Age and Gender Affect the Relation between Methylenetetrahydrofolate Reductase C677T Genotype and Fasting Plasma Homocysteine Concentrations in the Framingham Offspring Study Cohort

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ABSTRACT  The C677T variant of methylenetetrahydrofolate reductase (MTHFR), a key enzyme in the remethylation of homocysteine to methionine, is a frequent genetic cause of mild hyperhomocysteinemia among individuals with low folate status. However, little is known about the influence of subject characteristics, such as age and sex, on the relation between the C677T MTHFR polymorphism and fasting plasma total homocysteine (tHcy) concentrations. The aim of the present study was to explore the influence of age and gender, together with folate status, on the association between the C677T polymorphism and tHcy concentrations. The C677T genotype was determined for 1820 participants from the fifth examination of the Framingham Offspring Study. Mean age of the participants was 56 y (range 28–82 y). The allelic distribution was not different from the Hardy-Weinberg equilibrium, with a TT frequency comparable in men and women (14%). Geometric mean tHcy was 15% higher in men than in women (P < 0.001), and women had significantly higher plasma folate levels (P < 0.001). Geometric mean tHcy was significantly higher in TT participants (P = 0.001) than in participants with the CC and CT genotypes among those with plasma folate <12.5 nmol/L, but not among those with higher folate status. Because of a significant age and sex interaction (P = 0.02), we further stratified the low folate group by age and sex, and observed that the association between genotype and tHcy was confined to men <55 y old (P < 0.001). Our results suggest that age and sex modify the contribution of the MTHFR C677T mutation to fasting tHcy concentrations. J. Nutr. 133: 3416–3421, 2003.

KEY WORDS: • homocysteine • methylenetetrahydrofolate reductase • age • sex • folate • genetics

As circulating total homocysteine (tHcy) concentrations increase so does the risk of occlusive vascular disease (1,2). A recent meta-analysis demonstrated that a 3 μmol/L or 25% lower tHcy concentration was associated with an 11% lower risk of ischemic heart disease and a 19% lower risk of stroke (3). Many factors are related to circulating tHcy concentrations (4). Fasting plasma tHcy concentration is consistently higher in men than in women, and increases with age (5–10). The male-female difference has been attributed mainly to sex differences in muscle mass (6,11,12) and circulating sex hormones (10,11,13). tHcy concentrations are strongly dependent on renal function (6,14–17); thus, impaired renal function associated with aging may account in part for the increased plasma tHcy concentrations in older subjects. The nutritional status of vitamin B-12, B-6 and folate is a major determinant of tHcy concentrations (18), and other recently identified nutritional and lifestyle factors may also influence circulating tHcy levels (19–21).

Genetic background also affects tHcy concentrations. A common gene variant of methylenetetrahydrofolate reductase (MTHFR, EC 1.7.99.5), which synthesizes N5-methyltetrahydrofolate, the methyl donor for methylation of homocysteine to methionine, is the most frequent genetic cause of mild hyperhomocysteinemia. The molecular defect responsible for

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this MTHFR genetic variant, first described as “thermolabile” MTHFR by Kang et al. (22) because of its decreased stability under specific heat conditions, resides in a cytosine to thymine transition at nucleotide 677 (C677T) in the MTHFR gene locus (23,24). This, in turn, determines an alanine to valine substitution in the folate-binding site of the enzyme. Those bearing two copies of the mutant T allele have circulating tHcy concentrations that are on average between 1.5 and 2.6 μmol/L (~15–25%) higher than subjects carrying the C allele (25,26), but the association of the thermolabile variant with mild hyperhomocysteinemia appears to be contingent on folate availability (25–28). When folate status is low, homocysteines for this mutation have tHcy concentrations that range from 2.0 to 4.3 μmol/L (~25–50%) higher than concentrations in those without the T allele (25). If folate concentrations are adequate, however, MTHFR genotype does not affect tHcy levels. A recent study from our group (29) suggested that riboflavin, a cofactor for MTHFR, also interacts with the MTHFR genotype in determining plasma tHcy concentrations, but only in subjects with lower folate status.

