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Genetic variants in one-carbon metabolism genes and breast cancer risk in European American (EA) and African American (AA) women

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Abstract

Folate-mediated one-carbon metabolism plays critical roles in DNA synthesis, repair, and DNA methylation. The impact of single nucleotide polymorphisms (SNPs) in folate-metabolizing enzymes has been investigated in risk of breast cancer among European or Asian populations, but not among women of African ancestry. We conducted a comprehensive analysis of SNPs in eleven genes involved in one-carbon metabolism and risk of breast cancer in 1,275 European-American (EA) and 1,299 African-American (AA) women who participated in the Women's Circle of Health Study. Allele frequencies varied significantly between EA and AA populations. A number of these SNPs, specifically in genes including *MTR*, *MTRR*, *SHMT1*, *TYMS*, and *SLC19A1*, were associated with overall breast cancer risk, as well as risk by estrogen receptor (ER) status, in either EA or AA women. Associations appeared to be modified by dietary folate intake. Although single-SNP associations were not statistically significant after correcting for multiple comparisons, polygenic score analyses revealed significant associations with breast cancer risk. Per unit increase of the risk score was associated with a modest 19% to 50% increase in risk of breast cancer overall, ER positive or ER negative cancer (all $P < 0.0005$) in EAs or AAs. In summary, our data suggest that one-carbon metabolizing gene polymorphisms could play a role in breast cancer and that may differ between EA and AA women.

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Keywords

One-carbon metabolism; polymorphisms; breast cancer; African American; European American

Introduction

Breast cancer is the most common cancer and second leading cause of cancer death among U.S. women, accounting for approximately 29% of all new cancers and 14% of cancer deaths each year¹. Breast cancer incidence is higher in European American (EA) than African American (AA) women overall, yet AA women are more likely than EA women to be diagnosed with estrogen receptor (ER)-negative tumors, and to die from breast cancer²⁻⁴. The sources of these racial differences in breast cancer remain largely unknown, despite the importance of identifying risk factors that may modify the risk of breast cancer and contribute to these racial differences.

One-carbon metabolism is a complex network of interdependent reactions that facilitate the transfer of one-carbon units and ultimately provide various forms of precursors needed for DNA methylation, nucleotide synthesis, DNA replication and repair⁵ (Figure 1). Folate, a water-soluble B-vitamin found in leafy green vegetables and fruits, is the principle element of this metabolism pathway because inter-conversions of various forms of this nutrient are the foundation of one-carbon metabolism⁶. Other nutrients, including methionine and other B-vitamins, also play roles in this pathway⁷. Altered one-carbon metabolism due to deficiency of methyl-group nutrients and genetic polymorphisms of enzymes involved in the pathway can lead to aberrant DNA methylation patterns, disruption of DNA integrity and DNA repair, and increased DNA damage and gene mutations, and all of these mechanisms can ultimately contribute to genetic instability and can facilitate carcinogenesis⁸. A number of epidemiological studies have suggested an inverse association between dietary folate intake and breast cancer risk, although findings have not been consistent across studies^{9, 10}. Various factors could contribute to these inconsistent findings, including differences in study design, study populations, dietary assessment, range and classification of folate. Inconsistencies may also be due, in part, to genetic variations in genes coding key enzymes involved in one-carbon metabolism.

Genetic variants in the folate-mediated one-carbon metabolism pathway may influence their function in one-carbon supply and subsequently result in aberrant methylation and disruption of DNA synthesis and repair, thereby modifying breast cancer risk. A number of studies have examined one-carbon metabolism gene polymorphisms for association with breast cancer risk; however, most studies have considered only a small number of genes and functional polymorphisms, including *MTHFR* (e.g., C677T: rs1801133, A1298C: rs1801131), *MTR* (A2756G: rs1805087), and *MTRR* (A66G: rs1801394), with mixed results¹¹⁻¹⁹. Furthermore, none of these studies has examined these polymorphisms in AA women. Thus it is possible that differences in genetic variants in the one-carbon metabolism pathway could contribute to differential risks of developing breast cancer between EA and AA women.

In a large case-control study, we conducted a more comprehensive assessment of the one-carbon metabolism pathway, examining associations between genetic variants of multiple genes in the pathway and breast cancer risk in EA and AA women. We further considered if associations varied according to ER status, or were modified by folate intake.

Materials and Methods

Study population

The Women's Circle of Health Study (WCHS) is a case-control study designed to evaluate risk factors for early/aggressive breast cancer in AA and EA women; details of the study design, enrollment criteria, as well as collection of biospecimens and questionnaire data, have been previously described^{20, 21}. In brief, women with incident breast cancer were identified using hospital-based case ascertainment in targeted hospitals within four boroughs of the metropolitan New York City (NYC) area and by population-based rapid case ascertainment in seven counties in nearby New Jersey (NJ), through the NJ State Cancer Registry, a participant in the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program. Eligible cases were English speaking women who self-identified as AA or EA, 20-75 years of age, and were recently diagnosed with primary, histologically confirmed breast cancer, with no previous history of cancer other than non-melanoma skin cancer. Controls were frequency matched to cases by self-reported race and 5-year age groups and were recruited during the same time period as cases from the target population in the same residential area using random digit dialing supplemented by community recruitment efforts for AA women with the help of community partners and advocates²². Overall, the participation rate for women who were contacted and eligible was 82.4% and 52.5 % in AA cases and controls, respectively, and 79.1 and 49.0 % in Caucasian cases and controls, respectively. At the time of genotyping, data and samples from 1,275 EA (637 cases, 638 controls) and 1,299 AA (584 cases, 715 controls) participants were available from WCHS. Of these, 45 (22 EA and 23 AA) women were excluded due to missing dietary data, leaving 1,253 EA and 1,276 AA cases and controls in the gene-nutrient interaction analysis.

This study was approved by institutional review boards at Roswell Park Cancer Institute (RPCI), the Rutgers Cancer Institute of New Jersey (RCINJ), Mount Sinai School of Medicine (MSSM; now the Icahn School of Medicine at Mount Sinai), and participating hospitals in NYC. Signed informed consent was obtained from each participant prior to interview and biospecimen collection.

Data and sample collection

Detailed data on demographic characteristics, medical history, family history of cancer, and lifestyle factors, as well as anthropometric measures and biospecimens were collected by trained interviewers. Blood samples were initially collected for DNA extraction, but after enrollment of approximately 850 participants, saliva samples were collected using Oragene kits as a source of DNA. Pathology data including ER status, grade and stage, were collected and abstracted by trained study staff.

