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Human Genome Epidemiology (HuGE) Review

Meta- and Pooled Analyses of the Methylenetetrahydrofolate Reductase *C677T* and *A1298C* Polymorphisms and Gastric Cancer Risk: A Huge-GSEC Review

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Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the metabolism of folate, whose role in gastric carcinogenesis is controversial. The authors performed a meta-analysis and individual data pooled analysis of case-control studies that examined the association between *C677T* and *A1298C* polymorphisms (the former being associated with low folate serum levels) and gastric cancer (meta-analyses: 16 studies, 2,727 cases and 4,640 controls for *C677T* and seven studies, 1,223 cases and 2,015 controls for *A1298C*; pooled analyses: nine studies, 1,540 cases and 2,577 controls for *C677T* and five studies, 1,146 cases and 1,549 controls for *A1298C*). An increased risk was found for *MTHFR* 677 *TT* in the meta-analysis (odds ratio (OR) = 1.52, 95% confidence interval (CI): 1.31, 1.77) and pooled analysis (OR = 1.49, 95% CI: 1.14, 1.95). No association resulted for *MTHFR* 1298 *CC* (meta-OR = 0.94, 95% CI: 0.65, 1.35; pooled OR = 0.90, 95% CI: 0.69, 1.34). Results from the pooled analysis of four studies on *C677T* stratified according to folate levels showed an increased risk for individuals with low (OR = 2.05, 95% CI: 1.13, 3.72) versus high (OR = 0.95, 95% CI: 0.54, 1.67) folate levels. Overall, these findings support the hypothesis that folate plays a role in gastric carcinogenesis.

epidemiology; folic acid; genetic predisposition to disease; meta-analysis; *MTHFR*; stomach neoplasms

Abbreviations: CI, confidence interval; GSEC, Genetic Susceptibility to Environmental Carcinogens; HWE, Hardy-Weinberg equilibrium; MTHFR, methylenetetrahydrofolate reductase.

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GENE AND FUNCTION

The 5,10-methylenetetrahydrofolate reductase gene (*MTHFR*) maps to chromosome 1p36.3 (1). The complementary DNA sequence is 2.2-kb long and contains 11 exons (1). The gene product is a 77-kD protein, although a smaller isoform of approximately 70 kD has been observed in some tissues such as liver (2). MTHFR plays a central role in folate metabolism, together with other enzymes, by irreversibly catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate and a cosubstrate for homocysteine methylation to methionine (figure 1). In humans, folate plays the fundamental role of providing methyl groups for de novo deoxynucleotide synthesis and for intracellular methylation reactions (2, 3).

MTHFR enzyme function may influence cancer risk in two ways. The substrate of MTHFR enzyme, 5,10-methylenetetrahydrofolate, is involved in the conversion of deoxyuridylate monophosphate to deoxythymidylate monophosphate, and low levels of 5,10-methylenetetrahydrofolate would lead to an increased deoxyuridylate monophosphate/deoxythymidylate monophosphate ratio. In this situation, increased incorporation of uracil into DNA in place of thymine may follow, resulting in an increased chance of point mutations and DNA/chromosome breakage (3). A less active form of MTHFR would lead, all other factors being equal, to an accumulation of 5,10-methylenetetrahydrofolate, thus a lower deoxyuridylate monophosphate/deoxythymidylate monophosphate ratio, and a presumably lower cancer risk (3).

The second way in which impaired MTHFR activity might influence cancer risk is determined by the level of *S*-adenosyl-L-methionine, the common donor of methyl that is necessary for maintenance of the methylation patterns in DNA. Changes in methylation modify DNA conformation and gene expression. A less active form of MTHFR leads to lower *S*-adenosyl-L-methionine levels and consequently to hypomethylation; this phenomenon would be expected to increase the risk of some cancers (4) (figure 1). Similarly, low folate intake may modify cancer risk by inducing uracil misincorporation during DNA synthesis, leading to chromosomal damage, DNA strand breaks and impaired DNA repair, and DNA hypomethylation (5).

GENE VARIANTS

Twenty-nine rare mutations of the *MTHFR* gene have been described in homocystinuric patients, resulting in very low enzymatic activity (6), whereas two common polymorphisms are present in healthy individuals with lower enzyme activity: $C \rightarrow T$ in exon 4 at nucleotide 677, leading to Ala222Val (7); and $A \rightarrow C$ in exon 7 at nucleotide 1298, leading to Glu429Ala (8, 9). These polymorphisms are located 2.1-kb apart and have been investigated in association with the risk of gastric and other cancers (10). Three additional polymorphisms have been described, *T1059C*, *T1317C*, and *G1793A* (9, 11, 12). The *T1059C* polymorphism has been reported to be associated with increased neural tube defects in an Iowa population (11), while *T1317C* is a silent change with no effect on plasma homocysteine and folate concentrations (9). The variant allele of the *G1793A* polymorphism is least frequent among Ashkenazi Jewish individuals (1.3 percent) compared with Caucasians (6.9 percent) (12); it has been reported that individuals with the heterozygous genotype for the variant allele, compared with individuals with the wild genotype, have borderline or deficient folate concentrations (13).

Individuals who are homozygous for the *MTHFR* 677 less frequent variant (*TT*) have 30 percent of the expected enzyme activity in vitro compared with those who are homozygous for the common variant (*CC*), whereas heterozygous carriers have 65 percent activity (2). van der Put et al. (8) evaluated MTHFR activity according to the combination of *A1298C* and *C677T* genotypes, showing that individuals who are homozygous for the wild-type *MTHFR* 677 allele (*CC*) and contemporarily homozygous for the 1298 mutant allele (*CC*) have 60 percent activity compared with subjects carrying the *1298AA* genotype, while, in the same population, 80 percent activity was detected for the 1298 heterozygotes (*AC*). Enzyme activity for individuals who are heterozygous for both *C677T* and *A1298C* appears to be approximately 50–60 percent that for those without either variant (9). It has been reported that subjects who are *TT* homozygous for *MTHFR* 677 exhibit reduced folate concentrations and higher serum homocysteine levels compared with those who carry at least one *677C* allele (14–16). The evidence regarding the association of the 1298 variant allele with increased folate levels is less consistent (14, 15, 17, 18).

