Dietary Folate Intake, *MTHFR* Genetic Polymorphisms, and the Risk of Endometrial Cancer among Chinese Women

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Abstract

Folate plays an important role in carcinogenesis. The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR), encoded by the *MTHFR* gene, is involved in this process. We investigated both the independent and joint effects of dietary folate and other methyl-related nutrients, as well as three polymorphisms of *MTHFR* (677C>T, 1298A>C, and 1793G>A), on endometrial cancer risk in a population-based case-control study. Between 1997 and 2003, 1,204 newly diagnosed endometrial cancer cases and 1,212 controls were recruited among women between the ages of 30 and 69 years in urban Shanghai, China. Information on dietary intake of folate and other methyl-related nutrients, including vitamin B2 (riboflavin), vitamin B6, vitamin B12, and methionine, was derived from a validated food frequency questionnaire. Genotyping was completed on 1,041 cases and 1,030 controls for *MTHFR* 677C>T (rs1801133), 1298A>C (rs1801131), and 1793 G>A (rs22749746). Haplotype estimation of the three single-nucleotide polymorphisms was performed using PHASE software. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to evaluate associations of nutrients, *MTHFR* genotypes, and haplotypes with endometrial cancer risk. A significant inverse association between dietary folate intake and endometrial cancer risk was observed among all subjects and non–B vitamin supplement users. The greatest reduction in endometrial cancer risk was observed among non-users of supplements in the highest quartile of dietary folate intake (OR, 0.5; 95% CI, 0.4-0.7) as compared with those in the lowest quartile. Dietary intake of folate cofactors (methionine, vitamin B2, vitamin B6, and vitamin B12) was not related to risk of endometrial cancer. No association was observed between endometrial cancer and the *MTHFR* 677C>T, 1298 A>C, and 1793G>A polymorphisms or derived haplotypes. Among non-users of supplements, however, the 1298C and 1793A alleles were associated with a lower risk of endometrial cancer among women with high dietary folate intake but related to a higher risk among those with low dietary folate intake ($P_{interaction}$ = 0.08 and 0.03, respectively). Further analysis showed that the lowest risk (OR, 0.6; 95% CI, 0.4-1.1) was among women with the 1298C allele and the highest intake of both folate and riboflavin ($P_{interaction}$ = 0.04). A similar association was observed for the 1793A allele ($P_{interaction}$ = 0.03). Our findings suggest that folate intake may decrease the risk of endometrial cancer and modify the effect of *MTHFR* polymorphisms on risk. (Cancer Epidemiol Biomarkers Prev 2007;16(2):281–7)

Introduction

Folate, a B vitamin, is the major source of dietary methyl groups (1). It plays an important role in DNA methylation, synthesis, and repair. Other B vitamins, such as vitamin B2 (riboflavin), vitamin B6, and vitamin B12, are key cofactors in the folate metabolism pathway. Methionine, the methyl donor for DNA methylation, is also involved in this pathway and is an alternate dietary source of methyl groups. Folate deficiency may cause uracil misincorporation and subsequent DNA instability (2), retarded DNA repair capacity for oxidative or alkylating damage (3), and global and proto-oncogenic DNA hypomethylation (4). All of these effects have been reported to be involved in carcinogenesis (3), and higher intake of folate has been associated with a decreased risk of cancers, including colorectal and breast cancers (5, 6). The relationship between folate and endometrial cancer, however, is inconsistent based on previous reports. Adequate folate intake has been associated with a substantially decreased risk (7, 8), a marginally reduced risk (9), or unrelated to endometrial cancer risk (10-12).

5,10-Methylenetetrahydrofolate reductase (MTHFR), a key enzyme in folate metabolism, carries out the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which in turn directs the folic acid pool toward remethylation of homocysteine to methionine (1). Several single-nucleotide polymorphisms in the *MTHFR* gene have been reported. The most commonly described variant, an alanine-to-valine substitution at codon 222 (677C>T, rs1801133), is correlated with enzyme thermolability and reduced enzyme activity (13). Located in the COOH-terminal regulatory domain of the *MTHFR* gene is another common polymorphism, A to C in the 1298th bp, which causes a substitution of codon 429 glutamate to alanine (rs1801131). *MTHFR 1298A>C* (Glu>Ala) may also lead to lower activity of the enzyme (14). Another nonsynonymous single-nucleotide polymorphism of the *MTHFR* gene, Arg[594]Gln (1793G>A, rs2274976), has also been identified, but its functional significance is not known. The frequency of the variant allele is much higher in Chinese populations (10.0%) than in Caucasian (5.8%) or Yoruba populations (3.3%);[4] thus, we chose to evaluate whether it plays a role in endometrial cancer risk among Chinese women.