Details of assessment of dietary intake have been described previously²³. Briefly, a Food Frequency Questionnaire (FFQ) was used to collect data on usual frequency of intake and portion size (small, medium, or large with reference to a specified medium portion size for each item) for approximately 125 food and beverages consumed during the 12 months prior to diagnosis for cases and to a comparable reference date for controls. The average daily intake of each nutrient, including folate intake, was computed by multiplying the standard serving frequency of each food or beverage item by its nutrient content of the specified standard portion size and then summing the nutrient intake for all foods and beverages.

Sample collection and processing

Genomic DNA from blood and saliva samples was extracted using the FlexiGene™ DNA isolation kits (Qiagen Inc., Valencia, CA) and Oragene™ kits (DNA Genotek Inc., Kanata, Ontario, Canada) following the manufacturer's protocols. Genomic DNA was evaluated and quantitated by Nanodrop UV-spectrometer (Thermo Fisher Scientific Inc., Wilmington, DE) and PicoGreen-based fluorometric assay (Molecular Probes, Invitrogen Inc., Carlsbad, CA), and stored at -80°C until analysis.

SNP selection and genotyping

We included in our analysis eleven key genes involved in folate transport or intracellular one-carbon metabolism (summarized in Figure 1) and then surveyed the Human Genome Epidemiology (HuGE) Navigator to identify single nucleotide polymorphisms (SNPs) within each of these candidate genes²⁴. We then selected eighty-eight SNPs that were previously associated with cancer risk or outcomes, with a focus on those associated with known or putative functional changes. Selected SNPs were genotyped among cases and controls at the Genomics Core Facility at Roswell Park Cancer Institute using the Illumina GoldenGate assay (Illumina Inc., San Diego, CA). As a quality control measure, five percent duplicates and two sets of in-house trio samples were included across all plates. The concordance among blind duplicate pairs was greater than 99.9%. After excluding SNPs with a call rate less than 90%, in violation of Hardy-Weinberg equilibrium, or with a minor allele frequency (MAF) less than 5% for both AAs and EAs, seventy-four SNPs remained for the eleven one-carbon metabolism genes and were included in this analysis (Supplementary Table S1). To account for population admixture in the analysis, all samples were also genotyped for a panel of 100 ancestry informative markers (AIMs) that were previously validated in the Black Women's Health Study²⁵. Proportions of European Ancestry and African Ancestry of individual EA and AA women were computed quantitatively using the Bayesian Markov Chain Monte Carlo clustering algorithm implemented in STRUCTURE²⁶, based on data from the 100 genotyped AIMs.

Statistical analysis

Descriptive variables were compared between cases and controls using chi-square tests for categorical variables and t-tests for continuous variables. Multivariable unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of breast cancer associated with genotype, with adjustments for age at diagnosis, family history of breast cancer, body mass index, education, history of benign breast disease, menopausal status, and proportion of European ancestry. Other covariates

including alcohol intake did not significantly affect the risk estimates and thus were not included in the multivariable-adjusted analysis. All analyses were performed separately for EA and AA women. Participants with the most common homozygous genotype among EA controls were treated as the referent group. Genotypic (co-dominant) models were assumed for SNP effects. Based on the risk estimates, heterozygotes were combined with either homozygous rare or homozygous common genotypes to explore dominant and recessive models, respectively. When the number of the rare homozygote was small (i.e., <10) in both populations, ORs and 95% CIs were reported for the dominant model only (heterozygous and rare homozygous genotypes combined) for power considerations. Additive genotype coding based on the number of rare alleles (i.e., 0, 1, 2) was analyzed as an ordinal variable in tests for linear trend. Analyses also were conducted to examine associations stratified by ER status. Interactions by self-reported race (AA and EA) or dietary folate intake (low and high-intake based on the median intake among controls) were also tested by including an interaction term (SNP*self-reported race or SNP*dietary folate) in multivariable logistic models and performing the likelihood ratio test.

In addition to single SNP analysis, we also performed multi-marker analyses by using a weighted polygenic risk score as described by Speliotes and colleagues²⁷. The multi-marker risk score was calculated as the sum of the number of risk genotypes (dominant and recessive models) and risk alleles (additive model) of the final model chosen for each significant SNP, and weighted by the regression coefficients from the logistic regression model. The final genetic score was then standardized by dividing the sum of regression coefficients and multiplying the expected number of risk genotypes/alleles; therefore, each unit of the composite genetic score is equal to one risk genotype or allele. The score was analyzed as a continuous variable in the logistic regression model with adjustment for the same set of covariates as described above. For SNPs associated with a decreased risk, the reference and comparison groups were selected such that the genotypes or alleles of interest were associated with an increased risk. For SNPs located within 500kb on the same chromosome and in high linkage disequilibrium (LD; $r^2 \geq 0.8$), only the SNP with the strongest association was included in the computation of the polygenic score.

All analyses were conducted using SAS V9.3 (SAS Institute, Cary, CA) or PLINK program V1.07. LD was determined by calculating r^2 values between each SNP pair using the program Haploview V4.2. Statistical tests were two-sided and considered significant for uncorrected $P < 0.05$. All significant P -values were further adjusted for multiple comparisons using Bonferroni correction.

Results

Participant Characteristics

Characteristics of the study population are shown separately for EA and AA women in Table 1. Overall, AA women tend to have a higher BMI and to be less well-educated than EA women. Compared to EA controls, EA cases were more likely to have a family history of breast cancer, to have a history of benign breast disease, and to be less well educated. AA cases were more likely than AA controls to have a history of benign breast disease. Data on ER status were available for 71.9% of EA cases and 75.9% of AA cases. As expected, AA

cases were more likely than EA cases to be diagnosed with ER negative breast cancer (31.6% versus 17.5%).

Differences in allele frequencies of SNPs between EA and AA women

SNPs and MAF of the 74 SNPs among EA and AA controls are shown in Supplementary Table S1. For 39 of the 74 SNPs, allele frequencies differed significantly between EA and AA controls ($P < 0.05$), and for 12 of these SNPs, the rare allele variant was reversed between the two groups.

