MTHFR Polymorphisms, Dietary Folate Intake, and Breast Cancer Risk: Results from the Shanghai Breast Cancer Study

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Abstract

Folate plays an important role in DNA methylation, synthesis, and repair; intake has been associated with breast cancer. The folate-metabolizing enzyme, methylenetetrahydrofolate reductase (MTHFR) is polymorphic at nucleotides 677 (C→T) and 1298 (A→C), resulting in allozymes with decreased activity. We evaluated these two common polymorphisms and their effects on the folate intake and breast cancer risk association in a population-based case-control study of 1144 breast cancer cases and 1236 controls using a PCR-RFLP-based assay. All subjects completed in-person interviews, which included a food frequency questionnaire. Unconditional logistic regression models were used to calculate odds ratios and their 95% confidence intervals, after adjusting for potential confounding factors. Cases and controls were similar in the distribution of *MTHFR* polymorphisms at codons 677 (41.4% cases and 41.8% controls carried the *T* allele) and 1298 (17.6% cases and 17.5% controls carried the *C* allele). An inverse association of breast cancer risk with folate intake was observed in all genotype groups, particularly among subjects with the *677TT* genotype. Compared with those with the *677CC* genotype and high folate, the adjusted odds ratios (95% confidence intervals) associated with low folate intake were 1.94 (1.15–3.26), 2.17 (1.34–3.51), and 2.51 (1.37–4.60) for subjects who had *CC*, *CT*, and *TT* genotypes (*p* for interaction, 0.05). No modifying effect of A1298C genotypes on the association of folate intake with breast cancer risk was observed. Results of this study suggest that the *MTHFR C677T* polymorphisms may modify the association between dietary folate intake and breast cancer risk.

Introduction

Folate is involved in DNA methylation, synthesis, and repair. Low intake of folate may increase risk for several cancers, including breast cancer (1, 2). The enzyme methylenetetrahydrofolate reductase (MTHFR) irreversibly catalyzes 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the donor for the remethylation of homocysteine to methionine, the precursor for the universal methyl donor, *S*-adenosylmethionine (3, 4). Folate that is not converted through this pathway can be used for purine synthesis or the conversion of uracil to thymine, which is used for DNA synthesis and repair (5).

Two common polymorphisms in the *MTHFR* gene have been characterized (6, 7). The 677C → T polymorphism codes for an alanine to valine substitution in the N-terminal catalytic domain and results in an allozyme with ~65% and ~30% of the wild-type homozygote activity for heterozygotes and homozygotes of the variant allele, respectively (6, 8). The A→ C polymorphism at nucleotide 1298 codes for an alanine to glutamine substitution in the C-terminal regulatory domain (7). Individuals homozygous for the *1298C* allele have approximately the same enzyme activity as those heterozygous for the *677T* allele (7, 8).

The C677T polymorphism has been examined in relation to several cancers (2, 9). In most studies of colorectal neoplasms, the *MTHFR 677TT* genotype has been associated with an overall reduction in risk, reduced risk among those with higher intakes of folate (10–13), or increased risk among those with lower folate intakes (13–15). *MTHFR* has not been as well studied in relation to breast cancer risk. Only three small studies have evaluated the association between *MTHFR* genotype and breast cancer (16–18). The results from these studies have been inconsistent. Only one study assessed both the *C677T* and *A1298C* polymorphisms and their possible joint effect with folate intake (18). However, in that study, only 60 cases were included. We reported recently that folate intake was inversely associated with breast cancer risk in a large population-based, case-control study among Chinese women in Shanghai (19). In an extension of these results, we investigated whether this association may be modified by *MTHFR* genotypes.

Materials and Methods

The Shanghai Breast Cancer Study is a population-based, case-control study conducted in urban Shanghai, China during 1996–1998. This study was approved by the committees for the use of human subjects in all collaborating institutions. Detailed study methods have been published previously (20).

Subjects. All incident breast cancer cases newly diagnosed during the study period and meeting the eligibility criteria were identified through a rapid case-ascertainment system supple-

Received 8/8/03; revised 9/15/03; accepted 9/26/03.

Grant support: This research was supported by United States Public Health Service Grant RO1CA64277 from the National Cancer Institute. Dr. Shrubsole is supported, in part, by DAMD17-02-1-0606 from the United States Army Medical Research and Materiel Command.

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mented by the Shanghai Cancer Registry and were approached for participation in the study. Eligibility criteria for the study were as follows: 25–64 years of age, resident of urban Shanghai, no previous history of any cancer, and alive at the time of interview. In all, 1602 eligible cases were identified, of whom 1459 (91.1%) completed in-person interviews. The median interval from cancer diagnosis to the in-person interview was 64 days. With the exception of a breast cancer diagnosis, controls had inclusion criteria identical to those of the cases and were frequency matched on age (5 years intervals) to the expected age distribution of the cases. In all, 1724 eligible controls were randomly selected from the Shanghai Resident Registry. Of these, 1556 (90.3%) completed in-person interviews.

