</td>
<td>0.91</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.050</td>
<td>0.81</td>
<td>0.66</td>
<td>1.00</td>
</tr>
<tr>
<td>Vessels diseased</td>
<td>0.054</td>
<td>1.25</td>
<td>1.00</td>
<td>1.57</td>
</tr>
</tbody>
</table>

**Note:** Multivariate Cox predictive model involves backward stepwise logistic regression (P = 0.10 to remove). Twelve univariate predictors (all with P < 0.10) were initially entered (see Table 2). HDL (which had incomplete data, n=992) was also entered in preliminary analyses but was not selected as independent predictor and was not entered in final analysis.

Multivariate Cox predictive model involves backward stepwise logistic regression (P = 0.10 to remove). Twelve univariate predictors (all with P < 0.10) were initially entered (see Table 2). HDL (which had incomplete data, n=992) was also entered in preliminary analyses but was not selected as independent predictor and was not entered in final analysis.

### TABLE 4. Multivariate Cox Predictive Model of Mortality Entering Continuous tHcy Levels (per 5-μg/dL Increment) (n=1002 in Dataset, 118 Events)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance (P)</th>
<th>HR</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/10 y</td>
<td>&lt;0.0001</td>
<td>1.74</td>
<td>1.42</td>
<td>2.12</td>
</tr>
<tr>
<td>EF/10%</td>
<td>0.0008</td>
<td>0.83</td>
<td>0.75</td>
<td>0.93</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.0019</td>
<td>1.93</td>
<td>1.27</td>
<td>2.90</td>
</tr>
<tr>
<td>CRP/tertile</td>
<td>0.0033</td>
<td>1.42</td>
<td>1.12</td>
<td>1.80</td>
</tr>
<tr>
<td>tHcy/5 μmol/L</td>
<td>0.0046</td>
<td>1.16</td>
<td>1.05</td>
<td>1.29</td>
</tr>
<tr>
<td>History of hyperlipidemia</td>
<td>0.018</td>
<td>0.62</td>
<td>0.41</td>
<td>0.92</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.071</td>
<td>0.82</td>
<td>0.67</td>
<td>1.01</td>
</tr>
<tr>
<td>Vessels diseased</td>
<td>0.071</td>
<td>1.23</td>
<td>0.98</td>
<td>1.55</td>
</tr>
</tbody>
</table>

**Note:** See Table 4 for methods, except that tHcy was entered as absolute values (per 5-μmol/L increment).
persons with the highest (≥20 μmol/L) compared with the lowest (<9 μmol/L) concentration bracket, and tHcy remained predictive after adjustment for traditional risk factors.

Our findings confirm and extend the observations of Nygard et al.14 The present study was larger (by 2- to 3-fold), had greater power, and yielded a highly significant result (P≤0.001). Adjustment was made for multiple traditional clinical factors as well as laboratory variables (lipid levels, number of diseased vessels, and number of coronary lesions). Multivariate analyses also included newer factors (ie, CRP, an inflammatory marker, and MTHFR genotype, a determinant of homocysteine metabolism). In the fully adjusted model, the predictive value of tHcy was undiminished. The projected increase in relative hazard (1.64 for levels >16 μmol/L), although substantial, appears to be less than that reported by Nygard et al but may be quantitatively more accurate.

MTHFR and Risk

MTHFR plays a critical role in homocysteine metabolism. The C677T gene variant has received attention because it specifies a thermolabile enzyme with reduced enzymatic activity and is common (53% of our patients were carriers; 11% were homozygotes). Kluijtmans et al8 reported this variant to be a genetic risk factor for cardiovascular disease, but we and others did not find an association with angiographic CAD in cross-sectional studies.9,10

In the present study, MTHFR polymorphism was not associated with secondary risk (progression to death) in patients with established CAD. However, the analysis is limited by modest statistical power: the 95% CIs allow exclusion of an adverse relative risk of mortality no smaller than ∼50%. Our HR of 1.03 for each 1-μmol/L increase in tHcy projects to a relative hazard of only ∼1.1 for the average increment in homozygotes of 3 μmol/L. Thus, the apparent null finding for MTHFR homozygosity is not surprising. However, even if present, the risk must be small. Also, tHcy values substantially vary within and overlap between genotype groups. Thus, it seems clear that the measurement of tHcy will be more informative in individual risk assessment than will the determination of MTHFR genotype.

