MTHFR Polymorphisms, Diet, HRT, and Breast Cancer Risk: The Multiethnic Cohort Study

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Abstract

Methylenetetrahydrofolate reductase (MTHFR) is a key regulatory enzyme in the metabolism of folate, a nutrient which has recently been found to be inversely related to breast cancer in women who drink alcohol. Two common variants in the *MTHFR* gene (C677T and A1298C) have been associated with a reduced activity of this enzyme, thereby increasing the availability of folate for thymidylate and purine synthesis. We investigated the relationship of these variants with invasive breast cancer in a case-control study of 1,189 cases and 2,414 controls nested within the Multiethnic Cohort Study. The Multiethnic Cohort Study is a large prospective study of men and predominantly postmenopausal women of Japanese, White, African American, Latino, and Native Hawaiian origin, residing in Hawaii and Los Angeles. We found an overall nonsignificant, weak inverse association between breast cancer risk and the 677TT genotype and no association with the 1298C variant. The odds ratio [OR and 95% confidence interval (95% CI)] for the 677CC, 677CT, and 677TT genotypes were 1.00, 0.98 (0.83-1.15), and 0.86 (0.67-1.09), respectively. Those for the 1298AA, 1298AC, and 1298CC genotypes were 1.00, 0.93 (0.79-1.08), and 1.20 (0.88-1.65), respectively. However, the inverse association with the 677TT genotype was stronger (OR, 0.62; 95% CI 0.39-0.98) among women who were on hormone replacement therapy (HRT) at baseline, and the increased breast cancer risk due to HRT was not observed in women with the 677TT genotype. An increased breast cancer risk was suggested for alcohol intake >10 g/d, when compared with nondrinkers, but only among HRT users with the 677CC genotype (OR, 1.51; 95% CI, 0.96-2.37). Folate intake exhibited no modifying effect on the genotype-breast cancer relationship. These findings suggest that the *MTHFR* 677TT genotype may confer a 40% decreased breast cancer risk in postmenopausal women using HRT. This is consistent with the role of MTHFR in facilitating the flow of folate for thymidylate and purine synthesis and with the increased nucleic acid need resulting from the hyperproliferative effect of HRT on mammary epithelial cells. (Cancer Epidemiol Biomarkers Prev 2004;13(12): 2071–7)

Introduction

A common C677T substitution in the methylenetetrahydrofolate reductase (*MTHFR*) gene, which converts an alanine to a valine at codon 225, has been associated with a reduced enzyme activity and a decreased colorectal cancer risk (1, 2). More recently, a second polymorphism (A1298C) in *MTHFR* has also been related to decreased enzyme activity (3). The MTHFR enzyme plays a central role in one-carbon metabolism by catalyzing the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyl-tetrahydrofolate, the primary circulatory form of folate and the carbon donor for the remethylation of homocysteine to methionine, used in DNA methylation (4). However, the same substrate, 5,10-methylenetetrahydrofolate, is also required for thymidylate and purine synthesis (4). Thus, MTHFR activity and availability of folate may affect both gene expression, through DNA methylation, and genome integrity, through DNA synthesis and repair.

Recent investigations, including four large prospective studies, have shown that lower intake and/or plasma levels of folate may increase breast cancer risk, particularly among women who drink alcohol, which acts as a folate antagonist (5-9). To our knowledge, only five studies have examined the relationship between *MTHFR* and breast cancer. All were relatively small, hospital-based case-control studies that have yielded conflicting results (10-14). Thus, we explored the relationships of the C677T and A1298C *MTHFR* polymorphisms and breast cancer risk in a large case-control study nested within the Multiethnic Cohort Study, a prospective study with an unusually wide range of dietary intake (15).

Materials and Methods

The design and baseline characteristics of the Multiethnic Cohort Study were described in detail elsewhere

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(15, 16). In short, participants are Hawaii and California (primarily, Los Angeles) residents who entered the cohort from 1993 to 1996 by completing a 26-page mail questionnaire including demographic and lifestyle factors (such as diet and smoking), medical history, medication use, family history of common cancers and, for women, reproductive history and hormone use. The cohort included 118,441 women and 96,810 men ages 45 to 75 years at cohort creation in 1993. Among the women, 25% were Japanese American, 22% White, 21% Latino, 19% African American, 7% Hawaiian, and 6% of other ethnic or racial origin. A nested case-control study of breast cancer was done among women of the five main ethnic groups. Breast cancer cases were identified through the Rapid Reporting System of the Hawaii Tumor Registry and through quarterly linkages to the Los Angeles County Cancer Surveillance Program, two cancer registries that are members of the Surveillance, Epidemiology and End Results program of the National Cancer Institute. This was complemented by annual linkages to the State of California's cancer registry. A sample of cohort participants was randomly selected to serve as controls; the selection was stratified by sex and ethnicity. Incident breast cancer cases occurring since January 1995 and controls were contacted for donation of a blood sample. Samples were collected at the subjects' homes, processed within 8 hours and stored at −80°C. As of June 1999, the participation rate among cases was 74% and varied from 70% in African Americans to 81% in Latinos. The corresponding rate for controls was 66% and varied from 60% in African Americans to 71% in Whites.

