Folate Intake, MTHFR Polymorphisms, and Risk of Esophageal, Gastric, and Pancreatic Cancer: A Meta-analysis

SPECIAL REPORTS AND REVIEWS

Folate Intake, *MTHFR* Polymorphisms, and Risk of Esophageal, Gastric, and Pancreatic Cancer: A Meta-analysis

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Background & Aims: Increasing evidence suggests that a low folate intake and impaired folate metabolism may be implicated in the development of gastrointestinal cancers. We conducted a systematic review with meta-analysis of epidemiologic studies evaluating the association of folate intake or genetic polymorphisms in 5,10-methylenetetrahydrofolate reductase (MTHFR), a central enzyme in folate metabolism, with risk of esophageal, gastric, or pancreatic cancer. **Methods:** A literature search was performed using MEDLINE for studies published through March 2006. Study-specific relative risks were weighted by the inverse of their variance to obtain random-effects summary estimates. **Results:** The summary relative risks for the highest versus the lowest category of dietary folate intake were 0.66 (95% confidence interval [CI], 0.53–0.83) for esophageal squamous cell carcinoma (4 case-control), 0.50 (95% CI, 0.39–0.65) for esophageal adenocarcinoma (3 case-control), and 0.49 (95% CI, 0.35–0.67) for pancreatic cancer (1 case-control, 4 cohort); there was no heterogeneity among studies. Results on dietary folate intake and risk of gastric cancer (9 case-control, 2 cohort) were inconsistent. In most studies, the *MTHFR 677TT* (variant) genotype, which is associated with reduced enzyme activity, was associated with an increased risk of esophageal squamous cell carcinoma, gastric cardia adenocarcinoma, noncardia gastric cancer, gastric cancer (all subsites), and pancreatic cancer; all but one of 22 odds ratios were >1, of which 13 estimates were statistically significant. Studies of the *MTHFR A1298C* polymorphism were limited and inconsistent. **Conclusions:** These findings support the hypothesis that folate may play a role in carcinogenesis of the esophagus, stomach, and pancreas.

Folate is a water-soluble B vitamin found naturally in many foods, particularly in citrus fruits, green leafy vegetables, cruciferous vegetables, legumes, cereals, and liver. Evidence is mounting for a role of folate in carcinogenesis. There are 2 prominent mechanisms whereby folate deficiency may influence the risk of cancer: (1) by inducing misincorporation of uracil into DNA, which could lead to chromosomal breaks and mutations; and/or (2) by causing aberrant DNA methylation, resulting in altered expression of critical proto-oncogenes and tumor suppressor genes.[1–3]

Besides an inadequate folate intake, functional polymorphisms in folate-metabolizing genes may influence susceptibility to cancer. Among the several genetic polymorphisms in the folate metabolic pathway, polymorphisms in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene are the most extensively studied. MTHFR is a central enzyme in folate metabolism that irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant form of folate in the circulation (Figure 1). Thus, MTHFR acts as a critical juncture in folate metabolism by directing folate metabolites toward the DNA methylation pathway and away from the DNA synthesis pathway. Two common functional polymorphisms of the *MTHFR* gene, C677T and A1298C, have been identified.[4,5] Heterozygotes (*CT*) and homozygotes (*TT*) for the *C677T* polymorphism have about 65% and 30%, respectively, of the MTHFR activity of individuals with the wild-type (*CC*) genotype.[4] Individuals with the *TT* genotype also have significantly lower plasma folate levels and higher plasma homocysteine levels than those with the *CC* genotype.[6–8] For *A1298C*, homozygotes (*CC*) have about 60% of normal MTHFR activity.[5] Studies on the effect of the *A1298C* polymorphism on folate and homocysteine levels are inconsistent.[5,6,9]

Other nutrients (eg, vitamin B$_6$, vitamin B$_{12}$, and methionine) involved in the folate metabolic pathway as well as alcohol (a folate antagonist) and smoking (which impairs folate status) may interact with folate and the *MTHFR* polymorphisms in relation to cancer risk.[10,11] Vitamins B$_6$ and B$_{12}$ are coenzymes of serine hydroxymethyltransferase and methionine synthase, respectively, both of which are involved in folate metabolism (Figure 1). Alcohol may perturb folate metabolism by reducing folate absorption,[12] by increasing folate excretion,[12] or through inhibition of methionine synthase,[13] which may trap folate as 5-methyltetrahydrofolate (Figure 1). The inverse association between folate intake and plasma homocysteine has been shown to be modified by alcohol intake and *MTHFR 677* genotype but not by *MTHFR 1298* genotype.[14]

Previous meta-analyses[15,16] have shown inverse associations of dietary folate intake and the *MTHFR 677TT* genotype with risk of colorectal cancer. The aim of the present study was to assess the relationships of folate and the *MTHFR C677T* and *A1298C* polymorphisms with risk of esophageal, gastric, and pancreatic cancer by conducting meta-analyses of available case-
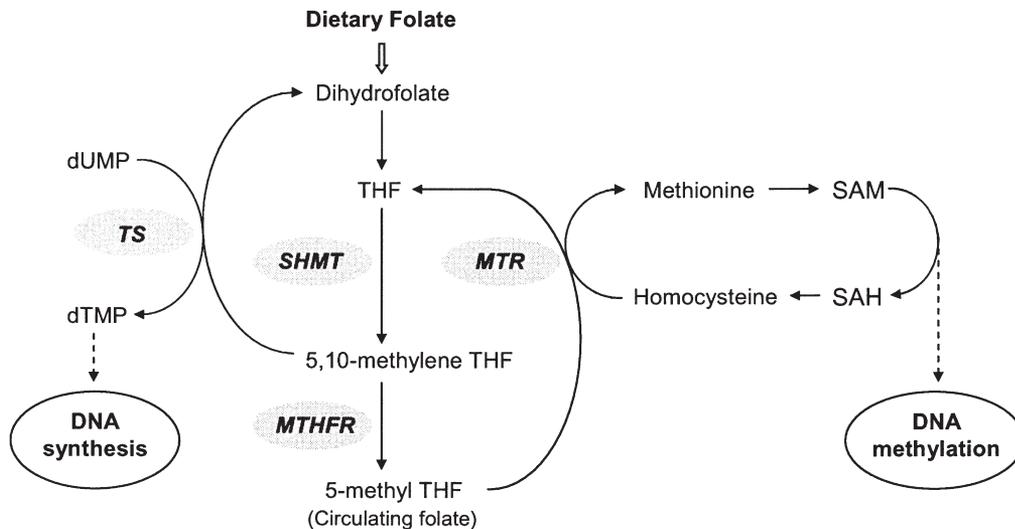
Dietary Folate

Figure 1. Simplified overview of folate metabolism involving DNA synthesis and DNA methylation. Enzymes: TS, thymidylate synthase; SHMT, serine hydroxymethyltransferase; MTHFR, 5,10-methylenetetrahydrofolate reductase; MTR, methionine synthase. Metabolites: THF, tetrahydrofolate; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.

control and cohort studies. We also examined whether the associations of folate and the *MTHFR* polymorphisms with cancer risk were modified by vitamins B$_6$ and B$_{12}$, methionine, alcohol, and smoking.

