Human Genome Epidemiology (HuGE) Review

Meta- and Pooled Analyses of the Methyleneetetrahydrofolate Reductase (MTHFR) C677T Polymorphism and Colorectal Cancer: A HuGE-GSEC Review


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Worldwide, over 1 million cases of colorectal cancer (CRC) were reported in 2002, with a 50% mortality rate, making CRC the second most common cancer in adults. Certain racial/ethnic populations continue to experience a disproportionate burden of CRC. A common polymorphism in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene has been associated with a lower risk of CRC. The authors performed both a meta-analysis (29 studies; 11,936 cases, 18,714 controls) and a pooled analysis (14 studies; 5,068 cases, 7,876 controls) of the C677T MTHFR polymorphism and CRC, with stratification by racial/ethnic population and behavioral risk factors. There were few studies on different racial/ethnic populations. The overall meta-analysis odds ratio for CRC for persons with the TT genotype was 0.83 (95% confidence interval (CI): 0.77, 0.90). An inverse association was observed in whites (odds ratio = 0.83, 95% CI: 0.74, 0.94) and Asians (odds ratio = 0.80, 95% CI: 0.67, 0.96) but not in Latinos or blacks. Similar results were observed for Asians, Latinos, and blacks in the pooled analysis. The inverse association between the MTHFR 677TT polymorphism and CRC was not significantly modified by smoking status or body mass index; however, it was present in regular alcohol users only. The MTHFR 677TT polymorphism seems to be associated with a reduced risk of CRC, but this may not hold true for all populations.

colorectal neoplasms; epidemiologic methods; epidemiology; folic acid; genetics; genetic variation; methylenetetrahydrofolate reductase (NADPH2); MTHFR C677T

BACKGROUND

Gene

The 5,10-methylenetetrahydrofolate reductase (MTHFR) gene is located at the end of the short arm of chromosome 1 (1p36.3) (1). As Figure 1 illustrates, the MTHFR enzyme plays a vital role in folate metabolism, which affects DNA methylation and synthesis. This occurs through the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (1-carbon metabolism), the dominant circulating form of folate. The 5-methyltetrahydrofolate product donates a methyl group to homocysteine in the generation of S-adenosylmethionine, a major source of methyl groups used for DNA methylation. The MTHFR gene product contributes to maintaining circulating levels of folate and methionine, thus preventing the accumulation of homocysteine (2, 3) (Figure 1).
Putative mechanistic basis for MTHFR to influence cancer risk

Polymorphisms that affect MTHFR enzyme activity may modify individual cancer risk. Two mechanisms have been reported in the literature—DNA hypomethylation and uracil misincorporation into DNA—both of which have been linked to the development of cancer (4–8). These mechanisms are illustrated in Figure 1. A lack of MTHFR enzyme function could decrease the availability of 5-methyltetrahydrofolate, causing a subsequent decline in the conversion of homocysteine to methionine (Figure 1). This folate depletion may result in DNA hypomethylation, which may in turn influence cellular development and function. Any aberration of DNA methylation may be involved in the carcinogenesis process (5, 9). Second, as a result of folate deficiency, low levels of 5,10-methylenetetrahydrofolate decrease the synthesis of thymidylate deoxynucleoside monophosphate, which increases the ratio of deoxyuridine monophosphate to deoxynucleoside monophosphate, inducing uracil misincorporation. This process causes DNA damage (e.g., point mutations and/or chromosomal breaks), which could be associated with carcinogenesis (4).

Gene variants

Although several single nucleotide polymorphisms in the MTHFR gene have been reported, this review focuses on the C677T polymorphism. The C-to-T transition at nucleotide 677 in exon 4 is a point change that converts a cytosine molecule (C) to a thymine molecule (T), resulting in an amino acid substitution of alanine to valine (10). Published papers refer to CC as the wild type, CT as the heterozygous form, and TT as the homozygous variant. Subjects with the TT or CT genotype have lower levels of enzyme activity (30% and 65%, respectively) than subjects carrying the CC genotype (11, 12).

Gene frequency

There is geographic and ethnic variation in the population frequency of the T allele. US Hispanics and Southern Europeans have a high frequency of the variant allele (>40%), whereas other European populations (e.g., British, Irish) and US whites have frequencies between 30% and 36%. US blacks have a low frequency of the variant allele (14%), while Asian populations have a frequency of 11% (13). A detailed review of population frequencies of the C677T polymorphism has been published (13), but available data on Latinos and populations of African descent are very limited.

Disease

Worldwide, over 1 million colorectal cancer cases were reported in 2002 (with 529,000 deaths from the disease), making it the second most common cancer (9.4%) (14). Incidence rates for colorectal cancer vary globally: North America, Australia/New Zealand, Western Europe, and men in Japan have the highest incidence; Africa and Asia have the lowest incidence; and South America and the Caribbean have intermediate incidence rates. These geographic differences could be partly explained by different dietary folate
Objective

Behavioral risk factors

Tobacco use, alcohol consumption, and excess body weight have been linked to an increased risk of developing colorectal cancer (10, 14, 19–21). Dietary practices, including consumption of vegetables and dietary fiber, physical activity, hormone replacement therapy, and nonsteroidal anti-inflammation drugs have been associated with a reduced risk of colorectal cancer (10, 14, 19–21). Smoking has been shown to inactivate both folate and vitamin B12 (22, 23), while alcohol is a folate antagonist that is responsible for folate malabsorption and increased excretion, as well as abnormalities in folate metabolism through the inhibition of methionine synthase (24, 25). Obesity has been indirectly linked to lower consumption of folate-enriched foods but also more directly to changes in insulin and glucagon, which are regulatory factors for methionine metabolism (26).