The food frequency questionnaire asked about the frequency and amount of consumption for more than 180 food items during the last year. Photographs of foods, showing three different portion sizes, were used to facilitate quantification of intakes. Nutrients were computed by applying a food composition table to the daily grams of each food item and summing across items. The food composition data were primarily based on the U.S. Department of Agriculture's nutrient database and were supplemented with data from other research and commercial publications. A calibration study that compared diet reported with the questionnaire with three 24-hour recalls suggested that folate intake was adequately measured, with correlation coefficients for women ranging between 0.4 and 0.6 across ethnic groups (17). Cohort members were also asked about their usage during the last year of multivitamins and/or minerals and seven single vitamins. Supplemental folic acid intake was assessed from multivitamin usage. A composite nutrient content was assumed for multivitamins (18).

DNA was purified from buffy coat fractions using the QIAamp 96 DNA Blood Kit (Qiagen, Valencia, CA). MTHFR genotypes were determined using the fluorogenic 5′-nuclease assay (Taqman Assay; ref. 19). The assays were done using a Taqman PCR Core Reagent kit (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. The oligonucleotide primers and probes (Taqman MGB Probes; Applied Biosystems) for amplification of the polymorphic region of MTHFR were A1298C, forward primer 5′-GGAGGAGCTGCTGAAGATGTG-3′, reverse primer 5′-CCCGAGAGGTAAAGAACAAAGACTT-3′, probe1 AGACACTT***G***CTTCACT, probe2 CAAAGACACTT***T***CTTC; C677T, forward primer 5′-GCACTTGAAGGAGAAGGTGTCT-3′, reverse primer 5′-TGTGTCAGCCTCAAAGAAAAGCT-3′, probe1 ATGAAATCG***A***CTCCCGC, probe2 ATGAAATCG***G***CTCCCGC. PCR amplification using ~5 ng/sample of genomic DNA was done in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems) with an initial step of 95C for 10 minutes followed by 50 cycles of 95C for 15 seconds and 68C for 1 minute. The fluorescence profile of each well was measured in an Applied Biosystems 7900HT Sequence Detection System and the results analyzed with Sequence Detection Software (Applied Biosystems).

Laboratory personnel were blinded to case-control status and ~5% of samples were included as duplicates. The concordance for the blinded samples was >99.5% for both polymorphisms. Sixty-eight cases and 105 controls exhibited low signal due to low DNA concentration and were excluded from the analysis. Another 34 cases and 71 controls (2.8% of cases and controls) were excluded due to noninterpretable results for either polymorphism. The remaining 1,189 cases and 2,414 controls were available for data analysis. An age- and ethnicity-adjusted comparison of these subjects with those who did not provide genotype data, because of not donating blood or inconclusive results, revealed no difference in age, age at menarche, age at first birth and parity. However, mean folate and alcohol intakes were somewhat higher among nonrespondents (385 versus 372 μg/d and 0.94 versus 0.41 g/d, respectively).

The statistical analysis used unconditional logistic regression to compute odds ratio (OR) and 95% confidence interval (95% CI) for exposures of interest (20). Genotypes were modeled as two dummy variables representing the three levels, or as a gene-dosage effect variable assigned a value of 1, 2, or 3 according to the number of variant alleles (zero, one, and two variant alleles, respectively). The final models were only adjusted for age at blood draw and ethnicity because further adjustment for other breast cancer risk factors (age at menarche, parity, age at first birth, and body mass index) did not materially change the risk estimates (see Table 2). The likelihood ratio test was used to determine the significance of the interaction among certain variables with respect to breast cancer risk. The test compares a main effects, no interaction model with a fully parameterized model containing all possible interaction terms for the variables of interest. Deviation from Hardy Weinberg equilibrium was tested with the χ^2 test. To test for nonrandom association of the two *MTHFR* alleles for each ethnic group among controls, the D' and r^2 statistics were calculated (21). These values were computed and tested for important deviations from zero with the χ^2 test for linkage equilibrium, using the genetics package in *R* (22). A statistically significant D' very close to 1 is a strong indication that minimal recombination has occurred between two single nucleotide polymorphisms, but intermediate values are difficult to interpret (23). Thus, we also used the r^2 to quantify the correlation between *SNP* alleles because the power to detect associations with marker single nucleotide polymorphisms directly depends on the r^2 value between the marker and the causal variant (23).