Materials and Methods

Study Selection

A computerized literature search was conducted in MEDLINE for studies published in any language from 1966 to March 2006 using the key words folate, folic acid, or MTHFR in combination with cancer, neoplasm, or the individual cancer sites. We also reviewed the reference lists of the relevant articles to identify additional studies. Because folate intake frequently was only one of several dietary factors studied, reports that had fruit, vegetables, vitamins, or nutrients as key words were scrutinized for findings on folate.

Studies were included if they (1) presented original data from case-control or cohort studies and (2) provided odds ratios (ORs) or rate ratios with their confidence intervals (CIs) for the association of dietary folate intake (ie, folate from foods), total folate intake (ie, folate from foods and dietary supplements), blood folate levels, or polymorphisms in the *MTHFR* gene with esophageal, gastric, or pancreatic cancer risk. Studies were excluded if they provided only a risk estimate with no means by which to calculate the CI or if the risk estimate was not adjusted by age. When there were multiple publications from the same population, only the most recently published report was included.

Data Extraction

We extracted the following data from each publication: the first author's last name, year of publication, country where the study was performed, study design, type of controls in case-control studies, sample size, measure of exposure, outcome, prevalence of the variant genotype in the study population, covariates adjusted for by matching or in the analysis, and the risk estimates with 95% CIs for the highest versus the lowest intake categories of folate or for the *MTHFR* variant genotypes.

From each study, we extracted the risk estimates that reflected the greatest degree of control for potential confounders.

Statistical Analysis

We weighted the study-specific log ORs for case-control studies and log rate ratios for cohort studies by the inverse of the variance to compute summary relative risk (RR) estimates with 95% CIs. Because the absolute risk of the cancers considered in this meta-analysis is low, ORs in case-control studies and rate ratios in cohort studies yield similar estimates of RR.[17] Studies were pooled with the DerSimonian and Laird random-effects model, which considers both within- and between-study variability.[18] When separate RRs were provided for the intestinal and diffuse types of gastric cancer,[19] for cardia and noncardia gastric cancer,[20] or for men and women,[21] we pooled the RRs (weighted by the inverse of their variance) to obtain one RR from each study.

Statistical heterogeneity among studies was assessed with the Q and I^2 statistics.[22] For the Q statistic, heterogeneity was considered present if $P < .1$. I^2 is the proportion of total variation contributed by between-study variability.[22] We used random-effects meta-regression to investigate sources of heterogeneity and to provide an estimate of unexplained heterogeneity, τ^2.[23,24] Study characteristics examined included study design (case-control vs cohort), type of controls in case-control studies (population-based vs hospital-based), and geographical area (United States, Europe, other). We used funnel plots and Egger's regression asymmetry test to evaluate publication bias[25] ($P < .1$ was considered representative of statistically significant publication bias). The potential influence that unpublished studies could have on the summary estimate was examined using trim and fill analysis.[26] All analyses were performed with Stata statistical software (version 9.0; StataCorp, College Station, TX).

Results

Folate Intake

Esophageal cancer. We identified 11 case-control studies[20,27–36] that evaluated the association between dietary

folate intake and risk of esophageal cancer. Four studies were excluded for the following reasons: no CIs,[33,34] duplicate publications,[35] or the RR was not adjusted by age.[36] The 7 remaining studies[20,27–32] were included in the meta-analysis (Table 1). All studies adjusted for potential confounding by smoking and alcohol intake, and 5 studies also controlled for body mass index. The summary RRs for individuals in the highest relative to the lowest category of dietary folate intake were 0.66 (95% CI, 0.53–0.83) for esophageal squamous cell carcinoma (total of 929 cases) and 0.50 (95% CI, 0.39–0.65) for esophageal adenocarcinoma (total of 501 cases) (Figure 2). When all 7 studies (including 1496 cases) were analyzed together, the summary RR of esophageal cancer (all types) was 0.62 (95% CI, 0.53–0.72) for high versus low dietary folate intake. There was no heterogeneity among these studies (Q = 5.27; P = .51; I^2 = 0%). The association was similar in population-based case-control studies (RR, 0.52; 95% CI, 0.42–0.65) and hospital-based case-control studies (RR, 0.74; 95% CI, 0.59–0.92). The Egger's test provided no indication of publication bias (P = .33 for all studies, P = .36 for esophageal squamous cell carcinoma, and P = .33 for esophageal adenocarcinoma).

Gastric cancer. We identified 11 case-control[19–21,31,37–43] and 2 cohort studies[44,45] that examined the association between dietary folate intake and risk of gastric cancer. Two case-control studies were excluded for the following reasons: no CI[37] or duplicate publications.[39] Eleven studies with 3205 cases met the predefined inclusion criteria (Table 1). Among these studies, 9 reported results on cardia and noncardia gastric cancers combined,[19,21,38,40–45] one presented results on both cancer sites,[20] and one included noncardia gastric cancer cases only.[31] In the study[20] that provided results for the 2 subsites, high versus low dietary folate intake was associated with a statistically significant lower risk of both gastric cardia adenocarcinoma (RR, 0.73; 95% CI, 0.55–0.97) and noncardia gastric cancer (RR, 0.67; 95% CI, 0.51–0.88). Overall, there was no significant association between dietary folate intake and risk of gastric cancer; however, there was significant heterogeneity among all studies and among the case-control studies but not among the cohort studies (Figure 3). In meta-regression analysis, geographical region but not study design was a predictor of between-study heterogeneity. The between-study variance (τ^2) was reduced from 0.070 to 0.000 when including geographical region in the meta-regression model. The summary RRs for individuals in the highest relative to the lowest category of dietary folate intake were 0.68 (95% CI, 0.58–0.80) for studies conducted in the United States (n = 4), 1.15 (95% CI, 0.91–1.45) for European studies (n = 4), and 0.89 (95% CI, 0.40–1.96) for studies (n = 3) conducted elsewhere. There was no heterogeneity among the US studies (Q = 1.06; P = .79; I^2 = 0%) or among the European studies (Q = 1.04; P = .79; I^2 = 0%). The Egger's test for publication bias was not statistically significant (P = .28).

