Clinical Investigation and Reports

Myocardial Infarction in Young Women in Relation to Plasma Total Homocysteine, Folate, and a Common Variant in the Methylenetetrahydrofolate Reductase Gene

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Background In a population-based study, we examined the relationship between the risk of myocardial infarction (MI) among young women and plasma total homocysteine (tHCY), folate, vitamin B_{12}, and a common cytosine (C) to thymine (T) polymorphism in the gene for 5,10-methylenetetrahydrofolate reductase (MTHFR).

Methods and Results In-person interviews and nonfasting blood samples were obtained from 79 women <45 years old diagnosed with MI and 386 demographically similar control subjects living in western Washington state between 1991 and 1995. Compared with control subjects, case patients had higher mean tHCY concentrations (13.4±5.2 versus 11.1±4.4 μmol/L, P=.0004) and lower mean folate concentrations (12.4±13.4 versus 16.1±12.2 nmol/L, P=.018). There was no difference in vitamin B_{12} concentrations between case patients and control subjects (346.8±188.4 versus 349.7±132.4 pmol/L, P=.90). After adjusting for cardiovascular risk factors, we found that women with tHCY ≥15.6 μmol/L were at approximately twice the risk of MI as women with tHCY <10.0 μmol/L (OR, 2.3; 95% CI, 0.94 to 5.64). Women with folate ≥8.39 nmol/L had an ≈50% lower risk of MI than women with folate <5.27 nmol/L (OR, 0.54; 95% CI, 0.23 to 1.28). There was no association with vitamin B_{12} concentration. Among control subjects, 12.7% were homozygous for the *MTHFR* T^{677} allele, and these women had higher plasma tHCY and lower plasma folate than women with other genotypes. Ten percent of case patients were homozygous for

te T^{677} allele, and there was no association of homozygosity for T^{677} with MI risk (OR, 0.90; 95% CI, 0.31 to 2.29).

Conclusions These data support the hypothesis that elevated plasma tHCY and low plasma folate are risk factors for MI among young women. Although homozygosity for *MTHFR* T^{677} is related to increased plasma tHCY and low plasma folate, this genetic characteristic is not a risk factor for MI in this population. (***Circulation.* 1997;96:412-417.**)

Key Words • myocardial infarction • women • genetics • homocysteine • folate

Homocysteine is a thiol-containing metabolite of methionine with atherogenic and thrombotic properties.[1] Studies conducted primarily among young to middle-aged men indicate that mildly elevated blood levels of tHCY—the sum of protein-bound or free forms of homocysteine and its disulfides, homocysteine and cysteine-homocysteine—are a risk factor for CHD, stroke, and possibly other forms of arterial and venous vascular disease.[2] Low blood levels of folate appear to be a particularly strong environmental determinant of tHCY levels in many populations.[2]

Genetic factors also contribute to tHCY levels, and recent studies have focused on a common inherited variation in the enzyme MTHFR. MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a reaction that contributes substrates for the remethylation of homocysteine to methionine by methionine synthase. Kang et al[3-5] reported that up to 5% of the population has an inherited thermolabile form of MTHFR, one that is associated with reduced enzyme activity and premature CHD. Thermolabile MTHFR reportedly accounts for ≈25% to 30% of elevated tHCY in patients with premature vascular disease.[6] A common single-base-pair change, cytosine (C) to thymine (T), at nucleotide 677 of the MTHFR gene was recently identified,[7] and persons homozygous for the T allele were more likely to have thermolabile MTHFR and elevated tHCY than persons with other genotypes. Thus, the $C^{677}\rightarrow T$ polymorphism in the MTHFR gene may be a genetic risk factor for premature cardiovascular disease.[7,8]

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Selected Abbreviations and Acronyms

CHD = coronary heart disease
CI = confidence interval
MI = myocardial infarction
MTHFR = 5,10-methylenetetrahydrofolate reductase
OR = odds ratio
tHCY = total homocysteine

Although the strong inverse correlation between folate and tHCY supports the hypothesis that reduced folate status may be a risk factor for CHD, those few studies that examined this relationship have yielded conflicting results.[9-14] Similarly, while some investigations have found the risk of vascular disease to be increased among persons homozygous for the *MTHFR* T^{677} allele,[15-17] others have not.[18-20] We studied the relationship of plasma tHCY, plasma folate, and the *MTHFR* $C^{677}\rightarrow T$ polymorphism to the risk of acute MI among young women in a population-based case-control study.