Three recent studies reported that the *MTHFR TT* genotype is related to DNA hypomethylation (19, 20), particularly in individuals with reduced plasma folate concentrations (21). Inconsistent results derive from studies of the *A1298C* polymorphism, plasma folate, and homocysteine levels (15, 18, 22, 23).

POPULATION FREQUENCIES

The *T* allele frequency (percentage) of the *MTHFR* 677 polymorphism is reported to be 24.5–43.8 in Europeans, 17.6–42.4 in Asians, 21.1–39.1 in US individuals, and 12.0–23.5 in African Americans (24). The frequency of homozygosity ranges from 1 percent (95 percent confidence interval (CI): 0.2, 2.0) in US African-American populations to more than 20 percent (95 percent CI: 14.6, 26.8) in US Latinos; 5 percent (95 percent CI: 1.2, 9.6) to 30 percent (95 percent CI: 21.4, 38.9) in White populations in Europe and North America; 32.2 percent (95 percent CI: 28.3, 36.4) in Mexico; 5.8 percent (95 percent CI: 3.5, 9.6) in White Canadians in Alberta to 14.3 percent (95 percent CI: 10.9, 17.6) in those in Quebec, Canada; 0.0 percent (95 percent CI: 0.0, 1.2) in Sub-Saharan Africa; 10.7 percent in Oceania (95 percent CI: 5.5, 19.7); and 11.5 percent (95 percent CI: 10.2, 12.7) in Japanese and 16 percent (95 percent CI:

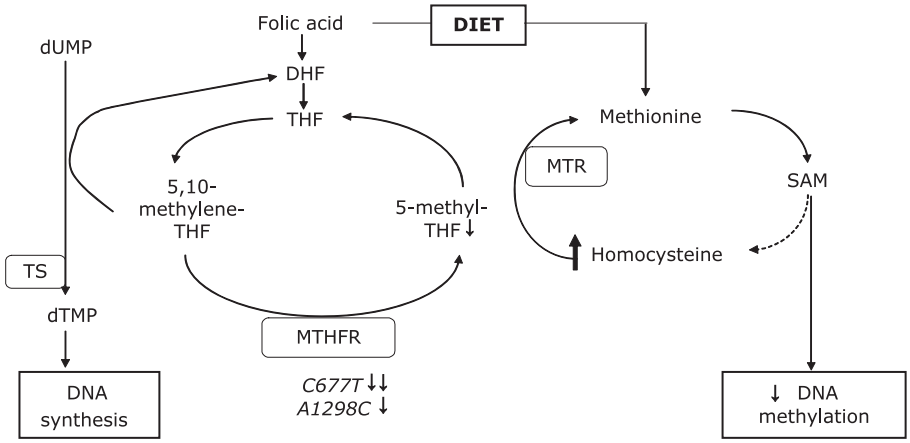

FIGURE 1. The folate pathway. Modified from Hung et al. (83). dUMP, deoxyuridine monophosphate; DHF, dihydrofolate; THF, tetrahydrofolate; MTR, methionine synthase; SAM, *S*-adenosyl-L-methionine; TS, thymidylate synthase; dTMP, deoxythymidine monophosphate; MTHFR, methylentrahydrofolate reductase.

8.0, 31.0) in Chinese (24–27). For *A1298C*, the variant allele frequency is reported to be 14.0–40.4 in Europeans and 11.1–17.0 in Asians. The frequency of homozygosity ranges from 10 percent (95 percent CI: 9.0, 11.0) in White populations in Europe and North America to 3.5 percent (95 percent CI: 0.2, 7.2) in Asians (28).

DISEASE

Gastric cancer is the second most common cause of cancer mortality, with 647,000 deaths reported worldwide in 2002 (29). In many populations, particularly in high-income countries, its incidence has gradually decreased in the last decades; however, it is still the fifth most common type of cancer in Europe and the fourth internationally (30). *Helicobacter pylori* infection is the single most common cause of adenocarcinoma of the distal stomach (31), but it is not a necessary or a sufficient cause. The development of gastric cancer appears in fact to be the result of a complex interaction between *H. pylori* infection, lifestyle, and genetic factors. Among the lifestyle risk factors, tobacco smoking, a high intake of salt, and lack of food refrigeration all seem to play a major role (32). Lastly, gastric cancer risk shows a familial clustering (33).

With regard to genetic factors, several single nucleotide polymorphisms might potentially alter individual susceptibility to gastric cancer (34). Among them are polymorphisms in genes involved in the protection of gastric mucosa against damaging agents and inflammatory response, genes that influence the ability to detoxify carcinogens (metabolic genes) and are involved in oxidative damage response and DNA repair, and oncogenes (35). Genes involved in folate metabolism have also been considered to play a role in gastric cancer risk (28).

GENE-ENVIRONMENT AND GENE-GENE INTERACTIONS

Some nutrients involved in the folate metabolic pathway (e.g., vitamins B_6 and B_{12}, methionine), alcohol (a folate antagonist), and smoking (which impairs folate level) may interact with plasma folate levels and the *MTHFR* polymorphisms in determining cancer risk (36, 37). It has been reported that alcohol perturbs folate metabolism by reducing folate absorption, increasing folate excretion, or inhibiting methionine synthase (38, 39). The inverse association between folate intake and plasma homocysteine levels can be modified by alcohol intake and by the *MTHFR* 677 but not the 1298 polymorphism (40). The inverse effect of smoking on folate status might be confounded by alcohol intake or dietary habits (41, 42), even though the association persists after adjusting for dietary folate and alcohol intake (42, 43). Additional studies have reported that elevated folate turnover in response to rapid tissue proliferation or DNA repair in aerodigestive tissues among individuals exposed to tobacco smoke might partially explain this phenomenon (44, 45).