Because tHcy concentrations are strongly modulated by gender and age, interactions among these factors, folate status and the C677T mutation in MTHFR gene locus may explain the inconsistent association of this polymorphism with the risk of atherothrombotic vascular disease (25,26). Moreover, age and sex interactions with polymorphic mutations were reported recently for several gene loci associated with cardiovascular disease, such as apolipoprotein (apo) E, apoA1, lipoprotein lipase and apoC3 (30–33).

The aim of the present study was to determine whether the association of the common MTHFR C677T mutation with plasma tHcy concentrations varies by age and gender in a large cohort of free-living U.S. Caucasian subjects, well characterized for folate status and other major determinants of plasma tHcy concentrations.

SUBJECTS AND METHODS

Subjects. The details of the design and methods of the Framingham Offspring Study were presented elsewhere (34). Briefly, the Framingham Heart Study, an epidemiologic study of heart disease, was established in Framingham, Massachusetts between 1948 and 1950 with a cohort of 5209 men and women, aged 30 to 59 y (35). By 1971, the original cohort included 1644 husband-wife pairs and 378 individuals, who had developed cardiovascular disease. The offspring of these subjects and the offsprings’ spouses were invited to participate in the Framingham Offspring Study, and 5135 of the 6838 eligible individuals participated in the first study examination (34). The offspring cohort has undergone repeat examinations at ~3- to 4-y cycles. Nearly all subjects were Caucasians (34,36). The fifth examination of the offspring cohort began in January 1991 and was completed in December 1994. This study was approved by the Human Investigations Review Committee at New England Medical Center and by the Institutional Review Board for Human Research at Boston University Medical Center.

Biochemical analyses. Twelve-hour fasting venous blood samples were collected in tubes containing 0.1% EDTA from subjects who attended the 5th examination visit of the Framingham Offspring Study. Plasma was separated from blood cells by centrifugation at 1500 × g for 30 min at 4°C and immediately used for the measurement of lipids. Plasma total cholesterol, HDL cholesterol and triglyceride concentrations were measured as previously described (37). HDL cholesterol was measured after precipitation of apo B-containing lipoproteins with heparin-manganese chloride (38). LDL cholesterol concentrations were estimated with the equation of Friedewald (41). Total cholesterol, HDL cholesterol and triglyceride measurements were each <5%. Serum creatinine levels were measured by the Jaffe method (40), adapted for autoanalyzers (Roche Diagnostics, Indianapolis, IN). The CV was 2.7% for creatinine.

RESULTS

Of the 3799 members of the Framingham Offspring cohort who attended the 5th examination cycle, 2369 had genotype information for the MTHFR C677T polymorphism. Information was missing for 549 participants on either plasma tHcy, folate or creatinine concentrations, leaving 1820 study participants (871 men and 949 women) for the present analyses. Demographic, biochemical and genotype characteristics of study participants according to gender are shown in Table 1.
The mean age for men and women at examination was 56 y (range 28–82 y), and 17.5% of the women were receiving hormone replacement therapy (HRT). Geometric mean tHcy concentration was higher in men than in women (P < 0.001), and women had higher plasma folate concentrations (P < 0.001). No gender differences were noted for plasma concentrations of vitamin B-12 or PLP. Plasma creatinine and triglyceride concentrations were higher in men (P < 0.001), whereas plasma total cholesterol and HDL cholesterol were higher in women (P < 0.0001). Plasma LDL cholesterol concentrations did not differ between genders. BMI and alcohol intake were higher in men compared with women (P < 0.001). A similar proportion of men and women were smokers.

In this mainly Caucasian population, we found 707 CC (338M; 369F), 853CT (401M; 452F) and 260 TT (132M; 128F) subjects (Table 1). The frequency of the less common allele (T) was comparable in men and women, and the allelic distribution did not differ from Hardy-Weinberg equilibrium, with an TT homozygous frequency of 15.1% in men and 13.5% in women.

