Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2009 September ; 18(9): 2453–2459. doi: 10.1158/1055-9965.EPI-09-0159.

Alcohol consumption and genetic variation in *MTHFR* and *MTR* in relation to breast cancer risk

Mary E. Platek[1,2], **Peter G. Shields**[3], **Catalin Marian**[3], **Susan E. McCann**[2], **Matthew R. Bonner**[1], **Jing Nie**[1], **Christine B. Ambrosone**[2], **Amy E. Millen**[1], **Heather M. Ochs-Balcom**[1], **Sylvia K. Quick**[1], **Maurizio Trevisan**[1,4], **Marcia Russell**[5], **Thomas H. Nochajski**[6], **Stephen B. Edge**[7], and **Jo L. Freudenheim**[1]

[1] Department of Social and Preventive Medicine, State University of New York at Buffalo, Buffalo, NY

[2] Department of Cancer Prevention and Control, Epidemiology, and Biostatistics, Roswell Park Cancer Institute, Buffalo, NY

[3] Division of Cancer Genetics and Epidemiology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC

[4] Nevada System of Higher Education, Las Vegas, NV

[5] Prevention Research Center, Berkeley, CA

[6] School of Social Work, University at Buffalo, Buffalo, NY

[7] Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY

Abstract

It has been hypothesized that effects of alcohol consumption on one-carbon metabolism may explain, in part, the association of alcohol consumption with breast cancer risk. The methylenetetrahydrofolate reductase (*MTHFR)* and 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR)* genes express key enzymes in this pathway. We investigated the association of polymorphisms in *MTHFR* (rs1801133 and rs1801131) and *MTR* (rs1805087) with breast cancer risk and their interaction with alcohol consumption in a case-control study, the Western New York Exposures and Breast Cancer (WEB) study. Cases (n=1063) were women with primary, incident breast cancer and controls (n= 1890) were frequency matched to cases on age and race. Odds ratios (OR) and 95% confidence intervals (CI) were estimated by unconditional logistic regression. We found no association of *MTHFR* or *MTR* genotype with risk of breast cancer. In the original case control study, there was a nonsignificant increased odds of breast cancer among women with higher lifetime drinking. In the current study, there was no evidence of an interaction of genotype and alcohol in premenopausal women. However, among postmenopausal women there was an increase in breast cancer risk for women who were homozygote TT for *MTHFR* C677T and had high lifetime alcohol intake (≥1161.84 ounces) (OR=1.92, CI=1.13–3.28) and for those who had a high number of drinks per drinking day (> 1.91 drinks/day) (OR=1.80, CI=1.03–3.28) compared to nondrinkers who were homozygote CC. Our findings indicate that among postmenopausal women, increased breast cancer risk with alcohol consumption may be as a result of effects on one-carbon metabolism.

Correspondence to: Dr. Mary Platek, Cancer Prevention and Population Sciences, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, New York 14263, Tel: 716-845-4394, mary.platek@roswellpark.org.

Keywords

breast cancer epidemiology; one carbon metabolism genes; alcohol

Introduction

Alcohol consumption is an established risk factor for breast cancer. Recently, Key et al conducted a meta analysis of studies that examined incidence of primary breast cancer and alcohol consumption (1). Ninety-eight studies were included and the results showed an excess risk of 22% for drinkers versus non-drinkers. Risk estimates did not significantly differ by beverage type or menopausal status. The authors concluded that the relationship between alcohol and breast cancer may be causal (1).

Furthermore, findings from international studies provide consistent evidence that breast cancer risk is higher for women who drink moderate to high levels of alcohol (approximately greater than 3 drinks/day) in comparison to those who do not drink alcohol at all and that there is a dose response relationship (2,3).

The mechanism for this association, however, is not well understood. One potential mechanism might be alcohol's effect on one-carbon metabolism. One carbon metabolism is critical for the synthesis of purines and pyrimidines and for methylation of macromolecules including DNA (4). Two key enzymes in this pathway are methylenetetrahydrofolate reductase, (*MTHFR)* and 5- methyltetrahydrofolate-homocysteine methyltransferase, also referred to as methionine synthase (*MTR)*(5). Both acute and chronic alcohol ingestion inhibit *MTR* directly (6,7). Inhibition of *MTR* can inhibit *MTHFR.* Furthermore, alcohol consumption adversely affects the availability of methyl groups necessary for one carbon metabolism (8).

