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-

Abbreviations used in this paper: CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; RR, relative risk.

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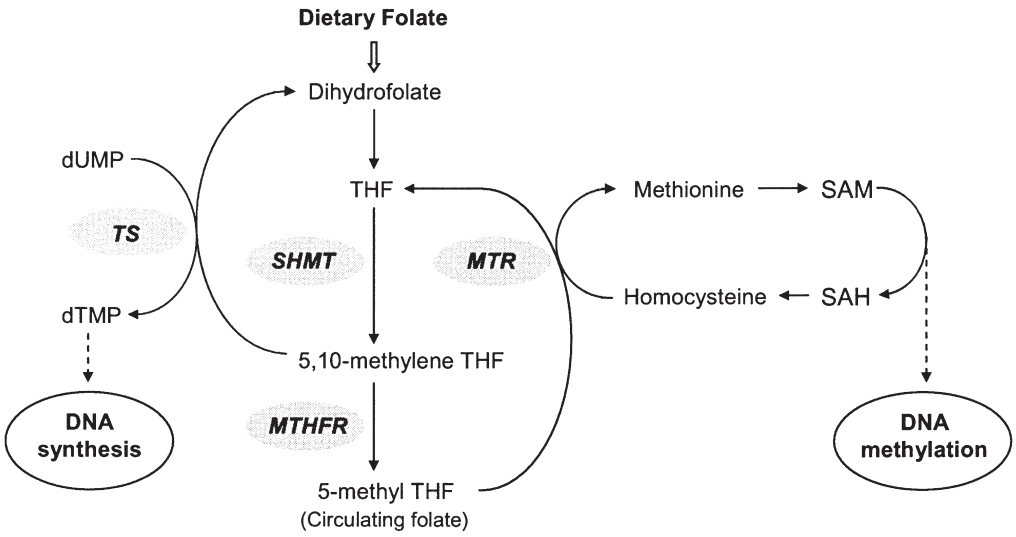

Figure 1. Simplified overview of folate metabolism involving DNA synthesis and DNA methylation. Enzymes: TS, thymidylate synthase; SHMT, serine hydroxymethyltransferase; MTHFR, 5,10-methylenetetrahydrofolate reductase; MTR, methionine synthase. Metabolites: THF, tetrahydrofolate; dTMP, deoxythymidine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