Objective

The metabolic enzyme MTHFR has been identified as a key factor in the metabolism of folate, and C677T, a common polymorphism of the MTHFR gene, has been associated with a lower risk of colorectal cancer. Few studies on the MTHFR C677T polymorphism have been conducted in minority populations—especially blacks and Latinos (27–29), 2 groups that have increased risk for colorectal cancer. In 2 previously published meta-analyses focusing on the MTHFR C677T polymorphism and colorectal cancer, the investigators were unable to stratify their results by ethnicity because of limitations of the available data (30, 31). Several colorectal cancer risk factors, including tobacco use, alcohol consumption, and excess body weight, may interfere with folate metabolism (27–42). Previous studies have not explored the potentially modifying effects of these behavioral risk factors on the association between MTHFR C677T and colorectal cancer.

The purpose of this meta- and pooled analysis was to identify the role of the MTHFR C677T polymorphism in colorectal cancer in a large sample of subjects of different ethnic backgrounds and to assess the impact of behavioral risk factors on the association between MTHFR C677T and colorectal cancer. Our aims in the study were to 1) investigate the link between the C677T polymorphism and colorectal cancer, 2) examine ethnic and geographic variation in the association between the MTHFR polymorphism and colorectal cancer, and 3) examine whether the association between the MTHFR polymorphism and colorectal cancer is modified by 3 behavioral risk factors—tobacco use, alcohol consumption, and body mass index.

MATERIALS AND METHODS

Selection criteria—meta-analysis

We conducted a meta-analysis of all published articles and a pooled analysis of published and unpublished studies investigating the relation between the MTHFR C677T variant and colorectal cancer. To be eligible for inclusion, studies had to be case-control or nested case-control studies published between November 1, 1996, and April 30, 2008, that reported genotype frequencies for both case and control populations. November 1, 1996, was the publication date of the first report on the MTHFR polymorphism and risk of colorectal cancer (32).

To identify all pertinent studies, we initially conducted a broad search using PubMed to access the MEDLINE database and Ovid to access the Cumulative Index to Nursing and Allied Health Literature (CINAHL) database. The keywords “MTHFR,” “colorectal cancer,” “colon cancer,” “rectal cancer,” and “genetic polymorphisms” were used to identify published studies in the 2 databases. PubMed identified the specific Medical Subject Headings “methylentetrahydrofolate reductase (NADPH2),” “rectal neoplasms,” and “polymorphism, genetic” and the title-and-abstract term “colorectal neoplasm.” These Medical Subject Headings were also applied to the CINAHL database. PubMed also used “MTHFR,” “colorectal cancer,” and “genetic polymorphisms” as text words within the search. There was overlap between the 2 databases; 71 studies were identified in both the CINAHL database and MEDLINE. We also evaluated 2 meta-analysis review articles (30, 31) and manually searched bibliographies to ensure that any relevant but previously omitted articles were included in the review process. The searches yielded 199 articles.

Titles and abstracts for the 199 articles were reviewed by 2 independent researchers (M. A. Garza (University of Pittsburgh Medical Center) and C. Mitchell-Miland (University of Pittsburgh School of Public Health)). After multiple reviews of these articles, 29 met the criteria for relevance and were selected. Two abstractors reviewed each of the articles using a standardized data collection form, which resulted in good agreement for the 29 selected articles. The remaining 170 articles were deleted, for the following reasons: They focused on colorectal adenomas/tumors (8, 43–54); they did not focus on the 677C allele of the MTHFR gene (55, 56); they did not specify case and control occurrences of the CC, CT, and TT genotypes (55, 57–59); they were not case-control studies; they did not deal with colorectal disease; they were reviews or editorials; or they focused on treatment of colorectal cancer.

Data collection—pooled analysis

We extracted individual data from the Genetic Susceptibility to Environmental Carcinogens (GSEC) database (www.gsec.net) to further assess the association between...
**MTHFR C677T** and colorectal cancer among ethnic groups and in relation to 3 behavioral risk factors—tobacco use, alcohol consumption, and body mass index. GSEC is a collaborative project that collects information from both published and unpublished case-control studies on metabolic gene polymorphisms and cancer. Details are given elsewhere (60). At the time of enrollment, each investigator completes a standardized questionnaire regarding (but not limited to) study design, laboratory methods used for genotyping, the selection and source of controls, and the source of DNA for genotype analysis. Each investigator receives a specific request to provide his or her data for this pooled analysis if he/she has not already responded to the initial request.

Data on smoking were available for 12 of the 14 studies used in the pooled analysis. However, only 9 of the 12 studies provided sufficiently detailed information for calculation of pack-years (packs of cigarettes smoked per day times years of smoking). Smoking status was categorized as never smoker (self-reported smoking of fewer than 100 cigarettes in one’s lifetime) or ever smoker (including current smokers and ex-smokers). No validation of smoking status with cotinine data was available in these studies.

Data on alcohol consumption were available for 10 of the 14 studies, with variable response categories (never drinker, ex-drinker, current drinker, or ever drinker). For purposes of comparison across studies, current drinkers, ex-drinkers, and ever drinkers were all assembled into the category “ever drinker.” No information on type of beverage or quantity consumed was available.

Body mass index (weight (kg)/height (m)²) was calculated from self-reported height and weight recorded at the time of interview and was available for 10 of the 14 studies.

**Statistical analysis**

**Meta-analysis.** Numbers of cases and controls by **MTHFR** genotype (CC, CT, and TT genotypes) were extracted from the published studies. The corresponding study-specific crude odds ratios and 95% confidence intervals for colorectal cancer risk were calculated.