MTHFR plays a role in the directing of one carbon moieties from nucleic acid synthesis to methionine synthesis and methylation reactions (4,5,9,10). Two common polymorphisms in this gene are C677T (rs1801133) and A1298C (rs1801131); both affect function. The homozygote variants of the 677 polymorphism and the 1298 polymorphism result in loss of enzyme activity of approximately 70% and 32% respectively (11,12).

MTR catalyzes the methylation of homocysteine to methionine with simultaneous conversion of 5-methyletetrahydrofolate to tetrahydrofolate (THF) (13). *MTR* is necessary for synthesis of S-adenosylmethionine (SAM), required for methylation reactions and of THF, required for nucleotide synthesis (13). There is a common variant of the *MTR* gene (rs1805087; A2756G) which results in an amino acid change from aspartic acid to glycine at codon 919 on chromosome 1q43. To date, no direct functional impact has been established for this variant, but several studies have shown some effect on human homocysteine levels (14–21).

Results of epidemiologic studies of the association of *MTHFR* C677T and *MTHFR* A1298C genotype with breast cancer risk have not been consistent; there are few studies including the *MTR* A2756G polymorphism (22–26). Most have either not included an extensive examination of alcohol intake or have not included alcohol intake at all. Additionally, many studies did not examine the potential modifying effect of menopausal status.

To better understand the role of one carbon metabolism in breast carcinogenesis, we examined these common polymorphisms in *MTHFR* and *MTR*, in a population based case control study, the Western New York Exposures and Breast Cancer (WEB) study. Specifically, we examined main effects of the polymorphisms and interactions with lifetime alcohol consumption in relation to breast cancer risk.

Methods

Study Subjects

Data used in this study were collected as part of a case-control study of breast cancer focused on alcohol consumption, the Western New York Exposures and Breast Cancer (WEB) Study, described in detail elsewhere (27). Subjects were accrued between 1996 and 2001. Briefly, cases were residents of Erie and Niagara counties (35 to 79 years old) with incident, primary, histologically confirmed breast cancer (n=1170; 72% participation rate). Controls (n=2115; 63% participation rate) were frequency matched to cases on age and race. Controls under the age of 65 were randomly selected from the Department of Motor Vehicle driver's license lists and women age 65 and over were randomly selected from the Health Care Financing Administration. Both cases and controls were limited to women who had no previous history of cancer other than non-melanoma skin cancer. Data on demographics, past medical history and other study variables were collected via questionnaires by trained interviewers or by self-administered questionnaires.

Alcohol Consumption

Data on alcohol consumption was collected by trained interviewers during in-person computer assisted interviews. Participants were asked about recent alcohol consumption (12 to 24 months prior to the interview for controls or diagnosis for cases), as well as throughout their lifetime (up to two years prior to interview or diagnosis). Intensity of alcohol consumption, the number of drinks per drinking day, for these same time periods, was also determined. Consumption (absolute and intensity) in the last 2–10 and 2–20 years as well for each age decade (20's, 30's, etc) were calculated.

Dietary and Supplement Intake

Recall of dietary and vitamin and mineral supplement intake 12 to 24 months prior to the interview was collected using a self-administered modified version of the Health Habits and History food frequency questionnaire (FFQ) (28). Nutrient intakes from food were calculated using the DietSys nutrient analysis software (version 3.7) developed specifically for the FFQ (29). Dietary intake was adjusted for total energy by using the residual approach (30,31) in models controlling for diet intake alone that we tested.

Immunohistochemistry

Immunohistochemical staining for estrogen receptor (ER) and progesterone receptor (PR) status was performed in sections of paraffin embedded breast tumor blocks. The Allred score was used to evaluate staining for ER and PR status (32).

Genotyping

A fasting blood sample was collected from all participants who agreed to a blood draw (78% of cases and 88% of controls). For some of the participants who did not agree or were unable to provide a blood draw, an oral rinse sample using the method of Lum and LeMarchand was collected (approximately 17% of cases and 8% of controls) (33). DNA extraction from blood or oral cells was done with the DNAQuik™ (BioServe, Beltsville, MD) extraction kit according to the manufacturer's instructions. Genotyping of *MTHFR* C677T (rs 1801133), *MTHFR* A1298C (rs 1801131) and *MTR* A2756G (rs 1805087) was performed by real time polymerase chain reaction (PCR) allelic discrimination with TaqMan probes in an ABI 7900HT real time PCR system using available probes and primers (Applied Biosystems, Foster City, CA). Quality control procedures were followed for all laboratory assays and included positive and negative controls in all runs and samples analyzed as blind duplicates (20%). The genotype assays were validated for confirming polymorphic Mendelian inheritance patterns in seven

human family cell lines and each encompassing at least three generations. All genotyping included cases and controls together in the runs and laboratory personnel were blinded to case-control status.