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[4] http://www.hapmap.org

Polymorphisms 677C>T and 1298A>C have been evaluated in relation to several cancers including breast cancer (15, 16). Results on endometrial cancer, however, are very limited and mixed (9, 17). In the only two studies evaluating the association between the *MTHFR* genotype and endometrial cancer risk, one hospital-based case-control study observed an increased risk of endometrial cancer comparing *MTHFR 677 T* allele carriers to *CC* homozygotes (17), but another nested case-control study detected neither an overall association between *MTHFR* genotype and endometrial cancer nor an *MTHFR*-folate intake interaction (9). Both of these previous studies were small and conducted in Western populations in which vitamin supplement use, use of folic acid fortified foods, and consumption of alcohol are frequent, and thus, the ascertainment of folate intake and the subsequent evaluation of interactions are more difficult.

In this large population-based case-control study conducted among Chinese women in which a significant folate-gene interaction with breast cancer has been reported (15), we examined the independent and joint effects of folate intake, other methyl-related nutrients, and *MTHFR* genotypes on the risk of endometrial cancer.

Materials and Methods

Study Population. The Shanghai Endometrial Cancer Study is a population-based case-control study. Eligible cases, identified from the Shanghai Cancer Registry, included all permanent female residents of urban Shanghai, between the ages of 30 and 69 years, who were newly diagnosed with endometrial cancer between January 1997 and December 2003. Study approval was garnered by the relevant institutional review boards for human research in both China and the United States. Of the 1,454 eligible cases identified, 1,204 (82.8%) participated in the study, 135 (9.3%) refused to participate, 66 (4.5%) died before study enrollment, 35 (2.5%) could not be located or were absent during study period, and 14 (1.0%) could not be interviewed due to health or communication problems.

Controls were randomly selected from the Shanghai Resident Registry and were frequency matched to cases. The number of controls in each 5-year group was determined in advance according to the ages of incident endometrial cancer cases in 1996. Women who had undergone a hysterectomy, as identified during the survey, were not eligible (n = 36). A total of 1,629 controls were identified for possible participation, and 1,212 (74.4%) were enrolled in the study. Of the controls not enrolled in the study, 340 (20.9%) declined to be interviewed, 61 (3.7%) did not live in Shanghai during the study period, and 16 (1.1%) did not participate due to health or communication problems.

Questionnaire Data. In-person interviews were conducted with participants by retired medical professionals to gather information on demographics, dietary intake, lifestyle factors, disease history, family history of any cancer, menstrual and reproductive history, and hormone use. Anthropometric measurements, including weight, standing and sitting height, and waist and hip circumferences, were taken during the interview. Calculation of body mass index (BMI) and physical activity in metabolic equivalent tasks, as well as the definition of menopausal status and years of menstruation, has been described in detail in our previous reports (18, 19).

Dietary information was gathered using a validated quantitative food frequency questionnaire, which included 71 common foods and food groups (20). The questionnaire covers more than 85% of foods commonly consumed in Shanghai. Intake of fruits and vegetables was calculated by the frequency and amount of intake and weighted by the length of time that the food was available during 1 year. Nutrient values were derived from the Chinese (folate and riboflavin; ref. 21) and U.S. Department of Agriculture (vitamin B6, vitamin B12, and methionine) food composition tables. Information on supplemental vitamin use and recent dietary change was also obtained.

Biological Samples and Genotyping. Among the study participants, 860 cases and 861 controls provided a blood sample. A buccal cell sample was collected from an additional 285 cases and 281 controls. As such, a DNA sample was available from 1,145 cases and 1,142 controls. All samples were stored at −70°C.

Genomic DNA was extracted from buffy coat fractions or buccal cells using QIAamp DNA mini kit (Qiagen, Inc., Valencia CA) following the manufacturer's protocol. The allelic discrimination of the *MTHFR* gene polymorphisms rs1801133 (677C>T), rs1801131 (1298A>C), and rs2274976 (1793G>A) was assessed using TaqMan genotyping assays (assay ID: C_1202883_20 for rs1801133, C_850486_20 for rs1801131, and C_16183363_10 for rs2274976) on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). The final volume for each reaction was 5 μL, consisting of 2.5 μL TaqMan Universal PCR Master Mix (Applied Biosystems), 0.25 μL of primers/TaqMan probe mix, and 5 ng genomic DNA. The PCR profile consisted of an initial denaturation step at 95°C for 10 min and 40 cycles at 92°C for 15 sec and 60°C for 1 min. The fluorescence level was measured with the ABI PRISM 7900HT sequence detector (Applied Biosystems). Genotypes were determined by ABI SDS software. The laboratory staff was blinded to the identity of the subjects. Quality control samples were included in the genotyping assays. Each 384-well plate contained four water, eight CEPH 1347-02 DNA, eight blinded quality control DNA, and eight unblinded quality control DNA samples. When comparing genotypes from the study samples to the quality control samples, there was 100% agreement for all three polymorphic sites.