Data and Biological Sample Collection. All subjects completed an in-person interview that used a structured questionnaire and incorporated anthropometric measurements. Dietary intakes were assessed using a 76-item food frequency questionnaire (FFQ). In a recent validation study of the FFQ among 200 women, we found that the FFQ captured >86% of food intake in Shanghai (21). Each subject was asked about the frequency that a specific food was eaten (daily, weekly, monthly, yearly, or never), followed by a question on the amount typically eaten. Dietary intakes of total folate and folate cofactors were derived from the FFQ by summing the product of the micronutrient content of each food item, usual portion eaten, and frequency of consumption. Because of the lack of folate data in the Chinese food composition database, an identical (82%) or equivalent (17%) item from the United States Department of Agriculture food composition database was used to determine micronutrient level (19). To assess the comparability of the Chinese and United States Department of Agriculture food composition databases, we evaluated the correlation of three other water-soluble vitamins (vitamin C, riboflavin, and niacin) and found excellent correlation; all Pearson correlation coefficients were $r \geq 0.91$ or higher, providing ancillary support for the validity of derived folate data in this study. In a validation study of the FFQ among 200 women, the FFQ was administered twice in a year and Pearson correlation for folate was $r = 0.36$. Blood samples were collected from 1193 (82%) cases and 1310 (84%) controls and used in this study for genotyping assays.

Laboratory Methods. Genomic DNA was extracted from blood samples with the Puregene DNA isolation Kit (Gentra Systems, Minneapolis, MN) following the protocol of the manufacturer. Genotyping for the *MTHFR C677T* and *A1298C* polymorphisms were performed using PCR-RFLP methods reported by Frosst *et al.* (6) and Weisberg *et al.* (7), with minor modifications. The primers for *C677T* analysis were 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ (exonic) and 5′-AGGACGGTGCGGTGAGAGTG-3′ (intronic). The primers for *A1298C* analysis were 5′-GGGAGGAGCTGACCAGTG-CAG-3′ and 5′-GGGGTCAGGCCAGGGGCAG-3′. The PCR reactions were performed in a Biometra TGradient Thermocycler. Each 20 μl of PCR mixture contained 10 ng DNA, 1× PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH 9.0)], 1.5 mM $MgCl_2$, 0.2 mM each of deoxynucleoside triphosphate, 0.5 mM of each primer, and 1 unit of *Taq*DNA polymerase. The reaction mixture was initially denatured at 94°C for 3 min. For *C677T* polymorphisms, PCR was performed in 30 cycles of 94°C for 45 s, 65°C for 45 s, and 72°C for 45 s. For A1298C polymorphisms, PCR was performed in 35 cycles of 94°C for 45 s, 65°C for 45 s, and 72°C for 45 s. The PCR was completed by a final extension cycle at 72°C for 7 min.

For *C677T* polymorphisms, each PCR product (10 μl) was digested with 10 units of *Hinf* I at 37°C for 3 h. The DNA fragments were then separated using 3% agarose gel and detected by ethidium bromide staining. The C→T substitution at nucleotide 667 creates a *Hinf* I digestion site. The PCR product (198 bp) with the *T* allele was digested to two fragments (175 bp and 23 bp), whereas the PCR product with wild-type C allele cannot be cut by *Hinf* I. For *A1298C* polymorphisms, each PCR product (10 μl) was digested with 5 units of *Fnu4H* I at 37°C for 3 h, followed by 3% agarose gel electrophoresis and ethidium bromide staining. The A→C substitution at nucleotide 1298 creates a *Fnu4H* I site. The PCR product (138 bp) with *C* allele was digested to two fragments (119 bp and 19 bp), whereas the PCR product with wild-type A allele cannot be cut by *Fnu4H* I.

Quality-control samples were included in various batches of samples assayed for the polymorphisms. The consistency rate was 98.5% in 119 quality-control samples that were repeated in the genotyping assays with their identities unknown to laboratory staff. Excluding a few subjects for whom sufficient DNA was not available or for whom the genotyping assay failed, genotyping data were obtained from 1112 cases and 1160 controls for *C677T* and 1121 cases and 1208 controls for *A1298C* polymorphisms. Because few women in the study consumed alcohol, a factor that may increase folate requirements, and because the data on the folate content of vitamins were not available, all analyses involving folate or its cofactors were limited to the cases (92.0%) and controls (91.1%) who were known not to consume alcohol regularly and not to take vitamin supplements.

Data Analysis. Odds ratios (ORs) were used to measure the association of breast cancer risk with *MTHFR* genotype. Unconditional logistic regression models were used to obtain maximum likelihood estimates of the ORs and their 95% confidence intervals (CIs), after adjusting for potential confounding variables. Risk factors previously identified as having an independent association with breast cancer were controlled in all models. These included age, personal history of fibroadenoma, age at first live birth, physical activity, waist-to-hip ratio, and daily meat intake. Age was included as a continuous variable throughout, and categorical variables were treated as indicator variables in the model. Quartile and tertile distributions of dietary intakes among controls were used to categorize all dietary intake variables. In the analyses including dietary factors, energy adjustment was performed using the standard multivariate method (22). Tests for trend were performed by entering categorical variables as continuous. Stratified analyses were used to evaluate the potential modifying effect of age, menopausal status, and folate and folate cofactor intakes on breast cancer risk associated with *MTHFR* genotypes and of *MTHFR* genotypes on breast cancer risk associated with folate intake. Tests for multiplicative interaction were done by including multiplicative variables in the logistic model and performing the likelihood ratio test. All statistical tests were based on two-sided probabilities using SAS, Version 8.2 (SAS Institute, Inc., Cary, NC).

