Association of Polymorphisms in One-Carbon Metabolism Genes and Postmenopausal Breast Cancer Incidence

Victoria L. Stevens, Marjorie L. McCullough, Alexandre L. Pavluck, Jeffrey T. Talbot, Heather S. Feigelson, Michael J. Thun, and Eugenia E. Calle

Department of Epidemiology and Surveillance Research, American Cancer Society, Atlanta, Georgia

Abstract

The interconversion of folates by the one-carbon metabolism pathway is essential for the synthesis of precursors used in DNA synthesis, repair, and methylation. Perturbations in this pathway can disrupt these processes and are hypothesized to facilitate carcinogenesis. We investigated associations of 25 candidate polymorphisms in nine one-carbon metabolism genes with risk of postmenopausal breast cancer using 502 cases and 505 controls from the Cancer Prevention II Nutrition Cohort. Four single nucleotide polymorphisms (SNP) in three different genes were significantly associated with breast cancer. The nonsynonymous R134K SNP in *methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthase* [*MTHFD1*; odds ratio (OR), 1.40; 95% confidence interval (95% CI), 1.06-1.85 for CT + TT] and an intronic SNP in *formyltetrahydrofolate dehydrogenase* (*FTHFD*; OR, 2.23; 95% CI, 1.09-4.54 for CC) were associated with a significant increase in risk. Significantly decreased risk was associated with an intronic SNP in *FTHFD* (OR, 0.75; 95% CI, 0.58-0.98 for CT + CC) and the A360A SNP in *cystathionine β-synthase* (*CBS*; OR, 0.63; 95% CI, 0.41-0.96 for TT). The presence of at least one variant from both the *methylenetetrahydrofolate reductase* (*MTHFR*) C677T and A1298C SNPs was also associated with increased risk (OR, 2.16; 95% CI, 1.34-3.48 for 677 CT + TT/1,298 AC + CC). Investigations into interactions of the associated SNPs with each other and with dietary factors yielded inconclusive results. Our findings indicate that genetic variation in multiple one-carbon metabolism genes may influence risk of postmenopausal breast cancer and may involve changes in methyl donor synthesis. However, larger studies are needed to further examine gene/gene and gene/diet interactions in this pathway. (Cancer Epidemiol Biomarkers Prev 2007; 16(6):1140–7)

Introduction

Breast cancer is the most common incident cancer and second most common fatal cancer among women in the United States. The American Cancer Society estimates that 178,480 new cases of invasive breast cancer and 40,460 deaths from breast cancer will occur in 2007 (1). Many established risk factors for breast cancer, which include age, family history, and other factors such as parity and age at menarche that influence estrogen exposure, are not readily modifiable. Therefore, there is considerable interest in identification of risk factors that can be modified to reduce risk of breast cancer. Among those for which evidence of protective role in this cancer has been accumulating is the B vitamin folate.

As an essential nutrient, folate is needed for DNA synthesis, repair, and methylation. A complex set of reactions (shown in Fig. 1), which cumulatively are known as one-carbon metabolism, convert folate into the forms needed for the various reactions by changing its oxidation status and adding or removing one-carbon groups. Other nutrients, including vitamin B_6, vitamin B_{12}, and methionine, are also required for this pathway. Because all of these reactions are interconnected, an insufficient supply of one substrate can alter the levels of numerous other metabolites generated by the pathway. Folate deficiency can compromise nucleotide synthesis, leading to an imbalance in the levels of dUMP and dTMP and the subsequent misincorporation of uracil into DNA in place of thymidine (2). This misincorporated uracil is poorly repaired and can lead to mutations and chromosomal breaks (3). Perturbations in folate metabolism can also compromise the synthesis of *S*-adenosylmethionine, the donor of the methyl group for DNA methylation. The DNA hypomethylation that results (4) from limiting availability of *S*-adenosylmethionine can potentially alter gene expression and decrease chromosomal stability. All of these mechanisms contribute to genetic instability and may facilitate carcinogenesis, thus leading to the hypothesis that imbalances in folate metabolism can influence cancer risk.

The findings of numerous studies that investigated the association of folate with breast cancer incidence were reviewed and subjected to meta-analyses (5). The summary odds ratio (OR) from 13 case-control studies [OR, 0.91; 95% confidence intervals (95% CI), 0.87-0.96] suggested that increased folate was associated with decreased risk of breast cancer (5). No evidence for any association with breast cancer was found from nine cohort studies (OR, 0.99; 95% CI, 0.98-1.01). However, five of the cohort studies found that higher folate intake attenuated the increased risk associated with alcohol consumption (6-10), which inhibits folate absorption (11). Numerous factors could contribute to variability in the findings of these studies, including differences in the study populations, variation in the detail of the dietary assessment and the ranges of folate intakes, unaccounted for effects of folate fortification, inconsistent consideration of the disparate bioavailability of various folates, or differential sensitivities to the consequences of low folate intakes because of genetic variation in the one-carbon metabolism enzymes.

Several studies have addressed this latter possibility, focusing primarily on two common nonsynonymous single nucleotide polymorphisms (SNP) in the *methylenetetrahydrofolate reductase* (*MTHFR*) gene: C677T and A1298C. Neither of these SNPs were found to be associated with breast cancer risk by a meta-analysis of 17 studies (5) or a more recent case-control study not included in the meta-analysis (12). Other

Received 12/11/06; revised 2/22/07; accepted 3/19/07.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Victoria L. Stevens, Department of Epidemiology and Surveillance Research, American Cancer Society, 1599 Clifton Road, Northeastern, Atlanta, GA 30329. Phone: 404-329-5197; Fax: 404-327-6450. E-mail: Victoria.Stevens@cancer.org

doi:10.1158/1055-9965.EPI-06-1037

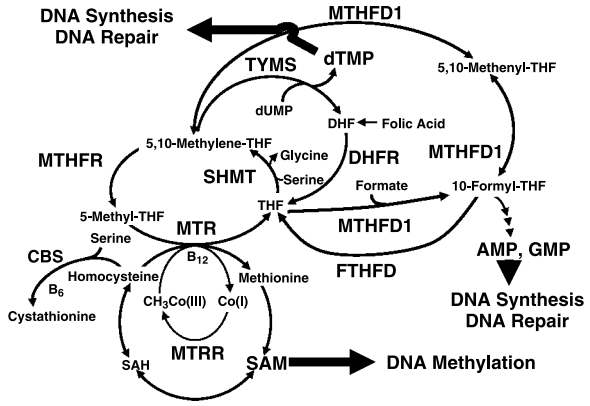

Figure 1. The major reactions in one-carbon metabolism. The nine genes investigated in this study are designated in capital letters next to the reactions catalyzed by the proteins they encode. Several nutrients involved in this pathway are also shown.

