Human Genome Epidemiology (HuGE) Review

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

E. Taioli*, M. A. Garza, Y. O. Ahn, D. T. Bishop, J. Bost, B. Budai, K. Chen, F. Gemignani, T. Keku, C. S. P. Lima, L. Le Marchand, K. Matsuo, V. Moreno, J. Plaschke, M. Pufulete, S. B. Thomas, G. Toffoli, C. R. Wolf, C. G. Moore, and J. Little

* Correspondence to Dr. Emanuela Taioli, SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203 (e-mail: emanuela.taioli@downstate.edu).

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

Abbreviations: CI, confidence interval; CINAHL, Cumulative Index to Nursing and Allied Health Literature; GSEC, Genetic Susceptibility to Environmental Carcinogens; HuGE, Human Genome Epidemiology; MTHFR, 5,10-methylenetetrahydrofolate reductase; OR, odds ratio.

Editor's note: This article is also available on the Web site of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/default.htm).

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-methyltetrahydrofolate 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).
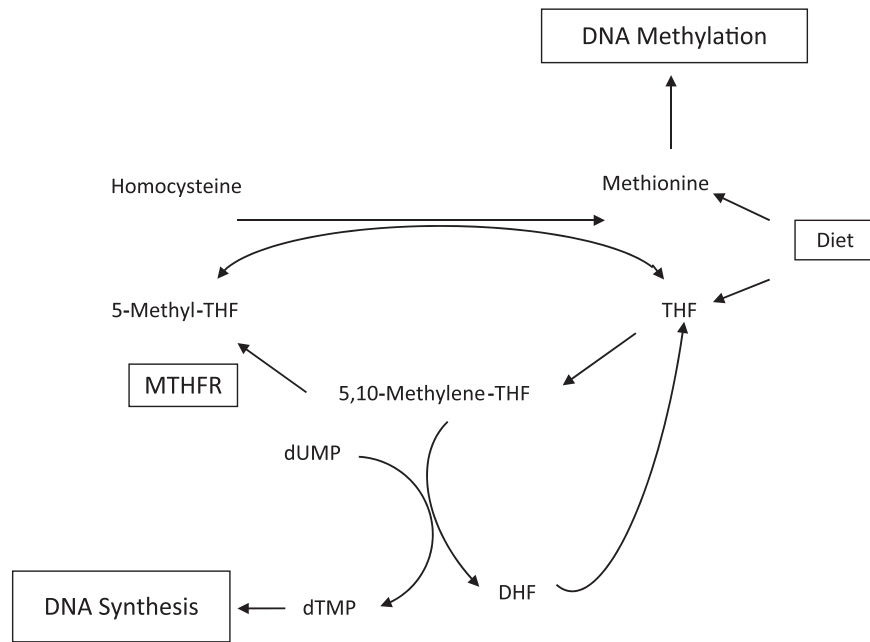
Figure 1. Schematic presentation of folate metabolism. DHF, dihydrofolate; dTMP, deoxythymidine monophosphate (deoxythymidylate); dUMP, deoxyuridine monophosphate (deoxyuridylate); MTHFR, methylenetetrahydrofolate reductase; THF, tetrahydrofolate. (Modified from Kono and Chen (8)).

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 deoxythymidine monophosphate, which increases the ratio of deoxyuridine monophosphate to deoxythymidine 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

intakes, different environmental exposures, or a combination of the 2 factors (14). Migrant studies show that subjects moving from a country with a low prevalence of colorectal cancer to a country with a high prevalence have an increased risk of developing colorectal cancer, similar to the prevailing level in the host country (14).

In the United States, colorectal cancer is the second-leading cause of cancer-related death and the third most common cancer in adults (15). Although there have been significant declines in overall cancer death rates, blacks and other minority populations continue to experience a disproportionate burden of cancer; this health disparity persists even after adjustment for environmental and social factors (16–18).