Results

Comparisons between cases and controls on select demographic factors, established risk factors, and dietary factors are presented in Table 1. Cases were, in general, more highly educated, more likely to have a history of fibroadenoma, younger at menarche, older at first live birth and menopause, less likely to be physically active, and more likely to have a higher body mass index and waist-to-hip ratio than controls.

Table 1 Comparison of cases and controls by selected descriptive characteristics, Shanghai Breast Cancer Study, 1996–1998

Subject characteristics	Cases (n = 1144)	Controls (n = 1236)	P^a
Age, yr (mean ± SD)	46.4 ± 9.9	46.7 ± 8.8	0.42
Education, %			
No formal education	3.8	6.0	
Elementary school	8.5	8.6	
Middle or high school	75.8	75.3	
College or above	12.0	10.1	<0.05
Breast cancer in first-degree relative, %	3.4	2.4	0.15
Ever had breast fibroadenoma, %	9.7	5.2	<0.01
Age at menarche (yr)	14.5 ± 1.6	14.7 ± 1.7	<0.01
Ever had a live birth, %	94.9	95.9	0.27
Number of live births, mean ± SD	1.5 ± 0.8	1.5 ± 0.9	0.19
Age at first live birth, yr (mean ± SD)	26.8 ± 4.1	26.2 ± 3.8	<0.01
Postmenopausal, %	33.3	36.3	0.13
Age at menopause, yr (mean ± SD)	48.2 ± 4.6	47.4 ± 5.0	0.03
Physically active past 10 yr, %	19.3	25.8	<0.01
Body mass index, kg/m^2 (mean ± SD)	23.6 ± 3.4	23.2 ± 3.4	0.02
Waist-to-hip ratio, mean ± SD	0.81 ± 0.06	0.80 ± 0.06	<0.01
Daily animal food intake, g (mean ± SD)	90.4 ± 61.8	79.4 ± 50.1	<0.01
Daily plant food intake, g (mean ± SD)	501 ± 275	496 ± 278	0.73
Daily folate intake, μg (mean ± SD)	287 ± 141	303 ± 179	0.02
Daily methionine intake, g (mean ± SD)	1.72 ± 0.60	1.65 ± 0.56	<0.01
Daily vitamin B_{12} intake, μg (mean ± SD)	4.77 ± 4.11	4.69 ± 4.20	0.66
Daily vitamin B_6 intake, mg (mean ± SD)	1.83 ± 0.60	1.77 ± 0.57	0.03
Daily energy intake, kcal (mean ± SD)	1875 ± 467	1852 ± 459	0.23

[a] For χ^2 test (categorical variables) or t test (continuous variables).

Cases also had higher average daily intakes of animal foods, methionine, and vitamin B_6 and lower average daily intake of folate than controls.

The frequencies of *MTHFR* alleles and genotypes by case-control status and the association between *MTHFR* genotypes and breast cancer risk are presented in Table 2. The frequencies of the *677T* and *1298C* alleles were 0.41 and 0.18, respectively, among the controls. These were virtually identical to the frequency among the cases. Among the controls, the distributions of the *MTHFR* genotypes did not differ from the predicted distribution under Hardy-Weinberg equilibrium (P = 0.44 for the *C677T* polymorphisms and P = 0.58 for the *A1298C* polymorphisms). Risk of breast cancer did not differ statistically for the *C677T* or *A1298C* genotypes or for their combination. Similar associations were observed in analyses stratified by age and menopausal status (data not shown in table).

The joint association of *MTHFR* genotype and dietary folate intake with breast cancer risk is presented in Table 3. Low intake of folate was associated with an increased risk of breast cancer among all genotypes, particularly subjects with the *TT* genotype (OR = 2.51; 95% CI: 1.37–4.60) There was a significant multiplicative interaction between folate intake and *C677T* polymorphism in relation to breast cancer risk (P = 0.05). Elevated ORs were observed to be associated with folate

Table 2 *MTHFR* genotype frequencies and adjusted odds ratios (ORs) for breast cancer among Chinese women, Shanghai Breast Cancer Study, 1996–1998

Genotype[a]	Cases, n (%)	Controls, n (%)	Age-adjusted OR (95% confidence interval)	Multi-adjusted OR (95% confidence interval)
C677T				
CC	374 (33.6)	387 (33.4)	1.00 (reference)	1.00 (reference)
CT	555 (49.9)	577 (49.7)	1.00 (0.83–1.20)	1.01 (0.84–1.22)
TT	183 (16.5)	196 (16.9)	0.97 (0.76–1.24)	0.97 (0.76–1.25)
A1298C				
AA	768 (68.5)	824 (68.2)	1.00 (reference)	1.00 (reference)
AC	311 (27.7)	344 (28.5)	0.97 (0.81–1.16)	0.96 (0.80–1.16)
CC	42 (3.8)	40 (3.3)	1.13 (0.72–1.75)	1.14 (0.73–1.79)
Combined				
A1298C-AA				
C677T-CC	196 (18.0)	180 (15.9)	1.00 (reference)	1.00 (reference)
C677T-CT	375 (34.4)	410 (36.2)	0.84 (0.66–1.07)	0.85 (0.66–1.09)
C677T-TT	179 (16.4)	184 (16.3)	0.89 (0.67–1.19)	0.89 (0.67–1.20)
A1298C-AC/CC				
C677T-CC	171 (15.7)	203 (17.9)	0.77 (0.58–1.03)	0.77 (0.57–1.02)
C677T-CT/TT	168 (15.4)	155 (13.7)	0.99 (0.74–1.34)	1.01 (0.75–1.36)

[a] The frequencies of the *677T* allele were 41.4% in cases and 41.8% in controls (P = 0.81) and the frequencies of the *1298C* allele were 17.6% in cases and 17.5% in controls (P = 0.95).
[b] All ORs are adjusted for age, waist-to-hip ratio, age at first live birth, physical activity, menopausal status, and total meat intake.

