Age Dependence of the Influence of Methylene tetrahydrofolate Reductase Genotype on Plasma Homocysteine Level

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Received for publication December 30, 2002; accepted for publication May 8, 2003.

An elevated plasma homocysteine level is a risk factor for cardiovascular disease and is often observed in other common disorders, including neural tube defects, pregnancy complications, and Alzheimer’s disease. Plasma homocysteine level is affected by vitamin intake and by sequence variation in enzymes of homocysteine metabolism. One such enzyme, methylenetetrahydrofolate reductase (MTHFR), synthesizes 5-methyltetrahydrofolate, utilized in homocysteine remethylation to methionine. A variant of the MTHFR gene at base pair 677 is associated with reduced activity, increased thermolability, and hyperhomocysteinemia. This variant has been reported to increase risk of the aforementioned disorders. However, not all studies examining disease risk with respect to MTHFR genotype have reported a statistically significant relation. The current authors hypothesized that the effect of the variant might be stronger in younger age groups, as is the case with other genetic risk factors. Thus, the authors examined data from three North American studies: a study of mothers of spina bifida children and control mothers (1995–1996; n = 136); the National Heart, Lung, and Blood Institute Family Heart Study (1994–1995; n = 537); and a Mayo Clinic study of patients undergoing coronary angiography (1998–1999; n = 504). In each study, the effect of MTHFR genotype on plasma homocysteine level was statistically significant only in younger age groups. Failure to examine younger patients separately may explain why some studies have found no association between the genotype and cardiovascular disease.

Abbreviations: FHS, Family Heart Study; MCS, Mayo Clinic study; MTHFR, methylenetetrahydrofolate reductase; SBS, spina bifida study.

Numerous studies have documented an association between elevated total plasma homocysteine level and vascular disease (1, 2). Extracranial carotid-artery stenosis (3), cardiovascular disease mortality (4), and stroke (5) have each been associated with plasma homocysteine level in large studies. Plasma homocysteine levels are affected by nutritional and genetic factors. Both folic acid and vitamin B12 status influence plasma homocysteine levels. Higher plasma homocysteine levels have been associated with low vitamin status in the Framingham offspring cohort (6), the National Heart, Lung, and Blood Institute Family Heart Study (FHS) (7), the elderly (8), and children (9).

Several enzymes are responsible for modulating intracellular levels of homocysteine, and an excess of this potentially toxic sulfur-containing amino acid spills into the bloodstream. One of these enzymes, methylenetetrahydrofolate reductase (MTHFR), catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a
major methyl donor in the remethylation of homocysteine to methionine by methionine synthase. In earlier work, we identified a common variant of the MTHFR gene (677C→T, or A222V) that has been observed in 35–40 percent of alleles in many Caucasian populations (10). This variant encodes a thermolabile enzyme with lower specific activity (10). We and other investigators have demonstrated an association between the homozygous mutant genotype and elevated plasma homocysteine levels (7, 10, 11). The effect of genotype on homocysteine levels is dependent on folate status, since homozygous mutant individuals with a higher plasma folate level do not display hyperhomocysteinemia (7). This phenomenon may relate to the folate-dependent stabilization of the mutant enzyme, as demonstrated for human and bacterial MTHFR (12, 13). These studies also demonstrated a stabilizing effect of flavin adenine dinucleotide, the MTHFR cofactor, prompting clinical studies of the influence of riboflavin on homocysteine levels (14, 15).

The mutant MTHFR genotype has been reported to increase the risk of several common disorders, including cardiovascular disease (16–18), neural tube defects (19, 20), pregnancy complications (21), and neuropsychiatric illnesses (22). Although many studies of MTHFR and coronary artery disease have demonstrated a clear increase in risk, other studies have shown no statistically significant increase in risk (2, 11, 23). However, many of these negative studies have not examined homocysteine or folate levels, despite the fact that modulation by MTHFR genotype is folate-dependent. The authors of one review (2) suggested that the clinical studies may not have had enough power to detect the modest role of MTHFR genotype on such a complex phenotype as coronary artery disease. Another variable that may influence risk is age, since a genetic modifier is likely to have a greater role in the young. The processes of growth and development place maximal demands on metabolic functions. Therefore, abnormalities in enzymes involved in the metabolism of nutrients might be expected to be of greater consequence in younger persons. In general, the risk of multifactorial diseases is thought to peak at a certain age and then decline (24). Cardiovascular disease risk, conferred by MTHFR genotype, may be influenced by age, as demonstrated in a study of people stratified by age of onset (25). The investigators suggested that MTHFR genotype was associated with risk in persons with an earlier age of onset of disease (25). Since MTHFR presumably alters disease risk through homocysteine levels, we evaluated the effect of the relation between age and genotype on plasma homocysteine levels in three different study populations: a small study of mothers of spina bifida cases and two larger studies, the FHS and a study of cardiac catheterization carried out at the Mayo Clinic in Rochester, Minnesota.