Table 1. Characteristic of breast cancer cases and controls at baseline

	Cases (n = 2,414)*	Controls (n = 2,414)*
Japanese American (%)	26.7	16.9
White (%)	26.9	17.2
African American (%)	20.7	26.5
Latino (%)	19.8	27.5
Hawaiian (%)	5.8	11.8
Age at baseline (y)	60.8 ± 8.4	58.3 ± 8.6
Age at blood draw (y)	65.7 ± 8.5	63.0 ± 8.7
Age at menarche (y)	12.6 ± 1.5	12.7 ± 1.5
Ever pregnant (%)	89.9	91.6
Age at first birth† (y)	23.9 ± 5.0	22.9 ± 4.7
Parity†	2.6 ± 1.8	3.0 ± 1.9
Menopausal at baseline (%)	87.2	82.3
Ever used HRT (%)	57.4	47.7
Ever smoked (%)	46.2	45.2
Current drinker (%)	41.8	40.9
Family history‡ (%)	17.6	11.8
Body mass index (kg/m^2)	26.3 ± 6.1	27.0 ± 6.6
Folate from foods (g/d)	356 ± 204	380 ± 252
Total folate§ (g/d)	505 ± 327	519 ± 354
Ethanol‖ (g/d)	4.9 ± 15.6	4.3 ± 14.6

NOTE: Mean ± standard deviation, except when specified as percentages.
*No. subjects was smaller for some risk factors due to missing data.
†Among parous women only.
‡Family history of breast cancer in mother and sisters.
§Folate from foods and supplements.
‖Among drinkers the means were 11.7 ± 22.2 and 10.4 ± 21.2 g/d among cases and controls, respectively.

Results

Table 1 shows the characteristics of breast cancer cases and controls at baseline. On the average, cases tended to be older, to have had their first child at an older age, and to have fewer children. They also more often had a positive family history of breast cancer and used hormone replacement therapy (HRT). Cases tended to consume less folate but drank somewhat more alcohol. Among controls, the frequency for the *677T* allele was 0.42 for Japanese Americans, 0.36 for Whites, 0.12 for African Americans, 0.43 for Latinos, and 0.20 for Hawaiians. The corresponding frequencies for the *1298C* allele were 0.19, 0.29, 0.18, 0.20, and 0.27. The ethnic-specific genotype distributions in controls were all in Hardy Weinberg equilibrium (all Ps > 0.5, except for C677T in Whites for whom P = 0.11). The two *MTHFR* variants were in linkage disequilibrium among the controls with D' values of 0.99 in all groups, except in African Americans for whom the D' was 0.80. However, the r^2 values were low, with values ranging from 0.2 in Japanese Americans, Whites, and Latinos to 0.02 in African Americans.

Table 2 presents the breast cancer odds ratios for the MTHFR genotypes adjusting for age at blood draw and ethnicity and after further adjustment for additional breast cancer risk factors. The age- and ethnicity-adjusted analysis shows no statistically significant association between breast cancer and either the 677T or 1298C variant. The OR for the 677TT genotype, compared with the 677CC genotype, was 0.86 (95% CI, 0.67-1.09) and the OR for the 1298CC versus 1298AA genotype was 1.20 (95% CI, 0.88-1.65). The OR for subjects heterozygous for both variant alleles (*677T* and *1298C*) was 0.89 (95% CI, 0.69-1.09), compared with subjects homozygous for both common alleles (with the 677CC and 1298AA genotypes). [As found in our other study in Hawaii (24), no individuals were homozygous for both variant alleles.] Further adjustment for age at menarche, age at first birth, parity, and body mass index did not modify these risk estimates. Thus, this additional adjustment was not carried out in the rest of the analyses. Table 2 also examines the same associations by menopausal status at baseline. No association was apparent among premenopausal women, although the number of cases in this group was small. For women who were postmenopausal at baseline, the OR

Table 2. Breast cancer ORs (95% CIs) for the *MTHFR* C677T and A1298C polymorphisms