Table 3 Joint association of *MTHFR* genotype and folate intake with breast cancer risk among Chinese women, Shanghai Breast Cancer Study, 1996–1998

Genotype	Daily Folate Intake[a]								*P* for trend
	Q_4 (High)		Q_3		Q_2		Q_1		
	Cases/ controls	Adjusted OR (95% CI)[a]	Cases/ controls	Adjusted OR (95% CI)[a]	Cases/ controls	Adjusted OR (95% CI)[a]	Cases/ controls	Adjusted OR (95% CI)[a]	
C677T[b]									
CC	69/88	1.00 (reference)	96/86	1.76 (1.12–2.77)	90/91	1.75 (1.08–2.83)	81/86	1.94 (1.15–3.26)	0.02
CT	103/117	1.16 (0.76–1.77)	135/142	1.50 (0.99–2.29)	133/137	1.73 (1.11–2.70)	145/136	2.17 (1.34–3.51)	0.06
TT	29/53	0.70 (0.40–1.23)	47/44	1.66 (0.97–2.85)	49/39	2.17 (1.23–3.81)	47/38	2.51 (1.37–4.60)	0.003
P for trend		0.51		0.71		0.49		0.31	
A1298C[c]									
AA	140/192	1.00 (reference)	184/192	1.59 (1.15–2.20)	195/185	1.94 (1.36–2.76)	194/186	2.18 (1.46–3.25)	0.0006
AC/CC	63/81	1.05 (0.70–1.57)	92/84	1.80 (1.22–2.67)	79/91	1.59 (1.04–2.44)	85/92	1.94 (1.23–3.05)	0.18
A1298C-AA[d]									
C677T-CC	42/45	1.00 (reference)	43/43	1.26 (0.68–2.34)	48/38	1.81 (0.95–3.44)	43/40	1.85 (0.94–3.67)	0.07
C677T-CT	67/81	0.86 (0.49–1.48)	94/101	1.18 (0.69–2.01)	90/98	1.35 (0.77–2.36)	100/98	1.74 (0.96–3.16)	0.08
C677T-TT	29/52	0.56 (0.30–1.05)	45/41	1.40 (0.75–2.60)	48/36	1.87 (0.98–3.57)	46/36	2.16 (1.09–4.28)	0.002
P for trend		0.13		0.63		0.81		0.70	

[a] All ORs are adjusted for age, waist-to-hip ratio, age at first live birth, physical activity, and total energy, meat, vitamin B_{12}, vitamin B_6, and methionine intakes. OR, odds ratio; CI, confidence interval.
[b] *P* for interaction, 0.048.
[c] *P* for interaction, 0.71.
[d] *P* for interaction, 0.06.

intake regardless of *A1298C* genotype, although the trend was statistically significant in only the *AA* group. To examine further the *C677T* association, analyses were restricted to *1298AA* individuals because of a small sample size for the *AC* and CC genotypes. Again, low intake of folate was associated with increased risk for all genotypes, and the increased risk was greatest among those with *677TT* genotype (OR = 2.16, 95% CI: 1.09–4.28, p for trend = 0.002, p for interaction = 0.06). There was a suggestive reduced risk associated with the *TT* genotype among those with high folate intake, although the OR was not statistically significant.

The joint associations of *MTHFR C677T* genotypes and folate intake with breast cancer risk are presented in Table 4, after stratifying by folate cofactor intake. With the exception of the high vitamin B_6 stratum, low folate intake was associated with an elevated risk of breast cancer, and the association appeared stronger among subjects with the *TT* genotypes. None of the tests for multiplicative interactions, however, was statistically significant, perhaps because of a small sample size in these stratified analyses.

Discussion

We found in this case-control study that there was no statistically significant association between the risk of breast cancer and *MTHFR C677T* or *A1298C* genotypes. However, *MTHFR C677T* genotype was a statistically significant effect modifier of the association between folate intake and breast cancer risk. Among those with the *677TT* genotype, low folate intake was associated with a more substantial increased risk than those with other genotypes. These are novel findings consistent with the possible role of MTHFR and folate in the etiology of cancer.