MATERIALS AND METHODS

Subjects

The data analyzed in this report were derived from three independent study populations. The first population, from a spina bifida study (SBS), consisted of mothers of children with spina bifida recruited from the Montreal Children’s Hospital ambulatory Spina Bifida Clinic and control mothers of children from the Pediatric Test Centre, Montreal Children’s Hospital (November 1995–April 1996) (26). These women ranged in age from 16 years to 53 years. Levels of red blood cell folate, serum folate, serum cobalamin, and plasma homocysteine were determined in case mothers (n = 79) and control mothers (n = 79) (26). The second population (7, 27) consisted of volunteers from both the random and high-risk groups of the FHS, for whom earlier results have been reported (27). Here we give results from 537 phase II participants aged 25–69 years who fasted for more than 10 hours and for whom data on plasma folate, plasma pyridoxal 5′-phosphate, plasma cobalamin, serum creatinine, and plasma homocysteine levels and MTHFR genotype were available (February 1994–December 1995) (7). The third population consisted of 504 patients aged 18–75 years who were undergoing clinically indicated coronary angiography at the Mayo Clinic (July 1998–January 1999) (28). In this Mayo Clinic study (MCS), serum folate, creatinine, serum cobalamin, and plasma homocysteine levels and MTHFR genotype were determined in all patients. Biochemical measurements were performed as described in the initial reports of the first two studies (7, 26). Total plasma homocysteine measurements in the MCS were performed by high performance liquid chromatography following reduction of the disulfide bonds.

Genotype determination

DNA was isolated from peripheral blood leukocytes using the Puregene kit (Gentra Systems, Inc., Minneapolis, Minnesota) in the FHS and MCS and phenol-chloroform in the SBS. All three studies used polymerase chain reaction followed by restriction digestion with HinfI for determination of MTHFR genotype (10). The three genotype groups were designated C/C for homozygous normal, C/T for heterozygous, and T/T for homozygous mutant.

Statistical analysis

The effects of age and MTHFR genotype on plasma homocysteine values were examined using a general linear model (SAS, version 8; SAS Institute, Inc., Cary, North Carolina) that included the relevant covariates available for each study population. In the SBS, covariates were case/control status, age, serum folate, serum cobalamin, and red blood cell folate; in the FHS, covariates were age, sex, institutional site, plasma folate, serum creatinine, plasma pyridoxal 5′-phosphate, and plasma cobalamin; and in the MCS, covariates were age, sex, serum folate, and serum creatinine. Only persons for whom complete data were available were included. Because plasma homocysteine values were skewed, a natural logarithmic transformation was applied to the data. Consequently, geometric means and their 95 percent confidence intervals are presented. Each study population was subdivided into three approximately equal-sized age groups based on the age distribution in each study. For the SBS, the age groups were <34 years, 34–40 years, and >40 years; in the FHS, the age groups were <45 years, 45–59 years, and ≥60 years; and in the MCS, the age groups were...
<56 years, 56–67 years, and ≥67 years. Least-square mean values were calculated for determination of the effect of age in each genotype group and the effect of genotype in each age group. Genotypes were coded as 1 for \(C/C\), 2 for \(C/T\), and 3 for \(T/T\), and \(p\) values for trend across genotype were based on these ordinal codes. Pairs of mean values within each age group were compared using a \(t\) test, with adjustment for multiple comparisons made by the Tukey-Kramer test. Statistical significance was defined as \(p < 0.05\).