genes in the one-carbon metabolism pathway have been studied much less thoroughly, if at all. Investigations of an insertion-deletion and a tandem repeat polymorphism in the *thymidylate synthase* (*TYMS*) gene (13, 14), a nonsynonymous SNP in the *methyltetrahydrofolate homocysteine methyltransferase* (*MTR*) gene (14, 15), and a nonsynonymous SNP in the *methyltetrahydrofolate homocysteine methyltransferase reductase* (*MTRR*) gene (15) found no associations with breast cancer risk.

To gain a more comprehensive assessment of the potential importance of variation in one-carbon metabolism genes, we have examined the association of polymorphisms in nine genes in this pathway with postmenopausal breast cancer. These genes were *MTHFR, MTR, MTRR, TYMS, serine hydroxymethyltransferase* (*SHMT1*), *cystathionine β-synthase* (*CBS*), *dihydrofolate reductase* (*DHFR*), *methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthase* (*MTHFD1*), and *formyltetrahydrofolate dehydrogenase* (*FTHFD*; also known as *ALDH1L1*). This study was done using cases and controls nested in the American Cancer Society Cancer Prevention Study II (CPS-II) Nutrition Cohort. In this cohort, folate intake was previously found to attenuate the risk of postmenopausal breast cancer associated with high alcohol consumption but not to be associated with altered risk of this cancer itself (16). Therefore, potential effect modification by dietary factors that influence one-carbon metabolism and gene-gene interactions was also examined.

Materials and Methods

Study Population. The women in this study were drawn from participants in the CPS-II Nutrition Cohort, a prospective study of cancer incidence of ~184,000 U.S. adults. Nutrition Cohort participants, who were from 21 states and ranged from 50 to 74 years old at enrollment in 1992 or 1993, completed a mailed questionnaire that included questions on demographics, diet, and other lifestyle factors. The recruitment and characteristics of this cohort have been described previously (17). Follow-up questionnaires were sent to all living Nutrition Cohort members in 1997 and every 2 years afterwards to update exposure information and to ascertain newly diagnosed cases of cancer. Incident cases reported via questionnaire response were verified through medical records, linkage with state cancer registries, or death certificates. Blood samples were collected from a subset of Nutrition Cohort participants (21,965 women and 17,411 men) between June 1998 and June 2001. Blood samples were fractionated into serum, plasma, buffy coat, and RBC and stored in liquid nitrogen vapor phase at −130°C until needed for analysis.

From among the women who had provided a blood sample, 509 postmenopausal women who were diagnosed with breast cancer between 1992 and 2001 and had no history of any other cancer (except nonmelanoma skin cancer) were identified. An equal number of controls were matched to cases on age (±6 months), race/ethnicity (White, African American, Hispanic, Asian, or other/unknown), and date of blood collection (±6 months). Controls were selected from women who were cancer-free at the time of diagnosis of the matching case using risk-set sampling (18). Seven of the cases and four of the controls originally selected were excluded from the analysis because, on more detailed review, they were found to have a compromised DNA sample, to not be postmenopausal, or to not have breast cancer (if a case). Thus, 502 cases and 505 controls were included in this study. Of the cases, 375 women had invasive breast cancer (stage I or higher); the remainder had *in situ* cancer.

Because the questionnaire information on demographic characteristics, reproductive history, medication use, and personal and family history was collected at enrollment in 1992 to 1993, exposure information predated the cancer diagnosis for all cases.

Polymorphism Selection and Genotyping. Polymorphisms were selected from those available from either the dbSNP[1] or Celera databases (information accessed through Applied Biosystems, Inc.) in June, 2004 using the following criteria: (*a*) all had a minor allele frequency in Caucasians > 5%, and (*b*) they caused nonsynonymous changes in the amino acid sequence, or (*c*) they had been previously studied and found to be associated with disease. For some genes for which no or only one polymorphism were identified using these criteria, one or two additional SNPs were selected if available frequency information indicated they occurred in at least 10% of Caucasians. A total of 25 polymorphisms, of which one was a tandem repeat, and the remainder were SNPs, were genotyped in this study.

Genotyping of the SNPs was done using Taqman at Applied Biosystems, Inc. The *TYMS* 28-bp tandem repeat was genotyped at Applied Biosystems by PCR amplification of the repeat sequences followed by gel electrophoresis to separate the different-sized products as described by Hishida et al. (19). The success rate for all the genotyping was >96%, and the genotype distributions of the controls for all the SNPs except the *MTHFR* C677T (rs1801133) were in Hardy-Weinberg equilibrium ($P > 0.05$). Because no evidence of a genotyping error was found after examining the Taqman scatter plots, and because the genotype distribution of the *MTHFR* C677T SNP (Hardy-Weinberg equilibrium, $P = 0.014$) could arise by chance, the results for this variant were not excluded from the study.

Dietary Assessment. Dietary intakes of folate, ethanol, and other nutrients were assessed at enrollment using a semiquantitative, 68-item Food Frequency Questionnaire (FFQ), which was a modification of the brief ''Health Habits and History Questionnaire'' developed by Block et al. (17, 20). The FFQ inquired about portion size and frequency of consumption of a variety of foods and use and frequency of vitamin supplements. Daily nutrient intakes from diet and supplements were estimated using the Diet Analysis System, version 3.8a (21). Dietary folate was derived primarily from leafy green vegetables, fruit juice, bran and granola cereals, and fortified cereals. Total folate was estimated by combining dietary and supplemental folate intake, assuming that each multivitamin

[1] http://www.ncbi.nlm.nih.gov/SNP/

contained 400 μg folic acid. Nutrient values were energy adjusted using the residuals method (22).

The FFQ was validated among 441 Nutrition Cohort participants who completed four 24-h dietary recall interviews and a repeat FFQ. The validity coefficient in women for dietary folate between the FFQ and dietary recall interviews was 0.43, and the reproducibility between the baseline FFQ and repeat FFQ was 0.66 (23).