Associations of SNPs with breast cancer risk in EA and AA women

Associations between each SNP and overall breast cancer risk in EA and AA women are shown separately in Supplementary Table S2, and ORs (95% CIs) for SNPs with significant associations ($P < 0.05$) are shown in Table 2. Among EA women, three SNPs were associated with breast cancer. *MTR*-rs1805087 was associated with decreased risk (OR= 0.44, 95% CI: 0.24-0.80) in a recessive model. *MTR*-rs2275565 was in LD ($r^2=0.86$) with *MTR*-rs1805087 and associated with similar decreased risk (Table S2). An increased risk also was observed for *MTRR*-rs10520873 (OR=1.37, 95% CI: 1.08-1.73) and *TYMS*-rs2612100 (OR=1.63, 95% CI: 1.12-2.37) in dominant and recessive models, respectively. Among AA women, four SNPs were associated with increased risk, including *SHMT1* (rs2168781, rs4925180), *TYMS*-rs2853533, and *SLC19A1*-rs3788189, with ORs ranging from 1.29 (95% CI: 1.02-1.63) to 1.49 (95% CI: 1.06-2.10) in dominant models. These genotype associations with breast cancer risk differed in strength between AA and EA women, but the SNP by race interaction was statistically significant only for *TYMS*-rs2612100 in the recessive model (p -interaction=0.03), with a significant increased risk associated with AA genotype among EA women (OR=1.63, 95% CI: 1.12-2.37), but not in AA women (OR=1.01, 95% CI: 0.80-1.26). Although there was no association between *MTHFR*-rs1801133 (C677T) and overall breast cancer risk in either EAs or AAs, in a post-hoc analysis, we found a significant increased risk among premenopausal AA women (OR=1.59, 95% CI: 1.07-2.36) (data not shown). Further, none of above associations remained significant after correction for multiple comparisons.

Associations stratified by ER status

Associations between each SNP and risk of ER positive and ER negative breast cancer were examined separately (Supplemental Table S3). Although the majority of associations were similar by ER status, some did differ in stratified analyses (Table 3).

Among EAs, *BHMT*-rs7700970 and *DHFR*-rs1643658 were both inversely associated with ER positive breast cancer (OR=0.61, 95% CI: 0.37-0.98 and OR=0.76, 95% CI: 0.57-0.99, respectively) in recessive and dominant models, respectively. *MTHFR*-rs2274976 was positively associated with ER negative breast cancer (OR=2.33, 95% CI: 1.07-5.08), whereas *BHMT*-rs567754 was inversely associated (OR=0.60, 95% CI: 0.37-0.98). *MTRR*-rs10520873 was associated with increased risk of both ER positive (OR=1.38, 95% CI: 1.05-1.82) and negative breast cancers (OR=1.69, 95% CI: 1.04-2.76).

Among AAs, seven SNPs were associated with ER positive breast cancer (Table 3). Of these, four SNPs, including *MTR*-rs2275565, *SHMT1*-rs2168781, *TYMS*-rs2853533, and *SLC19A1*-rs3788189, were associated with overall breast cancer risk in AAs, with relationships strongest with risk of ER positive cancer, with ORs ranging from 1.46 (95% CI: 1.08-1.96) to 1.87 (95% CI, 1.19-2.95). *MTR*-rs6668344 and *MTHFD1*-rs2236225, while not associated with overall breast cancer risk in AAs, did appear to be associated with decreased risk of ER positive disease (OR=0.76, 95% CI: 0.57-1.01 and OR=0.75, 95% CI: 0.56-1.00, respectively). We found no SNPs associated with ER negative breast cancer in AA women.

Associations with polygenetic risk score

The SNPs with designated risk alleles or genotypes, expected range of the polygenetic score, mean and standard deviation of the score in cases and controls, and risk estimates associated with per unit of the score are shown in Table 4. In each subgroup, breast cancer cases had higher polygenetic risk score than controls, and per unit increment of the score (refer to one risk allele or genotype) was associated with significantly increased risk. Each one-unit increase of the polygenetic score was associated with an 18% –50% increased risk for breast cancer overall, by ER status in EAs, or in ER positive disease in AAs.

Effect modifications by dietary folate

We examined interactions between each SNP and one-carbon nutrients, specifically folate intake from natural food sources that was previously found to be inversely associated with breast cancer in this study population²³. Associations of several SNPs with risk of breast cancer differed by level of folate intake (low- and high-intake by median) in EAs or AAs (*P*-interaction <0.05, Table 5).

Among EAs, variants for *MTR*-rs6668344 and *MTRR*-rs1801394 were associated with increased risk among women with high folate intake (p-interaction=0.06 and 0.03, respectively). A reduced risk associated with GG genotype of *SLC19A1*-rs3788189 was also observed among women with high-intake (p-interaction=0.01), with similar patterns found for the other four *SLC19A1* SNPs that were in LD with rs3788189 (data not shown). Among AAs, a significant interaction was observed for *MTHFR*-rs7533315, with TT genotype associated with increased risk in women with low-intake, but a non-significant reduced risk in women with high-intake (p-interaction=0.004). We also examined interactions between the polygenetic risk score and intake of other one-carbon nutrients such as vitamin B2, B6, B12 and methionine with overall cancer risk in EAs and AAs separately, and found no significant interactions (data not shown).

Discussion

In this case-control study, we conducted an analysis of a panel of genetic variants in eleven genes involved in folate-mediated one-carbon metabolism and risk of breast cancer in 1,275 EA and 1,299 AA women. Allele frequencies of SNPs in these genes varied significantly between EA and AA control populations. A number of these SNPs, especially in genes such as *MTR*, *MTRR*, *SHMT1*, *TYMS*, and *SLC19A1*, were found to be associated with overall

breast cancer risk, as well as breast cancer risk by ER status, in either EA or AA women. Our results also indicate that SNP associations may be modified by level of dietary folate intake. Although the single-SNP associations were not statistically significant after adjustment for multiple comparisons, the polygenetic risk score analyses which allowed us to examine the combined effect of all significant SNPs observed in each subgroup, revealed significant associations with breast cancer risk. To our knowledge, this is the first study to examine associations of one-carbon metabolism genes with breast cancer in both EA and AA populations, specifically with a large sample of AA women.

MTHFR is the most studied enzyme in one-carbon metabolism. As illustrated in Figure 1, it catalyzes the irreversible reduction of 5, 10-methylene-THF, a common substrate to both nucleotide synthesis and methylation reactions, to 5-methylene-THF, which is the primary circulating form of folate and provides methyl groups for reactions leading to DNA methylation⁸. The two non-synonymous polymorphisms in *MTHFR*, rs1801133 (C677T) and rs1801131 (A1298C), have been the most extensively studied SNPs because their variant alleles have been linked to reduced *MTHFR* activity^{28, 29}. Results from studies exploring associations between these *MTHFR* variants and breast cancer risk have been inconsistent, with meta-analyses failing to support an overall association, although some studies suggested an increased risk associated with *MTHFR* 677TT genotype in premenopausal women^{9, 30, 31}. Consistent with the literature, neither of the two SNPs was associated with breast cancer overall in EAs or AAs in our study, but a significant increased risk was observed among premenopausal AA women who carry the 677T allele. Thus, reduced *MTHFR* activity of the 677T allele could result in altered (less) availability of methyl groups and impaired DNA methylation, and subsequently lead to cancer development. We found no associations for other *MTHFR* polymorphisms except for the rs2274976 (1793G>A) polymorphism, which was associated with an increased risk for ER negative breast cancer in EA women. This SNP was not associated with breast cancer in one study³², but has been linked to increased risk of nonsyndromic cleft lip and endometrial cancer^{33, 34}.