**RESULTS**

The three study populations differed with respect to the biochemical variables measured (table 1). However, circulating folate and creatinine levels were highly significant covariates of plasma homocysteine level in both the FHS and the MCS (\(p < 0.0001\)); red blood cell folate was highly significant in the SBS (\(p < 0.0001\)) and circulating cobalamin levels were highly significant in the SBS and FHS (\(p < 0.001\)) and in the MCS (\(p < 0.0001\)). Both age and \(MTHFR\) genotype were significant in each of the study populations.

The age \(\times\) genotype interaction was significant in both the SBS (\(p = 0.015\)) and the FHS (\(p < 0.001\)) but not in the MCS (\(p = 0.62\)). Neither case/control status (SBS) nor institutional site (FHS) was a significant variable.

Geometric mean plasma homocysteine levels for each genotype and age group in each study population are presented in tables 2, 3, and 4. Consistent with the earlier reports from these three studies as well as many other reports in the literature, the \(C/C\) group had the lowest homocysteine level of the three genotype groups, with the \(T/T\) group showing the highest level.

Mean plasma homocysteine values were significantly different among the three genotype groups in the entire SBS population (\(p = 0.0007\); table 2) and in the youngest age group (\(p = 0.0004\)). Within the youngest age group, the mean plasma homocysteine level was almost twice as high in the \(T/T\) genotype group (15.3 \(\mu\)mol/liter) as in the \(C/C\) genotype group (8.0 \(\mu\)mol/liter); the \(C/T\) genotype group had an intermediate level of plasma homocysteine. Although the mean plasma homocysteine levels were higher in the \(T/T\) genotype group for the intermediate age group, the difference across groups was not statistically significant. The effect of age on plasma homocysteine level was not significant in the entire population, although plasma homocysteine levels appeared to increase with age in the \(C/C\) genotype group (\(p = 0.034\)). However, plasma homocysteine levels appeared to decrease with age in the other two genotype groups, although the differences were not statistically significant.

Similarly, in the FHS subjects (table 3), mean plasma homocysteine levels were significantly different among the three genotype groups in the whole study population (\(p = 0.004\)) and in the youngest age group (\(p = 0.002\)). In contrast to the SBS population, age was significantly related to plasma homocysteine in the FHS population overall (\(p < 0.001\)) and in the \(C/C\) and \(C/T\) genotype groups (\(p = 0.01\) and \(p < 0.0001\), respectively). The highest mean plasma homocysteine levels were observed in the youngest group with the \(T/T\) genotype.

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**TABLE 1. Clinical characteristics of participants in three North American studies**

<table>
<thead>
<tr>
<th>Study and ref. no.</th>
<th>Spina bifida study (26) (n = 136)</th>
<th>Family Heart Study (7) (n = 537)</th>
<th>Mayo Clinic study (28) (n = 504)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean or % SD†</td>
<td>Mean or % SD</td>
<td>Mean or % SD</td>
</tr>
<tr>
<td>Sex (% males)</td>
<td>37.6‡ 7.3</td>
<td>50.5‡ 13.3</td>
<td>60.0‡ 10.9</td>
</tr>
<tr>
<td>Folate level (nmol/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>15.5 8.9</td>
<td></td>
<td>15.8‡,§</td>
</tr>
<tr>
<td>Plasma</td>
<td>12.7‡ 10.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine level (mg/dl)</td>
<td>ND†</td>
<td>0.96‡ 0.19</td>
<td>1.1‡ 0.2</td>
</tr>
<tr>
<td>Plasma pyridoxal 5′-phosphate level (nmol/liter)</td>
<td>ND</td>
<td>74.4‡ 66.1</td>
<td>ND</td>
</tr>
<tr>
<td>Cobalamin level (pmol/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>327.8‡ 160.5</td>
<td>416.1‡ 181.4</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>295.5‡ 137.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red blood cell folate level (nmol/liter)</td>
<td>629.5‡ 299.8</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Data were obtained from Jacques et al. (7), Christensen et al. (26), and Singh et al. (28).
† SD, standard deviation; ND, not determined.
‡ The effect of the covariate on serum plasma homocysteine levels within each study was significant in the general linear model (\(p < 0.05\)).
§ Serum folate level in the Mayo Clinic study was reported as the median value (interquartile range, 4.15–14.15). All folate values greater than 30 were recorded as 30.
In the MCS subjects (table 4), as in the FHS, plasma homocysteine levels increased with age in the population as a whole \((p < 0.0001)\) and in the \(C/C\) and \(C/T\) genotype groups \((p < 0.0001\) and \(p < 0.001,\) respectively). Age was not significantly associated with plasma homocysteine level in the \(T/T\) genotype group \((p = 0.88)\). Genotype was significantly associated with plasma homocysteine levels in the whole study population and in the youngest group \((p = 0.002\) and \(p = 0.005,\) respectively) but not in the two older age groups.