Genotype	Bivariate*		Multivariate†		Premenopausal*,‡		Postmenopausal*,‡	
	n§	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
677								
CC	573/1211	1.00	534/1148	1.00	70/186	1.00	429/849	1.00
CT	479/920	0.98 (0.83-1.15)	441/866	0.97 (0.82-1.14)	51/146	1.01 (0.64-1.59)	365/648	0.96 (0.80-1.16)
TT	137/283	0.86 (0.67-1.09)	131/271	0.85 (0.66-1.09)	18/40	1.27 (0.65-2.47)	103/207	0.80 (0.60-1.06)
P		0.28[a]		0.25		0.57		0.16
1298								
AA	741/1493	1.00	685/1405	1.00	78/236	1.00	562/1048	1.00
AC	371/801	0.92 (0.79-1.08)	349/764	0.92 (0.78-1.08)	51/119	1.36 (0.89-2.07)	274/568	0.88 (0.73-1.06)
CC	77/120	1.20 (0.88-1.65)	72/116	1.18 (0.85-1.62)	10/17	1.96 (0.84-4.55)	61/88	1.18 (0.83-1.69)
P		0.93		0.96		0.06		0.78

NOTE: [a]P value for a gene dosage term assigned 1, 2, and 3 for 0, 1, and 2 variant alleles, respectively.
*Adjusted for age at blood draw and race and/or ethnicity.
†Further adjusted for age at menarche, age at first birth, parity, and body mass index (83 cases and 129 controls were excluded due to missing covariates).
‡Premenopausal status was defined as still menstruating at baseline. Postmenopausal status was defined as menstruation stopping and natural menopause, bilateral oopherectomy, or age >55 years at cohort entry. Women with a simple hysterectomy with age ≤55 years or who could not be classified into these groups were excluded (153 cases and 338 controls).
§No. cases/no. controls.

Table 3. Breast cancer ORs (95% CIs) by *MTHFR* C677T or A1298C genotype and ethnicity

Ethnicity	*n**	OR	*n*	OR (95% CI)	*n*	OR (95% CI)	*P*†
	C677T						
	CC		CT		TT		
Japanese American	135/140	1.00	140/196	0.75 (0.53-1.03)	43/74	0.60 (0.39-0.94)	0.02
White	131/178	1.00	151/176	1.11 (0.81-1.54)	38/61	0.80 (0.50-1.29)	0.65
African American	196/490	1.00	47/140	0.83 (0.57-1.20)	3/9	0.86 (0.23-3.23)	0.34
Latino	70/218	1.00	117/319	1.20 (0.85-1.70)	49/127	1.19 (0.78-1.83)	0.36
Hawaiian	41/185	1.00	24/89	1.25 (0.71-2.21)	4/12	1.40 (0.42-4.69)	0.38
	A1298C						
	AA		AC		CC		
Japanese American	224/271	1.00	83/126	0.81 (0.58-1.13)	11/13	1.13 (0.49-2.58)	0.44
White	160/211	1.00	118/166	0.91 (0.66-1.25)	42/38	1.39 (0.85-2.28)	0.48
African American	171/433	1.00	68/187	0.92 (0.67-1.29)	7/19	0.96 (0.40-2.34)	0.68
Latino	146/423	1.00	77/212	1.07 (0.78-1.48)	13/29	1.24 (0.63-2.47)	0.50
Hawaiian	40/155	1.00	25/110	0.85 (0.48-1.49)	4/21	0.73 (0.23-2.26)	0.46

NOTE: Adjusted for age at blood draw.
*No. cases/no. controls.
†*P* value for gene dosage term assigned 1, 2, and 3 for 0, 1, and 2 variant alleles, respectively.

for the 677TT versus 677CC genotype was 0.80 (95% CI, 0.60-1.06).

Table 3 presents the ORs for the *MTHFR* C677T and A1298C genotypes by race and/or ethnicity. There was an inverse association with the 677TT genotype that was statistically significant and dose dependent ($P = 0.02$) among Japanese Americans. No other association was observed for C677T or A1298C in these ethnic-specific analyses, although the power was limited for some of these comparisons.

Additional analyses stratified by stage at diagnosis (localized and regional or distant), body mass index (<25, ≥25 kg/m^2) and smoking status (never and ever) did not suggest that any of these factors modified the relationship between *MTHFR* and breast cancer (data not shown). Table 4 compares the ORs for MTHFR genotypes and breast cancer by HRT use at baseline. No association was detected among never and past users. However, a significant inverse association with the *677T* allele was found among women who were on HRT at baseline, with an OR of 0.62 (95% CI, 0.39-0.98) for the 677TT versus CC genotype. The corresponding ORs for women on estrogen alone and on estrogen + progesterone were similar [0.63 (95% CI, 0.32-1.25) and 0.61 (95% CI, 0.33-1.14), respectively].