In the study sample as a whole, plasma tHcy and folate concentrations were significantly related to MTHFR genotype (Table 2). tHcy concentrations in those with the CC genotype (9.4 μmol/L) were lower than concentrations in those with the CT (9.8 μmol/L, P = 0.02) and TT (10.2 μmol/L, P < 0.001) genotypes, after adjusting for sex, age and serum creatinine. Geometric mean plasma folate concentrations were higher in the CC (14.3 nmol/L) genotype category than in the CT (12.6 nmol/L, P < 0.001) and the TT (12.3 nmol/L, P = 0.007) genotype categories. Plasma PLP, vitamin B-12, creatinine and lipid concentrations were not associated with MTHFR genotype.

To confirm the previously reported effect of folate status on the relation between the C677T polymorphism and tHcy concentrations, we tested for the presence of an interaction between folate status and the mutation. Because this interac-

### Table 1
Characteristics and methylenetetrahydrofolate reductase (MTHFR) C677T genotype distribution of Framingham Offspring Study participants according to gender

|                     | Men (n = 871) | Women (n = 949) | P-value
|---------------------|--------------|----------------|--------
| Age, y [range]      | 56.3 (55.7–57.0) | 55.6 (54.9–56.2) | 0.10
| Homocysteine, μmol/L| 10.5 (10.3–10.8) | 8.9 (8.7–9.1) | <0.001
| Homocysteine > 13 μmol/L, % | 20.9 (18.5–23.3) | 11.6 (9.3–13.9) | <0.001
| Folate, nmol/L      | 12.3 (11.7–12.9) | 14.1 (13.5–14.8) | <0.001
| Folate < 12.5 nmol/L,% | 52.7 (49.4–56.0) | 46.9 (43.7–50.1) | 0.02
| Vitamin B-12, μmol/L| 307 (296–318) | 311 (300–322) | 0.65
| Pyridoxal phosphate, nmol/L | 59.9 (57.5–62.5) | 57.1 (54.9–59.4) | 0.11
| Creatinine, μmol/L  | 98.9 (97.4–100.4) | 84.7 (83.6–86.0) | <0.001
| Cysteine, μmol/L    | 255 (253–258) | 248 (245–251) | <0.001
| Cholesterol, mmol/L | 5.16 (5.0–5.23) | 5.39 (5.3–5.45) | <0.001
| HDL cholesterol, mmol/L | 1.09 (1.07–1.11) | 1.39 (1.37–1.41) | <0.001
| LDL cholesterol, mmol/L | 3.25 (3.19–3.31) | 3.22 (3.17–3.29) | 0.66
| Triglyceride, mmol/L | 1.48 (1.42–1.53) | 1.35 (1.30–1.40) | <0.001
| BMI, kg/m²           | 28.2 (27.9–28.5) | 26.8 (26.5–27.1) | <0.001
| Smoke, %            | 18.3 (15.7–20.9) | 20.0 (17.5–22.5) | 0.37
| Alcohol intake, g/d  | 15.8 (14.6–17.0) | 6.7 (5.6–7.8) | <0.001
| Hormone replacement therapy, % | 0 | 17.5 (15.7–19.2) | —
| MTHFR C677T Genotype, n (%) | 338 (38.8) | 369 (38.9) | 0.98
| CC                   | 401 (46.0) | 452 (47.6) | 0.50
| CT                   | 132 (15.2) | 128 (13.5) | 0.31
| TT                   | 665 (38.2) | 708 (37.3) | —

1 Values are means or percentages (95% CI).
2 P-value based on t test for differences between means.
3 Geometric mean (95% CI).
4 Square root-transformed mean (95% CI).