The proportion of variation in plasma homocysteine levels explained by the covariates of each study, including genotype, age group, and age \(\times\) genotype interactions, was 0.39, 0.33, and 0.31 in the SBS, FHS, and MCS, respectively.

**DISCUSSION**

In this analysis, we used three independent data sets that differed in ascertainment scheme and selection criteria and focused on two different types of disorders (a birth defect and a chronic adult-onset disease). However, there were several similarities in the original designs of the studies, as well as in the underlying biology. The original reports on these study populations examined the influence of a common

**TABLE 2.** Geometric mean plasma homocysteine levels* (µmol/liter) in three age groups and three methylenetetrahydrofolate reductase genotype groups from a study of mothers of spina bifida cases and control mothers, 1995–1996†

<table>
<thead>
<tr>
<th>Total (n = 136)</th>
<th>&lt;34 (n = 44)</th>
<th>34–40 (n = 44)</th>
<th>&gt;40 (n = 48)</th>
<th>p for trend‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean§</td>
<td>9.8</td>
<td>9.1, 10.8</td>
<td>8.7</td>
<td>8.0, 9.5</td>
</tr>
<tr>
<td>MTHFR¶ genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C/C) (n = 58)</td>
<td>8.4a</td>
<td>7.9, 9.1</td>
<td>8.0a</td>
<td>7.1, 9.1</td>
</tr>
<tr>
<td>(C/T) (n = 58)</td>
<td>9.3a</td>
<td>8.7, 10.0</td>
<td>10.3a</td>
<td>9.1, 11.9</td>
</tr>
<tr>
<td>(T/T) (n = 20)</td>
<td>11.0b</td>
<td>9.8, 12.5</td>
<td>15.3c</td>
<td>12.1, 19.5</td>
</tr>
<tr>
<td>(p) for trend#</td>
<td>0.0007</td>
<td></td>
<td>0.0004</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* Mean values were adjusted for serum folate, serum cobalamin, and red blood cell folate levels and case/control status.
† Data were obtained from Christensen et al. (26).
‡ \(p\) for trend was determined using age as a continuous variable within each age group.
§ Mean values within the same column that do not have the same alphabetic superscript were significantly different \((p < 0.05)\) after correction for multiple testing using the Tukey-Kramer test.
¶ CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase.
# \(p\) for overall trend was determined using genotype as an ordinal variable.

**TABLE 3.** Geometric mean plasma homocysteine levels* (µmol/liter) in three age groups and three methylenetetrahydrofolate reductase genotype groups from phase II of the National Heart, Lung, and Blood Institute Family Heart Study, 1994–1995†

<table>
<thead>
<tr>
<th>Total (n = 537)</th>
<th>&lt;45 (n = 190)</th>
<th>45–59 (n = 206)</th>
<th>≥60 (n = 141)</th>
<th>p for trend‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean§</td>
<td>8.5</td>
<td>8.1, 8.9</td>
<td>8.8</td>
<td>8.4, 9.2</td>
</tr>
<tr>
<td>MTHFR¶ genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C/C) (n = 215)</td>
<td>8.4a</td>
<td>8.1, 8.7</td>
<td>7.9a</td>
<td>7.4, 8.5</td>
</tr>
<tr>
<td>(C/T) (n = 253)</td>
<td>8.6a</td>
<td>8.3, 8.9</td>
<td>8.2a</td>
<td>7.7, 8.7</td>
</tr>
<tr>
<td>(T/T) (n = 69)</td>
<td>9.6b</td>
<td>9.0, 10.3</td>
<td>10.4b</td>
<td>9.2, 11.8</td>
</tr>
<tr>
<td>(p) for trend#</td>
<td>0.004</td>
<td>0.002</td>
<td>0.37</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* Mean values were adjusted for serum folate, serum creatinine, plasma folate, and plasma pyridoxal 5′-phosphate levels.
† Data were obtained from Jacques et al. (7).
‡ \(p\) for trend was determined using age as a continuous variable within each age group.
§ Mean values within the same column that do not have the same alphabetic superscript were significantly different \((p < 0.05)\) after correction for multiple testing using the Tukey-Kramer test.
¶ CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase.
# \(p\) for overall trend was determined using genotype as a nominal variable.
TABLE 4. Geometric mean plasma homocysteine levels* (µmol/liter) in three age groups and three methylenetetrahydrofolate reductase genotype groups from a Mayo Clinic study of cardiac catheterization, 1998–1999†