Statistical Analysis. The statistical significance of the difference in allele distribution of each polymorphism between cases and controls was calculated using the χ^2 test with 1 degree of freedom. OR and 95% CI for the association between the folate gene polymorphisms and breast cancer incidence were determined using unconditional logistic regression. All models were adjusted for age (in single year categories), race (White, other), date of blood draw (in single year categories), history of breast cyst (yes, no, missing), hormone replacement therapy use (none, former estrogen replacement therapy, current estrogen replacement therapy, former combined hormone replacement therapy, current combined hormone replacement therapy, unknown), and combined age at first birth and parity (nulliparous, age 15 to <25 and 1 or 2 births, age ≥25 and 1 or 2 births, age 15 to <25 and 3-9 births, age ≥25 and 3-9 births, unknown).

Effect modification of the association between the SNPs and breast cancer was examined using the likelihood ratio test for dietary and total folate, vitamin B_{12}, vitamin B_6, methionine, fruit and vegetable intake (each using cutoffs below versus at or above median), multivitamin use (non-use versus any use), and alcohol intake (none versus any). We also examined polymorphisms according to ''high-risk'' diet group (below median dietary folate and methionine intake and consumers of any alcohol) versus ''low-risk'' diet group (above median dietary folate and methionine intake and a nondrinker).

Gene/gene interactions between pairs of SNPs with significant main effects were evaluated using the likelihood ratio test. The association of combinations of variants with breast cancer incidence was evaluated by classifying each subject by the total number of risk or protective alleles present. ''Risk'' alleles were defined as those alleles that were independently associated with an increased risk (*MTHFR* C677T T, *MTHFR* A1298C C, *MTHFD1* rs1950902 T, and *FTHFD* rs2276731 C), whereas protective alleles were those associated with a statistically significant decreased risk of breast cancer (*CBS* rs1801181 T and *FHTFD* rs2002287 C). OR and 95% CI were then calculated using unconditional logistic regression. The reference groups for these analyses were subjects with no protective alleles and, because very few women had no alleles associated with increased risk, a combination of women with 0 or 1 risk allele.

Results

The 25 polymorphisms genotyped in the nine folate pathway genes, their location in the gene, the associated amino acid change they caused, and the frequency of the minor allele in cases and controls are listed in Table 1. No significant difference in allele frequencies were found for the majority of the variants. However, as indicated by the unadjusted *P* values shown in this table, four SNPs differed significantly in prevalence of the minor allele between cases and controls. The multivariate-adjusted associations of these SNPs and the two frequently studied SNPs in the *MTHFR* gene with breast cancer incidence among all cases and invasive cases are shown in Table 2. The T allele of the *MTHFD1* R134K SNP and the CC genotype of the *FTHFD* rs2276731 (T/C) SNP were associated with significantly higher breast cancer risk (OR, 1.40; 95% CI, 1.06-1.85 for *MTHFD1* R134K CT + TT and OR, 2.23; 95% CI, 1.09-4.54 for *FTHFD* CC). The C allele of the *FTHFD* rs2002287 (T/C) SNP and the homozygous TT genotype of the *CBS* A360 SNP were associated with significantly lower breast cancer risk (OR, 0.75; 95% CI, 0.58-0.98 for *FTHFD* rs2002287 CT + CC and OR, 0.63; 95% CI, 0.41-0.96 for *CBS* A360A TT). Findings were similar in analyses restricted to invasive breast cancer.

Both the *MTHFR* C677T and A1298C SNPs alter the amino acid sequence of this protein and affect its enzymatic activity, and many studies have investigated the association of these polymorphisms with breast cancer risk (5, 12, 24). We found that the presence of the minor alleles for the C677T and A1298C SNPs in the *MTHFR* gene were associated with increased risk that was not statistically significant (Table 2). The associations of combinations of these polymorphisms with breast cancer risk are shown in Table 3. The presence of at least one variant allele of both SNPs (CT + TT/AC + CC) was associated with a higher risk of breast cancer (OR, 2.16; 95% CI, 1.34-3.48) than those with one or two copies of the variant allele of only one SNP. A similar association was observed when the analysis was limited to women with invasive breast cancer (data not shown).

We were unable to assess interactions among the six SNPs listed in Table 2 considering each polymorphism separately because many of the subgroups contained few cases and controls. As an alternative approach, we grouped alleles associated with increased (risk alleles) or decreased (protective alleles) of breast cancer to determine if having more of each type of allele strengthened the association (Table 4). The ''protective'' alleles were *FTHFD* rs2002287 (T/C) C and *CBS* A360A T, and women could have zero to four of these alleles. An increasing number of ''protective'' alleles was inversely associated with breast cancer risk (OR, 0.63; 95% CI, 0.24-1.66 for four alleles; P_{trend} = 0.008). For risk alleles, the *MTHFR* rs1801133 (C677T) T, *MTHFR* rs1801131 (A1298C) C, *MTHFD1* rs1950902 (R134K) T, and *FTHFD* rs2276731 (T/C) C were considered. Although it was theoretically possible to have up to 8 copies of these alleles, no women had more than five risk alleles. Women with zero and one risk allele were combined to increase the stability of the reference group. An increased number of risk alleles was found to be associated with greater risk of breast cancer than any individual SNP alone (OR, 2.13; 95% CI, 0.57-7.96 for five alleles; P_{trend} = 0.0001). Additional adjustment for the number of alleles of the opposite type had little effect on these associations.

A number of substrates and cofactors involved in one-carbon metabolism, including folate, vitamin B_{12}, vitamin B_6, and methionine, are obtained from the diet. Therefore, the influence of different levels of intake of these nutrients as well as other dietary factors that can affect their availability (i.e., alcohol) on the associations of the various SNPs with breast cancer risk was investigated. The *MTHFR* C677T/folate interactions and three other gene/diet interactions found to be statistically significant out of the 48 examined are shown in Table 5. Neither dietary nor total folate modified the association of the *MTHFR* C677T SNP with postmenopausal breast cancer. However, significant interactions were found for the *MTHFR* A1298C SNP and both total folate and alcohol. The presence of the variant allele was associated with higher risk only among women with total folate intake at or above the median (OR, 1.53; 95% CI, 1.12-2.09) and those who consumed alcohol (OR, 1.70; 95% CI, 1.15-2.52). The other significant interaction found was between the *FTHFD* rs2002287 (T/C) SNP and methionine. The protective effect of this SNP was greater in women who consumed the median level or higher of this amino acid (OR, 0.62; 95% CI, 0.45-0.85). No statistically significant interactions were found for the any of the other SNPs.