We imported data into STATA, version 9.2 (Stata Corporation, College Station, Texas), and calculated pooled estimates of the odds of the occurrence of a CT polymorphism as compared with a CC polymorphism and the odds of the occurrence of a TT polymorphism as compared with a CC polymorphism. To determine whether to use the fixed- or random-effects model, we measured statistical heterogeneity between and within groups using the $Q$ statistic (61). We used fixed-effects methods if the result of the $Q$ test was not significant. Otherwise, we calculated pooled estimates and confidence intervals assuming a random-effects model with inverse-variance weighting, using the DerSimonian and Laird method (61). The proportion of total variability attributed to between-study heterogeneity, the $I^2$ statistic, and its corresponding 95% confidence (uncertainty) interval were calculated (62, 63). This statistic is useful when deciding whether there is too much heterogeneity to combine the studies and derive a pooled estimate. While publication bias was not expected, we assessed this possibility using Begg funnel plots and Egger’s bias test (64).

We assessed the cumulative effect of each study on the pooled prevalence estimate by adding studies one at a time, ordered by publication date. To evaluate the weight of particular studies on the pooled estimate, we performed influence analysis. This method recalculates the pooled prevalence estimate while omitting 1 study at a time. We calculated separate pooled estimates for different ethnic groups (white, Asian) and geographic regions (United States, Europe, and Asia). We performed meta-regression analyses to determine whether the pooled estimates differed significantly by ethnicity or geographic region.

**Pooled analysis.** We combined individual data on controls across all studies included in the pooled analysis to assess the relative frequency of the **MTHFR** C677T polymorphism by ethnicity.

We calculated crude odds ratios and their 95% confidence intervals to assess the association between the **MTHFR** C677T polymorphism and colorectal cancer by ethnicity (white, African-American, Asian, Latino), geographic region (United States, Europe, Asia), type of controls (hospital, healthy), and available behavioral risk factors (tobacco use, alcohol consumption, and body mass index). For tobacco use, subjects were divided into 3 categories defined by pack-years of smoking, with persons who had ever smoked being classified as above or below the median of the controls (never, $\leq$14.2 pack-years, $>$14.2 pack-years). Alcohol consumption was recoded into 2 categories (never drinker and ever drinker). Body mass index was calculated by self-reported height and weight and categorized into 4 groups previously defined by the Centers for Disease Control and Prevention (<18.5, 18.5–24.9, 25.0–29.9, and $\geq$30.0; http://www.cdc.gov/nccdphp/dnpa/obesity/defining.htm). For exploratory purposes, we tested the multiplicative interaction between the **MTHFR** polymorphism and each behavioral risk factor (smoking status, alcohol, and body mass index) using the Wald test, to assess whether the relation between the gene and risk of colorectal cancer was modified by the behavioral risk factor. The CC genotype was treated as the referent group. Adjusted odds ratios were calculated using multiple logistic regression, adjusting for study, age category (18–49, 50–59, 60–69, or $\geq$70 years), sex, and ethnic group, using fixed-effects models. SAS/STAT software, version 9.1 (SAS Institute Inc., Cary, North Carolina), was used to conduct all pooled analyses.

**RESULTS**

**Meta-analysis**

Twenty-nine studies were included in the meta-analysis, for a total of 30,650 subjects (11,936 cases and 18,714 controls). Table 1 provides general characteristics of the studies. Of these studies, 5 reported on blacks (27–29, 65, 66), 3 reported on Latinos (27, 28, 66), and 9 reported on Asians (28, 67–74). Some included mixed populations, but data on individual ethnicities could not be disentangled.

For associations between colorectal cancer and both the CT and TT genotypes, the $Q$ test of homogeneity was not significant; thus, the fixed-effects pooling method was used.
(for CT, $Q = 32.887$, $P = 0.240$, and $I^2 = 15\%$ (95% confidence interval (CI): 0, 46); for TT, $Q = 37.602$, $P = 0.106$, and $I^2 = 26\%$ (95% CI: 0, 53)). Publication bias was not
evident, as assessed by both Begg’s plot and Egger’s test. For both odds ratios, no study had undue influence on the
summary estimates.

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<table>
<thead>
<tr>
<th>Authors (Ref. No.)</th>
<th>Year of Publication</th>
<th>No. of Cases</th>
<th>No. of Controls</th>
<th>Country</th>
<th>Ethnic Group(s) and %</th>
<th>Source of Controls</th>
<th>CT vs. CC OR 95% CI</th>
<th>TT vs. CC OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park et al. (72)</td>
<td>1999</td>
<td>200</td>
<td>460</td>
<td>South Korea</td>
<td>Korean, 100</td>
<td>Healthy persons</td>
<td>0.94 0.65, 1.36</td>
<td>0.81 0.48, 1.38</td>
</tr>
<tr>
<td>Yin et al. (69)</td>
<td>2004</td>
<td>685</td>
<td>778</td>
<td>Japan</td>
<td>Japanese, 100</td>
<td>Hospital patients</td>
<td>0.93 0.74, 1.16</td>
<td>0.66 0.48, 0.91</td>
</tr>
<tr>
<td>Kim et al. (33)b</td>
<td>2004</td>
<td>243</td>
<td>225</td>
<td>South Korea</td>
<td>Korean, 100</td>
<td>Healthy persons</td>
<td>1.08 0.73, 1.61</td>
<td>1.02 0.58, 1.80</td>
</tr>
<tr>
<td>Jiang et al. (71)b</td>
<td>2005</td>
<td>126</td>
<td>343</td>
<td>China</td>
<td>Chinese, 100</td>
<td>Healthy persons</td>
<td>1.08 0.70, 1.69</td>
<td>0.64 0.33, 1.22</td>
</tr>
<tr>
<td>Matsuo et al. (67)b</td>
<td>2005</td>
<td>257</td>
<td>771</td>
<td>Japan</td>
<td>Japanese, 100</td>
<td>Hospital patients</td>
<td>0.89 0.66, 1.21</td>
<td>0.73 0.48, 1.13</td>
</tr>
<tr>
<td>Otani et al. (68)</td>
<td>2005</td>
<td>121</td>
<td>245</td>
<td>Japan</td>
<td>Japanese, 100</td>
<td>Hospital patients</td>
<td>0.69 0.39, 1.19</td>
<td>0.70 0.37, 1.33</td>
</tr>
<tr>
<td>Wang et al. (70)</td>
<td>2006</td>
<td>302</td>
<td>291</td>
<td>India</td>
<td>Indian, 100</td>
<td>Healthy persons</td>
<td>1.19 0.74, 1.91</td>
<td>NA  NA</td>
</tr>
<tr>
<td>Chang et al. (73)</td>
<td>2007</td>
<td>195</td>
<td>195</td>
<td>Taiwan</td>
<td>Taiwanese, 100</td>
<td>Hospital patients</td>
<td>1.07 0.70, 1.63</td>
<td>1.62 0.81, 3.26</td>
</tr>
<tr>
<td>Matsuo et al. (74)</td>
<td>2002</td>
<td>142</td>
<td>241</td>
<td>Japan</td>
<td>Japanese, 100</td>
<td>Healthy persons</td>
<td>1.36 0.84, 2.18</td>
<td>1.27 0.66, 2.44</td>
</tr>
</tbody>
</table>