Methods

Subjects

The data for this report were drawn from a study of incident cardiovascular disease (MI and stroke) among women 18 to 44 years old residing in King, Pierce, and Snohomish counties, Washington state. Eligible MI case patients were women diagnosed with a first fatal or nonfatal MI between July 1, 1991, and February 28, 1995, who had no prior history of major CHD or stroke. We identified potential case patients through monthly review and abstraction of discharge diagnoses of acute ischemic heart disease provided by all hospitals within the study region, incident reports from emergency medical service systems, and death certificates listing out-of-hospital deaths from cardiovascular disease and related conditions. We identified 208 eligible MI patients with definite or probable MI on the basis of the criteria used by the Cardiovascular Health Study,[21] of whom 161 were living at the time that we initiated recruitment. We recruited 107 of these women into the study, 4 were not approached at the request of their physicians, and the remainder either refused to participate (n=40) or could not be located (n=10).

We used random-digit telephone dialing to identify a sample of women 18 to 44 years old who were residents of King, Pierce, or Snohomish counties during the case diagnosis period. Briefly, telephone numbers were generated at random with a computer algorithm, and a household census to ascertain women meeting the eligibility criteria was completed for 94.9% of the residences contacted. Among the eligible women identified, we attempted to enroll 691 at random, frequency matched to the combined age distribution of all cardiovascular disease patients recruited for the study. Only 1 woman from each household was selected for recruitment. Seven of the 691 women were excluded because of a prior history of major cardiovascular disease (n=6) or inability to communicate in English (n=1). Of the remaining 684 women, 526 were recruited into the study, for an estimated overall response rate of 72.8% (94.9% of 526/684).

Data Collection

Participating case patients and control subjects were interviewed in person regarding histories of known or suspected cardiovascular risk factors, including histories of physician-diagnosed diabetes, hypertension, or high cholesterol; cigarette smoking; height and weight; menstrual history; contraceptive practices; alcohol consumption; physical activity; history of MI in first-degree relatives; and demographic characteristics. No information was collected on dietary or nutritional supplement sources of vitamins. The structured interview elicited information only from the time period before the MI in each case patient. Hence, in this report we use the term "current" to describe characteristics of each patient as of the date that she had her MI (or equivalent date for control subjects). In addition to the in-person interview, we also obtained a 30-mL venous blood specimen from 79 MI case patients and 391 control subjects into EDTA-treated vacuum tubes; aliquots of plasma and buffy coat were frozen at −70°C. Case blood samples were obtained at least 3 months after the event (mean, 8 months; median, 6.5 months).

Laboratory Analyses

Plasma tHCY concentrations were determined by high-pressure liquid chromatography and electrochemical detection as previously described.[22] Plasma folate and vitamin B_{12} concentrations were measured with the Quantaphase II Assay system (Bio-Rad Laboratories). Genomic DNA was extracted from buffy-coat aliquots by established methods.[23] The $C^{677}\rightarrow T$ variation in the MTHFR gene was determined as described by Frosst et al.[7] tHCY measurements were available for 79 case patients and 386 control subjects, folate and vitamin B_{12} measurements were available for 77 case patients and 382 control subjects, and MTHFR genotyping for 79 case patients and 379 control subjects. We also measured HDL cholesterol, LDL cholesterol, and triglycerides, by standard methods, on a subset of case patients (n=63) and control subjects (n=140) selected at random.[24] All laboratory analyses were conducted blind as to whether a sample came from a case patient or control subject.