Genotyping data were obtained from 1,041 cases and 1,030 controls. Genotype frequencies were similar for both blood and buccal cell sources. The major reasons for incomplete genotyping were insufficient DNA used for the assay and unsuccessful PCR amplification.

Statistical Analysis. Statistical analysis was conducted using SAS statistical software 9.1 (SAS Institute, Inc., Cary, NC). χ^2 statistics was used to test whether the genotype frequencies among cases and controls deviated from Hardy-Weinberg equilibrium. Dietary nutrient intakes were converted to nutrient density variables (per day per 1,000 kcal) and categorized into four groups according to quartile distributions among controls. Unconditional logistic regression analysis was conducted to estimate odds ratios (OR) and their 95% confidence intervals (95% CI) for associations between intake of B vitamins, *MTHFR* genotypes, and endometrial cancer risk. Potential confounders were identified according to a priori knowledge and were adjusted for in the model. Confounders adjusted for in the analysis include age (as continuous variable), education (no formal education/elementary/junior high school/high school/post-high school/college, as a dummy variable), menopausal status (premenopausal/postmenopausal), BMI (by quartile, as a dummy variable), alcohol consumption (never/ever), diagnosis of diabetes (ever/never), total energy intake (by quartile, as a dummy variable), animal food intake (by quartile, as a dummy variable), and total fruit and vegetable intake (by quartile, as a dummy variable). Additional adjustment for years of menstruation, number of pregnancies, oral contraceptive use, and physical activity did not substantially change the results and were not included in the model. Linkage disequilibrium between the three polymorphisms was examined using R programming language software. Multiplicative interaction between genotype and

nutrient intake was evaluated by comparing the difference of the log likelihoods between a model with the main effects and a model with both the main effects and the interaction terms. Haplotypes for three polymorphic sites (in the order: 1793G>A, 1298A>C, and 677C>T) were reconstructed via a Bayesian approach using PHASE software (22, 23). The differences of haplotype frequencies between cases and controls were tested with the permutation test (24). Logistic regression was used to assess the association of endometrial cancer risk with each haplotype as compared with others under dominant, recessive, and additive genetic models using the weighted probability method.

Results

Comparisons of demographic and nongenetic factors between cases and controls for all study participants have been reported in our previous study (25). Table 1 shows the comparisons of cases and controls with genotyping data. Cases were significantly more likely to be premenopausal, have a younger age at menarche, an older age at menopause, a greater number of years of menstruation, a first-degree relative with cancer, a higher BMI, to be nulliparous, and less likely to have ever used oral contraceptives, to consume alcohol regularly, or to engage in regular physical activity as compared with controls (Table 1). Cases also had a higher intake of total calories, animal foods, and fruits and vegetables. Additionally, cases had lower folate intake and higher riboflavin, vitamin B6, vitamin B12, and methionine intakes. No differences for any of the above-mentioned demographic or risk factors were observed between women with genotype data and the entire study population (data not shown).

Risk estimates associated with dietary folate and other nutrient intakes are shown in Table 2. Increased intake of folate was associated with a decreased risk for endometrial cancer in a dose-dependent manner (OR, 0.6; 95% CI, 0.4-0.7, for the highest versus the lowest folate quartile; P_{trend} <0.01). This pattern was true and seemed to be more evident among those who did not use a vitamin supplement containing folic acid (OR, 0.5; 95% CI, 0.4-0.7, for the highest folate quartile versus the lowest). Intake of riboflavin, vitamin B6, vitamin B12, and methionine was not associated with risk for endometrial cancer, regardless of vitamin supplement use, although there was a suggestion that riboflavin intake may be inversely related to risk.