Table 5 presents the joint effect of the C677T polymorphism and HRT use at baseline (never, 1-3, >3 years) on the risk of breast cancer. Use of HRT for more than 3 years significantly increased risk among women with the CC and CT genotype, but not among those with the TT genotype. However, the test for interaction was not statistically significant ($P = 0.35$). Exclusion of women who were premenopausal at baseline from the analyses in Tables 4 and 5 did not materially change the risk estimates.

Table 6 examines the joint effects of the C677T genotype with intake of folate from foods, total folate, or alcohol on the risk of breast cancer among ever HRT users at baseline. Since in past studies, an interaction between folate and alcohol in relation to breast cancer risk was found among women who drank at least 1 drink per day (10-15 g/d), we categorized ethanol intake as 0, 1-10, and >10 g/d for this analysis. Food folate and total folate were categorized into quartiles. No statistically significant interactions were found with the dietary variables. Although not significant, the effect of alcohol

Table 4. Breast cancer ORs (95% CIs) for the *MTHFR* C677T and A1298C polymorphisms and HRT use at baseline

Genotype	Never		Past		Current*	
	n†	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
677						
CC	240/619	1.00	103/221	1.00	181/295	1.00
CT	170/418	1.00 (0.78-1.28)	81/150	1.06 (0.72-1.58)	187/282	0.95 (0.72-1.26)
TT	63/137	1.07 (0.75-1.54)	27/50	1.07 (0.60-1.92)	37/74	0.62 (0.39-0.98)
P		0.77‡		0.76		0.09
1298						
AA	294/730	1.00	141/274	1.00	250/384	1.00
AC	148/383	0.99 (0.78-1.26)	59/129	0.88 (0.60-1.29)	124/235	0.74 (0.56-0.99)
CC	31/61	1.23 (0.77-1.96)	11/18	1.21 (0.53-2.75)	31/32	1.28 (0.74-2.21)
P		0.61		0.85		0.46

NOTE: Adjusted for age at blood draw and race and/or ethnicity.
*Current: Current use of hormonal replacement therapy at baseline (100 cases and 168 controls were excluded due to missing information on HRT use).
†No. cases/no. controls.
‡*P* value for a gene dosage term assigned 1, 2, and 3 for 0, 1, and 2 variant alleles, respectively.

Table 5. Joint effects of the *MTHFR* C677T polymorphism and HRT use at baseline on breast cancer risk

Duration (y)	CC		CT		TT	
	*n**	OR (95% CI)†	*n*	OR (95% CI)	*n*	OR (95% CI)
HRT use						
Never	240/619	1.00	170/418	0.95 (0.74-1.21)	63/137	1.01 (0.71-1.43)
1-3	146/325	1.07 (0.83-1.38)	143/262	1.14 (0.87-1.48)	32/65	0.96 (0.60-1.53)
>3	129/180	1.35 (1.01-1.79)	121/147	1.48 (1.10-1.99)	31/53	0.94 (0.58-1.54)
P				0.35‡		

*No. cases/no. controls (in comparison with Table 4, an additional 14 cases and 40 controls were excluded due to missing information on HRT duration).
†ORs (95% CIs) adjusted for ethnicity and age at blood draw.
‡*P* value derived from a likelihood ratio test comparing a model with main effects for the *MTHFR* C677T genotype and the HRT use (Never, 1-3 years of use, >3 years of use), and a model with main effects and interaction terms (*df*, 4).

on breast cancer risk did vary by genotype. We observed an increased breast cancer risk of marginal significance for alcohol consumption of >10 g/d (OR, 1.51; 95% CI, 0.96-2.37), compared with nondrinkers, among HRT users with the 677CC genotype. The lowest risk observed was for HRT users with the TT genotype who did not drink. Similar but weaker patterns were suggested when all subjects were included in these interaction analyses (data not shown).

Discussion

In this large case-control study nested within a cohort of predominantly postmenopausal women, we found only a weak overall inverse association between the *MTHFR* 677TT genotype and breast cancer. However, this association was stronger and statistically significant among Japanese and among women on hormone replacement therapy at baseline. Interestingly, the well-established direct association between HRT use and breast cancer was observed among women with the 677CC and CT genotype but not among those with the TT genotype. An association between alcohol and breast cancer was suggested among HRT users with the 677CC genotype only. No effect modification was detected for folate intake on the relationship between breast cancer risk and genotype. Finally, no parallel findings were observed in our study for the A1298C variant. This may be due to the fact that the reduction in MTHFR enzyme activity resulting from this variant is thought to be less than that for the *677T* allele (3).