Statistical Analysis

For initial analyses of the relationship between MI risk and concentrations of tHCY, folate, and vitamin B_{12}, we compared case patients and control subjects with respect to the mean concentrations of these compounds. The distributions of folate and vitamin B_{12} were skewed, but because case-control comparisons of geometric means and arithmetic means yielded identical results, only the latter are presented. For the risk of MI associated with tHCY, folate, and vitamin B_{12}, we classified the data into approximate quartiles based on the distribution of these compounds among case patients. We then used unconditional logistic regression models to estimate ORs and 95% CIs for each quartile.[25] For each of these compounds, we investigated potential confounding by age (in years); education (less than college, college, postcollege); ethnicity (non-Hispanic white, black, other); cigarette smoking (current, past, never); obesity (body mass index $\geq$27.3 kg/m^2 versus <27.3 kg/m^2); currently receiving medical treatment for hypertension, diabetes, or high cholesterol; menopausal status (postmenopausal versus premenopausal); average frequency of alcohol use in the previous year (three or more times per week, less than three per week, none); average frequency of vigorous exercise in the previous year (once per week, less than once per week, never); and current use of oral contraceptives (yes, no). Potential confounding by plasma lipid measures was investigated within the subset of case patients and control subjects with data available on those characteristics. Except for age, terms for confounders were retained in the model if they produced an important change in the coefficient for a particular plasma measurement. To evaluate the extent to which the pattern of ORs for tHCY, folate, or vitamin B_{12} were consistent with a linear trend in risk, we computed the difference in the log-likelihoods between hierarchical models containing three indicator terms and models containing a single term with four levels (0, 1, 2, 3) corresponding to each quartile. In this approach, the smaller the difference in log-likelihoods, the stronger the evidence that the patterns of ORs are consistent with a linear trend. We also examined the extent to which associations with tHCY, folate, and vitamin B_{12} varied by

TABLE 1. Distribution of Demographic Characteristics and Cardiovascular Risk Factors Among MI Case and Control Subjects

Characteristic	Case Patients (n=79)	Control Subjects (n=386)
Age, y		
18-29	1 3	8 0
30-34	12 7	13 2
35-39	22 8	32 1
40-44	63 3	46 6
Ethnicity		
White, not Hispanic	87 3	89 1
Black	6 3	2 3
Other	6 3	8 6
Education		
Less than college	58 2	27 5
College	35 4	55 7
Postcollege	6 3	16 8
Cigarette smoking		
Current	69 6	21 1
Past	16 5	23 7
Never	13 9	55 2
Currently receiving treatment for		
Hypertension	16 5	2 3
Diabetes	6 3	0 5
High cholesterol	2 5	0 5
Body mass index ≥27 3 kg/m^2	58 2	27 2
Postmenopausal	32 9	11 1
Current oral contraceptive use	5 1	10 6
First-degree relative with history of MI	54 4	29 8
Frequency of vigorous exercise*		
≥3 times/wk	7 7	24 2
Some but <3 times/wk	23 1	39 8
None	69 2	35 9
Frequency of alcohol consumption*		
≥1 time/wk	32 4	38 7
Some but <1/wk	49 4	39 5
None	18 2	21 8

Values are percent Percents may not add up to 100 because of rounding

*Average over year before diagnosis or reference date

whether a woman was a current cigarette smoker, obese, or had a first-degree relative with a history of MI, these characteristics were the only established cardiovascular risk factors for which we had sufficient numbers of case patients and control subjects to investigate heterogeneity in risk Likelihood ratio tests were used to estimate the extent to which chance might account for any differences we observed in associations between analytes and MI risk according to smoking, obesity, or family history status

We examined the distribution of MTHFR genotypes among control subjects and the relationship of genotype to tHCY, folate, and vitamin B_{12} concentrations ORs for the association of homozygous T^{677} and heterozygous T^{677} genotypes, compared with homozygous C genotypes, were estimated by the Mantel-Haenszel method All analyses of MTHFR genotypes were restricted to non-Hispanic whites to reduce the influence of genetic heterogeneity on our results

Results

The study population was largely white, not of Hispanic origin, but blacks were overrepresented among the case patients (Table 1). Case patients were much more likely than control subjects to be currently receiving medications for hypertension, diabetes, or high cholesterol; to be postmenopausal; and to have a first-degree relative with a history of MI. Cigarette smoking, obesity, and family history of MI were extremely common among case patients. ≈70%, 60%, and 54% of the patients reported these characteristics, respectively, compared with ≈21%, 27%, and 30% of control subjects. Case patients also tended to have less formal education and were less likely to be current users of oral contraceptives and to participate in regular vigorous physical activity than control subjects

Compared with control subjects, case patients had higher mean tHCY concentrations (13.4±5.2 versus 11.1±4.4 μmol/L, P=.0004) and lower mean folate concentrations (12.4±13.4 versus 16.1±12.2 nmol/L, P=.018) There was no difference in vitamin B_{12} concentrations between case patients and control subjects (346 8±188.4 versus 349.7±132.4 pmol/L, P=.90). Among control subjects, tHCY concentrations were inversely associated with concentrations of folate after adjustment for vitamin B_{12} (r=−.397, P=.0001), whereas vitamin B_{12} concentrations were not related to tHCY after adjustment for folate (r=−.082, P=.11).