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 drug use have been associated with a reduced risk of colorectal cancer (10, 14, 19–21). Smoking has been shown to inactivate both folate and vitamin B_{12} (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 glucagone, 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 co-lorectal 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 genotypic 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 "methyl-enetetrahydrofolate reductase (NADPH2)," "colorectal 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, ≤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 ≥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 ≥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

Table 1. Relation Between Methylenetetrahydrofolate Reductase (*MTHFR*) C677T Genotype and Colorectal Cancer: General Characteristics of Studies Included in a Meta-Analysis[a] and a Pooled Analysis

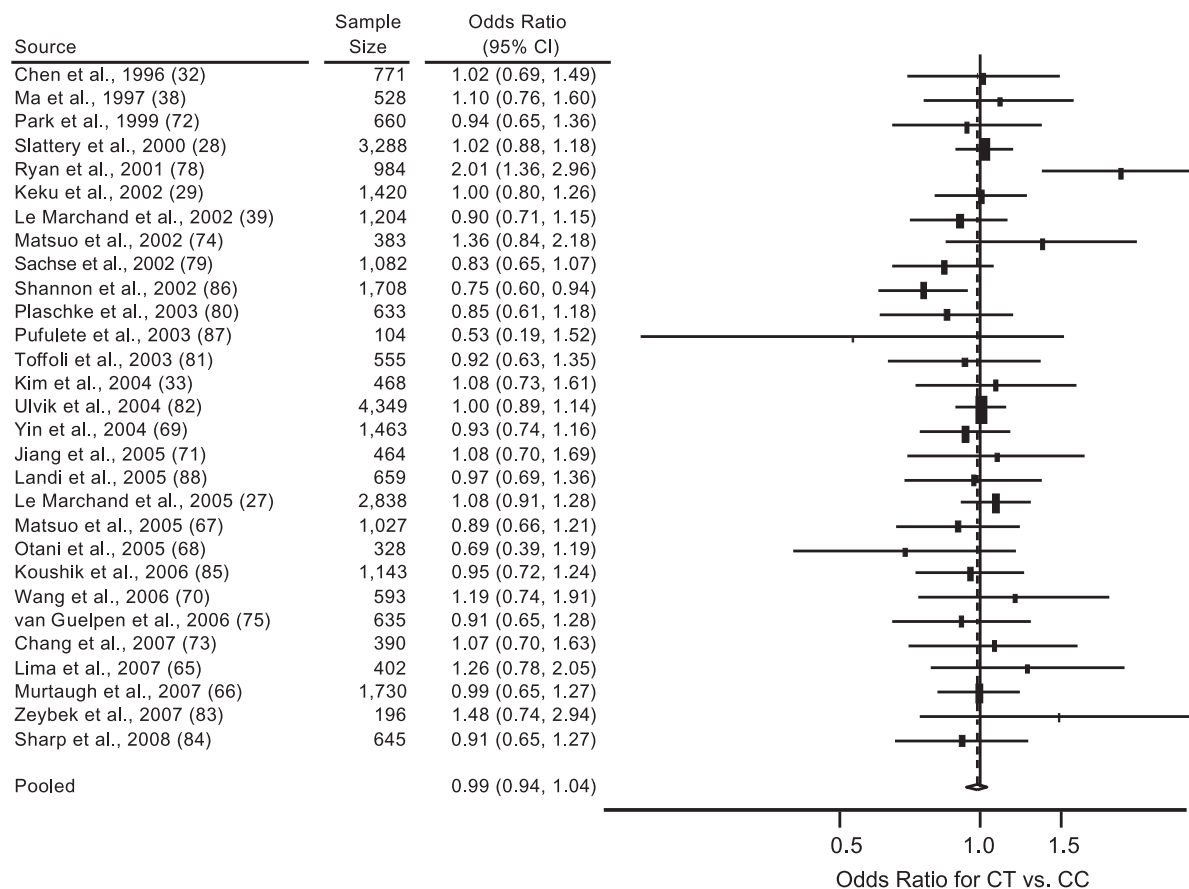| Authors (Ref. No.)	Year of Publication	No. of Cases	No. of Controls	Country	Ethnic Group(s) and %	Source of Controls	CT vs. CC OR	CT vs. CC 95% CI	TT vs. CC OR	TT vs. CC 95% CI
North and South Asian Countries										
Park et al. (72)	1999	200	460	South Korea	Korean, 100	Healthy persons	0.94	0.65, 1.36	0.81	0.48, 1.38
Yin et al. (69)	2004	685	778	Japan	Japanese, 100	Hospital patients	0.93	0.74, 1.16	0.66	0.48, 0.91
Kim et al. (33)[b]	2004	243	225	South Korea	Korean, 100	Hospital patients	1.08	0.73, 1.61	1.02	0.58, 1.80
Jiang et al. (71)[b]	2005	126	343	China	Chinese, 100	Healthy persons	1.08	0.70, 1.69	0.64	0.33, 1.22
Matsuo et al. (67)[b]	2005	257	771	Japan	Japanese, 100	Hospital patients	0.89	0.66, 1.21	0.73	0.48, 1.13
Otani et al. (68)	2005	121	245	Japan	Japanese, 100	Hospital patients	0.69	0.39, 1.19	0.70	0.37, 1.33
Wang et al. (70)	2006	302	291	India	Indian, 100	Hospital patients	1.19	0.74, 1.91	NA	NA
Chang et al. (73)	2007	195	195	Taiwan	Taiwanese, 100	Hospital patients	1.07	0.70, 1.63	1.62	0.81, 3.26
Matsuo et al. (74)	2002	142	241	Japan	Japanese, 100	Hospital patients	1.36	0.84, 2.18	1.27	0.66, 2.44
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, 1.35	0.61	0.36, 1.01
Pufulete et al. (87)[b]	2003	28	76	United Kingdom	White, 100	Hospital patients	0.53	0.19, 1.52	2.56	0.72, 9.13
Ulvik 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
Keku 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).