Table 3 presents the *MTHFR* genotype and haplotype distributions and ORs for the association of genotypes and haplotypes with the risk of endometrial cancer. Allele frequencies were similar between cases and controls for all three polymorphisms (data not shown). None of the genotype frequencies for the rs1801133 (677C>T), rs1801131 (1298A>C), and rs2274976 (1793G>A) polymorphisms deviated significantly from Hardy-Weinberg equilibrium among cases or controls (data not shown in table). We found no overall association of the three polymorphisms with endometrial cancer risk. Adjustment by education, menopausal status, diagnosis of diabetes, BMI, alcohol consumption, and energy

Table 1. Comparison of cases and controls by selected demographic factors and major risk factors for endometrial cancer from the Shanghai Endometrial Cancer Study, 1997-2003

	Subjects with genotyping data		*P*
	Cases (*n* = 1,041)	Controls (*n* = 1,030)	
Age at diagnosis (y)*	54 (48, 61)	54 (48, 62)	0.75
Education >high school (%)	40.4	39.8	0.04
Marital Status (%)			
Single	1.4	1.2	0.92
Married	87.0	87.7	
Widowed/separated/divorced	11.6	11.0	
Regular smoker (%)	3.2	3.5	0.68
Regular alcohol consumption (%)	3.1	5.5	<0.01
First-degree relative with cancer (%)	35.3	29.2	<0.01
Age at menarche (y)*	14 (13, 16)	15 (13, 16)	<0.001
Premenopausal (%)	43.3	38.4	0.02
Age at menopause (y)*,†	50.3 (48.6, 52.5)	49.5 (47.3, 51.1)	<0.001
Years of menstruation*	33.2 (30.1, 36.0)	31.4 (28.1, 34.2)	<0.001
No. pregnancies (%)			
None	7.1	3.8	<0.001
1	17.1	13.4	
2	25.8	26.3	
3	22.5	25.7	
4	16.1	17.1	
5 or more	11.3	13.7	
Oral contraceptive use ever (%)	18.4	25.1	<0.001
HRT use ever (%)	4.6	4.3	0.71
No regular physical activity (%)	71.9	66.2	0.04
BMI*	25.3 (23.0, 28.2)	23.4 (21.4, 25.9)	<0.001
Diagnosis of diabetes	15.0	6.9	<0.01
B vitamin supplement use (%)	8.0	11.6	<0.01
Folate intake (μg/d)*	288.9 (231.0, 370.0)	293.1 (227.7, 367.7)	0.98
Riboflavin intake (mg/d)*	0.89 (0.68, 1.12)	0.88 (0.67, 1.10)	0.11
Vitamin B6 intake (mg/d)*	2.09 (1.64, 2.63)	1.99 (1.57, 2.49)	<0.01
Vitamin B12 intake (μg/d)*	3.41 (2.27, 4.89)	3.01 (1.95, 4.46)	<0.01
Methionine intake (g/d)*	1.88 (1.45, 2.41)	1.75 (1.33, 2.26)	<0.01
Total calorie intake (kcal/d)*	1,739 (1,474, 2,048)	1,711 (1,423, 2,012)	0.04
Total animal food intake (g/d)*	176.7 (120.7, 241.9)	155.8 (103.8, 221.5)	<0.01
Total fruit and vegetable intake (g/d)*	510.3 (341.6, 699.0)	488.4 (338.3, 684.1)	0.32

Abbreviation: HRT, hormone replacement therapy.
*Median (25th, 75th percentile) is presented.
† Among postmenopausal women.

intake did not appreciably change the ORs; thus, only age-adjusted estimates are presented.

The three single-nucleotide polymorphisms under study are in linkage disequilibrium ($r^2 = 0.39$, $D' = 0.95$, $P < 0.001$ for 1298A>C and 1793G>A; $r^2 = 0.15$, $D' = 0.999$, $P < 0.001$ for 1298A>C and 677C>T; $r^2 = 0.06$, $D' = 0.94$, $P < 0.001$ for 677C>T and 1793G>A). To assess the potential effect of the multiple polymorphisms in the *MTHFR* gene, haplotypes and their frequencies were estimated and evaluated for their association with endometrial cancer using dominant and recessive modeling (Table 3). The most frequent haplotype was GAC (41.4%), followed by the GAT (41.0%) haplotype. The other common haplotypes (GCC and ACC) occurred at 9.6% and 8.4%, respectively. There were no significant differences in haplotype frequencies between cases and controls ($P = 0.64$), and no significant associations were observed for the major haplotypes under additive, recessive, or dominant models (Table 3).

The joint associations of *MTHFR* genotypes and dietary folate intake with endometrial cancer risk are presented in Table 4. Low folate intake was associated with an increased risk of endometrial cancer regardless of the various 677C>T, 1298A>C, and 1793G>A genotypes. Among women who never took B vitamin supplements, there was a significant multiplicative interaction between folate intake and 1793G>A in relation to endometrial cancer risk ($P_{interaction} = 0.03$), such that the presence of the variant allele was related to lower risk when folate intake was high and to elevated risk when folate intake was low.