The risk of MI adjusted for age, diabetes, cigarette smoking, and obesity among young women increased with increasing quartile of tHCY and decreased with increasing quartile of folate (Table 2), the patterns of

TABLE 2. Adjusted Risk of MI Among Young Women Associated With Quartiles of tHCY, Folate, and Vitamin B_{12} Concentrations

	Quartile			
Plasma Measure	I	II	III	IV
tHCY, μmol/L				
No cases No controls	19 187	20 106	21 57	19 36
Range/median	4 05 9 99/8 23	10 00-12 59/11 17	12 60-15 59/13 91	15 60 65 85/21 18
OR (95% CI)*	1 00	1 34 (0 63, 2 82)	1 98 (0 89, 4 43)	2 30 (0 94, 5 64)
Folate, nmol/L				
No cases No controls	19 40	19 76	19 100	20 166
Range/median	1 90-5 26/4 43	5 27-8 38/6 65	8 39-13 92/9 83	13 93-82 80/19 78
OR (95% CI)*	1 00	0 72 (0 29, 1 71)	0 47 (0 20, 1 12)	0 54 (0 23, 1 28)
B_{12}, pmol/L				
No cases No controls	19 75	19 92	19 72	20 142
Range/median	67-229/181	230-311/289	312-384/342	385-1492/504
OR (95% CI)*	1 0	0 92 (0 38, 2 2)	1 23 (0 50, 3 0)	0 66 (0 29, 1 56)

*Adjusted for age (continuous), cigarette smoking (current, former, never), currently taking medicine for diabetes, and body mass index (<27 3 kg/m^2 vs ≥27 3 kg/m^2)

risk for both tHCY and folate were consistent with a monotonic trend based on likelihood ratio tests (tHCY, $\chi^2_{2df}=0.401$, $P=.818$; folate, $\chi^2_{2df}=1.68$, $P=.432$). When adjusted for tHCY, the association with folate was weakened; the ORs for folate of ≥13.93 nmol/L, 8.39 to 13.92 nmol/L, and 5.27 to 8.38 nmol/L were 0.76 (95% CI, 0.29 to 1.96), 0.56 (95% CI, 0.23 to 1.38), and 0.79 (95% CI, 0.32 to 1.96), respectively. There was little relationship between vitamin B_{12} concentration and MI risk. These results were essentially unchanged when we adjusted for menopausal status, race, treated hypertension, treated high cholesterol, exercise, alcohol consumption, or oral contraceptive use. Furthermore, among the women for whom we had measures of plasma lipid concentrations, controlling for these measures did not alter the relationships with tHCY, folate, or vitamin B_{12}. The results also did not differ after exclusion of the few women who were being treated for hypertension or were taking oral contraceptive pills. We also conducted analyses in which we (1) excluded the 20% of case patients for whom the blood draw was performed relatively close to the event (3 to 4 months) or relatively distant from the event (≥14 months) and (2) estimated associations separately for case patients with blood drawn within 6.5 months of the event and for case patients with blood drawn after this interval; these analyses resulted in trivial changes in the associations.

The ORs for elevated tHCY (≥12.6 μmol/L) among smokers (55 case patients and 83 control subjects) and nonsmokers (24 case patients and 303 control subjects) were similar: 1.92 (95% CI, 0.91 to 4.96) and 1.86 (95% CI, 0.66 to 5.22) (heterogeneity, $\chi^2_{1df}=0.059$, $P=.808$), as were the ORs among women with (43 case patients and 112 control subjects) and without (34 case patients and 266 control subjects) a family history of MI: 2.07 (95% CI, 0.89 to 4.82) and 2.07 (95% CI, 0.91 to 4.73) (heterogeneity, $\chi^2_{1df}=0.000$, $P=.999$). Among obese women (46 case patients and 104 control subjects), there was no association with tHCY ≥12.6 μmol/L (OR, 1.14; 95% CI, 0.48 to 2.71), whereas among nonobese women (33 case patients and 279 control subjects), the OR was elevated (OR, 3.20; 95% CI, 1.35 to 7.57) (heterogeneity, $\chi^2_{1df}=4.451$, $P=.035$). The ORs for elevated folate (≥8.39 nmol/L) did not vary according to whether or not the woman smoked, was obese, or had a family history of MI.