(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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Source	Sample Size	Odds Ratio (95% CI)
Chen et al., 1996 (32)	771	1.02 (0.69, 1.49)
Ma et al., 1997 (38)	528	1.10 (0.76, 1.60)
Park et al., 1999 (72)	660	0.94 (0.65, 1.36)
Slattery et al., 2000 (28)	3,288	1.02 (0.88, 1.18)
Ryan et al., 2001 (78)	984	2.01 (1.36, 2.96)
Keku et al., 2002 (29)	1,420	1.00 (0.80, 1.26)
Le Marchand et al., 2002 (39)	1,204	0.90 (0.71, 1.15)
Matsuo et al., 2002 (74)	383	1.36 (0.84, 2.18)
Sachse et al., 2002 (79)	1,082	0.83 (0.65, 1.07)
Shannon et al., 2002 (86)	1,708	0.75 (0.60, 0.94)
Plaschke et al., 2003 (80)	633	0.85 (0.61, 1.18)
Pufulete et al., 2003 (87)	104	0.53 (0.19, 1.52)
Toffoli et al., 2003 (81)	555	0.92 (0.63, 1.35)
Kim et al., 2004 (33)	468	1.08 (0.73, 1.61)
Ulvik et al., 2004 (82)	4,349	1.00 (0.89, 1.14)
Yin et al., 2004 (69)	1,463	0.93 (0.74, 1.16)
Jiang et al., 2005 (71)	464	1.08 (0.70, 1.69)
Landi et al., 2005 (88)	659	0.97 (0.69, 1.36)
Le Marchand et al., 2005 (27)	2,838	1.08 (0.91, 1.28)
Matsuo et al., 2005 (67)	1,027	0.89 (0.66, 1.21)
Otani et al., 2005 (68)	328	0.69 (0.39, 1.19)
Koushik et al., 2006 (85)	1,143	0.95 (0.72, 1.24)
Wang et al., 2006 (70)	593	1.19 (0.74, 1.91)
van Guelpen et al., 2006 (75)	635	0.91 (0.65, 1.28)
Chang et al., 2007 (73)	390	1.07 (0.70, 1.63)
Lima et al., 2007 (65)	402	1.26 (0.78, 2.05)
Murtaugh et al., 2007 (66)	1,730	0.99 (0.65, 1.27)
Zeybek et al., 2007 (83)	196	1.48 (0.74, 2.94)
Sharp et al., 2008 (84)	645	0.91 (0.65, 1.27)
Pooled		0.99 (0.94, 1.04)

Odds Ratio for CT vs. CC

Figure 2. Study-specific (squares) and meta-analysis (diamond) odds ratios for colorectal cancer among persons with the methylenetetrahydrofolate reductase (*MTHFR*) 677CT genotype—CT versus CC. Meta-analysis estimate for the CT genotype versus the CC genotype: $P = 0.57$; $Q = 32.887$; $P = 0.240$; $I^2 = 15\%$, 95% confidence interval (CI): 0, 46. Horizontal lines, 95% CI.