Sample Selection and Missing Data

Participants without DNA (n=255), diet (n=51) or alcohol (n=26) information were omitted from these analyses (n=332). This provided a sample size for this study consisting of 1063 cases and 1890 controls. For missing values of other variables, the median value for cases or for controls, stratified by menopausal status was imputed. For missing values of variables that could not be imputed such as family history of breast cancer or history of benign breast disease, an additional "missing" category was created for that variable.

Statistical Analysis

Statistical analyses were performed using SPSS statistical package (Version 15, SPSS Inc., Chicago, IL). Breast cancer risk factors for those who were genotyped and not genotyped for the entire WEB study were compared by chi square analysis for categorical variables and the Student's t test for continuous variables.

Hardy Weinberg equilibrium (HWE) of genotype frequency was tested with the Pearson goodness-of-fit statistic among cases and controls for all of the polymorphisms (34) and all conformed to Hardy Weinberg proportions.

All analyses were stratified by menopausal status. Odd ratios (OR) and 95% confidence intervals (CI) were estimated with unconditional logistic regression for the main effect of each genotype as well as for alcohol-genotype effects. Breast cancer risk factors were included in the adjusted model for main effect of genotypes: age, education, age at menarche, age at first birth, parity, age at menopause (postmenopausal women only), body mass index (BMI) (postmenopausal women only), family history of breast cancer and history of benign breast disease. Due to the number of stratifications required to examine a joint effect of genotype and alcohol intake, a more parsimonious model was used. Parameter estimates for nutrients and supplements related to one carbon metabolism and affected by alcohol intake (folate and vitamins B6, B12 and riboflavin) did not meet our inclusion level of p=0.10 and were therefore not included in the parsimonious model. For examination of multiplicative interactions, p values for interaction were determined by including a multiplicative term in the regression model and deemed significant at $p<0.05$.

Main Effect of Genotype

Logistic regression was performed with breast cancer as the outcome for strata defined by genotype. Strata of the genotype were examined as dominant, co-dominant and recessive models. Additionally, main effects were examined stratified by ER status, PR status and by hormone replacement therapy (HRT) use among postmenopausal women.

Interactions of Genotypes

In exploratory examination of interactions of genotypes or alleles, pre- and postmenopausal women were combined and menopausal status was included in the regression model. The number of variant genotype combinations for the *MTHFR* (*C677T and A1298C*) and *MTR* genotypes were examined in a model with women who did not have a variant genotype as the referent. Additionally, risk for breast cancer based on the number of variant alleles for all three genotypes was examined with women who did not have any risk alleles as the referent.

Interactions of Alcohol Intake and Genotype

Genotype was examined in relation to several measures of alcohol consumption including both absolute intake and intensity of intake. The alcohol variables were categorized into three levels, lifetime nondrinkers, low drinkers and high drinkers. Nondrinkers or abstainers were individuals who reported less than 12 drinks in any one year throughout their lifetime. Low and high drinker categories were based on the median intake in the distribution of the pre- and postmenopausal controls separately, excluding the lifetime nondrinkers. Analysis of drinks per drinking day was further adjusted for total lifetime ounces of alcohol consumed.

RESULTS

In this study of genotype, alcohol and breast cancer risk, we did not find significant differences in characteristics between those who were genotyped and those who were not. Characteristics for cases and controls genotyped in this study are summarized in Table 1.

ORs and 95% CIs for breast cancer risk according to genotype are shown in Table 2. Genotype was not associated with breast cancer risk for any of the three polymorphisms for either pre or postmenopausal women and for all of the genetic models tested (dominant, recessive and co dominant) (results not shown for the different genetic models). Crude odds ratios were similar to adjusted odds ratios; only adjusted estimates are shown in Table 2. Additionally, within strata of ER status and PR status for all women or of hormone replacement therapy use among postmenopausal women, there was also no association of genotype with risk (results not shown).