About one eighth (12.7%) of the 338 non-Hispanic white control subjects were homozygous and 41.7% were heterozygous for the *MTHFR* T^{677} allele (Table 3). Folate concentrations were 30% lower and tHCY concentrations were 25% higher among women homozygous for *MTHFR* T^{677} compared with women possessing at least one copy of the C^{677} allele. The excess tHCY concentration among *MTHFR* T^{677} homozygotes was present only among women with low plasma folate (<8.39 nmol/L). Among all non-Hispanic white control subjects, 30% of women with tHCY ≥15.6 μmol/L (the 90th percentile among control subjects), compared with 10% of women with tHCY below this level, were homozygous for *MTHFR* T^{677}. Among the non-Hispanic white MI case patients, *MTHFR* T^{677} homozygotes had tHCY levels that were similar to other case patients (mean±SD, 13.2±6.9 versus 13.4±4.9 μmol/L, respectively) but had lower folate levels (mean±SD, 10.3±5.9 versus 13.0±14.8 μmol/L, respectively).

TABLE 3. Plasma Folate and tHCY Concentrations Among Control Subjects* by *MTHFR C/T*^{677} Genotype

	Genotype		
Plasma Measurement	***CC* (n=154)**	***CT* (n=141)**	***TT* (n=43)**
Folate, nmol/L	16.43 (11.81)	16.36† (13.02)	11.08‡ (9.56)
tHCY, μmol/L	10.85 (3.79)	10.76† (3.91)	13.48‡ (7.00)
By folate concentration, nmol/L	***CC+CT***		
<8.39 (n=100)	12.71 (4.50)		18.12§ (8.36)
8.39-15.59 (n=108)	11.45 (4.13)		10.96§ (2.41)
≥15.60 (n=109)	9.18 (2.50)		7.35§ (2.05)

Values are mean (SD) plasma concentration.
*Restricted to non-Hispanic whites.
†Compared with *CC* genotype, $P=.95$ for *t* test on difference in mean folate and $P=.70$ for *t* test on difference in mean tHCY.
‡Compared with *CC* genotype, $P=.02$ for *t* test on difference in mean folate and $P=.02$ for *t* test on difference in mean tHCY.
§Compared with *CC* and *CT* genotypes combined, $P=.013$ for folate <8.39 nmol/L, $P=.513$ for folate 8.39-15.59 nmol/L, $P=.063$ for folate ≥15.60 nmol/L.

The distribution of *MTHFR* $C^{677}\rightarrow T$ genotype was similar among non-Hispanic white case patients and control subjects, and the risk of MI was not associated with homozygosity or heterozygosity for the T^{677} allele (Table 4). This result was unchanged when we excluded the women who reported currently receiving medication for hypertension, diabetes, or high cholesterol. No association was observed after the population was stratified according to plasma folate level (data not shown).

Discussion

We observed an increasing risk of MI among young women with increasing plasma tHCY concentration, and in particular an approximately twofold increased risk for women with plasma tHCY concentrations of ≥15.6 μmol/L (the 90th percentile of the distribution among control subjects in our study). This association is consistent with the accumulated evidence supporting tHCY as a risk factor for CHD but is somewhat weaker than has been observed in the few, relatively small previous studies that presented results for women.[11,12,26,27] Important features of these earlier studies that differ from the present report are the inclusion of (1) older women (predominantly or entirely postmenopausal),[12,26] (2) case patients (but not control subjects) required to have a strong family history of CHD,[27] and (3) patients who have CHD but not necessarily MI.[11,12,26,27] That traditional risk factors such as cigarette smoking and obesity appear to account for the great majority of MI case patients in very young women could also contribute to our finding a weaker association between MI and tHCY than might be predicted from previous studies. Regarding the possible modifying role of these important estab-

TABLE 4. Risk of MI Among Young Women* According to *MTHFR C/T*^{677} Genotype

Genotype	Case Patients	Control Subjects	OR (95% CI)
CC	28 (40.6)	154 (45.6)	1.00
CT	34 (49.3)	141 (41.7)	1.33 (0.74, 2.39)
TT	7 (10.1)	43 (12.7)	0.90 (0.31, 2.29)

Values are No. of women (%).
*Restricted to non-Hispanic whites.

lished risk factors, neither of the two previous studies of tHCY and CHD that investigated whether the relationship varies among persons with and without other CHD risk factors reported results by obesity,[13,28] whereas Verhoef et al[13] found no variation by smoking status.