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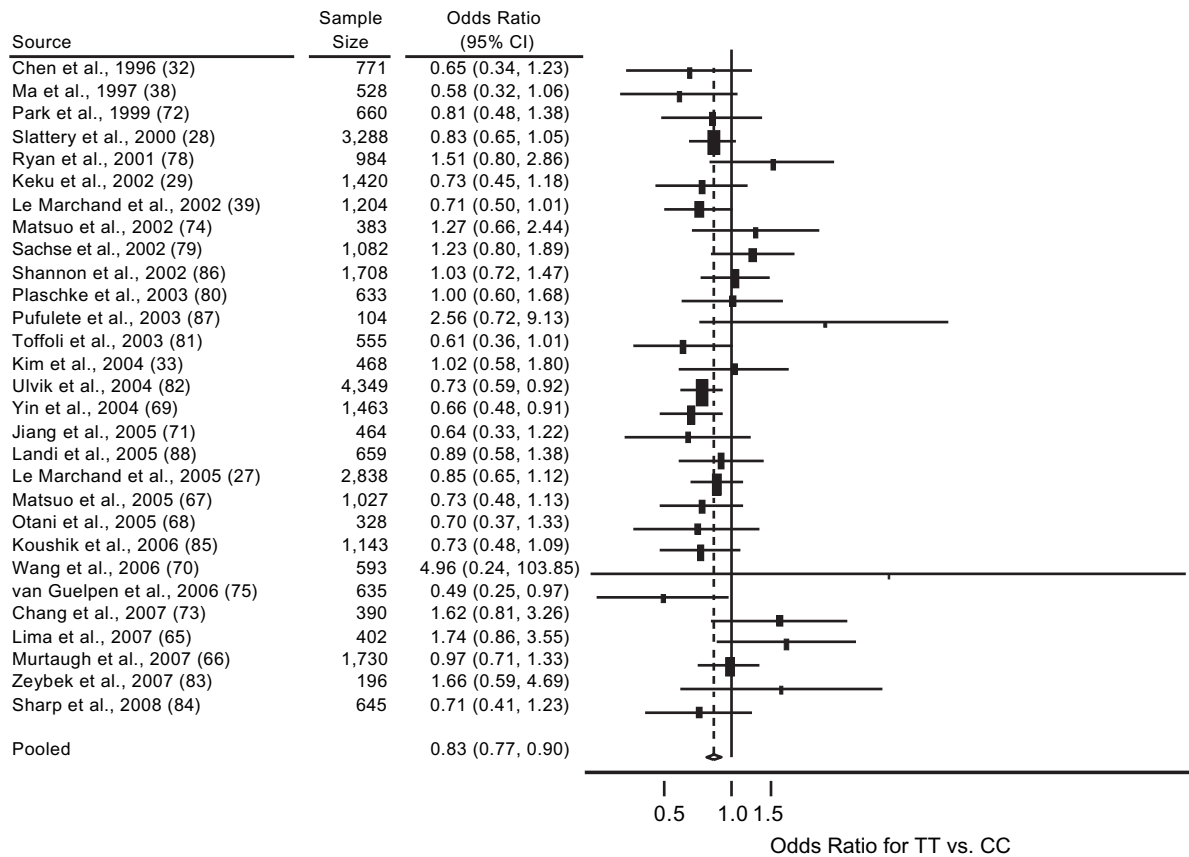
Colorectal cancer and *MTHFR* C677T—ethnic/geographic variation