According to recent reports, alcohol drinkers carrying the *MTHFR* 677 *TT* genotype had about a fivefold increased risk of gastric cancer compared with drinkers carrying the wild homozygous variant, namely, odds ratios of 5.36 (95 percent CI: 1.94, 14.83) reported by Graziano et al. (19) and 5.32 (95 percent CI: 1.66, 17.02) by Stolzenberg-Solomon et al. (46), whereas others did not show such interaction (47, 48). Additionally, Gao et al. (49) reported that smokers carrying the *MTHFR* 677 *T* allele had a 7.7-fold increased risk (OR = 7.72, 95 percent CI: 2.23, 26.79) of gastric cancer compared with nonsmokers with the *CC* genotype. To our knowledge, no published study has ever explored whether the effect of the *MTHFR* 677 *TT* genotype on gastric cancer is modified by individual folate intake or by plasma folate levels. Finally, the interaction between alcohol, smoking, or folate status and the *MTHFR A1298C* polymorphism has never been known to be tested in gastric cancer.

The effect of the combination of the two common *MTHFR* polymorphisms on gastric cancer was investigated by Miao et al. (50) and Boccia et al. (51). Both reported no interaction between them.

OBJECTIVE

A meta-analysis of prospective studies showed an inverse association between fruit and vegetables intake, the main dietary source of folate, and gastric cancer risk, particularly after 10 or more years of follow-up (52). Discrepant results, however, recently emerged from a large European cohort study, showing no association between fresh fruit intake and gastric cancer and a slight protective effect of total vegetable intake for the intestinal histotype only (53). Results from a meta-analysis of prospective and retrospective studies specifically focusing on dietary folate intake and risk of gastric cancer also reported no clear effect of dietary folate intake, with no differences between cohort or case-control studies (54).

On the other hand, two recent meta-analyses showed that the *MTHFR* 677 *TT* genotype is associated with an increased risk of gastric cancer, suggesting an important role of folate levels and subsequent impaired chromosomal DNA synthesis and aberrant DNA methylation in gastric carcinogenesis (28, 54). However, neither meta-analysis included all published reports available when the meta-analyses were published and specifically included either eight (28) or nine (54) studies compared with 16 studies in the present meta-analysis. In addition, these two provided unadjusted overall estimates, and the results were not stratified according to potential factors affecting folate status and *MTHFR* polymorphisms because of the nature of already published data. We accomplished both of the last two points by also carrying out a pooled analysis of individual-level data.

With the present meta- and pooled analyses, we aimed to assess the overall effect of the *MTHFR C677T* and *A1298C* polymorphisms on gastric cancer by including all available published papers and to help clarify the interrelations between these polymorphisms with folate, alcohol, and smoking and gastric cancer risk.

METHODS

We assessed the association between the *MTHFR C677T* and *A1298C* polymorphisms and gastric cancer by conducting meta-analyses of all published papers and pooled analyses of individual-level data when available.

Meta-analysis

Selection criteria. The papers were identified by searching the MEDLINE (National Library of Medicine, National Institutes of Health, Bethesda, Maryland) and EMBASE (Elsevier, Amsterdam, the Netherlands) databases up to January 2007 using the following terms: (''methylenetetrahydrofolate reductase'' or MTHFR) and (gastric or stomach) and (cancer or carcinoma), without any restriction on language. Our research produced 35 articles. A cited reference search of the retrieved articles was carried out, and publications were also identified by reviewing their bibliographies. Eligible were community-based studies that reported the frequency of the *MTHFR C677T* and/or *A1298C* polymorphisms as number of individuals with gastric cancer and controls according to the three variant genotypes of both polymorphisms. Studies whose allele frequencies in the control population deviated from Hardy-Weinberg equilibrium (HWE) at a p value of ≤ 0.05 were excluded from the meta-analysis. If more than one article was published from the same case series, we used the one that included the most individuals in the analysis.

Of the 35 articles retrieved, 21 studies were eligible for the analysis (19, 46–50, 55–69). Five reports (55–59) were excluded either because they concerned subjects included in an expanded series (50, 60) or because they partially overlapped with another study (49) eventually selected because it gave the absolute number of individuals according to the three variant genotypes of *MTHFR* 677 (57–59). Finally, one study was excluded from the meta-analysis for the association between *MTHFR C677T* and gastric cancer (60), and one from the analysis of *A1298C* (46), because of deviations from HWE. One study in press at the time (not yet published) was also included (51).

The final number of articles considered for our meta-analysis of the association between *MTHFR C677T* and gastric cancer risk included 16 case-control studies (19, 46–51, 61–69), of which three were written in the Chinese language (49, 61, 62), comprising a total of 7,367 subjects (2,727 cases and 4,640 controls). The studies are described in table 1. Ten of 16 were population based; one was a case-control study nested in a cohort (46). Among them, seven were also included in the meta-analysis of the association between *MTHFR A1298C* and gastric cancer risk (48, 50, 51, 60, 61, 63, 64), for a total of 3,238 subjects (1,223 cases and 2,015 controls).