Pancreatic cancer. The association between dietary folate intake and pancreatic cancer risk was examined in 2 case-control[46,47] and 4 cohort studies[48–50] (one study[49] reported data from 2 independent cohorts, which were included in the meta-analysis as 2 separate studies). One case-control study[46] was excluded because no CI was provided. All 5 analyzed studies (involving 722 cases) reported an inverse association between dietary folate intake and pancreatic cancer risk, and in 3 studies[47,48,50] the relationship was statistically significant (Table 1). The summary estimate indicates that individuals in the highest category of dietary folate intake have a significant 51% lower risk of pancreatic cancer compared with those in the lowest category; there was no heterogeneity among studies (Figure 4). Restricting the analysis to cohort studies yielded similar results (RR, 0.52; 95% CI, 0.36–0.75). There was no strong evidence of publication bias (P = .28 by Egger's test). Three cohort studies provided results on total folate intake and risk of pancreatic cancer, with a significant inverse association observed in one study (RR, 0.33; 95% CI, 0.15–0.72)[50] but not in the 2 other studies.[49]

Blood Folate Levels

In a case-control study in China, significantly (P < .0001) lower serum folate levels were found among cases with esophageal squamous cell carcinoma and gastric cardia carcinoma compared with healthy controls.[51] The ORs (adjusted for age, sex, and smoking) of squamous cell carcinoma and gastric cardia carcinoma were 13.73 (95% CI, 9.61–19.60) and 2.43 (95% CI, 1.64–3.60), respectively, for low (<3 ng/mL) versus high (≥3 ng/mL) serum folate status.[51]

In a nested case-control study within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort of male Finnish smokers, serum folate levels were significantly (P = .009) inversely associated with risk for pancreatic cancer.[52] Men in the highest tertile of serum folate levels had approximately half the risk of pancreatic cancer (RR, 0.45; 95% CI, 0.24–0.82) compared with men in the lowest tertile.[52]

Folate/Other Nutrients and Smoking Interactions

Galeone et al[29] reported that the inverse association of folate intake with risk of esophageal squamous cell carcinoma was stronger among individuals with high vitamin B_6 and methionine intake (above the median values). Two prospective studies showed no interaction between folate and methionine intake in relation to risk of gastric cancer[45] or pancreatic cancer.[49]

In an Italian case-control study, the inverse association between folate intake and risk of esophageal squamous cell carcinoma was stronger among heavy drinkers (RR for an increment in folate intake of 98 μg/day, 0.74) than among moderate drinkers (corresponding RR, 0.96).[29] Neither alcohol nor smoking was found to significantly modify the inverse relation between folate intake and pancreatic cancer in 3 prospective studies.[48–50]

MTHFR *Polymorphisms*

MTHFR C677T. We identified 18 publications[28,53–69] with data on the *MTHFR C677T* polymorphism in relation to esophageal squamous cell carcinoma, gastric cardia adenocarcinoma, noncardia gastric cancer, gastric cancer (all sites), or pancreatic cancer (some publications provided data on more than one outcome[54,57,60,61] or by ethnicity[56]) (Table 2). Two studies were excluded because of duplicate publications.[58,62] Among the 16 included studies, 10 were conducted in a Chinese population.[53–57,59,60,63,64,68] The prevalence of the *TT* genotype among controls varied considerably among studies, ranging from 6.5% (in US white patients)[67] to 44.0% (in a Chinese population).[56] The *MTHFR C677T* genotype frequency in controls was in Hardy–Weinberg equilibrium in all but one study.[60] Figure 5 shows the OR for the *TT* genotype compared with the

Table 1. Studies of Dietary Folate Intake and Risk of Esophageal, Gastric, and Pancreatic Cancer

Reference	Country, study design	Years of study	Cases	Controls[a]	Range of intake ($\mu g/day$)	Adjusted RR (95% CI)[b]	Adjustments
Esophageal squamous cell carcinoma							
Brown et al, 1988[27]	United States, case-control	1982–1984	207[c]	422 (H)	Highest vs lowest tertile[d]	0.7 (0.4–1.3)	Age, race, hospital, country of residence, smoking, moonshine use, and alcohol intake
Mayne et al, 2001[20]	United States, case-control	1993–1995	206	687 (P)	Highest vs lowest quartile[d]	0.58 (0.39–0.86)	Age, sex, race, education, proxy status, income, smoking, BMI, beer, wine, liquor, and energy intake
Yang et al, 2005[28]	Japan, case-control	2001–2004	165[e]	495 (H)	>400 vs <300	0.77 (0.45–1.31)	Age, sex, smoking, and alcohol intake
Galeone et al, 2006[29]	Italy and Switzerland, case-control	1992–1999	351	875 (H)	>305 vs <228	0.68 (0.46–1.00)	Age, center, education, smoking, BMI, and alcohol intake
Esophageal adeonocarcinoma							
Zhang et al, 1997[30]	United States, case-control	1992–1994	95[f]	132 (H)	Highest vs lowest quartile[d]	0.7 (0.3–1.8)	Age, sex, race, education, smoking, BMI, and intake of alcohol and energy
Mayne et al, 2001[20]	United States, case-control	1993–1995	282	687 (P)	Highest vs lowest quartile[d]	0.48 (0.36–0.66)	Age, sex, race, education, proxy status, income, smoking, BMI, and intake of beer, wine, liquor, and energy
Chen et al, 2002[31]	United States, case-control	1988–1994	124	449 (P)	Highest vs lowest quartile[d]	0.5 (0.3–1.0)	Age, sex, education, respondent type, smoking, family history, vitamin, BMI, supplement use, and alcohol intake
Esophageal carcinoma (all types)							
De Stefani et al, 1999[32]	Uruguay, case-control	1996–1997	66	393 (H)	Highest vs lowest tertile[d]	0.8 (0.5–1.1)	Age, sex, education, place of residence, urban/rural, smoking, BMI, and intake of alcohol and energy
Gastric cancer							
La Vecchia et al, 1994[38]	Italy, case-control	1985–1992	723	2024 (H)	>262 vs <163	1.33 (0.82–2.18)	Age, sex, education, family history, BMI, and intake of nitrites, nitrates, methionine, β-carotene, vitamin C, and energy
Harrison et al, 1997[19]	United States, case-control	1992–1994	91	132 (H)	High vs low[e]	0.60 (0.44–0.82)	Age, sex, race, education, smoking, BMI, and alcohol intake
López-Carrillo et al, 1999[40]	Mexico, case-control	1989–1990	220	752 (P)	≥466 vs <257	1.00 (0.45–2.27)	Age, sex, SES, history of peptic ulcer, smoking, and intake of salt, chili-pepper, and energy
Botterweck et al, 2000[44]	The Netherlands, cohort (NCS)	1986–1992	282	3123	384 vs 202 (median)	1.00 (0.60–1.40)	Age, sex, education, smoking, stomach disorders, and family history
Muñoz et al, 2001[41]	Venezuela, case-control	1991–1996	292	485 (P)	Highest vs lowest quartile[e]	1.51 (0.93–2.45)	Age, sex, SES, tobacco, and intake of alcohol and energy
Mayne et al, 2001[20]	United States, case-control	1993–1995	607	687 (P)	Highest vs lowest quartile[e]	0.70 (0.57–0.85)	Age, sex, race, education, proxy status, income, smoking, BMI, and intake of beer, wine, liquor, and energy
Chen et al, 2002[31]	United States, case-control	1988–1994	124[g]	449 (P)	Highest vs lowest quartile[e]	0.80 (0.40–1.40)	Age, sex, respondent type, tobacco, education, family history, vitamin supplement use, and alcohol intake
Nomura et al, 2003[21]	United States (Hawaii), case-control	1993–1999	300	446 (P)	>315 vs <236	0.76 (0.38–1.51)	Age, ethnicity, education, smoking, history of gastric ulcer, family history, NSAID use, and intake of fiber, β-carotene, vitamin C, vitamin E, and energy