The ≈50% reduction in risk we observed with folate concentrations ≥8.39 nmol/L (the 30th percentile of the distribution among control subjects) is consistent with most previous studies,[9,10,13,14] but other investigations have not observed any relationship between plasma folate and CHD.[11,12] The absence of a relationship between plasma folate and CHD in some previous studies[11,12] may reflect differences among populations in the importance of other vitamin determinants of tHCY concentrations, such as pyridoxal-5′-phosphate. It is possible that our findings are to some extent confounded by unmeasured nutritional factors (eg, vitamin E) that may influence CHD risk through other mechanisms. However, adjustment of folate for tHCY weakened the inverse relationship with folate, suggesting that the effect of folate on tHCY levels is responsible for the observed trend in risk with folate levels. We did not observe any difference in vitamin B_{12} concentrations between case patients and control subjects, consistent with most previous reports.[9,11-13]

Our findings for tHCY, folate, or vitamin B_{12} may not reflect the relationships with MI that would be observed had we used a prospective study design, but such an approach is not feasible given the extremely infrequent occurrence of MI in young women. We did not attempt to measure whether or how the diets of MI patients had changed after their events. If diets of patients improved and included higher intake of folate, our results would represent underestimates of the true associations. Alternatively, if the diets of patients included less folate than before the MI, our results might overestimate the true associations. Given the generally good agreement between results of previous prospective and retrospective studies of tHCY and CHD, it seems unlikely that the associations we observed are very different from those that we would have found had measurements been obtained before the MI. Our participation rates were relatively low, but our results would be biased only to the extent that case patients and control subjects would differ with respect to the association between participation and plasma tHCY, folate, and vitamin B_{12} concentrations. Among the women we interviewed, we did not identify any differences in demographic characteristics or cardiovascular risk factors between the women who did and did not provide a blood sample (data not shown). Finally, elevated tHCY could be part of the causal path through which smoking, diabetes, and obesity exert their respective causal effects on MI, and thus we may have underestimated the association with tHCY when we included these characteristics in our logistic models. However, there are other, more firmly established mechanisms through which smoking, diabetes, and obesity affect MI risk. In addition, inclusion of tHCY in the logistic regression models caused minimal change in the coefficients for these factors (data not shown), making it unlikely that elevated tHCY mediates more than a very small proportion of the effect of smoking, diabetes, and obesity on MI risk in our population. Hence, adjustment for smoking, diabetes, and obesity was necessary to avoid overestimating the independent association between tHCY and MI in young women.

Consistent with previous investigations in other populations,[7,15,20,29,30] we observed that young women in the general population carrying two copies of the *MTHFR* T^{677} allele had nonfasting plasma tHCY concentrations that were ≈25% higher than women with other genotypes. In addition, we found, as have others,[20,29] that the difference in plasma tHCY concentrations between *MTHFR* T^{677} homozygotes and persons with other genotypes is limited to individuals with low plasma folate levels. One previous study also reported that this pattern held when persons were classified according to use of multivitamins,[20] providing evidence that the *MTHFR* $C^{677}\rightarrow T$ polymorphism and folate intake interact to cause elevated tHCY. Randomized feeding studies, however, would be the strongest design to test the hypothesis that the relationship between folate intake and tHCY is modified by an individual's *MTHFR* C^{677}/T genotype.