Exploratory analysis investigating gene-gene interaction showed no increase in the estimated risk for breast cancer among women with more than one variant genotype for any of the three polymorphisms compared to those without any variant genotype (OR=1.40, 95% CI=0.56–3.47). The confidence interval however was wide due to small sample size. We also examined risk associated with the total number of variant alleles for any of the three genotypes; there was no difference in risk for women with one, two or three or more variant alleles, compared to those with none (OR=0.83 (CI=0.60–1.17), OR=0.97 (CI=0.70–1.35) and OR=0.72 (CI=0.50–1.04), respectively).

[*] In the original case control study, the unpublished results show that there was a nonsignificant increased odds of breast cancer among women with high compared to low self-reported lifetime drinking (total ounces during the lifetime or drinks per usual drinking day throughout the lifetime) for both pre and postmenopausal women. The breast cancer risk for those who drank one or more alcoholic beverages per day compared to those who were light drinkers was OR=1.23 (CI=0.80–1.89) for premenopausal women and OR=1.16 (CI=0.88–1.54) for postmenopausal women.

In this study, multiplicative interaction of genotype with lifetime alcohol consumption was examined (Table 3). Although the fully adjusted model estimates were similar to the crude estimates, a more parsimonious model to examine the joint effect was chosen and only adjusted estimates from this model are shown in Table 3. Neither the *MTHFR A1298C* nor *MTR A2756G* genotypes were associated with risk when examined in conjunction with alcohol consumption for either pre or postmenopausal women. The *MTHFR* C677T genotype was associated with breast cancer risk among postmenopausal women with the variant genotype and higher lifetime alcohol consumption compared to women who were non-drinkers with the CC genotype (OR=1.90, 95%, CI=1.09–3.28). Within sub-categories, other statistically significant results for postmenopausal women with the *MTHFR* C677T genotype included an

[*]Freudenheim, JL: Alcohol and Breast Cancer Risk: The Western New York Exposures and Breast Cancer Study

increased risk for nondrinkers and low drinkers with the CT genotype for lifetime total ounces and for nondrinkers with the CT genotype for lifetime drinks per drinking day. The p values for multiplicative interaction, however, were not significant for either drinking category.

Additionally, there was a statistically significant increase in risk for postmenopausal women with the TT genotype who reported more lifetime drinks per drinking days, even after adjusting for lifetime alcohol consumption (OR=1.80, 95%, CI=1.03–3.28). There was no evidence of an association for usual total intake or drinks per drinking day for the more recent time periods of 2 to 10 years or 10–20 years before diagnosis or interview among either pre- or postmenopausal women (data not shown).

We further examined the association with risk of the *MTHFR 677TT* genotype and alcohol consumption during each decade of life among postmenopausal women. While confidence intervals were wide and all included the null, point estimates for risk of breast cancer tended to be higher in the younger years of life for those with the variant genotype. The odds ratio for high drinkers with the TT genotype compared to nondrinkers with the CC genotype were as follows: 1.60 (CI=0.91–2.80) for alcohol consumption < 20 years old, 1.59 (CI=0.92–2.75) for 20–30 years old, 1.51 (CI=0.86–2.64) for 30–40 years old, 1.52 (CI=0.87–2.67) for 40–50 years old, 1.21 (CI=0.61–2.42) for 50–60 years old, 1.06 (CI=0.35–3.18) for 60–70 years old and 0.55 (CI=0.12–2.51) for > 70 years old.

DISCUSSION

In this study, we examined polymorphisms in the rate-limiting enzyme, *MTHFR* and a polymorphism in an associated enzyme, *MTR.* Both enzymes are important in one-carbon metabolism. None of these polymorphisms, however, were associated with an alteration in breast cancer risk in either pre- or postmenopausal women. We did find some evidence of an interaction of *MTHFR C677T* and alcohol with risk of breast cancer among postmenopausal women with the homozygote variant genotype (TT), but there was no evidence of that interaction for the other two polymorphisms.

The biochemical reactions involved in one carbon metabolism are necessary for the synthesis of purines and pyrimidines as well as for the formation of S-adenosylmethionine (SAM), important for the methylation of substrates such as DNA and therefore essential to gene regulation (4). The fundamental acceptor molecule for one carbon metabolism is folate (4). Alcohol consumption can affect the intake, absorption, activation and storage of folate and other nutrients that are methyl contributors (8). Abnormal folate status as a result of alcohol ingestion could potentially adversely affect methylation, both globally and at specific CpG sites in promoter regions of genes as well as nucleotide synthesis and consequently DNA synthesis and repair.