A similar and borderline interaction was observed for the 1298A>C polymorphism among non-users of supplements ($P_{interaction} = 0.08$).

Additional analyses were conducted to evaluate the interaction between *MTHFR* polymorphisms and the effect of folate intake and riboflavin with endometrial cancer risk. High and low intake of folate was classified by 75th percentile intake of folate per 1,000 kcal/d, whereas that of riboflavin was classified by median intake. Again, a significant interaction was observed for the 1298A>C polymorphism ($P_{interaction} = 0.04$). It seemed that adequate intake of both folate and riboflavin was needed to reduce the risk of endometrial cancer for women with the 1298C allele, although dietary intakes of riboflavin and folate were highly correlated ($r = 0.76$, $P < 0.0001$). The lowest risk was observed for women with the 1298AC/CC genotype and with higher folate and higher vitamin B2 intake (OR, 0.6; 95% CI, 0.3-1.0), whereas the highest risk was observed for women with the same genotype but higher folate and lower riboflavin intake (OR, 2.2; 95% CI, 0.9-5.7). A similar pattern was observed for 1793G>A polymorphisms, with $P_{interaction} = 0.03$. No significant interactions were found between folate intake and other cofactors (data not shown).

Discussion

We found in this case-control study that dietary folate intake was inversely associated with the risk of endometrial cancer. No overall associations were observed between *MTHFR* genotypes/haplotypes and endometrial cancer risk. However, an interaction between *MTHFR* polymorphisms at 1793G>A and 1298A>C and folate intake was noted. These two polymorphisms interacted with the joint effects of folate and riboflavin intake.

Folate has been suggested to play an important role in DNA methylation, synthesis, and repair, and thus may be involved in carcinogenesis. Folate deficiency may lead to reduced 5-methyltetrahydrofolate availability, which is used for the remethylation of homocysteine to methionine. This, in turn, may result in decreased levels of methionine, *S*-adenosyl-L-methionine, and global hypomethylation of DNA (3, 4, 26) and

Table 2. Multivariate-adjusted ORs and 95% CIs for association of dietary B vitamins with risk of endometrial cancer in the Shanghai Endometrial Cancer Study, 1997-2003

Nutrients	Quartile of dietary intake*				P_{trend}
	Q1 (low)	Q2	Q3	Q4 (high)	
Folate (μg/d/1,000 kcal)					
Cases/controls	334/303	329/303	278/303	263/303	
OR (95% CI)†	1.0	0.8 (0.7-1.1)	0.6 (0.5-0.8)	0.6 (0.4-0.7)	<0.01
No supplement use					
Cases/controls	313/281	307/268	258/266	232/267	
OR (95% CI)†	1.0	0.9 (0.7-1.1)	0.7 (0.5-0.9)	0.5 (0.4-0.7)	<0.01
Riboflavin (mg/d/1,000 kcal)					
Cases/controls	286/303	309/303	262/303	347/303	
OR (95% CI)†	1.0	0.9 (0.7-1.1)	0.7 (0.5-0.9)	0.8 (0.6-1.1)	0.10
No supplement use					
Cases/controls	276/282	283/287	240/265	311/248	
OR (95% CI)†	1.0	0.8 (0.6-1.1)	0.7 (0.5-0.9)	0.8 (0.6-1.2)	0.22
Vitamin B6 (mg/d/1,000 kcal)					
Cases/controls	251/303	307/303	332/303	314/303	
OR (95% CI)†	1.0	1.1 (0.8-1.5)	1.3 (0.9-1.8)	1.0 (0.7-1.6)	0.97
No supplement use					
Cases/controls	238/273	281/277	308/269	283/263	
OR (95% CI)†	1.0	1.1 (0.8-1.5)	1.2 (0.8-1.8)	1.0 (0.6-1.5)	0.89
Vitamin B12 (μg/d/1,000 kcal)					
Cases/controls	217/303	279/303	334/303	374/303	
OR (95% CI)†	1.0	1.1 (0.8-1.5)	1.1 (0.8-1.6)	1.1 (0.7-1.7)	0.65
No supplement use					
Cases/controls	210/286	261/272	306/272	337/252	
OR (95% CI)†	1.0	1.2 (0.8-1.6)	1.2 (0.8-1.7)	1.3 (0.8-2.0)	0.29
Methionine (g/d/1,000 kcal)					
Cases/controls	211/303	274/303	356/303	363/303	
OR (95% CI)†	1.0	1.1 (0.8-1.5)	1.2 (0.9-1.8)	1.1 (0.7-1.7)	0.84

*Quartile cut points for intake of folate, riboflavin, vitamin B6, vitamin B12, and methionine were 140.2,170.2, 205.8; 0.41, 0.51, 0.60; 0.98, 1.17, 1.40; 1.20, 1.77, 2.46; and 0.87, 1.02, 1.21, respectively.