<table>
<thead>
<tr>
<th>MTHFR genotype</th>
<th>Total (n = 504)</th>
<th>Age group (years)</th>
<th>p for trend‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean§</td>
<td>95% CI [¶]</td>
<td>Geometric mean§</td>
</tr>
<tr>
<td>All subjects</td>
<td>8.6</td>
<td>8.3, 9.0</td>
<td>9.3</td>
</tr>
<tr>
<td>C/C (n = 226)</td>
<td>8.8a</td>
<td>8.5, 9.1</td>
<td>8.0a</td>
</tr>
<tr>
<td>C/T (n = 224)</td>
<td>9.0a</td>
<td>8.7, 9.3</td>
<td>8.4c,d</td>
</tr>
<tr>
<td>T/T (n = 54)</td>
<td>9.9d</td>
<td>9.2, 10.5</td>
<td>9.5d</td>
</tr>
<tr>
<td>p for trend#</td>
<td>0.002</td>
<td>0.005</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* Mean values were adjusted for sex and serum creatinine, serum cobalamin, and serum folate levels.
† Data were obtained from Singh et al. (28).
‡ p for trend was determined using age as a continuous variable within each age group.
§ Mean values within the same column that do not have the same alphabetic superscript were significantly different (p < 0.05) after correction for multiple testing using the Tukey-Kramer test.
¶ CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase.
# p for overall trend was determined using genotype as a nominal variable.

It has been well established that homocysteine levels, with assessment of genotypes and with measurement of levels of homocysteine and the nutrients that play a role in its metabolism. The biologic link between these disorders is the elevation of levels of a potentially toxic compound (homocysteine) and the disturbance in folate-dependent methylation reactions that could alter gene expression. Both of these metabolic disturbances are commonly seen in the presence of the MTHFR variant, although it is not clear which of the above disturbances (or possibly other disruptions related to mild MTHFR deficiency) is likely to play a greater role in neural tube defects and in vascular disorders.

We observed that mild hyperhomocysteinemia associated with the MTHFR T/T genotype was greater in younger age groups. In the case of the SBS, mean plasma homocysteine level in the T/T genotype group was nearly twice that in the C/C group in the lowest group of the age distribution, that is, below the age of 34 years. Although the youngest age group in the FHS was older than that in the SBS (age <45 years), mean plasma homocysteine levels were approximately 25 percent higher in the T/T group than in the C/C group. Similarly, in the MCS, mean plasma homocysteine levels were approximately 16 percent higher in the T/T genotype group than in the C/C genotype group in the youngest age group (age <56 years). The smaller differences in the youngest age group of the MCS population could be a consequence of the older age of this population.

It has been well established that homocysteine levels increase with age (29). Findings in the two largest data sets analyzed here (FHS and MCS) support this. However, mean plasma homocysteine levels may not consistently increase with age within each genotype group. For example, mean plasma homocysteine levels did not increase significantly with age in the T/T genotype group in any of the three study populations, and there was a tendency for homocysteine levels to decrease with age in two of the studies.

The three study populations differed with respect to age distribution and the age group in which the greatest association with MTHFR genotype was observed. For example, the youngest age group of the FHS (age <45 years) and the oldest age group of the SBS (age >40 years) overlapped; however, mean plasma homocysteine levels were significantly different in the three genotype groups of the former but not the latter. In an attempt to account for this, we compared folate levels in these two age groups. Indeed, the oldest SBS age group had a higher mean folate level (17.1 nmol/liter) than the youngest FHS age group (10.0 nmol/liter). This could account for the apparent inconsistency of the effect of genotype in these two similar-aged groups.