Consistent with our findings regarding the association of *MTHFR* C677T genotype and breast cancer risk, a recent meta-analysis that included seventeen case control studies, found no association of *MTHFR* 677 genotype and breast cancer risk (22); the reported summary OR for TT homozygotes compared to CC homozygotes was 1.04 (95% CI=0.94–1.16) (22). More recently, however, Suzuki et al, reported an increase risk of postmenopausal breast cancer in Japanese women with the *MTHFR* 677 TT genotype (OR = 1.83, 95% CI: 1.08–3.11) (35).

For *MTHFR* A1298C, a number of epidemiological studies have found, as did we, no association of genotype with risk (23,24,36–40). The variant allele for *MTHFR* A1298C may have less impact on enzyme activity than the *MTHFR C677T* variant allele. It is also possible that the variant genotype for this polymorphism is only important in the presence of the variant genotype for the *MTHFR* 677 polymorphism (41). In our study, we did not have any participants who had both variant alleles.

There are few studies examining *MTR* and breast cancer risk (23,25,26) and results are inconsistent. We did not find any alterations in risk by *MTR* genotype for either pre or postmenopausal women in our population.

Gene-gene-environment interactions may also be important in understanding the role that one carbon metabolism plays in breast carcinogenesis. We did an exploratory examination of the significance of having one or more than one variant genotype in comparison to having the common genotype for either polymorphism for *MTHFR* C677T, *MTHFR* A1298C and *MTR* genotype. Additionally, we looked at the number of variant alleles for all three polymorphisms in relation to risk for breast cancer. Our study does not support an association for combined genotype or for multiple alleles of these specific genes.

It is possible that genetic variation in the enzymes involved in one carbon metabolism may not influence the development of breast carcinogenesis independent of other factors such as alcohol intake There is approximately a 10% increase in risk of breast cancer with consumption of one drink of alcohol per day (2,42), yet there have been only a few studies examining *MTHFR C677T* and breast cancer risk that included an examination of interactions with alcohol consumption (23,43–45). Additionally, in these studies the assessment of alcohol was not comprehensive and often limited to recent intake; results are not consistent (23,43–45). Although we did not find evidence for a multiplicative interaction, we found a significant increase in breast cancer risk among postmenopausal women with the TT genotype for *MTHFR* C677T whose lifetime total intake in ounces was above the median which is consistent with our hypothesis. Additionally, we saw an increase in risk for these women whose lifetime drinks per drinking day was above the median, about 1.91 drinks per drinking day, even with adjustment for total intake of alcohol. It is possible that there may be additive interaction without multiplicative interaction which may be of greater interest in the case of disease prevention.

When we examined alcohol consumption within decades, it appeared that drinking at younger ages among postmenopausal women with the *TT* genotype was more associated with risk possibly because of higher alcohol consumption in those years. These findings suggest that one carbon metabolism may play a role in the observed association of alcohol with breast cancer risk among postmenopausal women. We have no explanation for the significant findings among nondrinkers and low drinkers with the CT genotype other than that they may be spurious findings from small cells in subgroup analyses.

A strength of this study was the detailed assessment of lifetime alcohol consumption. Unlike most studies which assess only recent or usual alcohol intake, we were able to assess alcohol intake throughout life. Further, we assessed quantity and frequency separately, allowing us to examine both total consumption and intensity of consumption.

As with any case control study, there are limitations that need to be considered in the interpretation of the findings. There is a possibility of measurement error, particularly for measurement of lifetime alcohol. Nondifferential error in measurement of intake would likely bias results to the null. Despite this potential for error, our methods for measuring alcohol consumption were more comprehensive and detailed than previous assessments of alcohol intake (46). While recall bias regarding report of alcohol may also be a problem, there is evidence that bias in recall of alcohol consumption does not substantially alter results regarding alcohol (47).

To aid us in assessment of selection bias, we conducted a short interview with a subset of those refusing to participate and those who had agreed to participate in the case-control study. There was some tendency for both participating cases and controls to drink more than non-participants who agreed to the short interview; differences were small and not significant. There was some

tendency for participating cases to have lower stage disease than non-participants. It may be that our results are not generalizable to later stage disease. Among participants, we did not find important differences between those who were genotyped and those who were not. It is unlikely that genotype was related to participation in the study. We examined whether or not subjects, who were eliminated from this study due to the absence of DNA or diet information, were different based on alcohol consumption and whether or not this could have contributed a bias. We compared the risk estimates for breast cancer of those in the parent study with those included in this study for alcohol intake and risk estimates were the same between the groups for lifetime total ounces of alcohol intake and lifetime number of drinks per drinking days stratified by menopausal status.