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

Source	Sample Size	Odds Ratio (95% CI)
Chen et al., 1996 (32)	771	0.65 (0.34, 1.23)
Ma et al., 1997 (38)	528	0.58 (0.32, 1.06)
Park et al., 1999 (72)	660	0.81 (0.48, 1.38)
Slattery et al., 2000 (28)	3,288	0.83 (0.65, 1.05)
Ryan et al., 2001 (78)	984	1.51 (0.80, 2.86)
Keku et al., 2002 (29)	1,420	0.73 (0.45, 1.18)
Le Marchand et al., 2002 (39)	1,204	0.71 (0.50, 1.01)
Matsuo et al., 2002 (74)	383	1.27 (0.66, 2.44)
Sachse et al., 2002 (79)	1,082	1.23 (0.80, 1.89)
Shannon et al., 2002 (86)	1,708	1.03 (0.72, 1.47)
Plaschke et al., 2003 (80)	633	1.00 (0.60, 1.68)
Pufulete et al., 2003 (87)	104	2.56 (0.72, 9.13)
Toffoli et al., 2003 (81)	555	0.61 (0.36, 1.01)
Kim et al., 2004 (33)	468	1.02 (0.58, 1.80)
Ulvik et al., 2004 (82)	4,349	0.73 (0.59, 0.92)
Yin et al., 2004 (69)	1,463	0.66 (0.48, 0.91)
Jiang et al., 2005 (71)	464	0.64 (0.33, 1.22)
Landi et al., 2005 (88)	659	0.89 (0.58, 1.38)
Le Marchand et al., 2005 (27)	2,838	0.85 (0.65, 1.12)
Matsuo et al., 2005 (67)	1,027	0.73 (0.48, 1.13)
Otani et al., 2005 (68)	328	0.70 (0.37, 1.33)
Koushik et al., 2006 (85)	1,143	0.73 (0.48, 1.09)
Wang et al., 2006 (70)	593	4.96 (0.24, 103.85)
van Guelpen et al., 2006 (75)	635	0.49 (0.25, 0.97)
Chang et al., 2007 (73)	390	1.62 (0.81, 3.26)
Lima et al., 2007 (65)	402	1.74 (0.86, 3.55)
Murtaugh et al., 2007 (66)	1,730	0.97 (0.71, 1.33)
Zeybek et al., 2007 (83)	196	1.66 (0.59, 4.69)
Sharp et al., 2008 (84)	645	0.71 (0.41, 1.23)
Pooled		0.83 (0.77, 0.90)

Odds Ratio for TT vs. CC

Figure 3. Study-specific (squares) and meta-analysis (diamond) odds ratios for colorectal cancer among persons with the methylenetetrahydrofolate reductase (*MTHFR*) 677TT genotype—TT versus CC. Meta-analysis estimate for the TT genotype versus the CC genotype: $P < 0.001$; $Q = 37.602$; $P = 0.106$; $I^2 = 26\%$, 95% confidence interval (CI): 0, 53. Horizontal lines, 95% CI.

Table 2. Meta-Analysis Odds Ratios for the Relation Between Methylenetetrahydrofolate Reductase (*MTHFR*) 677TT Genotype and Colorectal Cancer, by Ethnic Group and Geographic Region[a]

Subgroup	No. of Studies	No. of Subjects	Reference Nos.	CT vs. CC OR	CT vs. CC 95% CI	TT vs. CC OR	TT vs. CC 95% CI
Ethnic group[b]							
White	14	13,992	32, 38, 75, 78–86, 87, 88	0.97	0.86, 1.09	0.83	0.74, 0.94
Asian[c]	8	5,183	33, 67–69, 71–74	0.97	0.86, 1.10	0.80	0.67, 0.96
Geographic region[d]							
United States	8	12,922	27–29, 32, 38, 39, 66, 85	1.01	0.94, 1.09	0.80	0.71, 0.91
Europe	10	9,842	75, 78–84, 87, 88	0.99	0.91, 1.08	0.90	0.72, 1.13
Asia[c]	9	5,776	33, 67–74	0.99	0.87, 1.11	0.81	0.68, 0.97

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

[a] 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, $P = 0.02$.

[b] 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).

[c] 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.

[d] Two studies (65, 86) were excluded because they did not fall within the 3 defined categories.

Table 3. Pooled Analysis Odds Ratios for the Relation Between Methylenetetrahydrofolate Reductase (*MTHFR*) 677TT Genotype and Colorectal Cancer Risk, by Ethnicity[a]