MTHFR polymorphisms have not been adequately investigated in relation to breast cancer risk. Only three previous small studies have examined *MTHFR* polymorphisms and breast cancer risk (16–18). In the first, a study among Jewish women, *MTHFR C677T* genotype was determined in 491 women with sporadic (n = 355) or hereditary (n = 136) breast and/or ovarian cancer and in 69 asymptomatic BRCA1/2 mutation carriers (16). The prevalence of the *T* allele was not significantly different between sporadic cases and the asymptomatic carriers, women diagnosed at a young and older age, and *BRCA1/2* carriers with and without cancer. The prevalence of the *T* allele was more frequent among women with bilateral breast cancer or with both breast and ovarian cancers than among women with only unilateral breast cancer. In the second study, a hospital-based, case-control study among postmenopausal Caucasian women (149 cases and 171 controls), it was reported that the *MTHFR 677T* (*val*) allele was more prevalent in cases than controls (17), which is in contrast to the results from the third case-control study conducted in the United Kingdom (62 cases, 66 controls), the only previous study that reported risk of breast cancer associated with both the *C677T* and *A1298C* polymorphisms (18). The British study reported breast cancer risk was reduced among those homozygous for the *677T* allele (OR = 0.39; 95% CI: 0.12–1.24) or *1298C* allele (OR = 0.24; 95% CI: 0.06–0.97). However, no modifying effect of the *MTHFR C677T* genotype was noted on the association between folate intake and breast cancer risk. There was some evidence of a joint association of folate and the *A1298C* genotype, but the sample size was not large enough to examine this association.

We did not find an overall reduced risk of breast cancer associated with *MTHFR 677TT* or *1298CC* genotypes, which is not consistent with the British study of breast cancer (17) and some of the previous studies for other cancers. Two of three case-control studies of colorectal cancer and the *MTHFR C677T* polymorphism observed an overall reduction in risk associated with the *TT* genotype (10, 11), as did studies of oral cancer (23) and adult acute lymphocytic leukemia (24). We also found a similar weak association among women with high intake of folate and folate cofactors. However, other studies of colorectal cancer (12), colorectal adenoma (13, 14, 25), gastric cancer (9), lung cancer (26), and acute myeloid leukemia (24) found no association or an increased risk of cancer for individuals with the *TT* genotype. Our observation for a stronger inverse association of folate intake and breast cancer risk

Table 4 Joint association of folate and *MTHFR C677T* genotype with breast cancer risk stratified by cofactor intake among Chinese women, Shanghai Breast Cancer Study, 1996–1998

Folate cofactor intake	Folate intake, odds ratio (95% confidence interval)[a]			*P* for trend	*P* for interaction
	T_3 (high)	T_2	T_1		
Vitamin B_{12}[b]					
Low					
CC	1.00 (reference)	1.71 (0.74–3.94)	1.77 (0.73–4.34)	0.25	
CT	1.62 (0.70–3.73)	1.36 (0.61–3.05)	1.74 (0.75–4.06)	0.79	
TT	0.83 (0.28–2.45)	0.86 (0.31–2.40)	2.36 (0.90–6.16)	0.11	0.56
Medium					
CC	1.00 (reference)	1.88 (0.97–3.67)	2.11 (0.97–4.59)	0.11	
CT	1.01 (0.56–1.81)	1.69 (0.92–3.11)	1.81 (0.89–3.68)	0.20	
TT	0.84 (0.38–1.87)	1.77 (0.83–3.83)	3.00 (1.18–7.61)	0.03	0.45
High					
CC	1.00 (reference)	0.96 (0.51–1.81)	1.10 (0.58–1.73)	0.39	
CT	1.00 (0.58–1.73)	1.22 (0.67–2.22)	1.26 (0.65–2.48)	0.88	
TT	0.61 (0.30–1.23)	2.33 (1.03–5.26)	1.25 (0.46–3.37)	0.12	0.02
Vitamin B_6[c]					
Low					
CC	1.00 (reference)	0.77 (0.16–3.86)	1.24 (0.26–5.91)	0.30	
CT	0.70 (0.09–5.37)	1.11 (0.23–5.41)	1.43 (0.31–6.76)	0.26	
TT	2.19 (0.09–52.31)	0.74 (0.13–4.27)	1.89 (0.38–9.39)	0.06	0.70
Medium					
CC	1.00 (reference)	1.45 (0.68–3.07)	1.67 (0.71–3.94)	0.24	
CT	0.84 (0.37–1.91)	1.31 (0.63–2.74)	1.18 (0.54–2.59)	0.57	
TT	0.66 (0.22–1.97)	1.37 (0.60–3.17)	1.74 (0.65–4.71)	0.02	0.70
High					
CC	1.00 (reference)	1.61 (0.88–2.96)	1.28 (0.29–5.59)	0.04	
CT	1.27 (0.84–1.92)	1.26 (0.75–2.11)	1.01 (0.33–3.04)	0.44	
TT	0.71 (0.41–1.22)	2.50 (1.11–5.63)	0.68 (0.12–3.73)	0.23	0.13
Methionine[d]					
Low					
CC	1.00 (reference)	2.17 (0.50–9.38)	1.87 (0.43–8.18)	0.88	
CT	2.82 (0.60–13.29)	2.00 (0.47–8.51)	2.06 (0.48–8.88)	0.98	
TT	3.24 (0.48–22.00)	1.56 (0.33–7.45)	2.89 (0.64–13.04)	0.41	0.98
Medium					
CC	1.00 (reference)	1.21 (0.61–2.40)	1.99 (0.91–4.37)	0.09	
CT	0.99 (0.48–2.05)	1.47 (0.76–2.84)	1.75 (0.85–3.64)	0.30	
TT	0.96 (0.37–2.48)	1.70 (0.77–3.73)	2.05 (0.74–5.67)	0.09	0.91
High					
CC	1.00 (reference)	1.71 (0.90–3.23)	1.57 (0.51–4.83)	0.16	
CT	1.08 (0.69–1.66)	1.31 (0.76–2.24)	1.16 (0.52–2.59)	0.82	
TT	0.59 (0.33–1.05)	1.84 (0.83–4.08)	1.53 (0.50–4.66)	0.03	0.19