Evaluation of effect modification by the collective influence of dietary folate, methionine, and alcohol intake using median intake cut points revealed no clear relationships, possibly due to the limited sample sizes in the low- and high-risk groups (data not shown).

Discussion

In this nested case-control study of nine one-carbon metabolism genes, which is the most comprehensive investigation of this pathway in breast cancer to date, we found significant associations for four SNPs in three genes. Using $P < 0.05$ to define statistical significance, we expected to find at least one significant association among the 25 polymorphisms genotyped simply due to chance. The P values for the four significant (at $P < 0.05$) SNPs ranged from 0.022 to 0.048, and correction of these values for multiple comparisons using any method would result in all of these associations being judged as not statistically significant. However, it seems unlikely that all four of these associations are false positives. Therefore, rather than presenting our results corrected for multiple comparisons, we have chosen to interpret our results as indicating that some, but not all, of the four SNPs with $P < 0.05$ reflect true associations with breast cancer. However, we are unable to conclude which of the four are true positives using this approach.

The R134K SNP in the *MTHFD1* gene was associated with increased risk of breast cancer. MTHFD1 is a trifunctional cytosolic enzyme whose three activities can cooperate to generate 10-formyltetrahydrofolate, which donates its formyl group in two different steps in purine biosynthesis. The methylenetetrahydrofolate dehydrogenase and methenyltetrahydrofolate cyclohydrolase activities, which reside together in one domain of the protein, catalyze the oxidation of 5,10-methylenetetrahydrofolate to 5,10-methenyltetrahydrofolate, which is then converted to 10-formyltetrahydrofolate. The synthetase activity resides in a separate protein domain and catalyzes the synthesis of 10-formyltetrahydrofolate from formate and tetrahydrofolate. The R134K SNP changes an arginine to a lysine in the dehydrogenase/cyclohydrolase domain of MTHFD1 and may affect these activities. However, no studies have investigated the functional consequences of this SNP, and the only study to investigate the R134K SNP in relation to disease did not find an association with altered risk of neural tube defects (25). Altered dehydrogenase/cyclohydrolase activity could either affect the forward reaction and alter supply of formyltetrahydrofolate for purine synthesis or perturb the reverse reaction and influence the availability of 5,10-methylenetetrahydrofolate, which either could be used by MTHFR and feed into homocysteine remethylation or by TYMS for dTMP synthesis. Substrate binding and kinetic studies have suggested that the forward and reverse reactions of the MTHFD dehydrogenase/cyclohydrolase activities are in equilibrium (26, 27). Therefore, whether the availability of 5,10-methylenetetrahydrofolate or 10-formyltetrahydrofolate is affected would depend on the tissue levels of these folates and how the dehydrogenase and cyclohydrolase activities are perturbed by the polymorphism.

Two SNPs in the *FTHFD* gene (also known as *ALDH1L1*) were significantly associated with altered risk of breast cancer. FTHFD catalyzes the conversion of 10-formyltetrahydrofolate to tetrahydrofolate and CO_2. Despite the fact that 10-formyltetrahydrofolate, the substrate for FTHFD, is required for purine biosynthesis, altered expression of this enzyme does not influence nucleotide metabolism (28). Rather, changes in *FTHFD* expression have been found to regulate the levels of

Table 1. Characteristics and genotype frequencies of the folate gene polymorphisms analyzed in this study among cases and controls

Gene/SNP*	Location	AA change	Minor allele	Minor allele frequency		P †
				Cases	Controls	
MTHFR						
rs1801133 (C677T)	Exon 5	Val → Ala	T	35.2	32.7	0.24
rs1801131 (A1298C)	Exon 8	Ala → Glu	C	31.6	28.5	0.14
rs2066470 (T118C)	Exon 2	None	T	10.0	7.8	0.094
MTR						
rs1806505 (C/T)	Intron 13	NA	T	40.8	43.0	0.26
rs1805087 (A2756G)	Exon 26	Asp → Gly	G	18.1	17.9	0.91
rs1050993 (G/A)	3′ UTR	NA	A	38.1	35.4	0.21
MTRR						
rs1801394 (A66G)	Exon 2	Met → Ile	A	43.3	43.6	0.90
rs10380 (C1785T)	Exon 14	Tyr → His	T	8.7	10.1	0.29
SHMT1						
rs643333 (C/A)	5′ UTR	NA	A	29.1	28.9	0.90
rs2273028 (C/T)	Intron 7	NA	T	31.2	32.2	0.66
rs1979277 (C1420T)	Exon 12	Leu → Phe	A	31.0	31.2	0.92
MTHFD1						
rs1076991 (A/G)	5′ UTR	NA	G	45.8	46.5	0.76
rs1950902 (R134K)	Exon 6	Arg → Lys	T	19.2	15.8	0.048
rs2236224 (C/T)	Intron 21	NA	T	38.4	41.0	0.24
rs2236225 (R653Q)	Exon 21	Arg → Gln	T	43.1	45.4	0.30
TYMS						
rs502396 (T/C)	Intro 1	NA	C	48.7	49.8	0.56
rs699517 (C/T)	3′ UTR	NA	T	33.5	33.3	0.91
28-bp Tandem repeat	5′ UTR	NA	2r	48.4	48.5	0.97
DHFR						
rs1677693 (C/A)	Intron 3	NA	A	26.4	27.1	0.71
rs1643638 (T/C)	Intron 4	NA	C	26.2	27.5	0.52
FTHFD (*ALDH1L1*)						
rs2276731 (T/C)	Intron 4	NA	C	20.4	16.4	0.022
rs2002287 (T/C)	Intron 13	NA	C	34.2	38.8	0.034
rs1127717 (A2380C)	Exon 21	Asp → Gly	G	18.9	19.6	0.70
CBS						
rs234706 (Y233Y)	Exon 8	None	A	34.7	31.4	0.12
rs1801181 (A360A)	Exon 12	None	T	32.4	36.9	0.040

Abbreviations: NA, not applicable; UTR, untranslated region.
*The designation by which the SNP is commonly referred to in the literature, when one exists, is shown in parenthesis.
†P for the difference in minor allele frequencies was determined using the χ^2 test with 1 degree of freedom.