### Table 2
Characteristics of the Framingham Offspring Study participants according to genotype

|                     | CC (n = 707) | CT (n = 853) | TT (n = 260) | P-value
|---------------------|--------------|--------------|--------------|--------
| Age, y              | 55.7 (54.9–56.4) | 55.9 (55.3–56.6) | 56.7 (55.6–57.9) | 0.31
| Homocysteine, μmol/L| 9.4 (9.1–9.6) | 9.8 (9.6–10.0) | 10.2 (9.9–10.6) | <0.001
| Homocysteine > 13 μmol/L, % | 14.4 (11.8–17.0) | 16.2 (13.8–18.5) | 20.9 (16.6–25.1) | 0.04
| Folate, nmol/L      | 14.3 (13.6–15.1) | 12.6 (12.0–13.2) | 12.3 (11.3–13.3) | <0.001
| Folate < 12.5 nmol/L,% | 45.0 (41.4–48.7) | 52.3 (49.0–55.6) | 54.4 (48.4–60.4) | 0.004
| Vitamin B-12, μmol/L| 309 (297–322) | 306 (295–318) | 317 (296–338) | 0.71
| Pyridoxal-S'-phosphate, nmol/L | 58.3 (55.7–61.1) | 58.5 (55.9–60.8) | 59.6 (55.2–64.4) | 0.87
| Creatinine, μmol/L  | 91.3 (89.8–92.8) | 92.2 (90.8–93.6) | 90.2 (87.8–92.7) | 0.36
| Cysteine, μmol/L    | 252 (250–255) | 252 (249–254) | 249 (245–254) | 0.53
| Cholesterol, mmol/L | 5.31 (5.24–5.38) | 5.25 (5.19–5.52) | 5.27 (5.15–5.59) | 0.56
| HDL cholesterol, nmol/L | 1.23 (1.21–1.26) | 1.24 (1.21–1.26) | 1.19 (1.15–1.23) | 0.11
| LDL cholesterol, mmol/L | 3.26 (3.19–3.32) | 3.23 (3.17–3.29) | 3.22 (3.11–3.33) | 0.79
| Triglyceride, mmol/L | 1.44 (1.38–1.49) | 1.37 (1.32–1.42) | 1.48 (1.39–1.58) | 0.08
| BMI, kg/m²           | 27.4 (27.0–27.7) | 27.6 (27.2–27.9) | 27.6 (27.0–28.2) | 0.70
| Smoking, %           | 19.0 (16.1–21.9) | 19.6 (17.0–22.2) | 18.0 (13.2–22.8) | 0.84

1 Values are means or percentages (95% CI), adjusted for sex, age, and creatinine. Creatinine was adjusted for sex and age. * Different from CT (P = 0.02) and TT (P < 0.001); † different from CT (P < 0.001) and TT (P = 0.007); ‡ different from TT (P = 0.03); § different from CT (P = 0.02) and TT (P < 0.003).
2 P-value based on ANCOVA test for differences between means.
3 Geometric mean (95% CI).
4 Square root-transformed mean (95% CI).
Different from TT, within folate strata. Different from TT, interaction was significant (P = 0.007), we stratified the study participants by the median folate level (12.5 nmol/L) (Table 3). Geometric mean plasma tHcy concentrations were unrelated to MTHFR C677T genotype in subjects with plasma folate ≥ the sample median, but for subjects with plasma folate <12.5 nmol/L, mean tHcy was higher in those with the TT genotype (P = 0.001) than in those with the CC or CT genotypes.