Ethnic Group[b]	No. of Studies	No. of Subjects	CC		CT		TT		CT vs. CC		TT vs. CC	
			No.	%	No.	%	No.	%	OR[c]	95% CI	OR[c]	95% CI
Overall	14											
Controls		7,441	3,356	45.10	3,105	41.73	980	13.17				
Cases		4,964	2,232	44.96	2,155	43.41	577	11.62	1.00	0.92, 1.08	0.85	0.75, 0.96
White	11											
Controls		3,622	1,554	42.90	1,599	44.15	469	12.95				
Cases		3,014	1,293	42.90	1,352	44.86	369	12.24	1.00	0.89, 1.11	0.91	0.77, 1.07
Black	3											
Controls		776	596	76.80	164	21.13	16	2.06				
Cases		418	323	77.27	86	20.57	9	2.15	1.05	0.77, 1.41	1.13	0.48, 2.63
Latino	1											
Controls		469	166	35.39	215	45.84	88	18.76				
Cases		166	54	32.53	84	50.60	28	16.87	1.14	0.76, 1.72	0.95	0.55, 1.62
Asian	5											
Controls		2,192	805	36.72	1,000	45.62	387	17.66				
Cases		1,228	481	39.17	587	47.80	160	13.03	0.95	0.82, 1.12	0.68	0.55, 0.85

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

[a] Wald χ^2 test (2 df) *P* value: overall—*P* = 0.0235; white—*P* = 0.49; black—*P* = 0.93; Latino—*P* = 0.70; Asian—*P* = 0.0024.

[b] In 4 studies (27, 29, 39, 65), investigators reported results for more than 1 ethnic group.

[c] Adjusted for study, age, and sex.

in 12,405 subjects' being available for analysis (4,964 cases and 7,441 controls).

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).

Table 4. Pooled Analysis Odds Ratios for Colorectal Cancer Risk Among Persons With the Methylenetetrahydrofolate Reductase (*MTHFR*) 677TT Genotype, by Geographic Location and Tumor Site[a]

Subgroup	No. of Studies	No. of Subjects	CC		CT		TT		CT vs. CC		TT vs. CC	
			No.	%	No.	%	No.	%	OR[b]	95% CI	OR[b]	95% CI
Geographic location[c]												
United States	3											
Controls		3,567	1,784	50.01	1,366	38.30	417	11.69				
Cases		1,934	980	50.67	771	39.87	183	9.46	1.01	0.90, 1.14	0.80	0.66, 0.97
Asia	3											
Controls		1,336	505	37.80	601	44.99	230	17.22				
Cases		624	243	38.94	295	47.28	86	13.78	0.98	0.79, 1.21	0.77	0.57, 1.04
Europe	7											
Controls		2,238	924	41.29	1,011	45.17	303	13.54				
Cases		2,304	968	42.01	1,043	45.27	293	12.72	0.99	0.86, 1.13	0.99	0.74, 1.10
Tumor site												
Colon	10											
Controls		6,042	2,755		2,486		801					
Cases		2,280	1,035		987		258		1.00	0.89, 1.11	0.82	0.69, 0.97
Rectum	8											
Controls		4,895	2,143		2,064		688					
Cases		1,593	694		706		193		1.00	0.88, 1.14	0.85	0.71, 1.03

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

[a] Wald χ^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 study, age, and sex.

[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

Table 5. Pooled Analysis Odds Ratios for Colorectal Cancer Risk Among Persons With the Methylenetetrahydrofolate Reductase (*MTHFR*) 677TT Genotype, by Tobacco Use[a]

Tobacco Use	No. of Subjects	CC		CT		TT		CT vs. CC		TT vs. CC	
		No.	%	No.	%	No.	%	OR[b]	95% CI	OR[b]	95% CI
Never smoking	4,321										
Controls		1,235	44.67	1,150	41.59	380	13.74				
Cases		694	44.60	665	42.74	197	12.66	1.03	0.90, 1.19	0.92	0.74, 1.13
≤14.2 pack-years of smoking	2,249										
Controls		708	47.84	589	39.80	183	12.36				
Cases		371	48.24	312	40.57	86	11.18	0.95	0.78, 1.16	0.84	0.62, 1.15
>14.2 pack-years of smoking	2,485										
Controls		677	45.84	606	41.03	194	13.13				
Cases		493	48.91	420	41.67	95	9.42	0.88	0.73, 1.05	0.60	0.45, 0.80

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

[a] Unadjusted *P* value for interaction between tobacco use and *MTHFR* genotype: *P* = 0.43. Wald χ^2 test (2 df) *P* value, by tobacco use: never smoking—*P* = 0.54; ≤14.2 pack-years—*P* = 0.55; >14.2 pack-years—*P* = 0.0021.