Table 2. Association of SNPs in folate pathway genes with incidence of breast cancer in all cases and invasive cases

Gene/SNP	All cases		Invasive cases*	
	No. cases/control	OR† (95% CI)	No. cases/control	OR† (95% CI)
MTHFD1				
rs1950902 (R134K)				
CC	316/353	1.00	241/353	1.00
CT	163/124	1.49 (1.12-1.99)	120/124	1.42 (1.04-1.93)
TT	13/16	0.77 (0.36-1.68)	11/16	0.88 (0.39-1.97)
CT + TT	176/140	1.40 (1.06-1.85)	131/140	1.35 (1.00-1.82)
FTHFD				
rs2276731 (T/C)				
TT	317/344	1.00	250/344	1.00
CT	157/136	1.23 (0.92-1.63)	109/136	1.10 (0.81-1.50)
CC	23/13	2.23 (1.09-4.54)	18/13	2.25 (1.06-4.76)
rs2002287 (T/C)				
TT	220/188	1.00	172/188	1.00
CT	209/229	0.76 (0.57-1.00)	156/229	0.72 (0.54-0.97)
CC	64/77	0.75 (0.50-1.11)	45/77	0.67 (0.43-1.03)
CT + CC	273/306	0.75 (0.58-0.98)	201/306	0.71 (0.54-0.94)
CBS				
rs1801181 (A360A)				
CC	216/194	1.00	168/194	1.00
CT	218/214	0.92 (0.70-1.22)	161/214	0.87 (0.65-1.18)
TT	47/69	0.63 (0.41-0.96)	33/69	0.56 (0.35-0.91)
MTHFR				
rs1801133 (C677T)				
CC	208/236	1.00	160/236	1.00
CT	224/193	1.34 (1.02-1.77)	167/193	1.27 (0.94-1.72)
TT	62/65	1.09 (0.72-1.63)	47/65	1.06 (0.68-1.64)
CT + TT	286/258	1.28 (0.98-1.66)	214/258	1.22 (0.92-1.61)
rs1801131 (A1298C)				
AA	224/252	1.00	167/252	1.00
AC	228/201	1.28 (0.97-1.67)	173/201	1.31 (0.97-1.75)
CC	42/40	1.09 (0.67-1.78)	35/40	1.25 (0.75-2.10)
AC + CC	270/241	1.25 (0.96-1.62)	208/241	1.30 (0.98-1.72)

*Invasive cases include all but *in situ* breast cancers.
†ORs were determined using unconditional logistic regression adjusting for age, race, date of blood draw, history of breast cyst, hormone replacement therapy, and parity.

5-methyltetrahydrofolate and the subsequent remethylation of homocysteine to methionine (28). One *FTHFD* SNP [rs2276731 (T/C)] was associated with increased risk (OR, 2.23; 95% CI, 1.09-4.54 for CC versus TT), whereas the other [rs2002287 (T/C)] was associated with decreased risk (OR, 0.75; 95% CI, 0.58-0.98 for CT + CC versus TT). Neither of these SNPs is expected to alter FTFHD activity directly because they are both in introns. Therefore, it is expected that they are in linkage disequilibrium with functional polymorphisms. These two SNPs are in linkage disequilibrium with each other ($D' = 1.0$, $r^2 = 0.082$) and with three nonsynonymous SNPs: rs4646750 (V812I), rs2276724 (G481S), and rs2886059 (F330V). Any of these SNPs may be responsible for the associations found here. The FTHFD protein has three separate domains responsible for different parts of the enzymatic reaction and its regulation (29). Therefore, our finding of two different SNPs in this gene being associated with breast cancer risk in disparate ways is not unreasonable because they could influence different properties of this enzyme. There have been no previous studies of the association of this gene with any cancer.

The A360A SNP in the *CBS* gene was found to be associated with a reduced risk of breast cancer. CBS catalyzes the vitamin B_6–dependent trans-sulfuration reaction between serine and homocysteine that results in the formation of cystathionine.

Table 3. Association of genotype combinations of the *MTHFR* C677T and A1298C SNPs with breast cancer

Genotypes		All cases	
C677T	A1298C	No. cases/control	OR* (95% CI)
CC	AA	49/76	1.00 (reference)
CC	AC + CC	155/157	1.53 (0.99-2.38)
CT + TT	AA	172/174	1.56 (1.02-2.40)
CT + TT	AC + CC	111/82	2.16 (1.34-3.48)

*ORs were calculated using unconditional logistic regression adjusting for age, race, date of blood draw, history of breast cyst, hormone replacement therapy, and parity.

Table 4. Combined effect of genotypes from protective or risk alleles on the risk of breast cancer in all cases

Allele type/number	Cases	Controls	OR* (95% CI)	OR† (95% CI)
Protective‡				
0	99	77	1.00 (reference)	1.00 (reference)
1	187	164	0.87 (0.60-1.27)	0.89 (0.61-1.30)
2	130	147	0.67 (0.46-1.00)	0.70 (0.47-1.04)
3	50	70	0.58 (0.36-0.94)	0.64 (0.39-1.05)
4	8	11	0.63 (0.24-1.66)	0.70 (0.26-1.89)
			$P_{trend} = 0.008$	$P_{trend} = 0.029$
Risk§				
0 + 1	132	183	1.00 (reference)	1.00 (reference)
2	178	172	1.49 (1.08-2.04)	1.44 (1.05-1.98)
3	129	94	1.89 (1.31-2.71)	1.83 (1.27-2.64)
4	33	24	1.93 (1.07-3.48)	1.75 (0.96-3.17)
5	6	4	2.13 (0.57-7.96)	1.95 (0.52-7.30)
			$P_{trend} = 0.0001$	$P_{trend} = 0.0005$

*ORs were adjusted for age, race, date of blood draw, history of breast cyst, hormone replacement therapy, and parity.
†ORs were adjusted for age, race, date of blood draw, history of breast cyst, hormone replacement therapy, parity, and the number of alleles of the opposite type (protective or risk).
‡Protective alleles: *CBS* rs1801181 (A360A) T and *FHTFD* rs2002287 (T/C) C.
§Risk alleles: *MTHFR* rs1801133 (C677T) T, *MTHFR* rs1801131 (A1298C) C, *MTHFD1* rs1950902 (R134K) T, and *FTHFD* rs2276731 (T/C) C.