We examined the influence of age and sex on the association between genotype and tHcy among participants with folate concentrations below the median. Because of a significant age and sex interaction (P = 0.02), we further stratified our sample by age (above and below 55 y) and gender (Table 4). In women with lower folate status, there were no differences in mean plasma tHcy concentrations among MTHFR genotypes in either age stratum. Exclusion of women receiving HRT did not affect this observation. Similarly, in older men (≥55 y old), the relationship between MTHFR genotype and plasma tHcy concentration was not significant in the low folate status group. However, in younger men (<55 y old) with low folate concentrations, those who were homozygous for the T allele had significantly higher tHcy concentrations than those carrying the C allele (P < 0.001). The prevalence of tHcy concentrations >13 μmol/L among the younger men with low folate concentrations was <20% for those with at least one C allele, whereas 45% of the homozygotes for the T allele had tHcy concentrations >13 μmol/L. The effect of genotype on the tHcy distribution in these younger men with low folate concentrations was not confined to the upper tail. For example, the 25th percentile value for tHcy in the homozygotes for the T allele, 10.5 μmol/L, was equal to the median tHcy value for those carrying the C allele.

### DISCUSSION

Age, sex, folate status and the common C677T polymorphism at the MTHFR gene locus are major determinants of plasma tHcy concentrations. In this study, we confirmed the previously reported folate-dependent association between this MTHFR mutation and plasma tHcy levels (25–29). As expected, an association between genotype and plasma tHcy concentration was confined to subjects with lower plasma folate concentrations. Our findings further suggest that this association is influenced by age and sex. In particular, a benefit

### TABLE 3

<table>
<thead>
<tr>
<th>Plasma folate</th>
<th>MTHFR Genotype</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma folate &lt; 12.5 nmol/L</td>
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<td></td>
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<tr>
<td>Mean total homocysteine, μmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>319</td>
<td>10.7 (10.4–11.1)*</td>
<td>10.7 (10.5–11.0)*</td>
</tr>
<tr>
<td>Plasma folate ≥ 12.5 nmol/L</td>
<td></td>
<td></td>
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<tr>
<td>Mean total homocysteine, μmol/L</td>
<td></td>
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</tr>
<tr>
<td>n</td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>388</td>
<td>8.4 (8.2–8.7)</td>
<td>8.7 (8.5–9.0)</td>
</tr>
</tbody>
</table>

1 Geometric mean total plasma homocysteine concentrations (95% CI), adjusted for age, sex, and creatinine and folate (as a continuous variable within folate strata). * Different from TT, P < 0.05.
2 P-value based on ANCOVA test for differences between means.

### TABLE 4

<table>
<thead>
<tr>
<th>Men &lt; 55 y old</th>
<th>MTHFR Genotype</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
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<td>Mean total homocysteine, μmol/L</td>
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<tr>
<td>n</td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>91</td>
<td>10.6 (10.0–11.3)*</td>
<td>10.7 (10.1–11.3)*</td>
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<tr>
<td>Men ≥ 55 y old</td>
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<tr>
<td>Mean total homocysteine, μmol/L</td>
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<tr>
<td>n</td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>77</td>
<td>12.5 (11.7–13.4)</td>
<td>12.1 (11.5–12.8)</td>
</tr>
<tr>
<td>Women &lt; 55 y old</td>
<td></td>
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<tr>
<td>Mean total homocysteine, μmol/L</td>
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</tr>
<tr>
<td>n</td>
<td>CC</td>
<td>CT</td>
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<tr>
<td>79</td>
<td>9.2 (8.8–9.7)</td>
<td>8.9 (8.5–9.3)</td>
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<tr>
<td>Women ≥ 55 y old</td>
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<tr>
<td>Mean total homocysteine, μmol/L</td>
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<tr>
<td>n</td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>72</td>
<td>10.9 (10.2–11.7)</td>
<td>11.5 (10.9–12.1)</td>
</tr>
</tbody>
</table>

1 Geometric mean total plasma homocysteine (95% CI), adjusted for age (as a continuous variable within age categories), creatinine and folate. * Different from TT, P < 0.05.
2 P-value based on ANCOVA test for differences between means.
to those with the CC genotype was demonstrated only for men <55 y old with low folate status, a subgroup that comprised only 11% of our sample. In this age/sex category, subjects with the CC genotype had tHcy concentrations that were ~25% (3.6 μmol/L) lower than those of subjects with the TT genotype. Meta-analyses from the Homocysteine Studies Collaboration (3) suggest that this difference in tHcy concentrations corresponds to an ~10–20% lower risk of ischemic heart disease and stroke, respectively.