Several SNPs in *MTR* were associated with breast cancer risk overall and selectively with risks for specific breast cancer subtypes (ER status) in EA and/or AAs. *MTR* catalyzes the remethylation of homocysteine to methionine with simultaneous demethylation of 5-methyl-THF to THF, thus is essential for maintaining adequate intracellular methionine for methylation reactions, and for the provision of THF for further use in nucleotide synthesis. We found that *MTR*-rs1805087, also known as A2756G or D919G, was associated with decreased risk of breast cancer among EA women carrying homozygous variant GG genotype, which is consistent with a recent meta-analysis indicating that the variant G allele is associated with reduced risk of breast cancer in European populations³⁵. The A2756G polymorphism occurs in the activation domain of *MTR*, and the variant GG genotype has been shown to be associated with lower homocysteine and higher serum folate concentrations^{36, 37}, suggesting that the GG genotype might increase enzyme activity. We also observed associations for ER positive breast cancer with two other *MTR* SNPs (rs6668344 and rs2275565) in AA women. The significance of these observations should be explored in future studies. *MTRR* has a crucial role to maintain the activity of *MTR*. We

observed an increased risk associated with *MTRR*-rs10520873 among EA women. This SNP is located at 3'UTR of *MTRR* gene, and has been significantly associated with increased risk of obesity in European adolescents³⁸. However, the biological function of this SNP remains unclear and needs to be determined in future studies. We did not observe an association for A66G (rs1801394), the most frequently studied *MTRR* polymorphism, which has been reported to affect plasma homocysteine concentrations³⁹. However, consistent with our results, previous studies that have examined this polymorphism in relation to breast cancer generally showed null results⁴⁰.

Our investigations of additional genes that have not been well studied in breast cancer led to some new findings. SHMT catalyzes the reversible conversion of serine to glycine and THF to 5, 10-methylene-THF, providing one carbon units for synthesis of methionine, thymidylate, and purines. We observed that two SNPs in the cytosolic form *SHMT* (*SHMT1* or *cSHMT*), rs2168781 and rs4925180, are associated with increased breast cancer risk overall and for ER positive cancer among AA women. The polymorphism, *SHMT1*-rs1979277 (C1420T) has been described and found to be associated with lower red blood cell and plasma folate levels⁴¹. However, we found no association with breast cancer for this polymorphism, which is consistent with findings from several previous studies^{12, 42, 43}. TYMS catalyzes the reductive methylation of dUMP to dTMP, thereby playing a central role in DNA synthesis and repair⁴⁴. We observed an increased risk associated with the AA genotype of *TYMS*-rs2612100 (G>A) among EA women. Another SNP, rs2853533, was associated with overall breast cancer risk and for ER positive cancer among AA women. These associations have not been observed in breast cancer, but a similar increased risk for colorectal cancer has been reported recently⁴⁵. In addition, although no association with overall risk was observed, several SNPs in *BHMT*, *DHFR*, and *MTHFD1* were associated with ER positive or negative breast cancer in either EAs or AAs.

Data on gene expression profiling support that ER positive and ER negative tumors are fundamentally distinct diseases⁴⁶. There is evidence showing that genetic and environmental factors differ in breast cancer by ER status, suggesting different etiological pathways for ER positive and ER negative breast cancer⁴⁷. Our findings that associations between several genetic polymorphisms of one-carbon metabolizing genes and breast cancer risk differ by ER status provide some further evidence, although the mechanisms underlying these associations are largely unknown. Some data have suggested that methylation of CpG islands on the ER gene is associated with a lack of ER expression, and that DNA methylation patterns in breast cancer tumors significantly differ by ER status⁴⁸. In addition, although results have been inconsistent, associations between dietary folate intake level and breast cancer tend to differ by ER status²³. Thus, it is biologically plausible that these genetic variants are associated with either ER positive or ER negative breast cancer through their differential effect on DNA methylation and other unknown mechanisms. The sample size is relatively small, especially in ER negative tumors, thus findings could be due to a chance. Confirmation of these results and future research on the underlying mechanisms are needed.

A number of studies have examined interactions between one-carbon SNPs, specifically on *MTHFR* C677T, and folate in relation to breast cancer⁴⁹, with few studies observing

significant interactions. Previous findings have also been conflicting, with the 677T variant allele associated with increased^{13, 50} or decreased risk¹⁵ among women with the lowest folate intake, and increased risk in women with high folate intake or plasma concentrations^{19, 51}. We did not observe interactions between *MTHFR* SNPs and dietary folate, but our results suggest that several SNP-breast cancer associations may differ by low- and high-folate intake, including SNPs in *MTR*, *MTRR*, *MTHFR*, and *SLC19A1* in either EAs or AAs. Our findings could be due to chance because none of the interactions remained significant after correcting for multiple comparisons. Results from previous studies, as well as our data, indicate that both folate intake and one-carbon gene SNPs are associated with breast cancer risk, which suggests the importance of continued investigation of possible interactions.

An interesting observation from our study is that associations of certain SNPs in relation to breast cancer differed between EA and AA populations, i.e., significant associations in either EAs or AAs, but not in both. We found that MAF frequencies differed among 39 out of 74 SNPs between EA and AA controls, with rare allele variant reversed among 12 SNPs. For example, in addition to the SNPs discussed above, the minor alleles of *SLC19A1* SNPs were reversed between EAs and AAs, and these SNPs were associated with breast cancer risk in AA women but not in EA women. *SLC19A1*, also known as the reduced folate carrier 1 (*RFC1*), is responsible for transporting folate compounds into cells, thus functional change in the activity of SLC19A1 could modify folate metabolism through increasing or decreasing intracellular folate availability. Although these differences in association between EAs and AAs could be chance findings, our results may reflect differences in genomic structures for these genes between the two populations. Associations between genetic factors and risk for breast cancer have been reported to differ by race in the literature. For example, similar racial differences in other genetic pathways in relation to breast cancer risk were observed in our study population, and these results have been published recently^{21, 52, 53}. Furthermore, studies have showed that GWAS-identified SNPs in breast cancer from EA and Asian populations could not be replicated in AA women^{54, 55}. These differences in associations by race could be due to the different allele frequencies, LD structure, etiological heterogeneity in AA and EA populations, as well as differences in environmental exposures. In summary, our findings suggest that different SNPs and gene networks of the one-carbon metabolism pathway may be associated with breast cancer in AA versus EA women, and highlight the importance of conducting etiology studies of breast cancer across different race/ethnicity populations.