Several epidemiologic investigations, including four prospective studies measuring intake and/or plasma levels, have suggested that adequate folate intake may be important in the prevention of breast cancer (5-9), particularly among women who consume alcohol. In the Canadian National Breast Screening Study (7) and the Shanghai Breast Cancer Study (25), this inverse association was only detectable among older women (8).

Table 6. Joint effects of the *MTHFR* C677T polymorphism and folate or ethanol intake on breast cancer risk among ever users of HRT at baseline

	CC		CT		TT	
	*n**	OR (95% CI)†	*n*	OR (95% CI)	*n*	OR (95% CI)
Food folate (μg/d)						
≤214	69/125	1.00	54/97	0.88 (0.55-1.40)	15/17	1.29 (0.59-2.82)
215-316	62/130	0.79 (0.51-1.23)	77/117	0.95 (0.61-1.46)	11/33	0.49 (0.22-1.05)
317-473	84/134	1.02 (0.67-1.54)	81/93	1.24 (0.79-1.93)	19/39	0.67 (0.35-1.28)
>473	62/115	0.98 (0.63-1.52)	50/113	0.73 (0.46-1.17)	17/31	0.68 (0.34-1.36)
P				0.34‡		
Total folate (μg/d)						
≤257	63/113	1.00	54/98	0.92 (0.57-1.48)	6/23	0.33 (0.12-0.87)
258-437	68/127	0.94 (0.61-1.47)	67/93	1.02 (0.64-1.61)	11/32	0.49 (0.22-1.07)
438-678	74/135	0.86 (0.56-1.34)	80/128	0.84 (0.54-1.31)	26/31	1.08 (0.68-2.42)
>678	72/129	0.89 (0.57-1.38)	61/101	0.92 (0.58-1.47)	19/34	0.63 (0.32-1.24)
P				0.13		
Ethanol (g/d)						
0	156/284	1.00	143/236	0.96 (0.71-1.30)	37/81	0.63 (0.40-1.00)
1-10	71/162	0.83 (0.58-1.19)	83/143	0.97 (0.68-1.38)	20/30	1.07 (0.57-2.02)
>10	50/58	1.51 (0.96-2.37)	36/41	1.34 (0.80-2.26)	5/9	0.85 (0.27-2.67)
P				0.48		

*No. cases/no. controls (15 cases and 28 controls were excluded due to unreliable dietary information).
†ORs (95% CIs) adjusted for ethnicity and age at blood draw.
‡*P* value derived from a likelihood ratio test comparing a model with main effects for the *MTHFR* C677T genotype and the dietary variable, and a model with main effects and interaction terms (*df*, 6 and 4 for folate analyses and alcohol analysis, respectively).

B vitamins are implicated in a number of important biological processes, which may explain their role in cancer. Dietary deficiencies in folate seem to mimic radiation in damaging DNA by causing double-strand breaks and/or oxidative damage (2). Folate also plays a role in DNA methylation, which may influence gene expression.

Because long-term folate status is difficult to assess, significant additional support for a relationship between folate and breast cancer would come from the association of this disease with genetic variants affecting some of the mechanisms mentioned above. The primary model for this is the inverse association observed between the *MTHFR* 677TT genotype and colorectal cancer, which was consistently found in a number of studies, including the Multiethnic Cohort Study,[3] to be stronger at high levels of folate intake and to be negated by alcohol consumption (26). These relationships are completely consistent with a causal protective role of folate against colorectal cancer through increased thymidylate and purine synthesis (2, 4). Previous attempts to explore the relationship of this polymorphism with breast cancer have brought mixed results. Gershoni-Baruch et al. (10) found the *677T* allele to be more common in Jewish women with bilateral breast cancer or with dual breast and ovarian cancers than among those with unilateral breast cancer. In a small study of 62 cases and 66 controls, Sharp et al. (11) found an inverse association for both the 1298CC and 677TT genotypes with breast cancer. In a study of 233 healthy women and 335 breast cancer cases selected on either age at onset <40 years, presence of bilateral breast cancer, or history of familial disease, Campbell et al. (12) found that the *677T* allele was associated with an OR of 1.43 (95% CI, 1.12-2.00). In a clinic-based study of 105 breast cancer cases and 247 controls with benign breast disease, Semenza et al. (13) reported an increased risk for premenopausal breast cancer (OR, 2.8; 95% CI, 1.02-7.51) with the presence of the T allele, whereas risk for postmenopausal breast cancer was slightly decreased (OR, 0.8; 95% CI, 0.4-1.4). Finally, in a hospital-based case-control study of 500 breast cancer cases and 500 controls in Austria, the OR for the TT versus CC genotype was 0.99 (95% CI, 0.68-1.43; ref. 14).