Table 5. Influence of dietary factors on the association of the *MTHFR* C677C, A1298C and *FTHFD* rs2002287 (T/C) SNPs with breast cancer

	Dietary folate			
	<Median		≥Median*	
Genotype	No. cases/control	OR† (95% CI)	No. cases/control	OR† (95% CI)
MTHFR C677T				
CC	100/116	1.00 (reference)	103/108	1.00 (reference)
CT + TT	124/121	1.17 (0.84-1.63)	145/129	1.23 (0.91-1.88)
		$P_{interaction}$ = 0.48		
	Total folate			
	<Median		≥Median*	
Genotype	No. cases/control	OR† (95% CI)	No. cases/control	OR† (95% CI)
MTHFR C677T				
CC	95/116	1.00 (reference)	108/108	1.00 (reference)
CT + TT	110/120	1.08 (0.77-1.52)	159/130	1.31 (0.97-1.78)
		$P_{interaction}$ = 0.067		
MTHFR A1298C				
AA	98/117	1.00 (reference)	113/126	1.00 (reference)
AC + CC	106/120	1.05 (0.75-1.48)	155/111	1.53 (1.12-2.09)
		$P_{interaction}$ = 0.03		
	Alcohol intake			
	None		Any	
Genotype	No. cases/control	OR† (95% CI)	No. cases/control	OR† (95% CI)
MTHFR A1298C				
AA	132/122	1.00 (reference)	92/130	1.00 (reference)
AC + CC	154/145	0.96 (0.68-1,36)	116/96	1.70 (1.15-2.52)
		$P_{interaction}$ = 0.03		
	Methionine intake			
	<Median		≥Median*	
Genotype	No. cases/control	OR† (95% CI)	No. cases/control	OR† (95% CI)
FTHFD rs2002287 (T/C)				
TT	107/90	1.00 (reference)	104/87	1.00 (reference)
CT + CC	141/143	0.83 (0.60-1.15)	119/154	0.62 (0.45-0.85)
		$P_{interaction}$ = 0.035		

*Above median groups include any individuals whose values are at the median.
†ORs were calculated using unconditional logistic regression adjusting for age, race, date of blood draw, history of breast cyst, hormone replacement therapy, and parity.

This reaction serves to metabolize excess homocysteine, and mutations in the *CBS* gene are the most common inborn errors of metabolism that result in hyperhomocysteinemia (30). The A360A SNP causes no change in the CBS protein and has no apparent functional consequence. However, this SNP has been inconsistently linked to changes in blood levels of homocysteine (31-33) and associated with a significant increase in risk of lung cancer (34). Thus, the A360A SNP has been proposed to be in linkage disequilibrium with another polymorphism, which has not yet been identified, that is actually responsible of changes in CBS activity (32, 34). No common nonsynonymous SNPs have been identified in the *CBS* gene; thus, the polymorphism responsible for the association of the A360A SNP may influence the expression of this gene.

No significant association was found for either of the two commonly studied SNPs in the *MTHFR* gene. However, a significant increase in risk was associated with the presence of at least one variant allele from both the C677T and A1298C SNPs. Whether the risk increases with additional copies of the variant alleles of these SNPs cannot be determined because the strong linkage disequilibrium between these polymorphisms (D' = 0.79-0.95 in Caucasian populations; ref. 35) limits the occurrence of these genotype combinations. MTHFR catalyzes the irreversible reduction of 5,10-methyltetrahydrofolate to 5-methyltetrahydrofolate, which is the folate used for the remethylation of homocysteine to methionine. The C677T SNP changes an alanine to valine and results in production of a ''thermolabile'' enzyme with reduced activity (36, 37). This variant allele has been associated with increased levels of serum or plasma homocysteine and numerous diseases, including several cancers (reviewed in ref. 38). The A1298C SNP leads to a substitution of an alanine for a glutamic acid (39) and also reduces MTHFR activity (40). Rather than indicate which SNP is more important, our findings suggest that reduced MTHFR activity is linked to increased risk of breast cancer. The null main effect found here for the C677T and A1298C polymorphisms independently is consistent with the results of many of the previous studies (5, 12). However, our finding of increased risk for the combined genotypes is different from all but one (41) of the five previous studies in which both *MTHFR* SNPs were investigated. One study found no association (42), whereas three observed a protective effect (14, 43, 44) when variant alleles from both SNPs were present. Why our results are different from the majority of other studies is not readily apparent but could be due to differences in ethnicity (42, 44), menopausal status (14, 42-44),

background dietary status (42, 43) of some or all of the study subjects, or chance. Furthermore, the value of comparison of our results for the *MTHFR* C677T and A1298C genotype combinations to previous findings is limited because only 5 of 18 studies of this gene in breast cancer (24) have evaluated both SNPs.

One of the major challenges for understanding how perturbations in one-carbon metabolism could influence cancer development is determining which of the cellular processes dependent on this pathway is responsible for the altered risk. Most studies have focused on polymorphisms in *MTHFR* and have suggested that changes in the generation of methyl donors for DNA methylation are responsible for the altered risk of cancer development (45). Our findings of increased risk associated with the *MTHFR* C677T/A1298C genotype combinations and reduced risk associated with the *CBS* gene are consistent with the hypothesis that reduced formation of methyl donors increases risk of breast cancer. For the *CBS* A360A SNP, methylation capacity should be increased in individuals with decreased activity of this enzyme because more homocysteine will be available for remethylation to methionine. Whether the associations of the *MTHFD1* and *FTHFD* SNPs with altered risk of breast cancer are consistent with this hypothesis is unclear because the effect of these polymorphisms on enzyme activity and metabolite levels is not known.