[a] All odds ratios are adjusted for age, waist-to-hip ratio, age at first live birth, physical activity, menopausal status, and total energy, meat, and intake of the other two cofactors.
[b] *P* for three-way interaction, 0.47.
[c] *P* for three-way interaction, 0.24.
[d] *P* for three-way interaction.

among women with the TT genotype is supported by the majority of studies examining a similar association for other cancers (10–12, 14, 15, 23), and is consistent with the role of folate in breast carcinogenesis. We, and others, have previously found a decreased risk of breast cancer among those with high intake level of folate (19, 27–33). Low folate intake is associated with an increased misincorporation of uracil and chromosome breaks (34, 35) and aberrant DNA methylation (35, 36). The critical factor in breast carcinogenesis may be an appropriate balance between the availability of for DNA methylation and 5,10-methylene-*S*-adenosylmethioninetetrahydrofolate for DNA synthesis. It is plausible that individuals with the 677TT genotype are particularly susceptible to the carcinogenic consequences of folate insufficiency. This genotype, in the presence of low folate, is associated with higher levels of homocysteine, lower levels of methylated folates and, therefore, reductions in genomic DNA methylation (37, 38). Our finding for a positive association of *C677T* genotype with low folate intake (OR = 2.51, 95% CI: 1.37–4.60) appears to support this notion. Conversely, in folate-replete conditions, the availability of 5,10-methylenetetrahydrofolate for nucleotide synthesis may be adequate or increased for these individuals because of the genetically determined decreased activity of MTHFR. This could explain the lower risk of this genotype among those with high folate levels in this (OR = 0.70, 95% CI: 0.40–1.23 for *677TT*) and other studies (10, 12, 14, 15). Therefore, the effect of *MTHFR* on breast cancer risk in a particular population may depend on the intake level of folate in that population. With increased folic acid fortification in the United States population, the general intake of folate may be higher than that from the Chinese, whose folate intake is primarily obtained from unfortified diets. This may explain, in part, the overall absence

of association of *MTHFR* genotype with breast cancer risk in our study.

The relationship between folate metabolism and carcinogenesis is likely to be a complex biological sum of genetic and nutritional differences. In our study, the association of folate and breast cancer risk was similar for all genotypes when intakes of vitamin B_{12}, B_6, or methionine were low. Vitamin B_{12}, vitamin B_6, and methionine all have important roles in one-carbon metabolism; vitamin B_{12} is a cofactor for the transfer of the methyl group from folate to methionine, vitamin B_6 is a coenzyme for the formation of 5,10-methylene-5,10-methylenetetrahydrofolate and the catabolism of homocysteine, and methionine is the precursor for *S*-adenosylmethionine. It is possible that below a certain intake threshold of vitamin B_{12} and methionine, the effect of *MTHFR C677T* genotype or folate intake is reduced or negated and that once this threshold is surpassed, both folate and *MTHFR* genotype have a greater impact on breast cancer risk. The inverse association with breast cancer risk among those with a high vitamin B_6 intake was unexpected and cannot be readily explained by the above rationale. This finding needs to be re-evaluated in future studies.

As with any case-control study, the potential for selection and recall biases must be considered. However, selection bias is unlikely to be a major issue in this study; both cases (91%) and controls (90%) had very high participation rates. Not only did this study have a high participation rate, it also had a high blood collection rate (>80%). Although it is possible that cases and controls may have had differentially recalled intakes of foods that contributed to the nutrients in this study, fruit and vegetable intake, the major contributors to folate, methionine, and vitamin B_6 intakes, did not significantly differ between cases and controls. Approximately 50% of cases were interviewed within 15 days of diagnosis and the majority (80%) were interviewed within 4 months, thus reducing potential recall bias attributable to dietary change related to a diagnosis of cancer. In addition, recall of diet would unlikely be related to *MTHFR* genotype and, therefore, could not account for the associations we observed in this study. Additionally, misclassification in the assessment of folate intake may have occurred from using the Unites States Department of Agriculture food composition database. However, any such misclassification would be nondifferential between cases and controls and would usually result in a bias toward the null. Confounding by other factors is always a concern in epidemiological studies. We observed little confounding when we carefully adjusted for known risk factors. Although it is possible that residual confounding may still exist, for example, from dietary factors not considered in this study, additional factors are not likely to explain the strength of the observed associations. Other strengths of our study include the population-based design, the estimation of folate intake in a population of nonusers of alcohol and vitamin supplements, and the large sample size that facilitated examination of modifying effects.

In summary, we found that, although there was no overall relationship between *MTHFR* genotype and breast cancer risk, women with low intake of folate and who are homozygous for the *MTHFR 677T* polymorphism may be at substantially increased risk for breast cancer. Our data also suggest this association may be further modified by vitamin B_{12}, vitamin B_6, and methionine intake. This study adds support to the literature that one-carbon metabolism and *MTHFR* polymorphisms have a role in carcinogenesis and may be important in breast carcinogenesis.