The lack of a clear relationship between the *MTHFR* TT genotype and breast cancer has been in sharp contrast to the inverse association that has consistently been observed between this genotype and colorectal cancer, especially at high levels of folate intake and low alcohol intake levels (4). This agrees with the markedly lower rates of cell division and, thus, lower nucleic acid biosynthesis need, of the postmenopausal breast, compared with the large bowel. Of particular interest is our finding of an inverse association of the *MTHFR* 677TT genotype and breast cancer among HRT users because exogenous estrogens have been shown to increase epithelial cell proliferation in the breast (27). Thus, although the main-effect ORs was of marginal statistical significance, our finding of a stronger inverse association of the *MTHFR* 677TT genotype with breast cancer in women on HRT is biologically plausible and consistent with the results for colorectal cancer mentioned above.

If replicated, the lack of association between HRT and breast cancer that we observed in women with the 677TT genotype would be of clinical significance given the cancer risks associated with HRT and its remaining indication for the treatment of menopausal symptoms (28). Whether there is a direct relationship between the effects of estrogens and folate on breast cancer remains unclear. HRT is known to decrease circulating homocysteine levels (29–31), but by a mechanism which seems to be independent of folate levels and *MTHFR* genotype (30, 31).

The strengths of the present investigation include its large size and the prediagnostic assessment of diet, HRT, and other breast cancer risk factors. Although not all cohort members were genotyped due to the case-control study design, participation was relatively high and no major difference was found between respondents and nonrespondents. Also, only <8 % of eligible patients died before contact and no modifying effect of stage at diagnosis was observed, making a survival bias unlikely. As mentioned in the Materials and Methods section, error in assessing folate intake was comparable to other studies and did not preclude us from detecting a modifying effect of folate on the association of the 677TT genotype with colorectal cancer in the Multiethnic Cohort Study.[3] However, because alcohol consumption is relatively low among women in this cohort, it may have reduced our ability to detect a stronger modifying effect of ethanol on the relationships of *MTHFR* with breast cancer. A number of subgroup analyses were conducted on the data, increasing the risk of chance findings, but these analyses addressed hypotheses that were formulated a priori. Finally, the current sample size does not allow for an adequate test of heterogeneity of effects across ethnic groups. Nevertheless, it is interesting to note that Japanese women, for whom the main effect of the 677TT genotype was significant, had the highest rates of current HRT use and alcohol abstinence at baseline, among all ethnic groups in the Multiethnic Cohort Study (16).

In conclusion, the present study provides evidence for only a weak overall association between the *MTHFR* 677TT genotype and postmenopausal breast cancer. However, the observation of a stronger and statistically significant effect among HRT users provides support for an inverse association among women with increased mammary epithelial cell proliferation. Replication of these findings and the investigation of *MTHFR* and other end points (e.g., cardiovascular diseases) among HRT users should be pursued in other large studies and in particular, in existing clinical trials on HRT.

[3] In preparation.

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References

1. Chen J, Giovannucci E, Kelsey K, et al. A methyltetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res 1996;56:4862–4.
2. Ames B. Cancer prevention and diet: help from single nucleotide polymorphisms. PNAS 1999;96:12216–8.