Table 1 (continued). Studies of Dietary Folate Intake and Risk of Esophageal, Gastric, and Pancreatic Cancer

Reference	Country, study design	Years of study	Cases	Controls[a]	Range of intake ($\mu g/day$)	Adjusted RR (95% CI)[b]	Adjustments
Lissowska et al, 2004[42]	Poland, case-control	1994–1996	274	463 (P)	Highest vs lowest quartile[e]	1.26 (0.81–1.98)	Age, sex, education, smoking, and energy intake
Kim et al, 2005[43]	Korea, case-control	1997–1998	136	136 (H)	≥354 vs <234	0.35 (0.13–0.96)	Age, sex, SES, family history, refrigerator use, and *H pylori* infection
Larsson et al, 2006[45]	Sweden, cohort (SMC)	1987–2004	156	61,433	≥260 vs <203	1.04 (0.61–1.86)	Age, education, and intake of alcohol, coffee, tea, β-carotene, vitamin C, and energy
Pancreatic cancer							
Baghurst et al, 1991[47]	Australia, case-control	1984–1987	104	253 (P)	Highest vs lowest quartile[e]	0.36 (0.18–0.74)	Age, sex, smoking, and intake of alcohol and energy
Stolzenberg Solomon et al, 2001[48]	Finland, cohort (ATBC)	1985–1997	157	29.133	>373 vs ≤280	0.52 (0.31–0.87)	Age, intervention group
Skinner et al, 2004[49]	United States, cohort (HPFS)	1986–2000	187	47,840	≥500 vs <300	0.66 (0.37–1.18)	Age, time period, smoking, height, BMI, diabetes, and energy intake
Skinner et al, 2004[49]	United States, cohort (NHS)	1984–1998	139	77,640	≥400 vs <200	0.65 (0.31–1.35)	Age, time period, smoking, height, BMI, diabetes, and energy intake
Larsson et al, 2006[50]	Sweden, cohort (SMC, COSM)	1998–2004	135	81,922	≥350 vs <200	0.25 (0.11–0.59)	Age, sex, education, smoking, BMI, exercise, diabetes, and intake of fruits, vegetables, carbohydrates, and energy

ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; BMI, body mass index; COSM; Cohort of Swedish Men; HPFS, Health Professionals Follow-up Study; NCS, Netherlands Cohort Study; NHS, Nurses' Health Study; NSAID, nonsteroidal anti-inflammatory drugs; SES, socioeconomic status; SMC, Swedish Mammography Cohort.

[a]Type of controls in parentheses (H, hospital-based; P, population-based).
[b]Relative risk for the highest vs the lowest intake category; the measure of RR is an OR in case-control studies.
[c]The histologic types of esophageal cancer were squamous cell carcinoma (85%), adenocarcinoma (13%), and carcinosarcoma (2%).
[d]The range of intake was not reported.
[e]Including 6 cases with esophageal adenocarcinoma.
[f]Adenocarcinoma of the esophagus and gastric cardia.
[g]Noncardia gastric cancer.

CC genotype in individual studies, stratified by cancer site. All but one of the 22 ORs were >1, suggesting that the *TT* genotype is associated with an increased cancer risk; 13 estimates were statistically significant. Overall, compared with individuals with the *CC* genotype, individuals with the *TT* genotype had a significantly higher odds of gastric cardia adenocarcinoma (OR, 1.90; 95% CI, 1.38–2.60) and gastric cancer (OR, 1.68; 95% CI, 1.29–2.19). There was no significant heterogeneity among the studies of gastric cardia adenocarcinoma (Q = 4.94; P = .29; I² = 19%) or among the studies of gastric cancer (Q = 8.86; P = .12; I² = 43.6%). However, substantial heterogeneity was present among the studies of esophageal squamous cell carcinoma (Q = 29.01; P < .001; I² = 79.3%) and among the studies of pancreatic cancer (Q = 15.97; P < .001; I² = 87.5%), which precluded calculation of a summary estimate. For esophageal squamous cell carcinoma, heterogeneity remained after excluding the only hospital-based case-control study[28] and when the analysis was restricted to the 5 studies conducted in a Chinese population.[53–57] There was no indication of publication bias in the literature on esophageal cancer (P = .80), gastric cardia adenocarcinoma (P = .51), or pancreatic cancer (P = .65). For gastric cancer, the funnel plot suggested a relative absence of small studies showing no association with the *TT* genotype (P =

.003 by Egger's test). According to the trim and fill analysis, 2 such studies may be missing. Adding those missing studies to the meta-analysis yielded a summary OR of gastric cancer of 1.48 (95% CI, 1.14–1.94) for *TT* versus *CC* genotype.