CRP and Risk

Ridker et al15 showed that CRP, a nonspecific marker of systemic inflammation, was a prospective predictor of cardiovascular events in healthy US physicians. We found CRP to be associated with CAD and a history of myocardial infarction in a cross-sectional angiographic study.16 In the present study, we demonstrate CRP to be independently predictive of progression to a fatal outcome (secondary risk) in patients with angiographic disease. Although the cause of relative CRP elevation is unknown, it was unassociated with tHcy elevation (correlation coefficient −0.06, P=0.04), suggesting that it does not reflect tHcy-related vascular injury.

tHcy: Risk Potential and Pathophysiologica l Considerations

Boushey et al24 estimated an increase of 5 μmol/L in basal tHcy to be associated with a 60% increase in the odds of coronary heart disease in men, an 80% increase in the odds of coronary heart disease in women, and a 50% increase in the relative risk of cerebrovascular disease. This magnitude of risk is similar to that of a nearly 20-mg/dL increase in cholesterol and could account for ∼10% of the risk of a primary CAD event. Our data suggest that secondary risk attributable to tHcy may be less than this but still highly significant.

Mild-to-moderate increases in tHcy levels in the general population may be related in part to dietary deficiencies of folic acid and vitamins B6 and B12.21,13 The role of common genetic factors, discussed above, remains unresolved.8–10 Perhaps MTHFR and other genetic variants act in concert, together with nutritional deficiencies and other as-yet-undetermined factors.

A causal relation between high tHcy levels and vascular disease is supported by observations from rare genetic diseases and experimental models: tHcy causes direct toxic damage to endothelial cells in both in vitro and in vivo models (including primates). As reviewed by Eikelboom et al,8 endothelial toxic damage may be caused by the generation of reactive oxygen species, impaired production of endothelium-derived NO, stimulation of smooth muscle cell proliferation, elevation of triglycerides and oxidation of LDL, and thrombogenicity associated with platelet adherence and coagulation factor activation.

In a recent study, oral methionine loading reduced flow-mediated brachial artery dilation (by 50%) in a time course paralleling increasing tHcy levels (from 8 to 23 μmol/L) in human subjects.23 In another study, methionine loading activated coagulation, modified adhesive properties of endothelium, and impaired vascular responses to L-arginine in association with moderate increases in tHcy (from 10.5 to 27 μmol/L).26 Pretreatment with vitamin E and ascorbic acid blocked these effects, suggesting an oxidative mechanism of vascular dysfunction.

Although folate with or without B vitamins is capable of decreasing tHcy levels,27,28 no well-designed studies have shown therapy to reduce vascular risk. Randomized clinical trials, currently under way, will better assess the causal role of tHcy in vascular disease and preventive strategies.2,29,30

Study Strengths and Limitations

The present study shares the limitations of nonrandomized observational studies (ie, unsuspected selection biases and

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**TABLE 5. tHcy Levels and Mortality by MTHFR Genotype**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wild Type</th>
<th>Heterozygote</th>
<th>Homozygote*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n (%)</td>
<td>245 (46.6)</td>
<td>221 (42.0)</td>
<td>60 (11.4)</td>
<td></td>
</tr>
<tr>
<td>tHcy, μmol/L</td>
<td>14.7</td>
<td>15.1</td>
<td>18.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Mean 95% CI</td>
<td>13.9–15.6</td>
<td>14.0–16.1</td>
<td>15.7–21.4</td>
<td></td>
</tr>
<tr>
<td>Mortality, n (%)</td>
<td>37 (15.1)</td>
<td>37 (16.7)</td>
<td>7 (11.7)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

tHcy levels were compared by ANOVA (P=0.003) and post hoc test (Dunnett T3, equality of variance not assumed, gives P=0.036 for comparison of mutant heterozygotes with homozygotes and P=0.073 for comparison of wild-type patients with mutant homozygotes). Mortality among groups was compared by Pearson χ² test.

*Homozygous for C677T variant polymorphism.
confounding), but it has the advantage of being large and prospective in design. The univariate association of tHcy with mortality was adjusted for multiple potentially confounding clinical and laboratory risk factors. Risk associations with tHcy were maintained undiminished in these multivariate analyses. Also, the presence and magnitude of associated risk with tHcy are in keeping with epidemiological observations from other2 or similar14 populations. The MTHFR results also are supported by the majority of association studies.9,10 Pathophysiological mechanisms cannot be directly assessed in epidemiological studies but require separate basic and clinical studies.

Conclusions
In patients with angiographically defined CAD, tHcy levels prospectively predicted mortality independent of traditional risk factors, CRP, and MTHFR genotype. This result extends the association between homocysteine and CAD to include mortality risk after CAD diagnosis, verifies the independent nature of its predictive value, and supports the performance of secondary intervention trials aimed at tHcy reduction.

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