Statistical analysis. Two researchers (S. Boccia and F. Gianfagna) extracted the data from each article by using a structured sheet and entered them into a database. The followings items were considered: year and location of the study, ethnicity, characteristics of the control group, tumor site (cardia/noncardia gastric cancer), and number of individuals heterozygous and homozygous for the *MTHFR* 677 and 1298 variant alleles in the compared groups. Heterogeneity was tested by the Q statistic (70). In carrying out the meta-analyses, random-effects models were used (71) to take into account the possibility of heterogeneity between studies. The summary odds ratios of gastric cancer associated with the *MTHFR* 677 *TT* and *CT* genotypes and the *MTHFR* 1298 *CC* and *CA* genotypes were estimated by using the homozygous wild type for each genotype as the reference group. To determine deviation from HWE, we used Fisher's exact permutation test with a Monte Carlo technique (72). A visual inspection of Begg's funnel plot and Begg and Egger asymmetry tests (70) was used to investigate for publication bias when appropriate (73).

Because two potential causes of heterogeneity among studies were ethnicity and tumor site, we calculated separate odds ratios in subgroups of studies performed among different ethnic groups (Asian/Europeans) and in subgroups of studies including cardia and noncardia gastric cancer cases, when genotype data were tabulated according to the tumor site specified in the published papers. A heterogeneity test was then performed to test for statistically significant differences among the strata estimates.

TABLE 1. Description of the studies included in the meta- and pooled analyses of the methylenetetrahydrofolate reductase *C677T* and *A1298C* polymorphisms and gastric cancer

First author (reference no.), year of publication	No. of cases	No. of controls	Country	Source of controls	Gastric tumor site	*MTHFR** 677 *TT* vs. *CC*				*MTHFR* 1298 *CC* vs. *AA*			
						Crude OR*	95% CI*	Adjusted OR†	95% CI	Crude OR	95% CI	Adjusted OR†	95% CI
Boccia et al. (51), 2007‡,§	102 (107¶)	254	Italy	Hospital	Cardia and noncardia	1.81	0.93, 3.52	1.95	1.01, 3.78	1.02	0.44, 2.37	0.44	0.16, 1.21
Wang Y et al. (65), 2007	467	540	China	Population	Cardia	1.63	1.15, 2.33	NA#					
Gotze et al. (66), 2007‡	103 (106)	106	Germany	Population	Cardia and noncardia	0.67	0.28, 1.58	0.59	0.21, 1.66				
Zhang et al. (48), 2007‡,§	295 (464)	399 (480)	Poland	Population	Cardia and noncardia	1.16	0.69, 1.95	1.17	0.70, 1.97	1.01	0.60, 1.69	1.06	0.62, 1.68
Weng et al. (63), 2006‡,§	38	34	China	Hospital	Noncardia	0.67	0.18, 2.54	0.84	0.18, 3.98	0.28	0.01, 7.30	—**	
Zeybek et al. (67), 2006	35	144	Turkey	Hospital	NS*	1.42	0.45, 4.53	NA					
Lacasana-Navarro et al. (47), 2006‡	201	427	Mexico	Hospital	NS	1.48	0.95, 2.31	1.50	0.96, 2.34				
Graziano et al. (19), 2006‡	162	164	Italy	Population	Cardia and noncardia	2.79	1.48, 5.23	3.03	1.60, 5.73				
Sarbia et al. (69), 2005	332	255	Germany	Population	Cardia and noncardia	0.96	0.57, 1.63	NA					
Si et al. (61), 2005	122	101	China	Hospital	Cardia and noncardia	1.50	0.61, 3.70	NA		0.79	0.22, 2.88	NA	
Kim et al. (64), 2005	133	445	South Korea	Population	NS	1.46	0.83, 2.57	NA		0.38	0.05, 3.11	NA	
Shen et al. (60), 2005§,††	320	313	China	Population	Cardia and noncardia					0.84	0.33, 2.17	0.83	0.32, 2.14
Wang LD et al. (68), 2005	129	315	China	Population	Cardia	1.78	1.02, 3.11	NA					
Mu et al. (62), 2004	194	390	China	Hospital	NS	1.79	1.06, 3.02	NA					
Stolzenberg-Solomon et al. (46), 2003‡,‡‡	90	398 (405)	China	Population	Cardia	1.14	0.60, 2.18	1.06	0.55, 2.05				
Miao et al. (50), 2002‡,§	217	468	China	Population	Cardia	2.02	1.28, 3.19	2.03	1.33, 3.36	1.30	0.31, 1.59	1.32	0.31, 5.71
Gao et al. (49), 2002‡	107 (155)	200 (223)	China	Population	NS	1.81	0.89, 3.66	1.27	0.68, 2.38				

* *MTHFR*, methylenetetrahydrofolate reductase gene; OR, odds ratio; CI, confidence interval; NS, not specified.
† Adjusted for age, gender, and smoking status (ever/never).
‡ Studies included in the pooled analysis of *MTHFR C677T*.
§ Studies included in the pooled analysis of *MTHFR A1298C*.
¶ Values in parentheses refer to the number of individuals included in the pooled analysis, when different from the number in the published study.
# NA, not applicable; not included in the pooled analysis.
** Study included only in the pooled analysis of *MTHFR* 1298 *CA* vs. *AA* (refer to the Methods section for details).
†† Study excluded from the meta- and pooled analysis of *MTHFR C677T*; not in Hardy-Weinberg equilibrium (refer to the Methods section for details).
‡‡ Study excluded from the meta- and pooled analysis of *MTHFR A1298C*; not in Hardy-Weinberg equilibrium (refer to the Methods section for details).