Several limitations of the study warrant consideration. First, although we investigated a large number of SNPs in multiple key genes thought to be important in cancer risk in one-carbon metabolism pathway, other potentially functional genetic variants may not be included in the current study. Second, none of the associations for single SNP analysis remained significant after correcting for multiple comparisons, thus we cannot exclude the possibility of false positive findings. However, significant associations observed between polygenetic risk score and breast cancer risk suggest that one-carbon metabolism gene polymorphisms contribute to the risk of breast cancer. Finally, although this is a study with a large number of AA and EA women, which allow us to examine racial differences for these

genetic variants with breast cancer risk, our sample size was limited when analyses were stratified by race and ER status, and may have been inadequate to detect a small effect size or interactions.

In conclusion, this case-control study provides the most comprehensive investigation to date regarding the role of SNPs in one-carbon metabolism genes as risk factors for breast cancer overall and by ER status in both EA and AA populations. Our study provides some evidence that genetic variants in one-carbon metabolism and gene-nutrient interactions may contribute to risk of breast cancer in EA and AA women, with susceptible and protective loci differing by race. Additional large scale studies with different populations and functional evaluations are warranted to confirm these findings and explore the underlying molecular mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What's new?

The impact of genetic variants in folate-metabolizing enzymes has been investigated in risk of breast cancer among European or Asian populations, but not among women of African ancestry. This is the first large study that specifically examined these associations in AA women and to involve a large number of both EA and AA cases and controls. Our results suggest that one-carbon metabolizing gene polymorphisms could play a role in breast cancer and that may differ between EA and AA women.

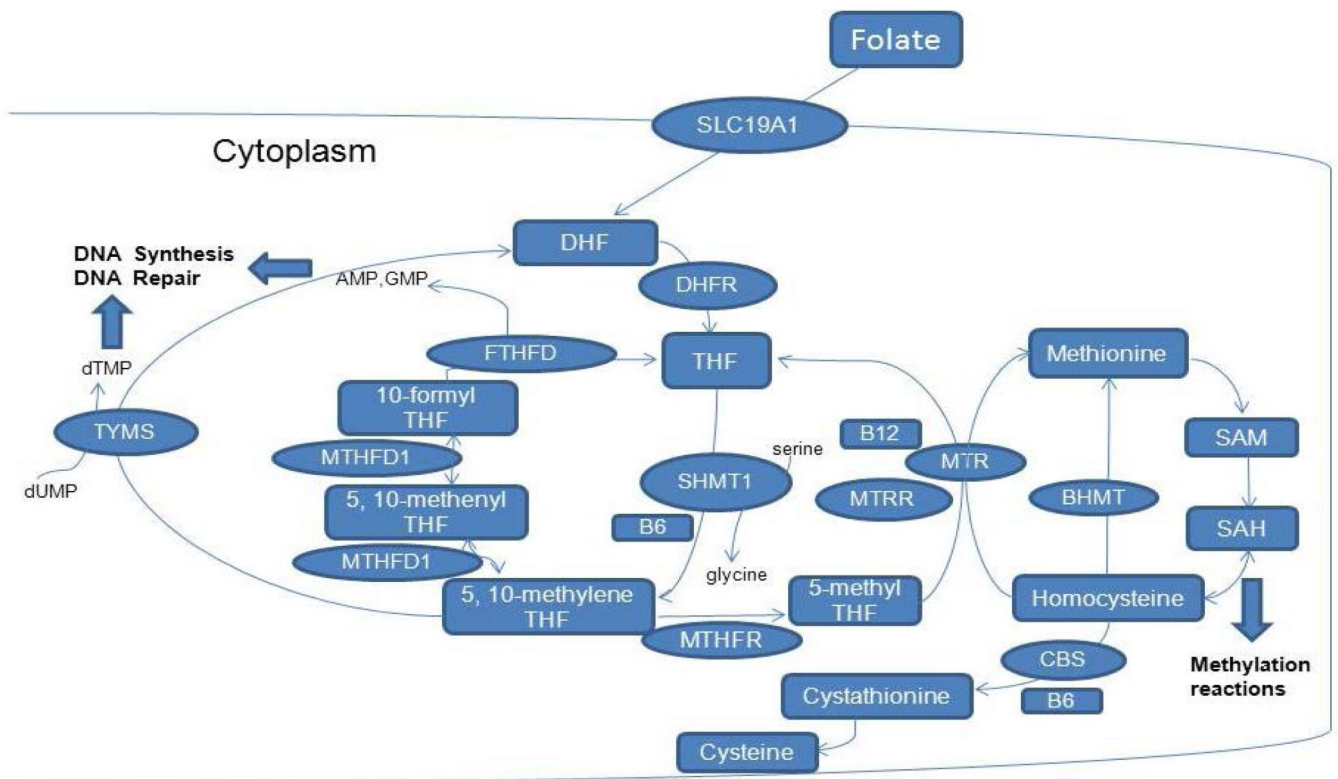


Figure 1.

Overview of folate-mediated one-carbon metabolism, with focus on enzymes investigated in this study. These enzymes include: BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine-beta-synthase; DHFR, dihydrofolate reductase; FTHFD, 10-formyltetrahydrofolate dehydrogenase; MTHFR, methylene tetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; MTHFD1, methylene tetrahydrofolate dehydrogenase; SLC19A1, solute carrier family 19; SHMT1, serine hydroxymethyltransferase 1; TYMS, thymidylate synthetase; The substrates and products include: DHF, dihydrofolate; THF, tetrahydrofolate; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; AMP, adenosine monophosphate; GMP, guanosine monophosphate; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate.