† Adjusted for age, education, menopausal status, diagnosis of diabetes, alcohol consumption, BMI, physical activity, total energy intake, total animal food intake, and total fruit and vegetable intake.

Table 3. ORs and 95% CI for *MTHFR* polymorphisms with risk of endometrial cancer in the Shanghai Endometrial Cancer Study, 1997-2003

	All subjects			Women never using B vitamin supplements		
	No. cases (%)	No. controls (%)	OR (95% CI)	No. cases (%)	No. controls (%)	OR (95% CI)
Genotypes						
rs1801133 (677C>T)	1,029	1,016		949	899	
677CC	356 (34.6)	337 (33.2)	1.0	332 (35.0)	301 (33.5)	1.0
677CT	506 (49.2)	521 (51.3)	0.9 (0.8-1.1)	465 (49.0)	458 (50.9)	0.9 (0.8-1.1)
677TT	167 (16.2)	158 (15.5)	1.0 (0.8-1.3)	152 (16.0)	140 (15.6)	1.0 (0.8-1.3)
	P_{trend}		0.80			0.74
rs1801131 (1298A>C)	1,036	1,019		954	900	
1298AA	699 (67.5)	705 (69.2)	1.0	640 (67.1)	619 (68.8)	1.0
1298AC	300 (29.0)	280 (27.5)	1.1 (0.9-1.3)	279 (29.2)	253 (28.1)	1.1 (0.9-1.3)
1298CC	37 (3.6)	34 (3.3)	1.1 (0.7-1.8)	35 (3.7)	28 (3.1)	1.2 (0.7-2.0)
	P_{trend}		0.42			0.38
rs2274976 (1793G>A)	1,030	1,013		949	898	
GG	856 (83.1)	855 (84.4)	1.0	788 (83.0)	756 (84.2)	1.0
AG/AA	174 (16.9)	158 (15.6)	1.1 (0.9-1.4)	161 (17.0)	142 (15.8)	1.1 (0.9-1.4)
Haplotypes						
	Cases (n = 1,029), %	Controls (n = 1,013), %	Dominant	Recessive	Additive	
GAC	41.0	41.4	1.0 (0.8-1.3)	1.0 (0.7-1.4)	1.0 (0.8-1.2)	
GAT	40.7	41.0	0.9 (0.7-1.2)	1.2 (0.8-1.7)	1.0 (0.8-1.2)	
GCC	9.6	9.3	1.0 (0.8-1.4)	1.0 (0.2-4.0)	1.0 (0.8-1.4)	
ACC	8.4	7.8	1.1 (0.8-1.5)	0.3 (0.1-3.2)	1.0 (0.8-1.4)	

NOTE: OR adjusted for age. Additional adjustment for education, menopausal status, diagnosis of diabetes, BMI, alcohol consumption, total energy intake, total animal food intake, and total fruit and vegetable intake did not change the results.

thereby result in increased mutation rates via genomic instability (27). Folate deficiency may also increase DNA replication errors through misincorporation of uracil into DNA resulting in higher levels of chromosomal breaks (2, 3, 26). Folate deficiency also seems to be associated with hypermethylation of CpG islands in the promoter regions of several tumor suppressor genes and DNA repair genes, reducing their expression (3, 28). Riboflavin (vitamin B2), vitamin B6, and vitamin B12 are key cofactors involved in folate and methionine metabolism (1).

Findings from our study lend support to the concept that folate may have anticarcinogenic properties in endometrial cancer risk. Our results are consistent with some previous reports (7-9, 29) in which higher folate or folic acid intake accounted for a 20% to 40% reduced risk for endometrial cancer, but not with others (10-12). Few of these previous studies took potential confounders, such as fruit and vegetable intake, into account. Folate intake is generally correlated with an intake of fiber and some other vitamins. Therefore, its effect may be easily confounded by other dietary factors that may themselves be protective for endometrial cancer. Moreover, these previous studies were conducted among populations with a frequent use of fortified or supplemental folic acid, but none of them accounted for this important source of folate intake. This may partly explain the mixed results from previous studies. It is also worth mentioning that previous studies applied different nutrient models [i.e., energy residual method (11, 12) and standard multivariate method (7-10)] in their data analyses, making it difficult to compare the results directly.