References

1. Kim, Y. I. Folate and carcinogenesis: evidence, mechanisms, and implications. J. Nutr. Biochem., *10:* 66–88, 1999.

2. Mason, J. B., and Choi, S. W. Folate and carcinogenesis: developing a unifying hypothesis. Adv. Enzyme Regul., *40:* 127–141, 2000.

3. Matthews, R. G., Sheppard, C., and Goulding, C. Methylenetetrahydrofolate reductase and methionine synthase: biochemistry and molecular biology. Eur. J. Pediatr., *157* (Suppl. 2)*:* S54–S59, 1998.

4. Fodinger, M., Horl, W. H., and Sunder-Plassmann, G. Molecular biology of 5,10-methylenetetrahydrofolate reductase. J. Nephrol., *13:* 20–33, 2000.

5. Bailey, L. B., and Gregory, J. F., III. Folate metabolism and requirements. J. Nutr., *129:* 779–782, 1999.

6. Frosst, P., Blom, H. J., Milos, P., Goyette, P., Sheppard, C. A., Matthews, R. G., Boers, G. J. H., den Heijer, M., Kluijtmans, L. A. J., van der Heuvel, L. P., and Rozen, R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat. Genet., *10:* 111–113, 1995.

7. Weisberg, I., Tran, P., Christensen, B., Sibani, S., and Rozen, R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol. Genet. Metab., *64:* 169–172, 1998.

8. Weisberg, I. S., Jacques, P. F., Selhub, J., Bostom, A. G., Chen, Z. T., Ellison, R. C., Eckfeldt, J. H., and Rozen, R. The 129XA -> C polymorphism in methylenetetrahydrofolate reductase (MTHFR): *in vitro* expression and association with homocysteine. Atherosclerosis, *156:* 409–415, 2001.

9. Shen, H., Xu, Y., Zheng, Y., Qian, Y., Yu, R., Qin, Y., Wang, X., Spitz, M. R., and Wei, Q. Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of gastric cancer in a Chinese population: a case-control study. Int. J. Cancer, *95:* 332–336, 2001.

10. Chen, J., Giovannucci, E., Kelsey, K., Rimm, E. B., Stampfer, M. J., Colditz, G. A., Spiegelman, D., Willett, W. C., and Hunter, D. J. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res., *56:* 4862–4864, 1996.

11. Ma, J., Stampfer, M. J., Giovannucci, E., Artigas, C., Hunter, D. J., Fuchs, C., Willett, W. C., Selhub, J., Hennekens, C. H., and Rozen, R. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res., *57:* 1098–1102, 1997.

12. Slattery, M. L., Potter, J. D., Samowitz, W., Schaffer, D., and Leppert, M. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. Cancer Epidemiol. Biomarkers Prev., *8:* 513–518, 1999.

13. Chen, J., Giovannucci, E., Hankinson, S. E., Ma, J., Willett, W. C., Spiegelman, D., Kelsey, K. T., and Hunter, D. J. A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms, and risk of colorectal adenoma. Carcinogenesis (Lond.), *19:* 2129–2132, 1998.

14. Ulrich, C. M., Kampman, E., Bigler, J., Schwartz, S. M., Chen, C., Bostick, R., Fosdick, Beresford, A. A., Yasui, Y., and Potter, J. D. Colorectal adenomas and the C677T MTHFR polymorphism: evidence for gene-environment interaction? Cancer Epidemiol. Biomarkers Prev., *8:* 659–668, 1999.

15. Levine, A. J., Siegmund, K. D., Ervin, C. M., Diep, A., Lee, E. R., Frankl, H. D., and Haile, R. W. The methylenettrahydrofolate reductase 677C–>T polymorphism and distal colorectal adenoma risk. Cancer Epidemiol. Biomarkers Prev., *9:* 657–663, 2000.

16. Gershoni-Baruch, R., Dagan, E., Israeli, D., Kasinetz, L., Kadouri, E., and Friedman, E. Association of the C677T polymorphism in the *MTHFR* gene with breast and/or ovarian cancer risk in Jewish women. Eur. J. Cancer, *36:* 2313–2316, 2000.

17. McGlynn, K. A., Wang, L., Patrick-Acevedo, N. Y., Strachan, S. D., and Kruger, W. D. Methylenetetrahydrofolate reductase, methionine synthase, folate, alcohol and breast cancer. The 91st Annual AACR Meeting, April 1–5, 2000, San Francisco, CA, p. 92.

18. Sharp, L., Little, J., Schofield, A. C., Pavlidou, E., Cotton, S. C., Miedzybrodzka, Z., Baird, J. O., Haites, N. E., Heys, S. D., and Grubb, D. A. Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). Cancer Lett., *181:* 65–71, 2002.

19. Shrubsole, M. J., Jin, F., Dai, Q., Shu, X. O., Potter, J. D., Hebert, J. R., Gao, Y. T., and Zheng, W. Dietary folate intake and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Res., *61:* 7136–7141, 2001.

20. Gao, Y. T., Shu, X. O., Dai, Q., Potter, J. D., Brinton, L. A., Wen, W., Sellers, T. A., Kushi, L. H., Ruan, Z., Bostick, R. M., Jin, F., and Zheng, W. Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai Breast Cancer Study. Int. J. Cancer, *87:* 295–300, 2000.