MTHFR A1298C. We identified 10 studies[53,54,58–60,65,67–70] on the *MTHFR A1298C* polymorphism (Table 3). One study[58] was excluded because it was superseded by a later report.[60] The prevalence of the *CC* genotype among controls ranged from 0% (in Chinese populations)[54,70] to 12.9% (in US white patients).[67] The *MTHFR A1298C* genotype frequency in controls was in Hardy–Weinberg equilibrium in all studies. In 2 studies, individuals with the *CC* genotype, compared with those with the *AA* genotype, had a significantly higher odds of esophageal squamous cell carcinoma[53] or pancreatic cancer.[69] The other studies either did not observe any significant associations[59,60,65,67,68] or had no cases or controls with the *CC* genotype.[54,70]

Gene/Environment Interactions

MTHFR C677T. Seven of 9 studies suggest interactions between alcohol intake or smoking and *C677T* in relation to cancer risk (Table 2). Stolzenberg-Solomon et al[54] reported a significant interaction (P = .03) between *C677T* and alcohol intake in relation to risk of gastric cardia adenocarcinoma; in
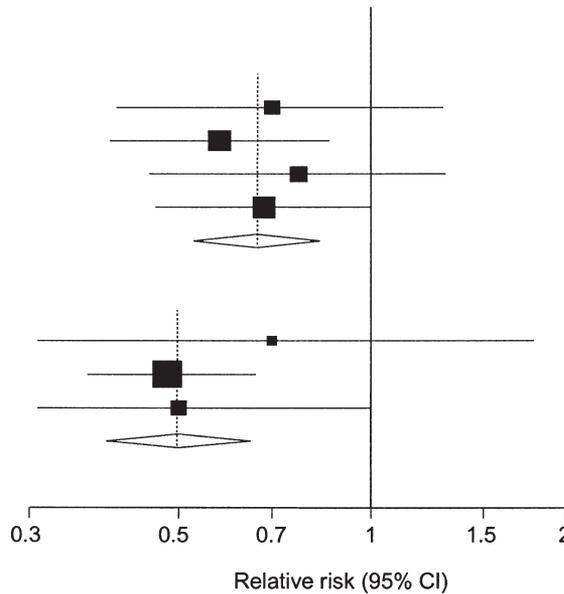
Esophageal cancer

Figure 2. RRs and 95% CIs of esophageal squamous cell carcinoma and esophageal adenocarcinoma for highest versus lowest dietary folate intake category in individual case-control studies and for all studies combined. The size of the data markers (*squares*) represents the statistical weight that each study contributed to the random-effects summary estimates; *horizontal lines* represent the 95% CIs. The summary RRs for each histologic subtype and their 95% CIs are indicated by the *diamonds*. Test for heterogeneity: esophageal squamous cell carcinoma: Q = 0.79, P = .85, I^2 = 0%; esophageal adenocarcinoma: Q = 0.61, P = .74, I^2 = 0%.

alcohol drinkers, those with the *TT* genotype had a significant 5.3-fold higher odds of gastric cardia adenocarcinoma compared with those with the *CC/CT* genotype. Likewise, Graziano et al[61] found that alcohol drinkers with the *TT* genotype had a 5.4-fold higher odds of gastric cancer compared with nondrinkers with the *CC* genotype. In 2 studies of pancreatic cancer,[67,68] the increased risk of pancreatic cancer associated with the *TT* or *TT/CT* genotypes was stronger in heavy drinkers and in heavy smokers than in nondrinkers and nonsmokers. In a study in China,[63] smokers with the *TT/CT* genotypes had a 7.7-fold higher odds of gastric cancer compared with nonsmokers with the *CC* genotype. Two studies found no significant interaction between dietary folate intake and *C677T* in relation to esophageal squamous cell carcinoma[28] or gastric cancer.[66]

MTHFR A1298C. Gao et al[70] found that alcohol drinkers with the C allele had a 2.9-fold higher odds of esophageal cancer compared with individuals with low alcohol con-

sumption and the *AA* genotype and that smokers with the C allele had a 3.5-fold higher odds of esophageal cancer compared with nonsmokers with the *AA* genotype. No significant interaction between *A1298C* and smoking in relation to pancreatic cancer was observed in a US study.[69]

Discussion

Results of this meta-analysis of observational studies support a significant inverse association of dietary folate intake with risk of esophageal squamous cell carcinoma, esophageal adenocarcinoma, and pancreatic cancer. Summary results indicate that individuals with a high dietary folate intake are about 40%–50% less likely to develop these cancers compared with those with low intake. In support of a role of folate in the etiology of esophageal and pancreatic cancer, most but not all studies reported increased risk of these cancers associated with

Figure 3. RRs and 95% CIs of gastric cancer for highest versus lowest dietary folate intake category in individual case-control and cohort studies and all studies combined. The size of the data markers (*squares*) represents the statistical weight that each study contributed to the random-effects summary estimate; *horizontal lines* represent the 95% CIs. The summary RR and the subtotals for each study design and their 95% CIs are indicated by the *diamonds*. Test for heterogeneity among all studies: Q = 24.27, P = .007, I^2 = 58.8%; case-control studies: Q = 22.67, P = .004, I^2 = 64.7%; cohort studies: Q = 0.01, P = .91, I^2 = 0%.
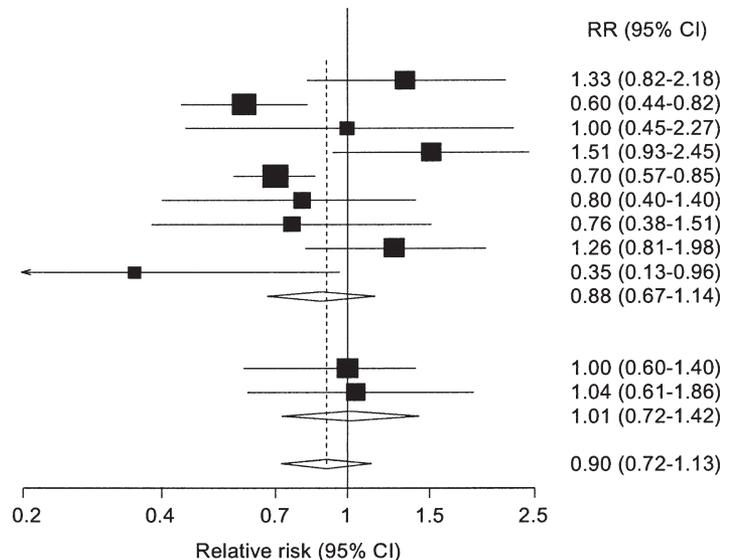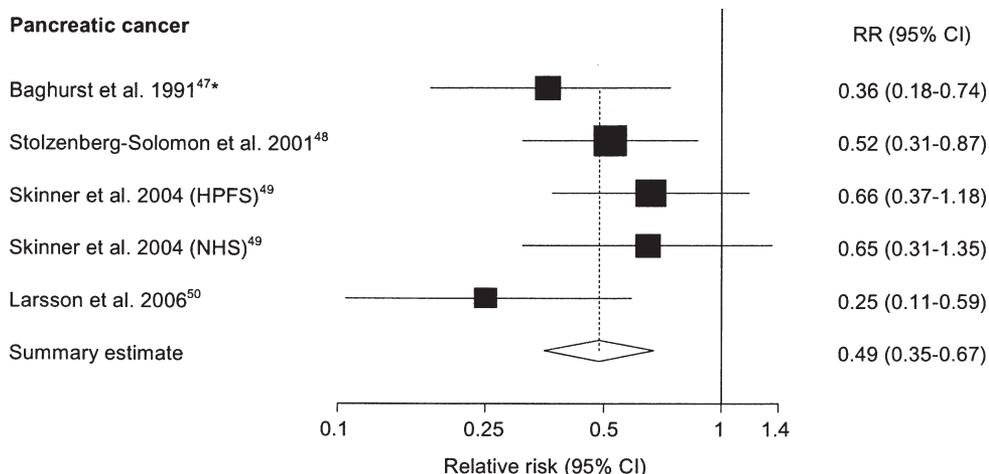
Figure 4. RRs and 95% CIs of pancreatic cancer for highest vs lowest dietary folate intake category in one case-control and 4 cohort studies and all studies combined. The size of the data markers (*squares*) represents the statistical weight that each study contributed to the random-effects summary estimate; *horizontal lines* represent the 95% CIs. The summary RR and its 95% CI are indicated by the *diamond*. Test for heterogeneity: Q = 4.83, P = .31, I² = 17.1%. *Case-control study; the remaining are cohort studies. HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study.