Pooled analysis

Data collection. The pooled analysis was performed by using the Genetic Susceptibility to Environmental Carcinogens (GSEC) database. The International Collaborative Study on GSEC (http://www.upci.upmc.edu/research/ccps/ccontrol/g_intro.html) project gathers information from both published and unpublished population-based studies on metabolic gene polymorphisms and cancer risk. The design of the GSEC study has been reported elsewhere (74). Apposite investigators were contacted and were asked to provide their data for the pooled analyses. A questionnaire was provided by e-mail to each investigator, collecting information on study design, selection and source of cases and controls, laboratory method used for genotyping, source of DNA used for the genotype analysis, and response rate for cases and controls. We contacted all authors of the identified published papers, including those whose control populations were not in HWE for the studied polymorphisms (46, 60). Of the 17 eligible data sets, we were able to obtain data from 10, with one of them (60) later excluded for the pooled analysis on *MTHFR* 677 and one more (46) on *MTHFR* 1298 because the allele frequency of the control population did not respect HWE. We finally included nine studies for *MTHFR* 677—four of Asians, four of Europeans, and one of Latinos—totaling 4,117 subjects (1,540 cases and 2,577 controls) (refer to table 1 for details). As for *MTHFR* 1298, five studies were included, totaling 2,695 subjects (1,146 cases and 1,549 controls; refer to table 1 for details).

Statistical analysis. To assess the association of the *MTHFR* 677 *TT* and 1298 *CC* genotypes with gastric cancer, the logistic regression model was used to estimate study-specific odds ratios and 95 percent confidence intervals in each single study. Adjusted odds ratios were obtained by including age, gender, and smoking status (ever/never) as covariates. In some studies, odds ratios estimated for individual studies and numbers of cases and controls did not precisely match those reported in the publications. A pooled odds ratio was estimated by inverse-variance weighting with the random-effects model (71), taking into account the possibility of heterogeneity between studies, which was tested with Q statistics (70). We could perform stratified analyses for only *MTHFR* 677 *TT*, since *MTHFR* 1298 *CC* was available in only four studies (table 1).

Results were stratified according to ethnicity (Asians/European descendants), alcohol drinking, tobacco smoking, and folate status. Because information on pack-years of smoking was available in only four studies (47, 48, 50, 51), subjects were classified as ever (current and former) and never smokers. For alcohol drinking, individuals were categorized as ever (current and/or former) and never (<1 glass of each alcoholic beverage/month) drinkers. Information on folate serum levels was obtained from Gotze et al. (66) and gastric mucosa folate levels from Weng et al. (63). The nutrient density of folate (dietary folate intake/total caloric intake: μg/kcal × 1,000) was obtained by Zhang et al. (48) and intake of fruit and vegetables portions by Boccia et al. (51). For the analysis, subjects were categorized in two classes based on the lower quartile of each variable estimated in the control population.

For each stratified analysis, a pooled odds ratio was estimated by inverse-variance weighting with the random-effects model (71), taking into account the possibility of heterogeneity between studies, which was tested with Q statistics. A heterogeneity test was performed to assess for statistically significant differences among the pooled strata estimates.

Statistical analyses were carried out by using the STATA software package v.8.2 (Stata Corporation, College Station, Texas).

RESULTS

Meta-analysis of *MTHFR C677T*

The odds ratios in 11 of 16 studies were above the unit; among them, five studies (19, 50, 62, 65, 68) reported a significant positive association between gastric cancer and the *MTHFR* 677 *TT* genotype (table 1). The meta-analysis produced overall odds ratios of 1.52 (95 percent CI: 1.31, 1.77) and 1.17 (95 percent CI: 0.99, 1.39) for gastric cancer and the *MTHFR TT* (figure 2) and *CT* genotypes, respectively. The heterogeneity test results were 0.37 for *TT* and 0.01 for *CT*. The funnel plot (not shown) and Begg's test provided no evidence of publication bias ($p = 0.72$) for the *MTHFR* 677 *TT* genotype, whereas the Egger test provided a p value of 0.007.

When stratifying the data by ethnicity, we observed odds ratios of 1.34 (95 percent CI: 0.90, 1.99) and 1.64 (95 percent CI: 1.36, 1.97) for the *MTHFR* 677 *TT* versus *CC* genotype in six studies of Europeans and nine studies of Asians, respectively (p for heterogeneity = 0.38). The analysis by anatomic tumor site showed that both gastric cardia cancer (11 studies) and noncardia cancer (six studies) were significantly associated with *MTHFR* 677 *TT*, with respective odds ratios of 1.51 (95 percent CI: 1.11, 2.05) and 1.57 (95 percent CI: 1.09, 2.24) (p for heterogeneity = 0.87). There was no evidence of heterogeneity in all subgroup meta-analyses performed.

Meta-analysis of *MTHFR A1298C*

All seven studies included reported odds ratios spread around the null effect (table 1). From the meta-analysis, the association between gastric cancer and *MTHFR* 1298 *CC* was 0.94 (95 percent CI: 0.65, 1.35) (figure 3); an odds ratio of 1.01 (95 percent CI: 0.86, 1.18) was found for the association with 1298 *AC*. There was no evidence of heterogeneity in the overall meta-analysis and in subgroup meta-analyses.

When we restricted the analysis of *MTHFR A1298C* to the five studies conducted among Asians (50, 60, 61, 63, 64), an overall odds ratio of 0.81 (95 percent CI: 0.43, 1.51) emerged. When the analysis was stratified by tumor site, an odds ratio of 0.99 (95 percent CI: 0.43, 2.28) resulted for cardia cancer, and an odds ratio of 0.81 (95 percent CI: 0.38, 1.74) was found for noncardia cancer (p for heterogeneity = 0.76).