In summary, we found that greater lifetime alcohol consumption among postmenopausal women with the *TT* variant genotype of *MTHFR* C677T was associated with increased risk of breast cancer. Further, we found that intensity of consumption may also contribute to this association. These findings indicate that one carbon metabolism may be important in the pathway leading to carcinogenesis and may explain, at least in part, the observed association of alcohol and breast cancer among postmenopausal women.

Acknowledgments

Funding: This work was funded in part by the Department of Defense grants number DAMD 170310446 and 170010417 and the NIH grants number RO1CA092040, P50AA09802 and R25CA114101.

References

1. Key J, Hodgson S, Omar RZ, et al. Meta-analysis of studies of alcohol and breast cancer with consideration of the methodological issues. Cancer Causes Control 2006;17:759–70. [PubMed: 16783604]
2. Longnecker MP. Alcoholic beverage consumption in relation to risk of breast cancer: meta-analysis and review. Cancer Causes Control 1994;5:73–82. [PubMed: 8123780]
3. Corrao G, Bagnardi V, Zambon A, Arico S. Exploring the dose-response relationship between alcohol consumption and the risk of several alcohol-related conditions: a meta-analysis. Addiction 1999;94:1551–73. [PubMed: 10790907]
4. Bailey LB, Gregory JF. Folate metabolism and requirements. J Nutr 1999;129:779–82. [PubMed: 10203550]
5. Bailey LB, Gregory JF. Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. J Nutr 1999;129:919–22. [PubMed: 10222379]
6. Barak AJ, Beckenhauer HC, Tuma DJ, Badakhsh S. Effects of prolonged ethanol feeding on methionine metabolism in rat liver. Biochem Cell Biol 1987;65:230–3. [PubMed: 3580171]
7. Halsted CH, Villanueva JA, Devlin AM, et al. Folate deficiency disturbs hepatic methionine metabolism and promotes liver injury in the ethanol-fed micropig. Proc Natl Acad Sci USA 2002;99:10072–7. [PubMed: 12122204]
8. Carmel, R. Folic Acid. In: Shils, M.; Shike, M.; Ross, A.; Caballero, B.; Cousins, R., editors. Modern nutrition in health and disease. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 470-81.
9. Bailey LB. Folate, methyl-related nutrients, alcohol, and the MTHFR 677C-->T polymorphism affect cancer risk: intake recommendations. J Nutr 2003;133:3748S–3753S. [PubMed: 14608109]
10. Schwahn B, Rozen R. Polymorphisms in the methylenetetrahydrofolate reductase gene: clinical consequences. Am J Pharmacogenomics 2001;1:189–201. [PubMed: 12083967]
11. Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). Thromb Haemost 1997;78:523–6. [PubMed: 9198208]
12. Weisberg IS, Jacques PF, Selhub J, et al. The 1298A-->C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis 2001;156:409–15. [PubMed: 11395038]