We were unable to investigate interactions between the genes found associated with breast cancer directly because of the small numbers for some genotype combinations. However, we found evidence for cumulative effects of either protective or risk alleles using an allele counting approach. Interpretation of these findings can be difficult because the same results used to define protective and risk alleles are used for the allele counting trend analysis. Increasing numbers of protective alleles from the *CBS* A360A and *FTHFD* rs2002287 (T/C) SNPs yielded a significant trend ($P = 0.008$) with an OR of 0.63 (95% CI, 0.24-1.66) for four of these alleles. This association was of a similar magnitude as found for the *CBS* A360A TT genotype alone and could potentially reflect this SNP alone. However, 53% of the women with three protective alleles did not have the *CBS* A360A TT genotype, suggesting that alleles from the *FTHFD* SNP contributed to the decreased risk observed in these women. Similarly, with the risk alleles, the trend found for increasing number of alleles was highly significant ($P = 0.0001$), but the magnitude of the association was similar to that found for the *FTHFD* rs2276731 (T/C) CC genotype and the *MTHFR* C677T CT + TT/A1298C AC + CC genotype combinations. The increased risk associated with four or five risk alleles could represent the influence of these associations alone. However, 49% of the women with four risk alleles and 20% of those with five risk alleles do not have either the *FTHFD* rs2276731 (T/C) CC genotype or *MTHFR* C677T CT + TT/A1298C AC + CC genotype combination. Therefore, the other risk alleles seem to contribute to this increased risk.

We found few significant interactions between the SNPs associated with altered risk of breast cancer and dietary factors expected to influence one-carbon metabolism. Bonferroni correction of the P values of the three interactions shown in Table 5 results in all having $P_{interaction} = 1$, indicating that these findings are likely to be due to chance. Previous studies that investigated dietary interactions with the *MTHFR* C677T and the A1298C SNPs in breast cancer have yielded no consistent findings (41-44, 46), and our findings provide no clarification as to whether dietary factors can modify the association of variants in one-carbon metabolism genes on breast cancer risk. Although it may be that there are no interactions of the dietary factors with the folate gene SNPs, results from studies of *MTHFR* SNPs in colorectal cancer (reviewed in ref. 45) and the fact that folate intake has been associated with altered risk of breast cancer (47-50) suggest that significant interactions are possible. Our analyses included fairly narrow ranges of intakes that limited our ability to examine very low or very high levels of a nutrient. Alternatively, multiple dietary factors may be interacting with the genetic variants at the same time. Assessment of a interaction involving multiple dietary factors, such as found by Giovannucci et al. (51) with colorectal cancer, was investigated but proved uninformative because too few women had dietary patterns expected to be either the best or worst for one-carbon metabolism.

This study is the most comprehensive of one-carbon metabolism genes in breast cancer done to date and, for the *FTHFD* gene and many of the SNPs, is the first to investigate their role in any cancer. An additional strength of this study is availability of prospectively collected information on potential dietary interactions.

A primary limitation of the study is the sample size, which limits our ability to detect statistically significant main effects after correction for multiple comparisons and to evaluate gene-gene and gene-environment interactions. An additional key limitation is the use of only two to four candidate polymorphisms per gene, which prohibits us from conclusively evaluating the role of these one-carbon metabolism genes in determining the risk of postmenopausal breast cancer. The lack of information on the functional consequences of many of the nonsynonymous SNPs also limits the interpretation of some of the results. Additional limitations to the study are the incompleteness of the dietary assessment with the 68-item FFQ and lack of validation of nutrient levels with blood biomarkers. Furthermore, the time frame of the study straddles the implementation of folate fortification in the United States (fully implemented in 1998), which may contribute to measurement error.

In summary, variants in four genes involved in one-carbon metabolism were associated with altered risk of postmenopausal breast cancer in our study. The increased risk associated with the *MTHFR* C677T and A1298C SNP combinations and the decreased risk for the *CBS* A360A SNP are consistent with the hypothesis that changes in the methyl donor synthesis can influence breast cancer development. Larger studies of one-carbon metabolism in which tag-SNPs are selected to comprehensively cover the genes of interest and gene/gene and gene/environment interactions can be assessed are needed.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43–66.
2. Wickramasinghe SN, Fida S. Bone marrow cells from vitamin B12- and folate-deficient patients misincorporate uracil into DNA. Blood 1994;83:1656–61.
3. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A 1997;94:3290–5.
4. Balaghi M, Wagner C. DNA methylation in folate deficiency: use of CpG methylase. Biochem Biophys Res Commun 1993;193:1184–90.
5. Lewis SJ, Harbord RM, Harris R, Smith GD. Meta-analyses of observational and genetic association studies of folate intakes or levels and breast cancer risk. J Natl Cancer Inst 2006;98:1607–22.
6. Baglietto L, English DR, Gertig DM, Hopper JL, Giles GG. Does dietary folate intake modify effect of alcohol consumption on breast cancer risk? Prospective cohort study. Br Med J 2005;331:807–11.
7. Zhang S, Hunter DJ, Hankinson SE, et al. A prospective study of folate intake and the risk of breast cancer. J Am Med Assoc 1999;281:1632–7.
8. Rohan TE, Jain MG, Howe GR, Miller, AB. Dietary folate consumption and breast cancer risk. J Natl Cancer Inst 2000;92:266–9.
9. 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.
10. Zhang S, Willet WC, Selhub J, et al. Plasma folate, vitamin B_6, vitamin B_{12}, homocysteine, and risk of breast cancer. J Natl Cancer Inst 2003;95:373–80.
11. Halsted CH, Villanueva JA, Devlin AM, Chandler CJ. Metabolic interactions of alcohol and folate. J Nutr 2002;132:2367–72S.
12. Martin YN, Olson JE, Ingle JN, et al. Methylenetetrahydrofolate reductase haplotype tag single-nucleotide polymorphisms and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2006;15:2322–4.