Although homozygosity for the *MTHFR* T^{677} allele was significantly associated with increased plasma tHCY in our population, we did not observe an increased risk of MI among women possessing this genotype. Three recent studies also did not find an association between the MTHFR polymorphism and CHD,[18-20] in contrast to three studies that have reported twofold to threefold increased risks of CHD or other vascular disease among those carrying two copies of the T^{677} allele.[15-17] The seven reports to date have examined different manifestations of vascular disease and different sexes and ages of patients, and varied considerably in size, yet these design features do not clearly distinguish studies that have observed an association from those that have not. Positive and negative studies also do not appear to differ consistently in the contribution of *MTHFR* T^{677} to elevated tHCY: 40% and 16% of subjects with elevated tHCY carried two copies of the *MTHFR* T^{677} allele in the Danish[15] and Irish[17] studies, respectively, compared with 21% and 30% in the Physicians Health Study[20] and our study, respectively. If the association between the MTHFR polymorphism and elevated tHCY depends on the amount of 5,10-methylenetetrahydrofolate available to the enzyme, it may be that an association between the homozygous T^{677} genotype will be observed only among persons with low folate intake. Two studies that reported no overall association with homozygosity for *MTHFR* T^{677} also did not find compelling evidence of an association among persons with low folate intake,[19,20] but the sample sizes were limited. Populations also may differ in the extent to which homozygosity for *MTHFR* T^{677} is associated with the thermolabile MTHFR phenotype. In that regard, it is interesting to note that those studies that observed an association were conducted in populations that potentially are more genetically homogeneous[15-17] than those studies (including ours) that failed to find an association.[18-20] Finally, given that clinical trials have not been conducted to determine whether lowering tHCY levels can reduce cardiovascular disease occurrence, the causal role of this risk factor has not been fully established. If tHCY in fact is not causally related to cardiovascular disease, then no association would be expected between MI risk and the $C^{677}\rightarrow T$ polymorphism in the MTHFR gene.

The growing evidence supporting a role for low folate in the occurrence of cardiovascular disease is likely to lead to randomized trials to provide definitive tests of this hypothesis [31] Whether individuals who carry two copies of the *MTHFR* T^{677} allele are particularly susceptible to the putative adverse cardiovascular effects of low folate is still unclear Thus, randomized trials should be complemented by additional experimental and observational studies designed to clarify the role of this genetic characteristic and other potential inherited influences on folate metabolism in the association between folate, tHCY, and cardiovascular disease risk