European Countries

| Ryan et al. (78)    | 2001                | 136          | 848            | Ireland   | White, 100           | Healthy persons   | 2.01 1.36, 2.96 | 1.51 0.80, 2.86 |
| Sachse et al. (79)b | 2002                | 490          | 592            | United Kingdom | White, 100       | Healthy persons   | 0.83 0.65, 1.07 | 1.23 0.80, 1.89 |
| Plaschke et al. (80)b| 2003                | 287          | 346            | Germany   | White, 100           | Healthy persons   | 0.85 0.61, 1.18 | 1.00 0.60, 1.68 |
| Toffoli et al. (81)b| 2003                | 276          | 279            | Italy     | White, 100           | Healthy persons   | 0.92 0.63, 1.55 | 0.61 0.36, 1.01 |
| Pufulete et al. (87)b| 2003                | 28           | 76             | United Kingdom | White, 100        | Healthy persons   | 0.53 0.19, 1.52 | 2.56 0.72, 9.13 |
| Ullik et al. (82)   | 2004                | 2,178        | 2,178          | Norway    | White, 100           | Healthy persons   | 1.00 0.89, 1.14 | 0.73 0.59, 0.92 |
| Landi et al. (88)b  | 2005                | 350          | 309            | Spain     | White, 100           | Hospital patients | 0.97 0.69, 1.36 | 0.89 0.58, 1.38 |
| Van Guelpen et al. (75) | 2006               | 254          | 415            | Sweden    | White, 100           | Healthy persons   | 0.91 0.65, 1.28 | 0.49 0.25, 0.97 |
| Zeybek et al. (83)  | 2007                | 86           | 144            | Turkey    | White, 100           | Hospital patients | 1.48 0.74, 2.94 | 1.66 0.59, 4.69 |
| Sharp et al. (84)b  | 2008                | 275          | 370            | Scotland  | White, 100           | Healthy persons   | 0.91 0.65, 1.27 | 0.71 0.41, 1.23 |

United States

| Chen et al. (32)    | 1996                | 144          | 627            | United States | White, 100         | Healthy persons   | 1.02 0.69, 1.49 | 0.65 0.34, 1.23 |
| Ma et al. (38)      | 1997                | 202          | 326            | United States | White, 100         | Healthy persons   | 1.10 0.76, 1.60 | 0.58 0.32, 1.06 |
| Slattery et al. (28)| 2000                | 1,467        | 1,821          | United States | Black, 4.2; Hispanic, 4.4; white, 91.4 | Hospital patients | 1.02 0.88, 1.18 | 0.83 0.65, 1.05 |
| Keu et al. (29)b    | 2002                | 643          | 1,048          | United States | Black, 39; white, 61 | Healthy persons   | 1.00 0.80, 1.26 | 0.73 0.45, 1.18 |
| Le Marchand et al. (39)b | 2002              | 548          | 656            | United States | Japanese, 58.8; white, 27.2; Hawaiian, 14.0 | Healthy persons   | 0.90 0.71, 1.15 | 0.71 0.50, 1.01 |
| Le Marchand et al. (27)b | 2005              | 822          | 2,021          | United States | Japanese-American, 26; white, 23; Latino American, 22; black, 16; Native American, 7; other, 6 | Healthy persons   | 1.08 0.91, 1.28 | 0.85 0.65, 1.12 |
| Koushik et al. (85) | 2006                | 349          | 794            | United States | White, 100          | Healthy persons   | 0.95 0.72, 1.24 | 0.73 0.48, 1.09 |
| Murtaugh et al. (66)| 2007                | 751          | 979            | United States | White, 84; Hispanic, 6; black, 4; other, 5 | Healthy persons   | 0.99 0.65, 1.27 | 0.97 0.71, 1.33 |

Other Countries

| Shannon et al. (86) | 2002                | 501          | 1,207          | Australia  | White, 100           | Healthy persons   | 0.75 0.60, 0.94 | 1.03 0.72, 1.47 |
| Lima et al. (65)b   | 2007                | 102          | 300            | Brazil     | White, 88.2; black, 9.8; unclassified, 2.0 | Hospital patients | 1.26 0.78, 2.05 | 1.74 0.86, 3.55 |

Abbreviations: CI, confidence interval; NA, not available; OR, odds ratio.

a One study published in Hungarian (58) could not be evaluated in the meta-analysis.
b Study was also included in the pooled analysis ($n = 13$ studies).
The overall meta-analysis odds ratio for colorectal cancer for persons with the CT genotype was 0.99 (95% CI: 0.94, 1.04) (Figure 2). Twenty-seven studies had odds ratios with 95% confidence intervals containing the value of 1, with 11 showing positive associations, 14 inverse associations, and 2 being equal to the null. Of the 2 studies with significant findings, 1 showed a positive association (odds ratio (OR) = 2.01, 95% CI: 1.36, 2.96) and 1 showed an inverse association (OR = 0.75, 95% CI: 0.60, 0.94).