Pancreatic cancer   RR (95% CI)

Baghurst et al. 1991[47]*	0.36 (0.18-0.74)
Stolzenberg-Solomon et al. 2001[48]	0.52 (0.31-0.87)
Skinner et al. 2004 (HPFS)[49]	0.66 (0.37-1.18)
Skinner et al. 2004 (NHS)[49]	0.65 (0.31-1.35)
Larsson et al. 2006[50]	0.25 (0.11-0.59)
Summary estimate	0.49 (0.35-0.67)

Relative risk (95% CI)

the *MTHFR 677TT* genotype, which disrupts folate metabolism. Although there was no overall relation between dietary folate intake and risk of gastric cancer, there was evidence that the *MTHFR 677TT* genotype is associated with an increased risk. Summary results indicate that individuals with the *TT* genotype are about 70% and 90% more likely to develop gastric cancer (all sites) and gastric cardia adenocarcinoma, respectively, compared with those with the *CC* genotype. Several studies suggested that the relationship between the *C677T* polymorphism and cancer risk may be modified by alcohol or smoking, which may interfere with folate metabolism.[10,12,71] The small number of subjects with the *MTHFR 1298CC* genotype in published studies limits the conclusion on this polymorphism.

The increased risk of esophageal, gastric, and pancreatic cancer associated with the *MTHFR 677TT* genotype suggests that aberrant DNA methylation may play a role in the development of these cancers. It has been shown that genomic DNA methylation is significantly lower in individuals with the *TT* genotype compared with those with the *CC* genotype and that the methylation status in individuals with the *TT* genotype is directly correlated with red blood cell folate levels.[72,73] Global genomic DNA hypomethylation, which is commonly observed in many cancers and in the early stages of carcinogenesis, may result in chromosomal instability, increased mutation rates, and activation of proto-oncogenes.[3,74–77]

As a meta-analysis of observational studies, our findings have several limitations. First, there is potential for recall bias from case-control studies, which represent the majority of studies included in this meta-analysis. Arguing against recall bias in case-control studies, however, for colorectal cancer (previous meta-analysis[15]) and pancreatic cancer, the results for dietary folate are very similar for cohort and case-control studies. A potential source of bias in studies of genotypes might be the inclusion of individuals from different ethnic backgrounds.[78]

Second, individual studies may have failed to control for potential confounders. A challenge when evaluating the association between folate intake and cancer risk relates to correlations between dietary folate and other nutrients, fiber, and phytochemicals in folate-rich plant foods. Greater consumption of folate-rich plant foods, such as fruits, vegetables, and whole grain cereals, has been associated with lower risk of esophageal, gastric, and pancreatic cancer in a number of case-control studies,[79–81] although cohort studies are less supportive of such associations.[80,82–85] High intake of folate and folate-rich foods may also reflect other factors related to a healthy lifestyle, such as never smoking, lower alcohol consumption, and lower body weight. Such healthy lifestyles have generally been associated with a reduced risk of gastrointestinal cancers. Although most studies controlled for these lifestyle factors, the possibility of residual confounding cannot be completely excluded. Only one study[43] on dietary folate intake adjusted for *Helicobacter pylori* infection, which is an important risk factor for noncardia gastric cancer.[86] In that study,[43] the inverse association between dietary folate intake and gastric cancer risk was similar in *H pylori*–infected individuals and in noninfected individuals. Although confounding is generally not anticipated in analyses of an association between a genotype and disease, there may be some imbalance in the distribution of risk factors by *MTHFR* genotypes. However, most studies controlled for potential risk factors. In addition, given the diversity of populations studied, the variety of cancers studied, and the different etiologies for cancers, uncontrolled confounding is unlikely to explain the strong associations with the *MTHFR 677TT* genotype.

A third limitation is that the method used to assess diet (food-frequency and recall questionnaires) will inevitably lead to some degree of misclassification of folate intake. Moreover, estimated folate intake from food may not accurately reflect the actual intake and absorption because folate is susceptible to heat, pH, and oxidation.[87] The impact of these measurement errors is most likely to be nondifferential misclassification, resulting in an underestimation of any true relationship. The few studies that have correlated reported folate intake with biochemical indicators of folate status have shown that food-frequency questionnaires can provide valid information on folate intake.[88,89] Since 1998, flour and uncooked cereal-grain products in the United States have been fortified with folate.[90] Although this could have resulted in misclassification of folate intake, only one of the US studies included years of study that covered prefortification and postfortification periods[49] (Table 1).