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References

1 Rees MM, Rodgers GM Homocysteinemia association of a metabolic disorder with vascular disease and thrombosis *Thromb Res* 1993,71 337-359
2 Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG A quantitative assessment of plasma homocysteine as a risk factor for vascular disease probable benefits of increasing folic acid intakes *JAMA* 1995,274 1049 1057
3 Kang SS, Wong PW, Zhou J, Sora J, Lessick M, Ruggie N, Grcevich G Thermolabile methylenetetrahydrofolate reductase in patients with coronary artery disease *Metabolism* 1988,37 611-613
4 Kang S S, Wong PWK, Susmano A, Sora J, Norusis M, Ruggie N Thermolabile methylenetetrahydrofolate reductase an inherited risk factor for coronary artery disease *Am J Hum Genet* 1991,48 536-545
5 Kang S-S, Passen EL, Ruggie N, Wong PWK, Sora H Thermo labile defect of methylenetetrahydrofolate reductase in coronary artery disease *Circulation* 1993,88 1463-1469
6 Engbersen AMT, Franken DG, Boers GHJ, Stevens EMB, Trijbels FJM, Blom HJ Thermolabile 5,10 methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia *Am J Hum Genet* 1995,56 142-150
7 Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer H, Kluijtmans LAJ, van den Heuvel LP, Rozen R A candidate genetic risk factor for vascular disease a common mutation in methylenetetrahydrofolate reductase *Nat Genet* 1995,10 110 113
8 Motulsky AG Nutritional ecogenetics homocysteine-related arteriosclerotic vascular disease, neural tube defects, and folic acid *Am J Hum Genet* 1996,58 17-20
9 Pancharuniti N, Lewis CA, Sauberlich HE, Perkins LL, Go RC, Alvarez JO, Macaluso M, Acton RT, Copeland RB, Cousins AL, Gore TB, Cornwell PE, Roseman JM Plasma homocyst(e)ine, folate, and vitamin B 12 concentrations and risk for early-onset coronary artery disease *Am J Clin Nutr* 1994,59 940-948
10 Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PWF, Belanger AJ, O Leary DH, Wolf PA, Schaefer EJ, Rosenberg IH Association between plasma homocysteine concentrations and extracranial carotid artery stenosis *N Engl J Med* 1995,332 286-291
11 Dalery K, Lussier Cacan S, Selhub J, Davignon J, Latour Y, Genest J Homocysteine and coronary artery disease in French Canadian subjects relation with vitamins B12, B6, pyridoxal phosphate, and folate *Am J Cardiol* 1995,75 1107-1111
12 Robinson K, Mayer EL, Miller DP, Green R, Lente F, Gupta A, Kottke-Marchant K, Savon SR, Selhub J, Nissen SE, Kutner M, Topol EJ, Jacobsen DW Hyperhomocysteinemia and low pyridoxal phosphate common and independent reversible risk factors for coronary artery disease *Circulation* 1995,92 2825-2830
13 Verhoef P, Stampfer MJ, Buring JE, Gaziano JM, Allen RH, Stabler SP, Reynolds RD, Kok FJ, Hennekens CH, Willett WC Homocysteine metabolism and risk of myocardial infarction relation with vitamins B6, B12, and folate *Am J Epidemiol* 1996, 143 845-859
14 Morrison HI, Schaubel D, Desmeules M, Wigle DT Serum folate and risk of fatal coronary heart disease *JAMA* 1996,275 1893-1896
15 Kluijtmans LA, van den Heuvel LPWJ, Boers GHJ, Frosst P, Stevens EMB, van Oost BA, den Heijer M, Trijbels FJ, Rozen R, Blom HJ Molecular genetic analysis in mild hyperhomocysteinemia a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease *Am J Hum Genet* 1996,58 35-41
16 DeFranchis R, Mancini FP, D'Angelo A, Sebastio G, Fermo I, DeStefano V, Margaglione M, Mazzola G, DiMinno G, Andria G Elevated total plasma homocysteine and 677C-T mutation of the 5,10-methylenetetrahydrofolate reductase gene in thrombotic vascular disease *Am J Hum Genet* 1996,59 262-264
17 Gallagher PM, Meleady R, Shields DC, Tan KS, McMaster D, Rozen R, Evans A, Graham IM, Whitehead AS Homocysteine and risk of premature coronary heart disease *Circulation* 1996,94 2154-2158
18 Wilcken DE, Wang XL, Sim AS, McCredie RM Distribution in healthy and coronary populations of the methylenetetrahydrofolate reductase (MTHFR) $C_{677}T$ mutation *Arterioscler Thromb Vasc Biol* 1996,16 878 882
19 Schmitz C, Lindpaintner K, Verhoef P, Gaziano JM, Buring J Genetic polymorphism of methylenetetrahydrofolate reductase and myocardial infarction a case-control study *Circulation* 1996,94 1812-1814
20 Ma J, Stampfer MJ, Hennekens CH, Frosst P, Selhub J, Horsford J, Malinow MR, Willett WC, Rozen R Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians *Circulation* 1996,94 2410-2416
21 Ives DG, Fitzpatrick AL, Bild DE, Psaty BM, Kuller LH, Crowley PM, Cruise RG, Theroux S Surveillance and ascertainment of cardiovascular events the Cardiovascular Health Study *Ann Epidemiol* 1995,5 278-285
22 Malinow MR, Kang SS, Taylor LM, Wong WK, Coull B, Inahara T, Mukerjee D, Sexton G, Upson B Prevalence of hyperhomocyst(e)inemia in patients with peripheral arterial occlusive disease *Circulation* 1989,79 1180-1188
23 Miller SA, Dykes DD, Polesky HF A simple salting out procedure for extracting DNA from nucleated cells *Nucleic Acids Res* 1988, 16 1215
24 Warnick GR Enzymatic methods for quantification of lipoprotein lipids In Albers JJ, Segrest JP, eds *Methods in Enzymology* New York, NY Academic Press Inc, 1986,129 101-123
25 Thompson WD Statistical analysis of case-control studies *Epidemiol Rev* 1994,16 33-50
26 Malinow MR, Sexton G, Averbuch M, Grossman M, Wilson D, Upson B Homocyst(e)inemia in daily practice levels in coronary artery disease *Coron Artery Dis* 1990,1 215-220
27 Wu LL, Wu J, Hunt SC, James BC, Vincent GM, Williams RR, Hopkins PN Plasma homocysteine as a risk factor for early familial coronary artery disease *Clin Chem* 1994,40 552-561
28 Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, Tishler PV, Hennekens CH A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians *JAMA* 1992,268 877 881
29 Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations *Circulation* 1996,93 7 9
30 van der Put NMJ, Steegers-Theunissen RP, Frosst P, Trijbels FJ, Eskes TK, van den Heuvel LP, Marinan EC, den Heyer M, Rozen R, Blom HJ Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida *Lancet* 1995,346 1070 1071
31 Stampfer MJ, Rimm EB Folate and cardiovascular disease why we need a trial now *JAMA* 1996,275 1929-1930