3. Weisberg I, Tran P, Christensen B, Sibani R, Rozen R. A second polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998; 64:169–72.
4. Choi S-W, Mason JB. Folate status: effects on pathways of colorectal carcinogenesis. J Nutr 2002;132:2413–8S.
5. Zhang S, Hunter DJ, Hankinson SE, et al. A prospective study of folate intake and the risk of breast cancer. JAMA 1999;281:1632–7.
6. Zhang SM, Willett WC, Selhub J, et al. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. J Natl Cancer Inst 2003;95:373–80.
7. Rohan TE, Jain MG, Howe GR, Miller AB. Dietary folate consumption and breast cancer risk. J Natl Cancer Inst 2000;92:266–9.
8. Sellers TA, Kushi LH, Cerhan JR, et al. Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. Epidemiology 2001;12:420–8.
9. Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR, Selhub J. A prospective study on folate, B12, and pyridoxal 5′-phosphate (B6) and breast cancer. Cancer Epidemiol Biomarkers Prev 1999;8:209–17.
10. Gershoni-Baruch R, Dagan E, Israeli D, Kasinetz L, Kadouri E, Friedman E. Association of the C677T polymorphism in the *MTHFR* gene with breast and/or ovarian cancer risk in Jewish women. Eur J Cancer 2000;36:2313–6.
11. Sharp L, Little J, Schofield AC, et al. Folate and breast cancer: the role of polymorphism in methylenetetrahydrofolate reductase (MTHFR). Cancer Lett 2002;181:65–71.
12. Campbell IG, Baxter SW, Eccles DM, Choong DYH. Methylenetetrahydrofolate reductase polymorphism and susceptibility to breast cancer. Breast Cancer Res 2002;4:R14.
13. Semenza JC, Delfino RJ, Ziogas A, Anton-Culver H. Breast cancer risk and *methylenetetrahydrofolate reductase* polymorphism. Breast Cancer Res Treat 2003;77:217–23.
14. Langsenlehner U, Krippl P, Renner W, et al. The common 677C>T gene polymorphism of methylenetetrahydrofolate reductase gene is not associated with breast cancer risk. Breast Cancer Res Treat 2003;81:169–72.
15. Kolonel LN, Henderson BE, Hankin JH, et al. A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. Am J Epidemiol 2000;151:346–57.
16. Pike MC, Kolonel LN, Henderson BE, et al. Breast cancer in a multiethnic cohort in Hawaii and Los Angeles: risk factor-adjusted incidence in Japanese equals and in Hawaiians exceeds that in Whites. Cancer Epidemiol Biomarkers Prev 2002;11:795–800.
17. Stram DO, Hankin JH, Wilkens LR, et al. Calibration of the dietary questionnaire for a multiethnic cohort in Hawaii and Los Angeles. Am J Epidemiol 2000;151:358–70.
18. Murphy SP, Wilkens LR, Hankin JH, et al. Comparison of two instruments for quantifying intake of vitamin and mineral supplements: a brief questionnaire versus three 24-hour recalls. Am J Epidemiol 2002;156:669–75.
19. Lee LG, Connell CR, Bloch W. Allelic discrimination by nick-translation PCR with fluorogenic probes. Nucleic Acids Res 1993; 21:3761–6.
20. Breslow NE, Day NE. Statistical methods in cancer research. Vol. 1. The analysis of case-control studies. IARC Sci Publ No. 32. Lyon: IARC; 1980.
21. Nei M. Molecular evolutionary genetics. New York: Columbia University Press; 1987.
22. Warnes GR. The genetics package. R News 2003;3:9–13.
23. Ardlie KG, Kruglyak L, Seielstad MK. Patterns of linkage disequilibrium in the human genome. Nat Rev Genet 2002;3:299–309.
24. Le Marchand L, Donlon T, Hankin JH, Kolonel LN, Wilkens LR, Seifried A. B-vitamin intake, metabolic genes and colorectal cancer risk. Cancer Causes Control 2002;13:239–48.
25. Shrubsole MJ, Jin F, Dai Q, et al. Dietary folate intake and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Res 2001;61:7236–42.
26. Little J, Sharp L, Duthie S, Narayanan S. Colon cancer and genetic variation in folate metabolism: the clinical bottom line. J Nutr 2003;133:3758–66S.
27. Conner P, Soderqvist G, Skoog L, et al. Breast cell proliferation in postmenopausal women during HRT evaluated through fine needle aspiration cytology. Breast Cancer Res Treat 2000;78:159–65.
28. Hulley SB, Grady D. The WHI estrogen-alone trial: do things look any better? JAMA 2004;291:1769–71.
29. Madsen JS, Kristensen SR, Klitgaard NA, et al. Effect of long-term hormone replacement therapy on plasma homocysteine in postmenopausal women: a randomized controlled trial. Am J Obstet Gynecol 2002;187:33–9.
30. Somekawa Y, Kobayashi K, Tomura S, Aaso T, Hamaguchi H. Effects of hormone replacement therapy and methylenetetrahydrofolate reductase polymorphism on plasma folate and homocysteine levels in postmenopausal Japanese women. Fertil Steril 2002;77:481–6.
31. Morris MS, Jacques PF, Selhub J, Rosenberg IH. Total homocysteine and estrogen status indicators in the Third National Health and Nutrition Examination Survey. Am J Epidemiol 2000;152:140–8.

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