13. Leclerc D, Odievre M, Wu Q, et al. Molecular cloning, expression and physical mapping of the human methionine synthase reductase gene. Gene 1999;240:75–88. [PubMed: 10564814]
14. Tsai MY, Bignell M, Yang F, Welge BG, Graham K, Hanson NQ. Polygenic influence on plasma homocysteine: association of two prevalent mutations, the 844ins68 of cystathionine beta-synthase and A(2756)G of methionine synthase, with lowered plasma homocysteine levels. Atherosclerosis 2000;149:131–7. [PubMed: 10704624]
15. Harmon DL, Shields DC, Woodside JV, et al. Methionine synthase D919G polymorphism is a significant but modest determinant of circulating homocysteine concentrations. Genet Epidemiol 1999;17:298–309. [PubMed: 10520212]
16. Dekou V, Gudnason V, Hawe E, Miller GJ, Stansbie D, Humphries SE. Gene-environment and gene-gene interaction in the determination of plasma homocysteine levels in healthy middle-aged men. Thromb Haemost 2001;85:67–74. [PubMed: 11204591]
17. Chen J, Stampfer MJ, Ma J, et al. Influence of a methionine synthase (D919G) polymorphism on plasma homocysteine and folate levels and relation to risk of myocardial infarction. Atherosclerosism 2001;154:667–72.
18. Morita H, Kurihara H, Sugiyama T, et al. Polymorphism of the methionine synthase gene: association with homocysteine metabolism and late-onset vascular diseases in the Japanese population. Arterioscler Thromb Vasc Biol 1999;19:298–302. [PubMed: 9974410]
19. van der Put NM, van der Molen EF, Kluijtmans LA, et al. Sequence analysis of the coding region of human methionine synthase: relevance to hyperhomocysteinaemia in neural-tube defects and vascular disease. QJM 1997;90:511–7. [PubMed: 9327029]
20. Jacques PF, Bostom AG, Selhub J, et al. Effects of polymorphisms of methionine synthase and methionine synthase reductase on total plasma homocysteine in the NHLBI Family Heart Study. Atherosclerosis 2003;166:49–55. [PubMed: 12482550]
21. Klerk M, Lievers KJ, Kluijtmans LA, et al. The 2756A>G variant in the gene encoding methionine synthase: its relation with plasma homocysteine levels and risk of coronary heart disease in a Dutch case-control study. Thromb Res 2003;110:87–91. [PubMed: 12893022]
22. 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. [PubMed: 17105984]
23. Lissowska J, Gaudet MM, Brinton LA, et al. Genetic polymorphisms in the one-carbon metabolism pathway and breast cancer risk: a population-based case-control study and meta-analyses. Int J Cancer 2007;120:2696–703. [PubMed: 17311260]
24. 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. [PubMed: 16365030]
25. Xu X, Gammon MD, Zhang H, et al. Polymorphisms of one-carbon-metabolizing genes and risk of breast cancer in a population-based study. Carcinogenesis 2007;28:1504–9. [PubMed: 17372271]
26. Pepe C, Guidugli L, Sensi E, et al. Methyl group metabolism gene polymorphisms as modifier of breast cancer risk in Italian BRCA1/2 carriers. Breast Cancer Res Treat 2007;103:29–36. [PubMed: 17151928]
27. Bonner MR, Han D, Nie J, et al. Breast cancer risk and exposure in early life to polycyclic aromatic hydrocarbons using total suspended particulates as a proxy measure. Cancer Epidemiol Biomarkers Prev 2005;14:53–60. [PubMed: 15668476]
28. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A databased approach to diet questionnaire design and testing. Am J Epidemiol 1986;124:453–69. [PubMed: 3740045]
29. Block, G.; Coyle, L.; Hartman, A.; Scoppa, S. HHHQ-DIETSYS analysis software. version 3. Bethesda, MD: National Cancer Institute; 1993.
30. Willett, W.; Stampfer, M. Implications of total energy intake for epidemiologic analysis. In: Willett, W., editor. Nutritional Epidemiology. New York: Oxford University Press; 1998. p. 273-301.
31. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65:1220S–1231S. [PubMed: 9094926]
32. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998;11:155–68. [PubMed: 9504686]