Our study is the first to examine the simultaneous influence of age and sex on the relation between tHcy and MTHFR C677T genotype. Only a few of the many earlier studies that evaluated the effect of MTHFR genotypes on plasma tHcy concentrations conducted gender (49–54) or age (55–57) comparisons, and the results were mixed. Two of these studies reported associations in young and middle-aged adult men but not in women of comparable ages (49,50). Three studies found no association in men or women aged ≥70 y (51), ≥65 y (52), or 19–90 y (53). One study of subjects aged 40–59 y demonstrated an association in both sexes (54). None of these studies took into account the effect of folate status on the relation between tHcy and genotype. Three studies examined the association between tHcy and genotype at different ages in mixed samples of men and women (55,56) and separate samples of men (57) and women (55). The association generally diminished with age (55,57) but it persisted in older coronary artery disease patients (56). Two of these studies examined the influence of folate status (56,57), demonstrating that the association was present only in those with low folate status.

Information on the association between tHcy and MTHFR C677T genotype might also be gleaned from studies limited to a single sex or restricted age range. For example, Passaro et al. (58) reported that the association was present in a group of women aged 55–80 y, but these women were highly selected to exclude anyone with known cardiovascular risk factors. Brown et al. (59) observed the association to be present in postmenopausal women receiving HRT, but not in similarly aged women who were not receiving therapy. In a study of women aged 18–44 y, the association was present in those with low folate status (<8.4 nmol/L), but not among women with adequate folate (≥15.6 nmol/L) (60). Gudnason et al. (61) provided evidence of a strong association in a cohort of young men aged 22–25 y, with genotype accounting for 12% of variance in tHcy concentrations.

The findings of Kauwell et al. (62) may help to explain some of the inconsistencies regarding the influence of age and sex on the relationship between MTHFR C677T genotype and tHcy concentrations. Consistent with our findings, Kauwell et al. observed no cross-sectional relationship between MTHFR genotype and tHcy concentrations in a sample of women aged 60–85 y. However, after 7 wk of low folate diet, the relation between genotype and tHcy concentrations was apparent. This observation suggests that the relationship between genotype and tHcy is present in women and persists into older ages. Cross-sectional studies such as ours may fail to find true relationships between genotype and tHcy concentrations in the elderly and women because of the increased influence in those population subgroups of age- and gender-related risk factors for hyperhomocysteinemia. There is an abundant literature on the relation between impaired renal function and elevated tHcy concentrations (14), and there is growing evidence that plasma tHcy concentration is influenced by estrogen status in women (8,10,13,63–65). The analytical consequence of additional determinants such as these might be a diminished amount of variance in tHcy concentrations attributable to genotype, which could affect the ability of cross-sectional studies to detect genotype–tHcy associations. Because the other tHcy determinants are essentially held constant in short-term intervention studies such as that of Kauwell et al. (62), the true genotype–tHcy associations may be more easily demonstrated.

Our understanding of the relationships between genetic and environmental determinants of homocysteine concentrations remains incomplete. Because fairly modest elevations in tHcy concentrations are associated with a higher risk of vascular disease, it is crucial to better understand the interplay among factors that affect tHcy levels. Whether the observed effect of age and sex on the relation between MTHFR and tHcy concentrations is real or a consequence of our inability to detect the association against a background of other tHcy determinants, this finding could be of particular relevance in the light of the renewed debate on the role of the MTHFR mutation as a candidate risk factor for coronary heart disease (28). In conclusion, our data suggest that the influence of folate status, age and sex on this relationship between the MTHFR C677T mutation and tHcy requires further examination, especially in elderly cohorts, to critically assess the influence of this mutation on the risk of vascular disease.

**LITERATURE CITED**