21. Shu, X. O., Yang, G., Jin, F., Liu, D., Kushi, L., Wen, W., Gao, Y. T., and Zheng, W. Validity and reproducibility of the food frequency questionnaire used in the Shanghai Women's Health Study. Eur. J. Clin. Nutr., *58:* 17–23, 2004.

22. Willett, W. C. Nutritional Epidemiology, Vol. 30, Ed. 2. New York, United States of America: Oxford University Press, pp. 291–292, 1998.

23. Weinstein, S. J., Gridley, G., Harty, L. C., Diehl, S. R., Brown, L. M., Winn, D. M., Bravo-Otero, E., and Hayes, R. B. Folate intake, serum homocysteine and methylenetetrahydrofolate reductase (MTHFR) C677T genotype are not associated with oral cancer risk in Puerto Rico. J. Nutr., *132:* 762–767, 2002.

24. Burbee, D. G., Forgacs, E., Zochbauer-Muller, S., Shivakumar, L., Fong, K., Gao, B., Randle, D., Kondo, M., Virmani, A., Bader, S., Sekido, Y., Latif, F., Milchgrub, S., Toyooka, S., Gazdar, A. F., Lerman, M. I., Zabarovsky, E., White, M., and Minna, J. D. Epigenetic inactivation of RASSF1A in lung and breast cancers and malignant phenotype suppression. J. Natl. Cancer Inst., *93:* 691–699, 2001.

25. Nomura, M., Watari, J., Yokota, K., Saitoh, Y., Obara, T., and Kohgo, Y. Morphogenesis of nonpolypoid colorectal adenomas and early carcinomas assessed by cell proliferation and apoptosis. Virchows Arch., *437:* 17–24, 2000.

26. Shen, H., Spitz, M. R., Wang, L. E., Hong, W. K., and Wei, Q. Polymorphisms of methylene-tetrahydrofolate reductase and risk of lung cancer: a case-control study. Cancer Epidemiol. Biomarkers Prev., *10:* 397–401, 2001.

27. Graham, S., Hellmann, R., Marshall, J., Freudenheim, J., Vena, J., Swanson, M., Zielezny, M., Nemoto, T., Stubbe, N., and Raimondo, T. Nutritional epidemiology of postmenopausal breast cancer in western New York. Am. J. Epidemiol., *134:* 552–566, 1991.

28. Freudenheim, J. L., Marshall, J. R., Vena, J. E., Laughlin, R., Brasure, J. R., Swanson, M. K., Nemoto, T., and Graham, S. Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients. J. Natl. Cancer Inst., *88:* 340–348, 1996.

29. Thorand, B., Kohlmeier, L., Simonsen, N., Croghan, C., and Thamm, M. Intake of fruits, vegetables, folic acid and related nutrients and risk of breast cancer in postmenopausal women. Public Health Nutr., *1:* 147–156, 1998.

30. Ronco, A., De Stefani, E., Boffetta, P., Deneo-Pellegrini, H., Mendilaharsu, M., and Leborgne, F. Vegetables, fruits, and related nutrients and risk of breast cancer: a case-control study in Uruguay. Nutr. Cancer, *35:* 111–119, 1999.

31. Zhang, S., Hunter, D. J., Hankinson, S. E., Giovannucci, E. L., Rosner, B. A., Colditz, G. A., Speizer, F. E., and Willett, W. C. A prospective study of folate intake and the risk of breast cancer. J. Am. Med. Assoc., *281:* 1632–1637, 1999.

32. Rohan, T. E., Jain, M. G., Howe, G. R., and Miller, A. B. Dietary folate consumption and breast cancer risk. J. Natl. Cancer Inst., *92:* 266–269, 2000.

33. Levi, F., Pasche, C., Lucchini, F., and La Vecchia, C. Dietary intake of selected micronutrients and breast-cancer risk. Int. J. Cancer, *91:* 260–263, 2001.

34. Blount, B. C., Mack, M. M., Wehr, C. M., MacGregor, J. T., Hiatt, R. A., Wang, G., Wickramasinghe, S. N., Everson, R. B., and Ames, B. N. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc. Natl. Acad. Sci. USA, *94:* 3290–3295, 1997.

35. Kim, Y. I., Pogribny, I. P., Basnakian, A. G., Miller, J. W., Selhub, J., and Mason, J. B. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the *p53* tumor suppressor gene. Am J. Clin. Nutr., *65:* 46–52, 1997.

36. Rampersaud, G. C., Kauwell, G. P., Hutson, A. D., Cerda, J. J., and Bailey, L. B. Genomic DNA methylation decreases in response to moderate folate depletion in elderly women. Am. J. Clin. Nutr., *72:* 998–1003, 2000.

37. Jacques, P. F., Bostom, A. G., Williams, R. R., Ellison, R. C., Eckfeldt, J. H., Rosenberg, I. H., Selhub, J., and Rozen, R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation, *93:* 7–9, 1996.

38. Friso, S., Choi, S. W., Girelli, D., Mason, J. B., Dolnikowski, G. G., Bagley, P. J., Olivieri, O., Jacques, P. F., Rosenberg, I. H., Corrocher, R., and Selhub, J. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc. Natl. Acad. Sci. USA, *99:* 5606–5611, 2002.