Table 1

Characteristics of 1,275 European American and 1,299 African American cases and controls in the Women's Circle of Health Study (WCHS)^a

Characteristics	European American		<i>P</i> -value ^c	African American		<i>P</i> -value ^c
	Cases (n=637)	Controls (n=638)		Cases (n=584)	Controls (n=715)	
Age (yr), mean (SD) ^b	52.2 (10.0)	49.7 (8.7)	<0.0001	51.7(10.4)	48.6 (9.5)	<0.0001
Body mass index, mean (SD) ^b	27.3 (6.6)	27.4 (7.1)	0.81	31.2 (6.7)	32.0 (7.9)	0.05
% European ancestry ^b	97 (8)	98 (4)	<0.0001	14 (16)	14 (14)	0.89
Menopausal status, n (%)			0.30			0.03
Premenopausal	331 (52.0)	350 (54.9)		286 (49.0)	393 (55.0)	
Postmenopausal	306 (48.0)	288 (45.1)		298 (51.0)	322 (45.0)	
Family history, n (%)			0.0004			0.13
No	481 (75.5)	533 (83.5)		498 (85.3)	630 (88.1)	
Yes	156 (24.5)	105 (16.5)		86 (14.7)	85 (11.9)	
Education, n (%)			<0.0001			0.33
<= high school	131 (20.6)	71 (11.1)		258 (44.2)	282 (39.4)	
Some college	140 (22.0)	113 (17.7)		159 (27.2)	201 (28.1)	
College graduate	198 (31.1)	208 (32.6)		102 (17.5)	139 (19.4)	
Post-graduate degree	168 (26.4)	246 (38.6)		65 (11.1)	93 (13.0)	
History of benign breast disease, n (%)			0.0006			<0.0001
No	368 (58.4)	431 (67.8)		399 (68.6)	564 (79.0)	
Yes	262 (41.6)	205 (32.2)		183 (31.4)	150 (21.0)	
Estrogen receptor (ER) Status, n (%) ^d						<0.0001
Positive	378 (82.5)			303 (68.4)		
Negative	80 (17.5)			140 (31.6)		

^aNumber may not add up to the total number due to missing values

^bSD: standard deviation.

^c*P*-value were from t-test for continuous variables and Chi-square test for categorical variables

^dER status were available for 458 (71.9%) EA cases and 443 (75.9%) AA cases

Table 2

Association between genetic polymorphisms in one-carbon metabolism genes and breast cancer risk in European American and African American women, WCHS

Gene	SNP	Type	Genotype	European American			African American			<i>P</i> ^e
				# Case/Control	OR (95% CI) ^{a,b}	<i>P</i> ^{c,d}	# Case/Control	OR (95% CI) ^{a,b}	<i>P</i> ^{c,d}	
MTR ^r	Rs1805087	Nonsynonymous	AA	410/408	1.00	0.27	325/387	1.00	0.52	0.77
			AG	201/191	1.09 (0.85-1.41)		215/273	0.95 (0.75-1.21)		
			GG	19/36	0.45 (0.24-0.83)		40/49	0.87 (0.55-1.38)		
MTRR	Rs10520873	3' region	GG vs. AA/AG	611/599	0.44 (0.24-0.80)	0.007	540/660	0.89 (0.57-1.39)	0.61	0.74
			AA	347/384	1.00	0.02	491/587	1.00	0.57	0.15
			AG	241/209	1.40 (1.09-1.79)		86/117	0.92 (0.67-1.26)		
SHMT1	Rs2168781	Intronic	GG	41/41	1.24 (0.77-2.00)		4/5	0.87 (0.22-3.43)		
			AG/GG	282/250	1.37 (1.08-1.73)	0.009	90/122	0.92 (0.67-1.25)	0.58	0.08
			GC	212/207	1.00	0.99	48/82	1.00	0.04	0.13
SHMT1	Rs4925180	Intronic	GC	301/307	0.98 (0.76-1.28)		237/294	1.37 (0.92-2.06)		
			CC	117/121	1.01 (0.72-1.41)		294/332	1.54 (1.03-2.31)		
			GC/CC	418/428	0.99 (0.77-1.27)	0.14	531/626	1.46 (1.00-2.16)	0.05	0.08
TYMS	Rs2612100	Intronic	GG	610/607	1.00	0.14	426/555	1.00	0.06	0.10
			GA/AA	17/23	0.60 (0.30-1.22)		141/144	1.32 (1.01-1.74)		
			GA	246/268	1.00	0.03	77/99	1.00	0.78	0.15
TYMS	Rs2853533	Nonsynonymous	GA	297/307	1.07 (0.83-1.36)		257/314	1.09 (0.77-1.55)		
			AA	85/57	1.68 (1.13-2.51)		246/297	1.08 (0.76-1.54)		
			AA vs. GG/GA	543/575	1.63 (1.12-2.37)	0.01	334/413	1.01 (0.80-1.26)	0.62	0.03
SLC19A1	Rs3788189	Intronic	GG	433/460	1.00	0.46	200/281	1.00	0.11	0.89
			GC	180/164	1.12 (0.86-1.46)		281/311	1.31 (1.02-1.68)		
			CC	11/10	1.02 (0.40-2.59)		96/115	1.22 (0.88-1.71)		
SLC19A1	Rs3788189	Intronic	GC/CC	191/174	1.11 (0.86-1.44)	0.42	377/426	1.29 (1.02-1.63)	0.04	0.65
			TT	177/197	1.00	0.55	64/112	1.00	0.24	0.47
			TG	343/315	1.13 (0.87-1.48)		278/311	1.56 (1.09-2.23)		

Gene	SNP	Type	Genotype	European American			African American			<i>P</i> ^e
				# Case/Control	OR (95% CI) ^{a,b}	<i>P</i> ^{c,d}	# Case/Control	OR (95% CI) ^{a,b}	<i>P</i> ^{c,d}	
			GG	110/122	0.85 (0.60-1.20)		238/287	1.42 (0.99-2.04)		
			TG/GG	453/437	1.05 (0.82-1.35)	0.45	516/598	1.49 (1.06-2.10)	0.02	0.23

^a OR, odds ratio; 95%CI, 95% confidence interval

^b Adjusted for age at diagnosis, education, body mass index, family history of breast cancer, history of benign breast disease, menopausal status, and proportion of European ancestry.

^c *P*-trend for genetic dose response determined by coding genotypes as having 0, 1, or 2 variant allele, which was subsequently analyzed as an ordinal variable.

^d *P* for heterogeneity from dominant or recessive models.

^e *P* for interaction was for the differences in ORs between African-American and European-American women.

^f rs2275565 was found in LD with rs1805087, with a similar association pattern.