The overall meta-analysis odds ratio for genotype TT was 0.83 (95% CI: 0.77, 0.90) (Figure 3). Twenty-six of the 29 studies had odds ratios with 95% confidence intervals containing the null value, with 10 odds ratios indicating a positive association and 15 an inverse association. All 3 studies with significant findings showed inverse associations between genotype TT and colorectal cancer.

**Colorectal cancer and MTHFR C677T—ethnic/geographic variation**

In 22 of the 29 studies, investigators presented separate data for white and Asian ethnic groups (Table 2). There was an inverse association between MTHFR C677T and colorectal cancer which was statistically significant when the TT variant was considered, with no differences between whites and Asians. In 27 studies, investigators conducted analyses by geographic region (United States, Europe, Asia), with consistent significant inverse associations between the TT genotype and colorectal cancer being observed across all geographic areas but Europe.

**Pooled analysis**

The 14 studies included in the pooled analysis (Table 1) covered a total of 12,944 subjects (5,068 cases and 7,876 controls). Of these, 13 studies were also included in the meta-analysis; 1 study published in Hungarian (58) could not be evaluated in the meta-analysis but was present in the GSEC database. Three studies included blacks (418 cases/766 controls), 1 study included Latinos/Hispanics (166 cases/469 controls), and 3 studies included Asians (626 cases/1,339 controls). A total of 223 subjects were excluded because of missing information on MTHFR genotype, and an additional 316 were missing data on age or sex, resulting...
Table 2. Meta-Analysis Odds Ratios for the Relation Between Methylenetetrahydrofolate Reductase (MTHFR) 677TT Genotype and Colorectal Cancer, by Ethnic Group and Geographic Region

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of Studies</th>
<th>No. of Subjects</th>
<th>Reference Nos.</th>
<th>CT vs. CC</th>
<th>TT vs. CC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Ethnic group&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>14</td>
<td>13,992</td>
<td>32, 38, 75, 78–86, 87, 88</td>
<td>0.97</td>
<td>0.86, 1.09</td>
</tr>
<tr>
<td>Asian&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8</td>
<td>5,183</td>
<td>33, 67–69, 71–74</td>
<td>0.97</td>
<td>0.86, 1.10</td>
</tr>
<tr>
<td>Geographic region&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>8</td>
<td>12,922</td>
<td>27–29, 32, 38, 39, 66, 85</td>
<td>1.01</td>
<td>0.94, 1.09</td>
</tr>
<tr>
<td>Europe</td>
<td>10</td>
<td>9,842</td>
<td>75, 78–84, 87, 88</td>
<td>0.99</td>
<td>0.91, 1.08</td>
</tr>
<tr>
<td>Asia&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9</td>
<td>5,776</td>
<td>33, 67–74</td>
<td>0.99</td>
<td>0.87, 1.11</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio.

<sup>a</sup> Meta-estimate P value: white—CT, P = 0.56; TT, P = 0.003; Asian—CT, P = 0.66; TT, P = 0.016; United States—CT, P = 0.78; TT, P < 0.001; Europe—CT, P = 0.77; TT, P = 0.35; Asia—CT, P = 0.80; TT, P = 0.02.

<sup>b</sup> Six studies were excluded because they contained mixed racial/ethnic groups or insufficient numbers of studies for grouping (e.g., the study population in the study by Wang et al. (70) was 100% Indian).

<sup>c</sup> The study by Wang et al. (70) was not included in the Asian ethnicity analysis because the study population was 100% Indian; however, the Wang et al. (70) study was included in the Asian geographic region because India is located in Asia.

<sup>d</sup> Two studies (65, 86) were excluded because they did not fall within the 3 defined categories.
The relative frequencies of the homozygous variant MTHFR C677T polymorphism in controls were similar in Latinos/Hispanics (19%) and Asians (18%), but the frequency was slightly lower in whites (13%); blacks had the lowest frequency (2%) (Table 3). The overall difference in TT frequency among the controls was statistically significant ($P < 0.0001$).

There was a significant inverse association between the TT genotype and colorectal cancer (adjusted OR = 0.85, 95% CI: 0.75, 0.96). Analysis stratified by ethnicity showed adjusted odds ratios for the TT genotype of 0.68 (95% CI: 0.55, 0.85) in Asians, 0.95 (95% CI: 0.55, 1.62) in Latinos, and 1.13 (95% CI: 0.48, 2.63) in blacks (Table 3).

When an analysis by tumor site was performed, the inverse association seemed to be restricted to colon cancer (Table 4).

For studies conducted in the United States, having the TT genotype appeared to significantly reduce the risk of colorectal cancer (OR = 0.80, 95% CI: 0.66, 0.97). The adjusted odds ratios for the TT genotype in studies conducted in Europe and Asia were 0.99 (95% CI: 0.74, 1.10) and 0.77 (95% CI: 0.57, 1.04), respectively (Table 4).