In the current study, we have taken vitamin supplement use into consideration and adjusted for total fruit and vegetable intake in the data analysis. We applied a multivariate nutrient density method, a standard multivariate method (data not shown), and a caloric residual method (data not shown) in our analysis and found a similar inverse association between endometrial cancer risk and folate intake. Finally, alcohol consumption is associated with folate malabsorption, which may lead to folic acid deficiency. However, the drinking of alcohol is not common in our study population (3.1% in cases and 5.5% in controls). Excluding those who reported ever drinking alcohol did not change the association substantially (data not shown). Therefore, our results are unlikely to be confounded by the above-mentioned factors.

MTHFR is a critical enzyme involved in folate metabolism. Two common polymorphisms described in the *MTHFR* gene, 677C>T and 1298A>C, have been reported to be related to the activity of the enzyme (14, 30). The 677TT genotype is associated with reduced enzyme activity. Individuals with the 677TT genotype have higher intracellular concentrations of 5,10-methylenetetrahydrofolate than individuals with the 677CC or 677CT genotypes who have predominantly 5-methyltetrahydrofolate intracellularly (30). 1298A>C is also associated with reduced enzyme activity, although not to the extent of the C677T site (14), and is related to lower global methylation and higher CpG island methylation (31). It has been suggested that these polymorphisms alter the balance of folate (30) and, thus, modify the association of folate intake with cancer risk (15, 16, 32). In the only two previous studies that evaluated these polymorphisms and endometrial cancer risk, one found an increased risk with the 677T allele (17) and the other did not (9). Similar to results from the Nurses' Health Study (9), we found no evidence of a significant association between these *MTHFR* polymorphisms and endometrial cancer risk.

This is, to our knowledge, the first study to evaluate another MTHFR polymorphism, 1793G>A, with endometrial cancer risk. The frequency of this rare allele is relatively higher in Chinese populations (10.0%) than in Caucasian (5.8%) or Yoruba (3.3%) populations.[5] Its function is not clear. In this study, there was no overall association between single-nucleotide polymorphism 1793G>A and risk of endometrial cancer. However, the rare allele interacted with folate intake on endometrial cancer risk. Among women who had never taken B vitamin supplements, the presence of the variant allele at this site was related to lower risk when folate intake was high and to elevated risk when folate intake was low.

We found that the single-nucleotide polymorphisms 1793G>A and 1298A>C in the *MTHFR* gene seemed to interact with folate and riboflavin in modulating cancer risk in our study population. Among women with one or two C alleles at 1298A>C or at least one A allele at 1793G>A, high folate and high riboflavin intake was related to the lowest risk, whereas high folate but low riboflavin intake was associated with the

[5] http://www.hapmap.org

Table 4. Joint association of *MTHFR* polymorphism and folate intake with endometrial cancer in the Shanghai Endometrial Cancer Study, 1997-2003

Genotype	Dietary folate intake (μg/d/1,000 kcal intake)								$P_{interaction}$
	Q4 (high)		Q3		Q2		Q1 (low)		
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	
All women									
rs1801133 (677C>T)									
677CC	68/90	1.0	93/77	1.9 (1.2-3.0)	98/93	1.9 (1.2-3.0)	97/77	2.8 (1.7-4.6)	
677CT	114/144	1.2 (0.8-1.8)	117/123	1.5 (1.0-2.3)	138/127	1.9 (1.3-3.0)	137/127	2.2 (1.4-3.5)	
677TT	42/37	1.5 (0.9-2.7)	38/41	1.8 (1.0-3.1)	43/45	1.7 (0.9-2.9)	44/35	2.4 (1.3-4.4)	0.63
rs1801131 (1298A>C)									
1298AA	161/187	1.0	156/173	1.2 (0.9-1.6)	193/180	1.5 (1.1-2.1)	189/165	2.0 (1.4-2.8)	
1298AC/CC	67/86	0.9 (0.6-1.3)	91/68	1.8 (1.2-2.7)	88/84	1.7 (1.1-2.5)	91/76	2.2 (1.4-3.4)	0.30
rs2274976 (1793G>A)									
GG	195/223	1.0	192/203	1.2 (0.9-1.6)	236/219	1.5 (1.1-2.1)	233/210	1.9 (1.4-2.7)	
AG/AA	33/46	0.8 (0.5-1.4)	53/37	2.1 (1.3-3.5)	43/44	1.6 (1.0-2.7)	45/31	2.9 (1.7-5.1)	0.15
Women never using B vitamin supplement									
rs1801133 (677C>T)									
677CC	61/80	1.0	90/66	2.2 (1.3-3.6)	88/82	1.9 (1.2-3.1)	93/73	2.8 (1.7-4.8)	
677CT	98/125	1.2 (0.7-1.8)	109/106	1.6 (1.0-2.4)	134/113	2.2 (1.4-3.4)	124/114	2.3 (1.4-3.8)	
677TT	38/32	1.6 (0.8-2.9)	31/37	1.6 (0.8-2.9)	41/38	1.8 (1.0-3.3)	42/33	2.5 (1.3-4.6)	0.48
rs1801131 (1298A>C)									
1298AA	141/157	1.0	143/151	1.2 (0.8-1.6)	182/156	1.5 (1.1-2.2)	174/155	1.8 (1.3-2.7)	
1298AC/CC	59/81	0.7 (0.5-1.1)	87/58	1.9 (1.2-2.9)	81/75	1.6 (1.1-2.5)	87/67	2.3 (1.5-3.7)	0.08
rs2274976 (1793G>A)									
GG	171/192	1.0	177/176	1.3 (0.9-1.7)	223/191	1.6 (1.2-2.2)	217/197	1.9 (1.3-2.6)	
AG/AA	29/43	0.7 (0.4-1.3)	51/33	2.2 (1.3-3.6)	39/41	1.6 (0.9-2.7)	42/25	3.6 (2.0-6.6)	0.03