Table 3

Association between genetic polymorphisms in one-carbon metabolism genes and breast cancer risk by estrogen receptor (ER) status in European American and African American women, WCHS

Gene	SNP	Genotype	European American				African American						
			ER positive OR (95%CI) ^{b,c}	P ^{d,e}	#Case/ Control	ER negative OR (95%CI) ^{b,c}	P ^{d,e}	#Case/ Control	ER positive OR (95%CI) ^{b,c}	P ^{d,e}	#Case/ Control	ER negative OR (95%CI) ^{b,c}	P ^{d,e}
MTHFR	Rs2274976	GG	1.00	0.34	65/580	1.00	0.03	278/636	1.00	0.62	126/636	1.00	0.90
		GA/AA	1.31 (0.78-2.32)		10/35	2.33 (1.07-5.08)		17/48	0.86 (0.48-1.55)		10/48	1.05 (0.51-2.16)	
MTR	Rs6668344	CC	1.00	0.37	25/260	1.00	0.15	167/353	1.00	0.04	64/353	1.00	0.93
		CT	1.28 (0.96-1.72)		37/283	1.39 (0.80-2.42)		108/278	0.79 (0.59-1.07)		63/278	1.29 (0.87-1.91)	
MTR	Rs2275565	TT	1.08 (0.71-1.64)		14/91	1.62 (0.79-1.82)		28/78	0.66 (0.40-1.06)		11/78	0.76 (0.38-1.53)	
		CT/TT	1.23 (0.93-1.63)	0.14	51/374	1.45 (0.86-2.44)	0.16	136/356	0.76 (0.57-1.01)	0.05	74/356	1.17 (0.81-1.71)	0.40
MTR	Rs2275565	CC	1.00	0.47	47/378	1.00	0.55	71/228	1.00	0.04	37/228	1.00	0.75
		CA	1.12 (0.83-1.50)		25/199	0.97 (0.57-1.65)		157/332	1.59 (1.13-2.22)		70/332	1.24 (0.80-1.94)	
MTRR	Rs10520873	AA	0.53 (0.27-1.03)		3/42	0.60 (0.17-2.08)		66/146	1.49 (0.99-2.25)		27/146	1.06 (0.61-1.84)	
		CA/AA	1.01 (0.77-1.34)	0.92	28/241	0.91 (0.55-1.52)	0.73	223/478	1.56 (1.13-2.14)	0.007	97/478	1.19 (0.78-1.81)	0.42
MTRR	Rs10520873	AA	1.00	0.02	39/384	1.00	0.04	263/587	1.00	0.16	120/587	1.00	0.54
		AG/GG	1.38 (1.05-1.82)		39/250	1.69 (1.04-2.76)		40/122	0.75 (0.51-1.10)		19/122	0.84 (0.49-1.44)	
SHMT1	Rs2168781	GG	1.00	0.49	29/207	1.00	0.36	22/82	1.00	0.02	15/82	1.00	0.86
		GC	1.00 (0.74-1.36)		37/307	0.84 (0.50-1.43)		118/294	1.50 (0.88-2.55)		57/294	1.03 (0.55-1.94)	
SHMT1	Rs2168781	CC	1.17 (0.79-1.72)		12/121	0.72 (0.34-1.51)		162/332	1.85 (1.10-3.11)		66/332	1.05 (0.56-1.97)	
		GC/CC	1.05 (0.79-1.39)	0.76	49/428	0.81 (0.49-1.34)	0.41	280/626	1.68 (1.01-2.77)	0.04	123/626	1.04 (0.58-1.89)	0.89
TXMS	Rs2853533	GG	1.00	0.92	53/460	1.00	0.31	97/281	1.00	0.03	52/281	1.00	0.97
		GC	1.02 (0.75-1.39)		23/164	1.28 (0.75-2.19)		149/311	1.46 (1.07-1.99)		68/311	1.17 (0.78-1.76)	
SILC19A1	Rs3788189	CC	0.73 (0.21-2.53)		2/10	1.61 (0.33-7.96)		54/115	1.46 (0.96-2.20)		19/115	0.90 (0.51-1.61)	
		GC/CC	1.00 (0.74-1.36)	0.99	25/174	1.30 (0.77-2.20)	0.33	203/426	1.46 (1.08-1.96)	0.01	87/426	1.10 (0.75-1.62)	0.63
SILC19A1	Rs3788189	TT	1.00	0.84	23/197	1.00	0.23	27/112	1.00	0.36	18/112	1.00	0.65
		TG	1.14 (0.83-1.55)		45/315	1.11 (0.64-1.92)		157/311	2.09 (1.30-3.35)		62/311	1.20 (0.67-2.13)	
BHMT	Rs567754	GG	0.92 (0.62-1.37)		10/122	0.54 (0.24-1.23)		119/287	1.64 (1.01-2.66)		58/287	1.19 (0.66-2.13)	
		TG/GG	1.07 (0.80-1.44)	0.63	55/437	0.95 (0.56-1.61)	0.84	276/598	1.87 (1.19-2.95)	0.007	120/598	1.19 (0.69-2.06)	0.52
BHMT	Rs567754	CC	1.00	0.60	44/267	1.00	0.04	254/618	1.00	0.30	124/618	1.00	0.62

Gene	SNP	Genotype	European American						African American					
			ER positive			ER negative			ER positive			ER negative		
			#Case/ Control	OR (95%CI) ^{b,c}	P ^{d,e}	#Case/ Control	OR (95%CI) ^{b,c}	P ^{d,e}	#Case/ Control	OR (95%CI) ^{b,c}	P ^{d,e}	#Case/ Control	OR (95%CI) ^{b,c}	P ^{d,e}
BHMT	Rs7700970	CT/TT	213/365	1.08 (0.82-1.42)	0.12	34/365	0.60 (0.37-0.98)	46/91	1.24 (0.82-1.88)	14/91	0.85 (0.45-1.61)			
		CC	183/297	1.00	42/297	1.00	97/233	1.00	39/233	1.00	0.30			
		CT	162/272	0.97 (0.73-1.29)		27/272	0.72 (0.43-1.21)		155/328	1.10 (0.80-1.51)		1.13 (0.73-1.75)		
DHFR	Rs1643658	TT	30/66	0.60 (0.36-0.99)	0.04	9/66	0.81 (0.36-1.80)	50/149	0.81 (0.54-1.22)	34/149	1.31 (0.79-2.19)			
		Vs. CC/CT	330/558	0.61 (0.37-0.98)	0.04	69/569	0.93 (0.43-2.02)	0.86	252/561	0.76 (0.53-1.10)	0.15	104/561	1.22 (0.79-1.88)	0.37
		TT	206/317	1.00	0.03	31/302	1.00	0.47	152/377	1.00	0.43	61/355	1.00	0.53
MTHFD1	Rs2236225	TC	137/257	0.78 (0.59-1.05)	0.04	42/254	1.66 (1.00-2.77)	130/276	1.21 (0.90-1.61)	63/282	1.27 (0.86-1.88)			
		CC	28/60	0.63 (0.38-1.06)	0.04	5/61	0.78 (0.28-2.14)	20/53	1.01 (0.57-1.77)	8/51	0.92 (0.41-2.06)			
		TC/CC	165/317	0.76 (0.57-0.99)	0.04	47/315	1.49 (0.90-2.44)	0.12	150/329	1.17 (0.89-1.55)	0.26	71/333	1.22 (0.84-1.79)	0.30
		CC	124/204	1.00	0.67	18/204	1.00	0.28	202/433	1.00	0.02	79/433	1.00	0.66
		CT	183/304	1.03 (0.76-1.39)		45/304	1.64 (0.91-2.96)		91/229	0.82 (0.60-1.11)		53/229	1.24 (0.84-1.84)	
		TT	68/127	0.90 (0.61-1.33)		15/127	1.41 (0.67-2.94)		10/48	0.42 (0.20-0.86)		7/48	0.85 (0.36-1.96)	
		CT/TT	251/431	0.99 (0.74-1.32)	0.95	60/431	1.58 (0.90-2.78)	0.11	101/277	0.75 (0.56-1.00)	0.05	60/277	1.18 (0.81-1.71)	0.40

^a Based on 458 European American (71.9%) and 443 (75.9%) African American cases who had available data on ER status.