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

Table 6. Pooled Analysis Odds Ratios for Colorectal Cancer Risk Among Persons With the Methylenetetrahydrofolate Reductase (*MTHFR*) 677TT Genotype, by Alcohol Consumption[a]

Alcohol Consumption	No. of Subjects	CC		CT		TT		CT vs. CC		TT vs. CC	
		No.	%	No.	%	No.	%	OR[b]	95% CI	OR[b]	95% CI
Never drinker	4,071										
Controls		1,295	48.90	1,019	38.48	334	12.61				
Cases		681	47.86	586	41.18	156	10.96	1.11	0.96, 1.29	0.93	0.74, 1.16
Ever drinker	5,657										
Controls		1,502	43.68	1,477	42.95	460	13.38				
Cases		1,023	46.12	941	42.43	254	11.45	0.89	0.79, 1.00	0.78	0.65, 0.93

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Unadjusted *P* value for interaction between alcohol use and *MTHFR* genotype: $P = 0.23$. Wald χ^2 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.

present study; 10 additional studies completed after the publication of the Hubner and Houlston paper were included in the present meta-analysis. Hubner and Houlston's meta-analysis showed an overall odds ratio for the relation of colorectal cancer to the TT genotype of 0.83 (95% CI: 0.75, 0.93) (30), very similar to the present finding.

The pooled analysis included 14 of the 29 studies included in the meta-analysis. The overall odds ratios were similar (OR = 0.85 (95% CI: 0.75, 0.96) and OR = 0.82 (95% CI: 0.75, 0.89), respectively).

A secondary analysis conducted on the individual data allowed us to study colon cancer separately from rectal cancer. The analysis suggested that the inverse association between *MTHFR* and colorectal cancer could be mainly attributed to colon cancer.

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 2 previous meta-analyses (30, 31). One of the limitations of

Table 7. Pooled Analysis Odds Ratios for Colorectal Cancer Risk Among Persons With the Methylenetetrahydrofolate Reductase (*MTHFR*) 677TT Genotype, by Body Mass Index[a] Category[b]

BMI Category	No. of Subjects	CC		CT		TT		CT vs. CC		TT vs. CC	
		No.	%	No.	%	No.	%	OR[c]	95% CI	OR[c]	95% CI
<18.5	299										
Controls		73	43.71	67	40.12	27	16.17				
Cases		61	46.21	55	41.67	16	12.12	1.08	0.62, 1.89	0.64	0.28, 1.46
18.5–24.9	4,365										
Controls		1,123	41.39	1,180	43.49	410	15.11				
Cases		719	43.52	730	44.19	203	12.29	0.97	0.85, 1.12	0.79	0.64, 0.96
25.0–29.9	3,355										
Controls		1,004	47.43	863	40.77	250	11.81				
Cases		588	47.50	509	41.11	141	11.39	0.97	0.82, 1.13	0.91	0.71, 1.17
≥30.0	1,566										
Controls		547	55.42	342	34.65	98	9.93				
Cases		313	54.06	221	38.17	45	7.77	1.02	0.81, 1.30	0.76	0.51, 1.14

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.
[a] Weight (kg)/height (m)^2.
[b] Unadjusted *P* value for interaction between BMI category and *MTHFR* genotype: $P = 0.47$. Wald χ^2 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.

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 populations. 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 polymorphism 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 previously published meta-analyses (30, 31), whereas no association 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-analysis were included in the pooled analysis, thus introducing some selection bias. In fact, when we restricted the meta-analysis in whites to the 6 studies that were also included in the pooled analysis, the association between colorectal cancer and *MTHFR* 677TT was not statistically significant. Thus, it is possible that the meta-analysis included a larger number of studies, most of which showed a significant inverse relation between the gene polymorphism and colorectal cancer.

Differences in allelic distribution by ethnicity could be partially responsible for the observed differences in the 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 previous 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. Differences 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 significance, 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 vegetables), thus decreasing their dietary folate intake, and to consume more alcohol than nonsmokers. Other unmeasured confounding factors associated with smoking could be partially 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 interaction 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 subjects who reported using alcoholic beverages regularly. These findings are consistent with those reported by Otani et al. (68), although their results were not statistically significant; 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). Unfortunately, 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 colorectal cancer persisted across strata of body mass index. This analysis included individual data from case-control studies; thus, information on body mass index prior to disease diagnosis was not available. In addition, the available information on weight and height was self-reported. These limitations 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* genotype 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 specific data available, as happens with meta- and pooled

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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