Finally, as with any meta-analysis, publication bias could be of concern. Tests for publication bias in the literature on dietary folate intake and risk of esophageal, gastric, and pancreatic cancer were not statistically significant. However, there are many studies on folate-rich foods, such as fruits and vegetables, in relation to these cancers[80] that were not included in this meta-analysis because they did not present results in terms of

Table 2. Studies of the *MTHFR C677T* Polymorphism and Risk of Esophageal, Gastric, and Pancreatic Cancer

Reference	Country	Cases	Controls[a]	MTHFR C677T genotype[b]		TT genotype (%)[c]	Adjustments	Gene-environment interaction
				CT	TT			
Esophageal squamous cell carcinoma								
Song et al, 2001[53]	China	240	360 (P)	3.14 (1.94–5.08)	6.18 (3.32–11.51)	17.2	Age, sex, smoking	In alcohol drinkers with *TT* vs *CC/CT*: RR, 1.92 (95% CI, 1.80–4.58); $P_{interaction}$ = .32
Stolzenberg-Solomon et al, 2003[54]	China	129	398 (P)	0.86 (0.68–2.26)	1.24 (0.68–2.26)	31.2	Age, sex, trial, BMI, smoking, alcohol	
Gao et al, 2004[55]	China	138[d]	223 (P)	—	1.56 (0.88–2.79)[e]	NA	Age, sex, smoking, and intake of alcohol, tea, meat, pickled vegetables, and raw vegetables	
Zhang et al, 2004[56]	China	189	141 (P)	2.69 (1.33–5.44)	2.02 (1.00–4.07)[f]	44.0	Age, sex, smoking	
Zhang et al, 2004[56]	Germany	241	256 (P)	1.15 (0.79–1.68)	1.04 (0.60–1.81)[f]	13.3	Age, sex, smoking	
Yang et al, 2005[28]	Japan	165	495 (H)	0.97 (0.63–1.49)	0.66 (0.35–1.25)	16.2	Age, sex, smoking, and intake of alcohol and folate	Significant interaction with heavy alcohol drinking; no significant interactions with folate or smoking
Wang et al, 2005[57]	China	275	315 (P)	0.96 (0.61–1.50)	1.58 (0.99–2.50)	31.1	Age, sex	
Gastric cardia adenocarcinoma								
Miao et al, 2002[59]	China	217	468 (P)	1.56 (1.03–2.36)	2.04 (1.28–3.26)	21.3	Age, sex, smoking	In alcohol drinkers with *TT* vs *CC/CT*: RR, 5.32 (95% CI, 1.66–17.02); $P_{interaction}$ = .03
Stolzenberg-Solomon et al, 2003[54]	China	90	398 (P)	0.67 (0.35–1.29)	1.17 (0.61–2.25)	31.2	Age, sex, trial, BMI, smoking, alcohol	
Shen et al, 2005[60]	China	125	313 (P)	0.95 (0.59–1.52)	2.60 (1.30–5.21)	8.9	Age, sex, residential area, smoking, and intake of alcohol and tea	
Wang et al, 2005[57]	China	129	315 (P)	0.81 (0.45–1.47)	1.58 (0.88–2.83)	31.1	Age, sex	
Graziano et al, 2006[61]	Italy	43	164 (H)	3.62 (1.46–8.98)	3.71 (1.30–10.57)	17.7	Age, sex	
Noncardia gastric cancer								
Graziano et al, 2006[61]	Italy	119	164 (H)	2.32 (1.31–4.12)	2.74 (1.39–5.42)	17.7	Age, sex	
Gastric cancer (all sites)								
Gao et al, 2002[63]	China	107	200 (P)	—	1.89 (1.08–3.32)[g]	NA	Age, sex	In smokers with *TT/CT* vs nonsmokers with *CC*: RR, 7.72 (95% CI, 2.23–26.79) In alcohol drinkers with *TT/CT* vs nondrinkers with *CC*: RR, 3.08 (95% CI, 1.30–7.23)
Mu et al, 2004[64]	China	194	391 (P)	1.44 (0.96–2.15)	1.80 (1.07–3.04)	14.6	Age	In smokers with *TT/CT* vs *CC*: RR, 2.83 (1.39–5.74); no interaction with alcohol
Shen et al, 2005[60]	China	320	313 (P)	1.07 (0.76–1.51)	1.79 (1.02–3.15)	8.9	Age, sex, residential area, smoking, and intake of alcohol and tea	
Kim et al, 2005[65]	Korea	133	445 (P)	0.92 (0.58–1.47)	1.15 (0.85–1.55)	14.2	Age, sex	

Table 2 (continued). Studies of the *MTHFR C677T* Polymorphism and Risk of Esophageal, Gastric, and Pancreatic Cancer

Reference	Country	Cases	Controls[a]	MTHFR C677T genotype[b]		TT genotype (%)[c]	Adjustments	Gene-environment interaction
				CT	TT			
Graziano et al, 2006[61]	Italy	162	164 (H)	(1.52–4.37)	(1.57–5.55)		Age, sex	In alcohol drinkers with *TT* vs nondrinkers with *CC:* RR, 5.36 (95% CI, 1.94–14.83); $P_{interaction}$ = .09
Lacasaña-Navarro et al, 2006[6]	Mexico	201	427 (H)	1.24 (0.81–1.90)	1.62 (1.00–2.59)	24.4	Age, sex, education, *H pylori,* energy and capsaicin intake	No significant interactions with folate (*P* = .16) or alcohol (*P* = .36)
Pancreatic cancer Li et al, 2005[67]	United States (white)	347	348 (H)	0.90 (0.63–1.27)	2.14 (1.14–4.01)	6.5	Age, smoking, history of pancreatitis and diabetes	In heavy smokers with *TT* vs never smokers with *CC/CT:* RR, 6.83 (95% CI, 1.91–24.38) In heavy alcohol drinkers with *TT* vs nondrinkers with *CC/CT:* RR, 4.23 (95% CI, 0.88–20.3)
Wang et al, 2005[68]	China	163	337 (P)	2.60 (1.61–4.29)	5.12 (2.94–9.10)	15.7	Age, sex, smoking, drinking status	In heavy smokers with *TT/CT* vs nonsmokers with *CC:* RR, 6.69 (95% CI, 3.39–13.63) In alcohol drinkers with *TT/CT* vs nondrinkers with *CC:* RR, 4.39 (95% CI, 2.25–8.78)
Matsubayashi et al, 2005[69]	United States (all ethnicities)	303	305 (H)	0.79 (0.56–1.11)[d]	1.10 (0.67–1.82)[d]	11.8	Age, sex, ethnicity	No significant interaction with smoking

BMI, body mass index; NA, not available.

[a]Type of controls in parentheses (H = hospital-based; P = population-based).

[b]The *CC* genotype is the reference. Values are ORs with 95% CIs.

[c]Prevalence of *TT* genotype among controls.