^b OR, odds ratio; 95%CI, 95% confidence interval

^c Adjusted for age at diagnosis, education, body mass index, family history of breast cancer, history of benign breast disease, menopausal status, and proportion of European ancestry.

^d P-trend for genetic dose response determined by coding genotypes as having 0, 1, or 2 variant allele, which was subsequently analyzed as an ordinal variable.

^e P for heterogeneity from dominant or recessive models.

Table 4

Polygenetic risk score^a and breast cancer risk in European American (EA) and African American (AA) women, WCHS

Subgroup	SNPs (risk genotype or allele)	Expected range of polygenetic risk score	Mean (SD) ^b of polygenetic score in cases	Mean (SD) ^b of polygenetic score in controls	OR (95% CI) ^c per unit of composite genetic score	P
Overall risk in EAs	rs1805087 (AA/AG), rs10520873 (AG/GG), rs2612100 (AA)	0-3.0	1.0 (0.6)	0.92 (0.6)	1.50 (1.24-1.81)	<0.0001
Overall risk in AAs	Rs2168781 (GC/CC), rs4925180 (GA/AA), rs2853533 (GC/CC), rs3788189 (TG/GG)	0-4.0	2.1 (1.0)	1.9 (1.0)	1.23 (1.10-1.374)	0.0003
ER+ in EAs	rs10520873 (AG/GG), rs7700970 (CC/CG), rs1643658 (T allele)	0-4.0	2.8 (1.4)	2.5 (1.4)	1.19 (1.08-1.31)	0.0005
ER+ in AAs	Rs6668344 (C allele), rs2275565 (CA/AA), rs2236225 (C allele), rs2168781 (GC/CC), rs2853533 (GC/CC), rs3788189 (TG/GG)	0-9.0	6.4 (2.3)	5.7 (2.3)	1.18 (1.10-1.25)	<0.0001
ER- in EAs	Rs2274976 (GA/AA), rs10520873 (AG/GG), rs567754 (T allele)	0-4.0	1.31 (1.1)	0.9 (0.9)	1.50 (1.20-1.88)	0.0004

^a Weighted composite genetic score is calculated as the sum of the number of risk allele or genotype weighted by their effect sizes of top ranked SNPs in each subgroup, which is then standardized to make 1 unit of the score equal to 1 risk allele or genotype, depending on the genetic model used for each SNP.

^b SD: standard deviation.

^c OR: odds ratio; CI: confidence interval; adjusted for age at diagnosis, education, body mass index, family history of breast cancer, history of benign breast disease, menopausal status, and proportion of European ancestry.

Table 5

Association between genetic polymorphisms in one-carbon metabolism genes and breast cancer risk stratified by dietary folate intake in European American (EA) and African American (AA) women, WCHS

Gene	SNP	Genotype	Low-intake (≤ 232 ug/day)			High-intake (>232 ug/day)			P^e
			# Ca/Co	OR (95% CI) ^{a,b}	$P^{c,d}$	# Ca/Co	OR (95% CI) ^{a,b}	$P^{c,d}$	
Overall EAs									
MTR	Rs6668344 ^f	CC	117/106	1.00	0.49	105/150	1.00	0.02	0.02
		CT	157/126	1.11 (0.76-1.63)		145/153	1.52 (1.06-2.17)		
		TT	46/49	0.76 (0.45-1.28)		46/40	1.61 (0.95-2.71)		
		CT/TT	203/175	1.01 (0.70-1.45)	0.96	191/193	1.54 (1.10-2.16)	0.01	0.06
MTRR	Rs1801394	AA	87/64	1.00	0.82	71/101	1.00	0.20	0.29
		AG	163/160	0.78 (0.51-1.20)		155/161	1.56 (1.05-2.33)		
		GG	70/57	1.08 (0.64-1.80)		70/81	1.34 (0.84-2.14)		
		AG/GG	233/217	0.86 (0.57-1.29)	0.46	225/242	1.49 (1.02-2.17)	0.04	0.03
SLC19A1	Rs3788189 ^g	TT	84/90	1.00	0.28	91/106	1.00	0.09	0.04
		TG	173/146	1.17 (0.79-1.73)		163/162	1.11 (0.76-1.61)		
		GG	64/44	1.32 (0.78-2.24)		44/75	0.60 (0.36-0.98)		
		GG vs. TT/TG	257/236	1.20 (0.75-1.91)	0.45	254/268	0.56 (0.36-0.87)	0.009	0.01
Overall AAs									
MTHFR	Rs7533315	CC	130/170	1.00	0.17	124/146	1.00	0.24	0.07
		CT	146/187	1.00 (0.72-1.38)		117/132	1.05 (0.73-1.51)		
		TT	34/23	1.83 (1.01-3.33)		18/38	0.58 (0.31-1.09)		
		TT vs. CC/CT	276/357	1.83 (1.04-3.25)	0.04	241/278	0.57 (0.31-1.04)	0.06	0.004

^aOR, odds ratio; 95%CI, 95% confidence interval

^bAdjusted for age at diagnosis, education, body mass index, synthetic folate (folic acid) from fortified foods, family history of breast cancer, history of benign breast disease, menopausal status, and proportion of European ancestry.

^c P -trend for genetic dose response determined by coding genotypes as having 0, 1, or 2 variant allele, which was subsequently analyzed as an ordinal variable.

^d P for heterogeneity from dominant or recessive models.

^e P for interaction was for the differences in ORs between low- and high-folate intake.

^fA similar association pattern observed for *MTR*-rs3795708 (in LD with rs6668344)

^gSimilar association patterns observed for other *SLC19A1* SNPs that were found in LD with this SNP: rs2838956, rs2838958, rs4819128, rs12659