**Behavioral risk factors**

**Smoking status.** Among subjects with more than 14.2 pack-years of cigarette smoking, subjects with the MTHFR TT genotype showed a statistically significant decrease in risk of developing colorectal cancer, while no association between MTHFR and colorectal cancer was observed among never smokers and persons with 14.2 pack-years or less (Table 5). However, no significant effect of the interaction between the MTHFR polymorphism and smoking status on colorectal cancer risk was observed ($P = 0.43$).

**Alcohol consumption.** Subjects who reported ever drinking alcohol and had the TT genotype had a 22% lower risk of developing colorectal cancer (Table 6). This inverse association was not observed among subjects who reported being never drinkers. However, the interaction term for the interaction between alcohol use and MTHFR was not significant ($P = 0.23$).

**Body mass index.** In general, there was an inverse association between the MTHFR polymorphism and colorectal cancer across strata of body mass index; however, no statistically significant effect of the interaction between body mass index and MTHFR on colorectal cancer risk was observed ($P$ for interaction = 0.47) (Table 7).

**DISCUSSION**

**Overall association between the MTHFR C677T genotype and colorectal cancer risk**

The present meta-analysis confirms that homozygosity for the T allele (MTHFR 677TT polymorphism) is associated with a significantly reduced risk of colorectal cancer, as previously reported in 2 other meta-analyses (30, 31).
In their meta-analysis, Huang et al. (31) examined the associations between MTHFR C677T and A1298C polymorphisms in both colorectal adenoma and colorectal cancer and included 20 studies; 18 of those studies were included in the present analysis, which also included an additional 11 studies primarily published between 2005 and 2008. Huang et al.’s meta-analysis showed a small but significant inverse association between colorectal cancer and the TT genotype (OR = 0.93, 95% CI: 0.89, 0.97) (31), similar to what we report here.

In the second meta-analysis, Hubner and Houlston (30) reported on 22 studies, 19 of which overlapped with the

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of Studies</th>
<th>No. of Subjects</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>CT vs. CC</th>
<th>TT vs. CC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Geographical location</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>3</td>
<td>3,567</td>
<td>1,784</td>
<td>50.01</td>
<td>1,366</td>
<td>38.30</td>
<td>417</td>
</tr>
<tr>
<td>Controls</td>
<td>3</td>
<td>1,934</td>
<td>980</td>
<td>50.67</td>
<td>771</td>
<td>39.87</td>
<td>183</td>
</tr>
<tr>
<td>Cases</td>
<td>3</td>
<td>1,336</td>
<td>505</td>
<td>37.80</td>
<td>601</td>
<td>44.99</td>
<td>230</td>
</tr>
<tr>
<td>Asia</td>
<td>3</td>
<td>624</td>
<td>243</td>
<td>38.94</td>
<td>295</td>
<td>47.28</td>
<td>86</td>
</tr>
<tr>
<td>Europe</td>
<td>7</td>
<td>2,238</td>
<td>924</td>
<td>41.29</td>
<td>1,011</td>
<td>45.17</td>
<td>303</td>
</tr>
<tr>
<td>Controls</td>
<td>7</td>
<td>2,304</td>
<td>968</td>
<td>42.01</td>
<td>1,043</td>
<td>45.27</td>
<td>293</td>
</tr>
<tr>
<td>Cases</td>
<td>7</td>
<td>2,280</td>
<td>1,035</td>
<td></td>
<td>987</td>
<td></td>
<td>258</td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>10</td>
<td>6,042</td>
<td>2,755</td>
<td></td>
<td>2,486</td>
<td></td>
<td>801</td>
</tr>
<tr>
<td>Controls</td>
<td>6,042</td>
<td>2,280</td>
<td>1,035</td>
<td></td>
<td>987</td>
<td></td>
<td>258</td>
</tr>
<tr>
<td>Cases</td>
<td>6,042</td>
<td>2,280</td>
<td>1,035</td>
<td></td>
<td>987</td>
<td></td>
<td>258</td>
</tr>
<tr>
<td>Rectum</td>
<td>8</td>
<td>4,895</td>
<td>2,143</td>
<td></td>
<td>2,064</td>
<td></td>
<td>688</td>
</tr>
<tr>
<td>Controls</td>
<td>4,895</td>
<td>2,143</td>
<td>2,064</td>
<td></td>
<td>688</td>
<td></td>
<td>688</td>
</tr>
<tr>
<td>Cases</td>
<td>4,895</td>
<td>2,143</td>
<td>2,064</td>
<td></td>
<td>688</td>
<td></td>
<td>688</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio.

a Wald $\chi^2$ test (2 df) $P$ value: United States—$P = 0.0500$; Asia—$P = 0.22$; Europe—$P = 0.56$; colon—$P = 0.058$; rectum—$P = 0.21$.

b Adjusted for age, sex, and study.

c One study from Brazil (65) was excluded because it did not fall within the 3 defined categories.

In their meta-analysis, Huang et al. (31) examined the associations between MTHFR C677T and A1298C polymorphisms in both colorectal adenoma and colorectal cancer and included 20 studies; 18 of those studies were included in the present analysis, which also included an additional 11 studies primarily published between 2005 and 2008. Huang et al.’s meta-analysis showed a small but significant inverse association between colorectal cancer and the TT genotype (OR = 0.93, 95% CI: 0.89, 0.97) (31), similar to what we report here.

In the second meta-analysis, Hubner and Houlston (30) reported on 22 studies, 19 of which overlapped with the
The consistency of the results from several meta-analyses and the pooled analysis seems to point to a predominant role of the MTHFR polymorphism in increasing the availability of 5,10-methylenetetrahydrofolate for DNA synthesis, which would be partially responsible for the reduced risk of colorectal cancer in subjects carrying the TT genotype.