[d]All histologic subtypes.

[e]Combined *TT* and *CT* genotypes. The RR (and its 95% CI) was derived by pooling the RRs across strata of the thymidylate synthase 3′-UTR polymorphism.

[f]Calculated from the provided data.
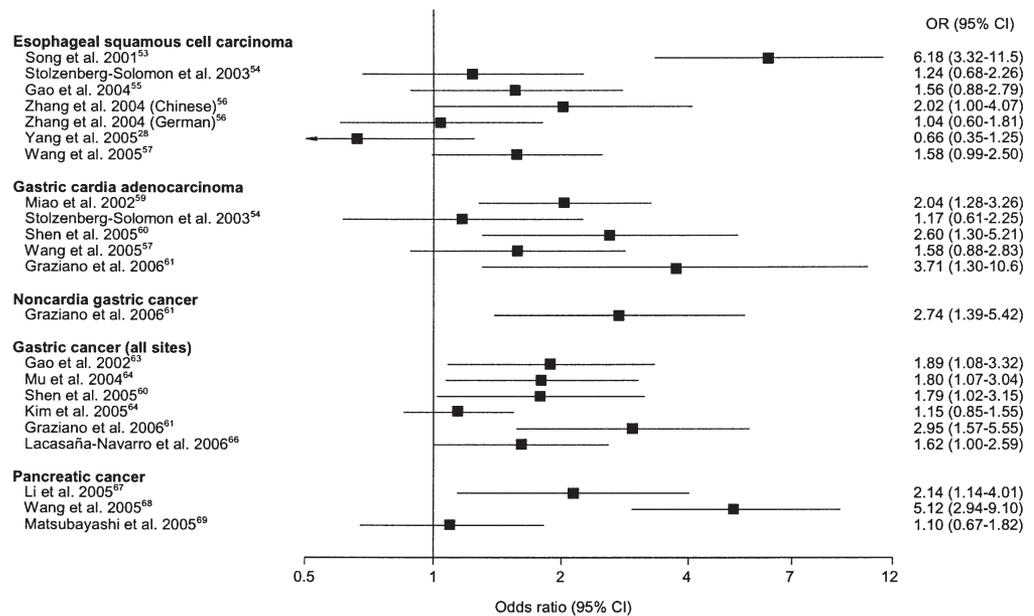
[g]Combined *TT* and *CT* genotypes.

Figure 5. ORs and 95% CIs of esophageal, gastric, and pancreatic cancer for *MTHFR 677TT* vs *CC* genotype in individual case-control studies.

Table 3. Studies of the *MTHFR A1298C* Polymorphism and Risk of Esophageal, Gastric, and Pancreatic Cancer

Reference	Country	Cases	Controls[a]	MTHFR A1298C genotype[b]		CC genotype (%)[c]	Adjustments
				AC	CC		
Esophageal squamous cell carcinoma							
Song et al, 2001[53]	China	240	360 (P)	0.88 (0.59–1.32)	4.43 (1.23–16.02)	1.4	Age, sex, smoking
Stolzenberg-Solomon et al, 2003[54]	China	129	398 (P)	0.92 (0.57–1.48)	—[d]	0	Age, sex, trial, BMI, smoking, alcohol
Gao et al, 2004[70]	China	141[e]	228 (P)	1.14 (0.72–1.79)	—[d]	0	Age
Gastric cardia cancer							
Miao et al, 2002[59]	China	217	468 (P)	1.19 (0.81–1.74)	2.41 (0.51–11.37)	1.1	Age, sex, smoking
Stolzenberg-Solomon et al, 2003[54]	China	90	398 (P)	0.78 (0.45–1.34)	—[d]	0	Age, sex, trial, BMI, smoking, alcohol
Shen et al, 2005[60]	China	125	313 (P)	0.77 (0.46–1.30)	—[d]	3.2	Age, sex, residential area, smoking, and intake of alcohol and tea
Gastric cancer (all sites)							
Kim et al, 2005[65]	Korea	133	445 (P)	1.11 (0.70–1.78)	0.83 (0.28–2.45)	1.8	Age, sex
Shen et al, 2005[60]	China	320	313 (P)	1.30 (0.90–1.87)	0.92 (0.35–2.42)	3.2	Age, sex, residential area, smoking, and intake of alcohol and tea
Pancreatic cancer							
Li et al, 2005[67]	United States (white)	347	348 (H)	1.12 (0.79–1.60)	0.77 (0.44–1.34)	12.9	Age, smoking, history of pancreatitis and diabetes
Wang et al, 2005[68]	China	163	337 (P)	0.86 (0.54–1.33)	0.48 (0.07–1.97)	2.4	Age, sex, smoking, drinking status
Matsubayashi et al, 2005[69]	United States (all ethnicities)	303	305 (H)	1.04 (0.74–1.45)[f]	1.86 (1.04–3.32)[f]	6.9	Age, sex, and ethnicity

BMI, body mass index.
[a]Type of controls in parentheses (H, hospital-based; P, population-based).
[b]The *AA* genotype is the reference. Values are ORs with 95% CIs.
[c]Prevalence of *CC* genotype among controls.
[d]There were no cases (or only one case[60]) or controls with this genotype.
[e]All histologic subtypes.
[f]Calculated from the provided data.

folate. Statistical testing suggested the presence of publication bias in the published literature examining the association of the *MTHFR C677T* polymorphism with gastric cancer risk. Adjusting for possible unpublished studies slightly attenuated the relation between the *TT* genotype and gastric cancer risk, but the association remained statistically significant.

Although findings of this meta-analysis suggest that increased consumption of foods naturally rich in folate may be beneficial, they do not encourage increased use of dietary supplements for cancer prevention. Three prospective studies have reported results on supplemental folate intake in relation to risk of pancreatic cancer.[48–50] All 3 studies observed an inverse association between dietary folate intake and pancreatic cancer risk, but none indicated an inverse relation with supplemental folate intake. Furthermore, a previous meta-analysis showed that intake of dietary folate, but not of total folate, was inversely associated with colorectal cancer risk.[15] Studies in animals suggest that the effect of folate on carcinogenesis may depend on the dose and timing of exposure, with folate deficiency enhancing carcinogenesis in normal cells but folate supplementation enhancing progression of existing tumor cells.[91]

In summary, this systematic review supports the hypothesis that folate may play a role in the etiology of esophageal, gastric, and pancreatic cancer. Large studies that assess the interrelations between folate, alcohol, smoking, and genetic polymorphisms in the folate metabolic pathway are needed to further clarify the role of folate deficiency and folate metabolism in the development of these cancers.

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