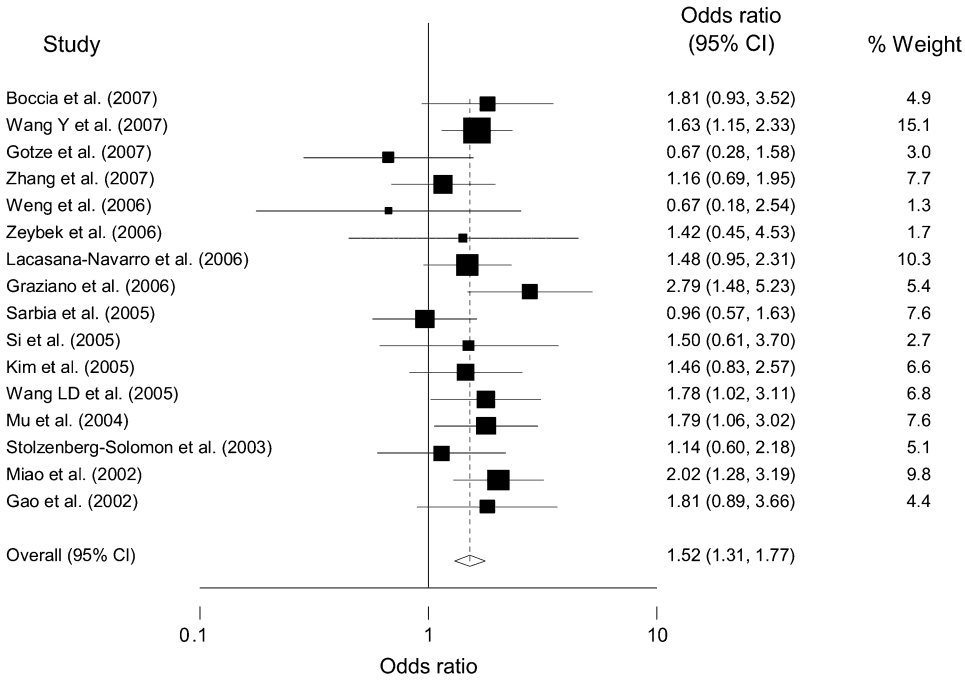

FIGURE 2. Forest plot of the odds ratios and 95% confidence intervals (CIs) of studies of the association between gastric cancer and the *MTHFR C677T* polymorphism (*TT* vs. *CC*). On the left, the first author of the study is followed by the publication year in parentheses. The size of the black box corresponding to each study is proportional to the sample size; the horizontal line shows the corresponding 95% CI of the odds ratio. The combined estimate is based on a random-effects model shown by the diamond. The solid vertical line represents the null result.

Pooled analyses

The study-specific adjusted odds ratios for *MTHFR* 677 *TT* are reported in table 1. Of the nine studies included in the pooled analysis, six had odds ratios above the unit; among them, three (19, 50, 51) reported a significant positive association between gastric cancer and the *MTHFR* 677 *TT* genotype (table 1). Results from the pooled analysis are shown in table 2. The overall odds ratio adjusted for age, gender, and smoking status was 1.49 (95 percent CI: 1.14, 1.95; p for heterogeneity = 0.06) for *MTHFR* 677 *TT*, whereas an odds ratio of 1.21 (95 percent CI: 0.90, 1.62; p for heterogeneity = 0.03) was detected for *MTHFR* 677 *CT*. Publication bias was not tested because of low statistical power of the tests when the number of studies is 10 or fewer (73, 75, 76).

The pooled odds ratio for *MTHFR* 677 *TT* among Asians was 1.54 (95 percent CI: 1.09, 2.15; p for heterogeneity = 0.34) and among Europeans was 1.52 (95 percent CI: 0.84, 2.76; p for heterogeneity = 0.03). The p for heterogeneity test result for Asians and Caucasians was 0.86 (table 2).

When we stratified on smoking habits, an odds ratio of 2.04 (95 percent CI: 1.27, 3.26) for *MTHFR* 677 *TT* resulted for ever smokers, whereas an odds ratio of 1.36 (95 percent CI: 1.03, 1.80) was found for never smokers, with a p for heterogeneity test result of 0.14 among them (table 2). The stratified analysis according to alcohol intake included six studies; similar risk estimates were found for ever drinkers and never drinkers (p for heterogeneity = 0.49; table 2). The stratified analysis according to estimated folate status showed an odds ratio for gastric cancer of 2.05 (95 percent CI: 1.13, 2.72) for *MTHFR* 677 *TT* individuals with low folate levels and an odds ratio of 0.95 (95 percent CI: 0.54, 1.67) for those with high folate levels (p for heterogeneity among the two estimates = 0.06) (table 2).

The study-specific adjusted odds ratios for *MTHFR* 1298 *CC* are reported in table 1. It was not possible to compute the adjusted odds ratio for the homozygous variant genotype in one study (60) because of the small number of subjects. The overall odds ratio adjusted for age, gender, and smoking status was 0.90 (95 percent CI: 0.69, 1.34; p for heterogeneity = 0.50, four studies) for *MTHFR* 1298 *CC*, whereas an odds ratio of 1.01 (95 percent CI: 0.83, 1.22; p for heterogeneity = 0.50, five studies) was found for *MTHFR* 1298 *AC*.

DISCUSSION

The results from the meta-analysis of 16 studies highlighted a higher risk of developing gastric cancer for subjects carrying the *MTHFR* 677 *TT* genotype. The results were confirmed by the pooled analysis including nine studies. No association was detected from either the meta-analysis or the pooled analysis between the *MTHFR* 1298 *CC* genotype and gastric cancer. Our results were consistent with two previously published meta-analyses by Zintzaras