### Ethnic variation in the association between the MTHFR C677T genotype and colorectal cancer risk

The meta-analysis stratified by ethnicity showed that the inverse association between the MTHFR C677T genotype and colorectal cancer was restricted to Asian and white populations. The same results were reported in the previous meta-analyses (30, 31). One of the limitations of

### Table 6. Pooled Analysis Odds Ratios for Colorectal Cancer Risk Among Persons With the Methylenetetrahydrofolate Reductase (MTHFR) 677TT Genotype, by Alcohol Consumptiona

<table>
<thead>
<tr>
<th>Alcohol Consumption</th>
<th>No. of Subjects</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>CT vs. CC</th>
<th>TT vs. CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never drinker</td>
<td>4,071</td>
<td>1,295</td>
<td>48.90</td>
<td>1,019</td>
<td>38.48</td>
<td>334</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>681</td>
<td>47.86</td>
<td>586</td>
<td>41.18</td>
</tr>
<tr>
<td>Ever drinker</td>
<td>5,657</td>
<td>1,502</td>
<td>43.68</td>
<td>1,477</td>
<td>42.95</td>
<td>460</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>1,023</td>
<td>46.12</td>
<td>941</td>
<td>42.43</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio.

a Unadjusted P value for interaction between alcohol use and MTHFR genotype: P = 0.23. Wald χ² test (2 df) P value, by alcohol consumption: never drinker—P = 0.17; ever drinker—P = 0.0129.

b Adjusted for age, sex, study, and ethnic group.

### Table 7. Pooled Analysis Odds Ratios for Colorectal Cancer Risk Among Persons With the Methylenetetrahydrofolate Reductase (MTHFR) 677TT Genotype, by Body Mass Indexa Categoryb

<table>
<thead>
<tr>
<th>BMI Category</th>
<th>No. of Subjects</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>CT vs. CC</th>
<th>TT vs. CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18.5</td>
<td>299</td>
<td>73</td>
<td>43.71</td>
<td>67</td>
<td>40.12</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>61</td>
<td>46.21</td>
<td>55</td>
<td>41.67</td>
</tr>
<tr>
<td>18.5–24.9</td>
<td>4,365</td>
<td>1,123</td>
<td>41.39</td>
<td>1,180</td>
<td>43.49</td>
<td>410</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>719</td>
<td>43.52</td>
<td>730</td>
<td>44.19</td>
</tr>
<tr>
<td>25.0–29.9</td>
<td>3,355</td>
<td>1,004</td>
<td>47.43</td>
<td>863</td>
<td>40.77</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>588</td>
<td>47.50</td>
<td>509</td>
<td>41.11</td>
</tr>
<tr>
<td>≥30.0</td>
<td>1,566</td>
<td>547</td>
<td>55.42</td>
<td>342</td>
<td>34.65</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>313</td>
<td>54.06</td>
<td>221</td>
<td>38.17</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

* Weight (kg)/height (m)².

b Unadjusted P value for interaction between BMI category and MTHFR genotype: P = 0.47. Wald χ² test (2 df) P value, by BMI category: BMI <18.5—P = 0.46; BMI 18.5–24.9—P = 0.0664; BMI 25.0–29.9—P = 0.73; BMI ≥30.0—P = 0.36.

c Adjusted for age, sex, study, and ethnic group.

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meta-analysis is the inability to stratify data for covariates of interest. In this case, no investigators reported results from 
subgroup analysis for blacks and Latinos; therefore, the 
association in these 2 groups could not be assessed.

Data from the pooled analysis also allowed us to further 
explore differences in the associations between MTHFR 
C677T and colorectal cancer among different ethnic popula-
tions. Individual data from 3 studies that included blacks 
(27, 29, 65), 1 of which also included Latinos, were 
available (27). In the pooled analysis, the MTHFR 677TT poly-
morphism was associated with a significantly reduced risk 
of colorectal cancer in Asian populations, thus confirming 
the findings of the present meta-analysis and the 2 previ-
ously published meta-analyses (30, 31), whereas no associ-
aton was observed in whites, Latinos, or blacks, although 
the sample sizes for the latter 2 categories were very small. 
The discrepancy between the results of the meta-analysis 
and the pooled analysis in white subjects could be due to 
the fact that not all of the studies included in the meta-
analyses were included in the pooled analysis, thus introduc-
ing some selection bias. In fact, when we restricted the 
meta-analysis to whites to the 6 studies that were also in-
cluded in the pooled analysis, the association between co-
lorectal cancer and MTHFR 677TT was not statistically 
significant. Thus, it is possible that the meta-analysis in-
cluded a larger number of studies, most of which showed 
a significant inverse relation between the gene polymor-
phism and colorectal cancer.

Differences in allelic distribution by ethnicity could be 
partially responsible for the observed differences in the as-
association between MTHFR C677T and colorectal cancer, as 
shown by the distribution of the gene polymorphism by 
ethnic group in Table 3; however, the influence of dietary 
habits associated with ethnicity cannot be discounted.

Geographic variation in the association between the 
MTHFR C677T genotype and colorectal cancer risk

In the present meta-analysis, studies conducted in each of 
the defined geographic regions—the United States, Europe, 
and Asia—showed a reduced risk of colorectal cancer with 
the MTHFR polymorphism. This is at variance with previ-
ous findings (30, 31), where the meta-analysis estimates 
from studies conducted in the United States and Asia were 
associated with reduced risk of colorectal cancer but the 
estimates from studies conducted in Europe were not. Dif-
fences in the studies’ inclusion criteria, as well as the 
addition of newly published studies to the present analysis, 
may explain the discrepancies. In the pooled analysis, only 
studies conducted in the United States showed a significantly 
reduced risk of colorectal cancer, while studies conducted in 
Asia and Europe, despite having the same direction of 
association as the US studies, did not reach statistical signif-
icance, probably because of statistical power issues.