13. Grieu F, Powell B, Beilby J, Iacopetta B. Methylenetetrahydrofolate reductase and thymidylate synthase polymorphisms are not associated with breast cancer risk or phenotype. Anticancer Res 2004;24:3215–9.
14. Justenhoven C, Hamann U, Pierl CB, et al. One-carbon metabolism and breast cancer risk: no association of MTHFR, MTR, and TYMS polymorphisms in the GENICA study from Germany. Cancer Epidemiol Biomarkers Prev 2005;14:3015–8.
15. Shrubsole MJ, Gao YT, Cai Q, et al. MTR and MTRR polymorphisms, dietary intake, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2006;15: 586–8.
16. Feigelson HS, Jonas CR, Robertson AS, McCullough ML, Thun MJ, Calle EE. Alcohol. folate, methionine, and risk of incident breast cancer in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. Cancer Epidemiol Biomarkers Prev 2003;12:161–4.
17. Calle EE, Rodriguez C, Jacobs EJ, et al. The American Cancer Society Cancer Prevention Study II Nutrition Cohort. Cancer 2002;94:2490501.
18. Rothman KJ, Greenland S. Modern Epidemiology. Baltimore: Lippincott, Williams and Wilkins; 1998.
19. Hishida A, Matsuo K, Hamajima N, et al. Association between polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and susceptibility to malignant lymphoma. Haematol 2003;88:159–66.
20. Block G, Hartman A, Naughton D. A reduced dietary questionnaire: development and validation. Epidemiology 1990;1:58–64.
21. Block G, Coyl L, Smucker R, Harlan L. Health habits and history questionnaire: diet history and other risk factors. Personal computer system documentation. Bethesda (MD): National Cancer Institute Division of Cancer Prevention and Control, National Institutes of Health; 1989.
22. Willet W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
23. Flagg EW, Coates RJ, Calle EE, Potischman N, Thun MJ. Validation of the American Cancer Society Cancer Prevention Study II Nutrition Survey cohort food frequency questionnaire. Epidemiology 2000;11:462–8.
24. Macis D, Maisonneuve P, Johansson H, et al. Methylenetetrahydrofolate reductase (MTHFR) and breast cancer risk: a nested-case-control study and a pooled meta-analysis. Breast Cancer Res Treat. Epub 2007 Apr 24.
25. Brody LC, Conley M, Cox C, et al. A polymorphism, R653Q, in the trifunctional enzyme methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase is a maternal genetic risk factor for neural tube defects: Report of the Birth Defects Research Group. Am J Hum Genet 2002;71:1207–15.
26. Pelletier JN, MacKenzie RE. Binding and interconversion of tetrahydrofolates at a single site in the bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase. Biochemistry 1995;34:12673–80.
27. Pwelek PD, MacKenzie RE. Methylenetetrahydrofolate cyclohydrolase is rate limiting for the enzymatic conversion of 10-formyltetrahydrofolate to 5,10-methylenetetrahydrofolate in bifunctional dehydrogenase-cyclohydrogenase enzymes. Biochemistry 1998;37:1109–15.
28. Anguera MC, Field MS, Perry C, et al. Regulation of folate-mediated one-carbon metabolism by 10-formyltetrahydrofolate dehydrogenase. J Biol Chem 2006;281:18335–42.
29. Reuland SN, Vlasov AP, Krupenko SA. Disruption of a calmodulin central helix-like region of 10-formyltetrahydrofolate dehydrogenase impairs its dehydrogenase activity by uncoupling the functional domains. J Biol Chem 2003;278:22894–900.
30. Selhub J. Homocysteine metabolism. Annu Rev Nutr 1999;19:217–46.
31. De Stefano V, Dekou V, Nicaud V, et al. Linkage disequilibrium at the cystathionine b synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. Ann Hum Genet 1998;62:481–90.
32. Aras O, Hanson NQ, Yang F, Tsai MY. Influence of 699C->T and 1080C->T polymorphisms of the cystathionine b-synthase gene on plasma homocysteine levels. Clin Genet 2000;58:455–9.
33. Lievers KJA, Kluijtmans LAJ, Heil SG, et al. Cystathionine b-synthase polymorphisms and hyperhomocysteinaemia: an association study. Eur J Hum Genet 2003;11:23–9.
34. Shen M, Rothman N, Berndt SI, et al. Polymorphisms in folate metabolic genes and lung cancer risk in Xuan Wei, China. Lung Cancer 2005;49: 299–309.
35. Shi M, Caprau D, Romitti P, Christensen K, Murray JC. Genotype frequencies and linkage disequilibrium in the CEPH human diversity panel for variants in folate pathway genes MTHFR, MTHFD, MTRR, RFC1, and GCP2. Birth Defects Res 2003;67:545–9.
36. Goyette P, Christensen B, Rosenblatt DS, Rozen R. Severe and mild mutation in cis for the methylenetetrahydrofolate reductase (MTHFR) gene, and description of five novel mutations in MTHFR. Am J Hum Genet 1996;59: 1268–75.
37. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3.
38. Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci 2001;22:195–201.
39. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998;64:169–72.
40. van der Put NMJ, Gabreels F, Stevens EMB, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 1998;62:1044–51.
41. Chen J, Gammon MD, Chan W, et al. One-carbon metabolism, MTHFR polymorphisms and risk of breast cancer. Cancer Res 2005;65:1606–14.
42. Shrubsole MJ, Gao YT, Cai Q, et al. MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev 2004;13:190–6.
43. Sharp L, Little J, Schofield AC, et al. Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). Cancer Lett 2002;181:65–71.
44. Chou YC, Wu MH, Yu JC, et al. Genetic polymorphisms of the methylenetetrahydrofolate reductase gene, plasma folate levels, and breast cancer susceptibility: a case-control study in Taiwan. Carcinogenesis 2006; 27:2295–300.
45. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol 2004;159:423–43.
46. Le Marchand L, Haiman CA, Wilkens LR, Kolonel LN, Henderson BE. MTHFR polymorphisms, diet, HRT, and breast cancer risk: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 2004;13:2071–7.
47. Chou YC, Lee MS, Wu MH, et al. Plasma homocysteine as a metabolic risk factor for breast cancer: findings from a case-control study in Taiwan. Breast Cancer Res Treat 2007;101:199–205. Epub 2006 Jul 19.
48. Negri E, La Vecchia C, Franceschi S. Re: dietary folate consumption and breast cancer risk. J Natl Cancer Inst 2000;92:1270–1.
49. 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;61:7136–41.
50. Lajous M, Lazano-Ponce E, Hernandez-Avila M, Willet WC, Romieu I. Folate, vitamin B_6, and vitamin B_{12} intake and the risk of breast cancer among Mexican women. Cancer Epidemiol Biomarkers Prev 2006;15:443–8.
51. Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willet WC. Alcohol, low-methionine-low-folate diets, and risk of colon cancer in men. J Natl Cancer Inst 1995;87:265–73.

Association of Polymorphisms in One-Carbon Metabolism Genes and Postmenopausal Breast Cancer Incidence

Victoria L. Stevens, Marjorie L. McCullough, Alexandre L. Pavluck, et al.

Cancer Epidemiol Biomarkers Prev 2007;16:1140-1147.