The interaction between MTHFR genotype and age has previously been observed to have an effect on plasma homocysteine levels (30) and on risk of coronary artery disease (25). In a prospective study of risk of myocardial infarction among US physicians (30), the effect of the homozygous mutant genotype on plasma homocysteine levels was significant in men under age 60 years (p < 0.01) but was not significant in men over the age of 60. In this study, the risk of myocardial infarction was not associated with genotype. In another study (25), the prevalence of the mutant genotype was 28 percent in early-onset coronary artery disease (age ≤45 years) as compared with 13 percent in late-onset coronary artery disease (p = 0.006) or 14 percent in healthy controls (p = 0.01). Plasma homocysteine levels were not measured in that study.

The differences in conclusions between studies that have examined the association between MTHFR genotype and vascular disease risk have been attributed to nutritional status in the study groups and to a lack of sufficient statistical power (2, 23). Our analysis in this report suggests that age
plays a significant role in modulating homocysteine levels among persons who are homozygous for the MTHFR mutation; consequently, failure to adjust for the interaction between age and genotype may have contributed to the discrepancies among studies. In contrast to variable results observed in studies of the risk of vascular disease associated with different genotypes, recent meta-analyses of MTHFR genotype and risk of neural tube defects (20) or risk of pregnancy complications (21) have demonstrated a significant increase in the risk of those conditions associated with the thermolabile MTHFR polymorphism. Botto and Yang (20) concluded that women with the mutant genotype had an odds ratio of 2.0 for having a child with a neural tube defect. Ray and Laskin (21) reported that pregnant women with the mutant genotype had odds ratios of 3.3, 2.3, and 2.6 for recurrent pregnancy loss, placental abruption, and preeclampsia, respectively. Since women in their reproductive years are considerably younger than patients with cardiovascular disease, the more consistent results for the relation between MTHFR genotype and birth defects or pregnancy complications may be due to the lower age of those study populations.

There are several hypotheses that might address why plasma homocysteine concentrations were higher in younger persons with the T/T genotype. First of all, each of the study samples was composed, at least in part, of persons who were at high risk for either heart disease (FHS, MCS) or having a child with spina bifida (SBS). Thus, our observations might not be duplicated in a community-based or randomly selected population. Secondly, there could be differential mortality, particularly in the older age groups. For example, if mild hyperhomocysteinemia puts a person at risk for cardiovascular or renal disease, then older persons with the T/T genotype may have already succumbed to the consequences of a lifetime of elevated plasma homocysteine levels. In addition, at an older age, the nongenetic causes of hyperhomocysteinemia may have a greater impact and overwhelm the effect of a genetic polymorphism. Finally, it is possible that additional regulatory mechanisms come into play in persons with the mutant genotype as they age or after chronic hyperhomocysteinemia; these as-yet-unidentified mechanisms may serve to readjust homocysteine metabolism and lower the levels of this toxic amino acid.

The results presented here suggest that the role of the MTHFR mutation, 677C→T, in determining plasma homocysteine levels after adjustment for folate status may be more critical in the young. In all three studies, the C/C genotype was associated overall with lower homocysteine levels, as previously reported in many other publications. Here we showed that this association was highly significant in the youngest group in all three studies, although similar nonsignificant trends were observed in the other age groups in the FHS and MCS. This underscores the importance of adequate intake of folate and other B vitamins for young people. It also suggests that higher folate status may be required in persons with the homozygous mutant genotype to prevent the deleterious effects of mild hyperhomocysteinemia.

ACKNOWLEDGMENTS

This work was supported by the Canadian Institutes for Health Research (R. R.), by the US National Institutes of Health under grant HD36606 (L. D. S.), by the US Department of Agriculture under agreement 58-1950-9-001 (P. J.), and by the National Institutes of Health and the National Heart, Lung, and Blood Institute under grants HL58955-01 and 53-3K06-01 and contract N01-HC-25106 (The Family Genetics Studies of Cardiovascular Disease) (R. C. E.).

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the US Department of Agriculture.

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