NOTE: Adjusted for age, education, menopausal status, diagnosis of diabetes, BMI, alcohol consumption, total caloric intake, total animal food intake, and total fruit and vegetable intake.

highest risk. Riboflavin plays an important role in folate metabolism, and, as a precursor for flavin adenine dinucleotide, it is a cofactor for MTHFR. It has been proposed that it acts jointly with folate to lower plasma homocysteine levels. One Japanese study reported that low riboflavin blunted the effect of folic acid, which resulted in a diminished reduction of total homocysteine (33). Conversely, high intake of folic acid supplements was observed to exacerbate a tendency toward riboflavin deficiency (34). Although these observations lend some support to our findings, the three-way joint effect could be a result of chance and requires confirmation in future studies.

In our study population, B vitamin supplement users had a higher level of dietary folate intake, which was consistent with the notion that supplement users are more health conscious and have a healthier lifestyle (35, 36). It is worth noting that we detected a significant interaction only among women who never took B vitamin supplements. This may be due to the fact that, among subjects who regularly use supplements, there is little variation of folate levels, or their folate level was sufficiently high enough to protect against carcinogenesis. Unfortunately, we were not able to verify this hypothesis due to a lack of folate supplement information. Misclassification of total folate intake among supplement users could also mask the diet-endometrial cancer association.

As with all case-control studies, the potential for recall bias cannot be completely dismissed. However, the median interval between diagnosis and interview for cases was 5.6 months, and we asked the participants to ignore any dietary change over the preceding year, which may have minimized recall bias. In addition, recall of dietary intake is unlikely to be related to any genotype. Selection biases are limited by the population-based design, the relatively high participation rate (82.8% for cases and 74.4% for controls), high DNA sample donation rate, and the low frequency of hysterectomy in our study population. The large sample size allowed us to evaluate gene-environment interactions and stratified analyses. Finally, the population is from a relatively homogeneous ethnic background (>98% Han Chinese). This decreases the potential confounding effect of ethnicity for genotyping data. In this study, we used Chinese food composition tables to estimate intake of dietary folate and riboflavin and the U.S. Department of Agriculture food composition tables for vitamin B6, vitamin B12, and methionine due to a lack of relevant data in the Chinese food tables. To evaluate the appropriateness of the method, we compared folate and riboflavin intakes derived from both Chinese and U.S. Department of Agriculture food composition tables and found excellent correlation (>0.91). Folate intake derived from the two sources yielded similar results on the association of dietary folate intake and endometrial cancer risk.

In summary, this population-based study suggests that dietary folate intake is inversely associated with the risk of endometrial cancer, particularly among women whose folate intake is derived solely from food. Whereas *MTHFR* genotypes do not seem to be directly linked to risk, they may interact with folate in the development of endometrial cancer.

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Correction

In an article (1) in the February 2007 issue, there was an error in the Abstract. The rs number given in line 17 of the Abstract should read ''rs2274967.''

Reference

1. Xu W-H, Shrubsole MJ, Xiang Y-B, et al. Dietary folate intake, *MTHFR* genetic polymorphisms, and the risk of endometric cancer among Chinese Women. Cancer Epidemiol Biomarkers Prev 2007;16: 281–7.

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Dietary Folate Intake, *MTHFR* Genetic Polymorphisms, and the Risk of Endometrial Cancer among Chinese Women

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