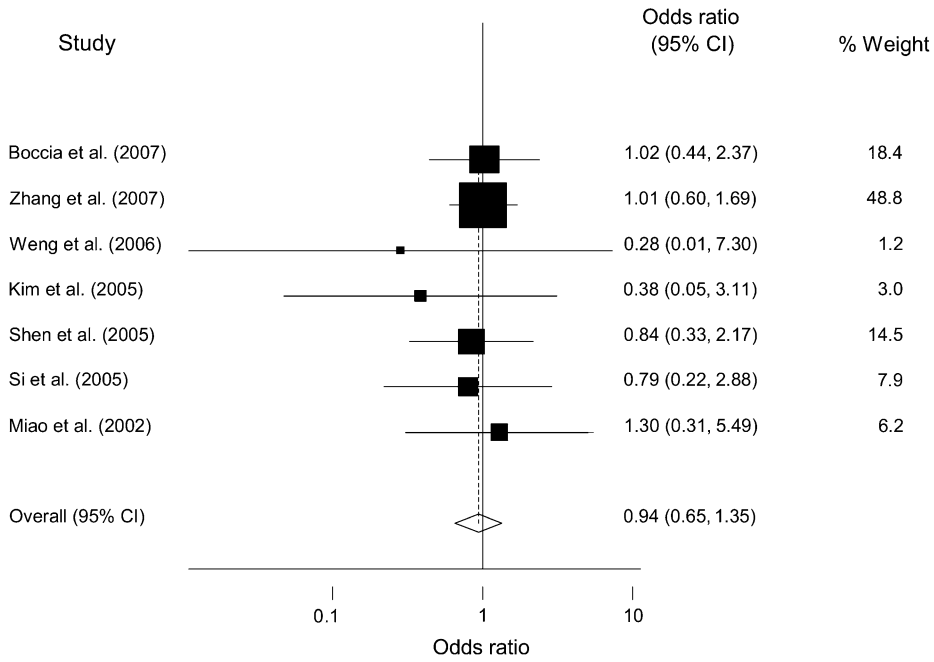

FIGURE 3. Forest plot of the odds ratios and 95% confidence intervals (CIs) of studies of the association between gastric cancer and the *MTHFR A1298C* polymorphism (*CC* vs. *AA*). On the left, the first author of the study is followed by the publication year in parentheses. The size of the black box corresponding to each study is proportional to the sample size; the horizontal line shows the corresponding 95% CI of the odds ratio. The combined estimate is based on a random-effects model shown by the diamond. The solid vertical line represents the null result.

(28) and Larsson et al. (54), which showed an increased risk of gastric cancer associated with only the *MTHFR* 677 *TT* genotype and an absence of risk for *MTHFR* 1298 *CC*. However, these two previously published meta-analyses included a smaller number of studies than ours did, and results were based on unadjusted estimates. In our pooled analysis of *MTHFR* 677, an increased risk of gastric cancer was observed for subjects with a low folate status compared with those with a high folate status. These results support our a priori hypothesis of a higher risk of gastric cancer for subjects carrying the variant *MTHFR* 677 homozygous variant who have low folate levels compared

TABLE 2. Odds ratios and 95% confidence intervals from the pooled analysis of the association between the *MTHFR *C677T* polymorphism and gastric cancer**

	No. of studies	No. of cases	No. of controls	No. of *MTHFR 677TT* cases	No. of *MTHFR 677TT* controls	OR*,†	95% CI*	*p* for heterogeneity within strata	*p* for heterogeneity across strata
All studies	9	1,540	2,577	309	502	1.49	1.14, 1.95	0.06	
Asians	4	507	1,147	138	278	1.54	1.09, 2.15	0.34	0.86
Europeans	4	832	1,003	111	120	1.52	0.84, 2.76	0.03	
Never smokers	8	616	1,189	135	274	1.36	1.03, 1.80	0.49	0.14
Ever smokers	7	834	1,225	163	219	2.04	1.27, 3.26	0.02	
Nonalcohol drinkers	6	527	995	107	228	1.37	0.97, 1.91	0.84	0.49
Alcohol drinkers	6	782	900	120	150	1.68	1.04, 2.73	0.05	
High folate status‡	4	403	433	31	50	0.95	0.54, 1.67	0.86	0.06
Low folate status	4	242	346	35	42	2.05	1.13, 3.72	0.96	

* *MTHFR*, methylenetetrahydrofolate reductase gene; OR, odds ratio (all were adjusted for age, gender, and smoking status); CI, confidence interval.

† The comparison is *MTHFR* 677 *TT* vs. *CC*.

‡ High folate status defined a nutrient density of folate (dietary folate intake/total caloric intake: μg/kcal × 1,000) >99 (48); eating at least two portions of fruit and vegetables/day for crude dietary folate intake (51); >5.5 ng/ml for serum folate (66); >4.0 ng/ml for gastric mucosa folate levels (63). Refer to the Methods, Pooled analysis, Statistical analysis subsection for details.

with subjects carrying the same variant but with high levels of folate.

A limitation common to both the meta- and the pooled analysis might be the presence of publication bias. In the meta-analysis of *MTHFR* 677 *TT*, we did not observe evidence of publication bias from visual inspection of the Begg's funnel plots and the results of the rank correlation statistical test. Results from Egger's regression method highlighted some publication bias; however, this method is usually more sensitive than Begg's test, reporting to provide evidence for bias (false-positive results) when in fact it is not present, especially when the number of studies is low (75, 76). We cannot rule out the possibility that the effect of *MTHFR* 677 *TT* on gastric cancer was overestimated in our meta-analysis, because negative results from small studies remained unpublished.

In the pooled analysis, we explored possible effect modification of the *MTHFR* 677 *TT* genotype on gastric cancer by stratifying on tobacco smoking and alcohol drinking, two factors that may affect folate levels. We were unable to observe any effect modification; however, in both instances, the information did not take into account the amount or duration of alcohol intake and tobacco smoking.

When the results from the pooled analysis were stratified according to folate status (available from four studies), a strong association between the *MTHFR* 677 *TT* genotype and gastric cancer was noted among subjects with a low folate status compared with a high folate status. The heterogeneity test showed that results were borderline significantly different; therefore, our result needs to be confirmed with a larger population. This result supports our hypothesis, suggesting that concomitant inadequate folate intake and impaired MTHFR activity might be important susceptibility factors for gastric cancer. A limitation is the heterogeneity regarding collection of folate information: gastric mucosa level (63), serum level (66), nutrient density of folate (48), or dietary fruit and vegetables intake (51).