Association between MTHFR C677T genotype and 
colorectal cancer risk by behavioral risk factors

Individual data from the pooled analysis were used to 
investigate modifying effects of behavioral risk factors— 
tobacco use, alcohol consumption, and body mass index— 
on the association between MTHFR C677T and colorectal 
cancer. The homozygous variant form of MTHFR C677T 
showed a protective effect on colorectal cancer across strata 
of smoking status; this effect was more evident in subjects 
who had smoked more than 14.2 packs of cigarettes per 
year, although there was no significant interaction effect 
between smoking dose and MTHFR C677T on colorectal 
cancer risk. This result is counterintuitive, since tobacco 
use acts as an antagonist of folate uptake, decreasing folate 
levels and affecting folate metabolism (34–37). In addition, 
smokers tend to have poorer diets (lower in fruits and vege-
tables), thus decreasing their dietary folate intake, and to 
consume more alcohol than nonsmokers. Other unmeasured 
confounding factors associated with smoking could be par-
tially responsible for the finding. For example, information 
on the use of oral contraceptives and estrogen replacement 
therapy was not collected in the pooled analysis, and these 
behavioral factors may be associated with a specific type of 
smoking behavior. The present findings could be due to 
chance; one must also bear in mind that the analysis of in-
teraction performed here had an explorative purpose and 
was meant to be hypothesis-generating.

The protective effect of the MTHFR C677T homozygous 
variant on colorectal cancer risk was also restricted to sub-
jects who reported using alcoholic beverages regularly. 
These findings are consistent with those reported by Otani 
et al. (68), although their results were not statistically sig-
nificant; in several other studies, investigators reported that 
the MTHFR polymorphism was associated with a lower risk 
of colorectal cancer in subjects with adequate-to-high levels 
of folate and low levels of alcohol use (27, 32, 33, 38, 39, 
69). Alcohol consumption interacts with folate metabolism, 
leading to folate depletion, which in turn contributes to 
abnormal DNA synthesis and methylation (40–42). Unfor-
natunately, no individual data on folate were available for the 
studies included in this pooled analysis. In addition, it is 
possible that some misclassification in alcohol intake was 
present in the original studies, given the self-reported nature 
of the information collected through questionnaires.

The inverse association between the TT genotype and co-
lorectal cancer persisted across strata of body mass index. 
This analysis included individual data from case-control stud-
ies; thus, information on body mass index prior to disease 
diagnosis was not available. In addition, the available infor-
mation on weight and height was self-reported. These limita-
tions may explain the results observed. Although obesity is 
a risk factor for colorectal cancer, investigators reported levels 
of body mass index only to compare cases and controls; no 
data are currently available on the effect of the MTHFR ge-
notype on such an association (27, 29, 34, 67, 68, 70, 75, 76).

Limitations

The database for the pooled analysis included limited 
numbers of studies on ethnic minority groups; only 3 studies 
collected data on blacks and only 1 study collected data on 
Latinos, reflecting the current lack of epidemiologic studies 
in these populations. There were also limitations of the spe-
cific data available, as happens with meta- and pooled

Am J Epidemiol 2009;170:1207–1221
analyses; for example, data on the duration of smoking and alcohol consumption, the type of tobacco smoked, and the type and amount of alcohol consumed were available in only a small fraction of the pooled data set. Despite the attempt to standardize the type of behavioral information collected by the various studies, it is possible that some differences still existed across studies in methods used for data collection, in questionnaire structure, etc. Such differences cannot be fully quantified and addressed in the reanalysis of existing data.

An additional limitation was the lack of information on folate intake or levels in the available studies; therefore, the role of folate in the association between MTHFR and colorectal cancer could not be addressed by this pooled analysis.

Another important issue that was not addressed was the role of the timing of folate consumption in the carcinogenesis process. As Ulrich and Potter (77) pointed out recently, administration of folate prior to the existence of preneoplastic lesions can prevent tumor development, whereas its consumption after precancerous lesions have become established may increase the chance of progression because of the nucleotide synthesis function of folate in rapidly proliferating tissues.

LABORATORY TESTS

Methods used for MTHFR C677T genotyping were described in detail in each article. In the majority of the studies, investigators reported using polymerase chain reaction (73%) and genomic DNA extracted from blood (96%).

POPULATION TESTING

At this point, an association between the MTHFR 677TT polymorphism and colorectal cancer has not been clearly established, and we need more data on racial and ethnic minorities. Thus, there is insufficient evidence to warrant population testing for the MTHFR C677T polymorphism in risk assessment for colorectal cancer.

CONCLUSIONS AND RECOMMENDATIONS FOR RESEARCH

To our knowledge, this is the first pooled analysis with sufficient individual data to stratify results by both ethnicity and 3 behavioral risk factors. This analysis supports conclusions that homozygosity for the T allele (MTHFR 677TT polymorphism) is associated with a reduced risk of colorectal cancer, but this association may not hold true for all populations. The inverse association between the MTHFR homozygous variant and colorectal cancer risk was not modified by smoking status or body mass index; however, it was restricted to regular alcohol users. Exploring the interaction between the MTHFR C677T polymorphism and behavioral and environmental determinants of colorectal cancer risk is necessary to better understand how this risk affects population subgroups. It is possible that C677T may contribute, in combination with environmental exposures, to the differences in colorectal cancer prevalence between different ethnic/racial populations. The low prevalence of the MTHFR 677TT genotype observed among blacks, in conjunction with dietary factors, folate intake, and smoking and drinking habits, may constitute a combination of risk factors for colorectal cancer that deserves further investigation. These findings warrant larger studies to clarify the role of the MTHFR 677TT polymorphism and evaluate gene-environment interactions for MTHFR.

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