To our knowledge, this pooled analysis is the first assessing the role of two common *MTHFR* polymorphisms in the risk of gastric cancer. In fact, the two previously published meta-analyses did not include individual-level data (28, 54); therefore, the authors were unable to calculate adjusted estimates and to stratify the results of the meta-analyses according to folate status, alcohol intake, or smoking habits. Because the data sets included information on age, gender, and cigarette smoking from all studies, it was possible to adjust for the potential confounding effect of these variables and to assess consistently the presence of gene-environment interactions for *MTHFR* 677, a factor that makes the pooled analysis preferable to the meta-analysis (77). The absence of publication bias and statistical heterogeneity among studies strengthens our results.

LABORATORY TESTS

Both *MTHFR C677T* and *A1298C* can be detected by means of polymerase chain reaction (followed by restriction fragment-length polymorphism) analysis with *Hinf*I and *Mbo*II for *C677T* and *A1298C*, respectively (7, 8). Other methods include direct DNA sequencing or TaqMan assays (48). Most studies did not report the success rate in extracting DNA from samples, the proportion of eligible subjects for whom genotyping failed, whereas 43.0 percent (7/16) of them reported the degree of genotyping reproducibility (19, 46, 48, 50, 51, 61, 68). HWE was tested in 87.5 percent (14/16) of the studies. All previously mentioned variables are important indicators of the analytical validity of the genotyping methods, also influencing potential nondifferential misclassification of the exposure. In addition, only 31.2 percent of the studies (5/16) clearly reported that the analysts were unaware of the clinical status of the subjects when genotyping the samples; therefore, differential exposure misclassification may not be ruled out.

POTENTIAL PUBLIC HEALTH IMPACT

At the moment, the potential public health impact of this issue is limited, given the small association between gastric cancer and homozygosis *TT* for *MTHFR* 677. Additional studies on the possible additional risk of gastric cancer for subjects who are 677 *TT* homozygous and have low folate levels are urgently needed, however. If this preliminary result is confirmed, proper evaluation of the clinical utility of *MTHFR C677T* testing for identifying gastric cancer susceptibility among populations with folate deficiency, followed by the introduction of specific folate supplementation (vs. no folate supplementation), would be warranted. Currently, however, population testing for the *MTHFR C677T* polymorphism to prevent gastric cancer is not indicated.

CONCLUSION AND RESEARCH PRIORITIES

MTHFR plays a central role in balancing DNA synthesis (which involves 5,10-methylentetrahydrofolate) and DNA methylation (which involves 5,10-methyltetrahydrofolate). Specifically, the *677T* allele contributes to DNA hypomethylation, which in turn may lead to altered gene expression; at the same time, this polymorphism might exert a protective effect, as observed for colorectal cancer (24), by increasing the levels of the MTHFR substrate, essential for DNA synthesis. Therefore, exact interpretation of the MTHFR-cancer association is not straightforward, although the observed increased risk of gastric cancer associated with the *MTHFR* 677 homozygous variant suggests that dietary folate might be protective in gastric carcinogenesis mainly by limiting aberrant DNA methylation when folate status is impaired. In general, studying the association between sequence variants of folate-related genes and cancer has the advantage of being less prone to the confounding effect exerted by dietary or lifestyle factors (78). The observed increased risk of gastric cancer for *MTHFR* 677 *TT* individuals strengthens the hypothesis of a protective effect of folate in gastric carcinogenesis. If this hypothesis holds true, it would be interesting to explore whether the introduction of folate fortification in some common food items (79) in North America beginning in 1998 actually contributed to the decreasing rates of gastric cancer (80). However, in view of the lag time regarding an effect of folic acid and the lengthy induction time

required for gastric cancer, this issue could probably be addressed in only the next decade.

The observation of a potential role of folate in gastric carcinogenesis is also strengthened by our results of an increased risk of gastric cancer for *MTHFR* 677–homozygous subjects with low folate levels. This observation suggests that concomitant inadequate folate intake and impaired MTHFR activity might be important susceptibility factors for gastric cancer.

Despite the limitations of this analysis in terms of comparable folate data, which requires confirmation from large prospective studies based on blood folate measurement, our results are in keeping with the model proposed by Friso et al. (21). With folate deficiency, a decrease in downstream MTHFR products results in a lower global DNA methylation status. Recently, aberrant methylation of proto-oncogenes has been explored as both a mechanism and a marker of carcinoma progression (81), with some studies reporting an altered methylation pattern particularly for diffuse gastric cancer (82). Additionally, it was recently reported that significant global DNA hypomethylation occurs in *MTHFR* 677 *TT* subjects when compared with those with the wild-type genotype (19, 20), especially when plasma folate level is reduced (21). Taken together, these results suggest that the increased risk of gastric cancer associated with the homozygous *MTHFR* 677 variant might be referable to the subsequent impaired folate levels affecting DNA methylation status. Therefore, the observed association between the homozygous variant *MTHFR* genotype and gastric cancer might be counterbalanced to some extent by adequate folate intake.

Other genes involved in folate metabolism should be considered for a more comprehensive understanding of the exact role of the folate pathway in gastric cancer susceptibility. Given the controversial evidence from nutritional studies on the effect of fruit and vegetables on gastric cancer, there is a need for large prospective cohort studies based on repeated serologic dosage of folate levels and/or detailed and repeated nutritional data that would further clarify the role of folate in gastric carcinogenesis. Such studies would lay the foundation for evaluating the possible benefits of preventive nutritional interventions for individuals at risk of gastric cancer.

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