33. Lum A, Le Marchand L. A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. Cancer Epidemiol Biomarkers Prev 1998;7:719–24. [PubMed: 9718225]
34. Xu J, Turner A, Little J, Bleecker ER, Meyers DA. Positive results in association studies are associated with departure from Hardy-Weinberg equilibrium: hint for genotyping error? Hum Genet 2002;111:573–4. [PubMed: 12516594]
35. Suzuki R, Ye W, Rylander-Rudqvist T, Saji S, Colditz GA, Wolk A. Alcohol and postmenopausal breast cancer risk defined by estrogen and progesterone receptor status: a prospective cohort study. J Natl Cancer Inst 2005;97:1601–8. [PubMed: 16264180]
36. Chen J, Giovannucci E, Hankinson SE, et al. A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms, and risk of colorectal adenoma. Carcinogenesis 1998;19:2129–32. [PubMed: 9886567]
37. Ergul E, Sazci A, Utkan Z, Canturk NZ. Polymorphisms in the MTHFR gene are associated with breast cancer. Tumour Biol 2003;24:286–90. [PubMed: 15004488]
38. 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. [PubMed: 15598763]
39. 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. [PubMed: 14973091]
40. Zintzaras E. Methylenetetrahydrofolate reductase gene and susceptibility to breast cancer: a meta-analysis. Clin Genet 2006;69:327–36. [PubMed: 16630166]
41. Stevens VL, McCullough ML, Pavluck AL, et al. Association of polymorphisms in one-carbon metabolism genes and postmenopausal breast cancer incidence. Cancer Epidemiol Biomarkers Prev 2007;16:1140–7. [PubMed: 17548676]
42. Smith-Warner SA, Spiegelman D, Yaun SS, et al. Alcohol and breast cancer in women: a pooled analysis of cohort studies. JAMA 1998;279:535–40. [PubMed: 9480365]
43. Beilby J, Ingram D, Hahnel R, Rossi E. Reduced breast cancer risk with increasing serum folate in a case-control study of the C677T genotype of the methylenetetrahydrofolate reductase gene. Eur J Cancer 2004;40:1250–4. [PubMed: 15110890]
44. Le Marchand L, Donlon T, Hankin JH, Kolonel LN, Wilkens LR, Seifried A. B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). Cancer Causes Control 2002;13:239–48. [PubMed: 12020105]
45. Semenza JC, Delfino RJ, Ziogas A, Anton-Culver H. Breast cancer risk and methylenetetrahydrofolate reductase polymorphism. Breast Cancer Res Treat 2003;77:217–23. [PubMed: 12602921]
46. Russell M, Marshall JR, Trevisan M, et al. Test-retest reliability of the cognitive lifetime drinking history. Am J Epidemiol 1997;146:975–81. [PubMed: 9400340]
47. Giovannucci E, Stampfer MJ, Colditz GA, et al. Recall and selection bias in reporting past alcohol consumption among breast cancer cases. Cancer Causes Control 1993;4:441–8. [PubMed: 8218876]

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2010 September 20.

Table 1

Characteristics of Genotyped (*MTHFR* 677, *MTHFR* 1298 and *MTR* A2756G) Women by Case Control and Menopausal Status: The Western New York Exposures and Breast Cancer Study (WEB)

Characteristics	Pre Menopausal		P Value	Post Menopausal		P Value
	Cases n = 296 Mean (SD)	Controls n = 549 Mean (SD)		Cases n = 767 Mean (SD)	Controls n = 1341 Mean (SD)	
Age (years) *	44.8 (4.6)	44.1 (4.6)	0.02	63.2 (8.4)	63 (8.8)	0.68
Education (years) *	13.4 (2.3)	13.5 (2.4)	0.68	13.3 (2.5)	13.4 (2.5)	0.59
Age at first birth (years) *	20.5 (10.1)	20.9 (9.5)	0.60	20 (9.9)	21.2 (9.0)	< 0.01
Age at menarche (years) *	12.6 (1.7)	12.7 (1.7)	0.43	12.6 (1.6)	12.7 (1.6)	0.19
Age at menopause (years) *	N/A	N/A	N/A	47.8 (5.4)	47.9 (6.2)	0.81
Parity *	2.4 (1.8)	2.6 (1.8)	0.07	2.4 (1.7)	2.7 (1.8)	< 0.01
BMI *	28.4 (7.0)	27.9 (6.4)	0.42	28.1 (6.2)	28.4 (6.1)	0.37
Lifetime Total oz. *	**2251.3 (3.4)**	**2417.6 (6.2)**	**0.36**	**3120.1 (6.2)**	**3490.7 (1.4)**	**0.31**
Lifetime Drinks per Drinking Day *	**2.7 (2.3)**	**2.95 (3.2)**	**0.40**	**2.00 (3.2)**	**2.3 (3.7)**	**0.05**
	Cases n= 296 Frequency (%)	**Controls n=549 Frequency (%)**	**P value**	**Cases n=767 Frequency (%)**	**Controls n=1341 Frequency (%)**	**P value**
Race **						
White	259 (87.5)	505 (92)		703 (91.7)	1215 (90.6)	
Non White	37 (12.5)	44 (8)	0.04	64 (8.3)	126 (9.4)	0.42
Ever Use HRT **	*§*	*§*	*§*	n=763	n=1335	
Yes				420 (55)	662 (50.9)	0.07
Have/Had Benign Breast Disease **	n=291	n=545				
Yes	82 (27.7)	130 (23.7)	< 0.01	213 (27.8)	326 (24.3)	0.05
Family History of Breast Cancer **	n=269	n=518		n=710	n=1236	
Yes	50 (18.6)	77 (14.9)	0.07	126 (16.4)	150 (11.2)	< 0.01
Lifetime Drinker Status **	n=293	n=544		n=762	n=1328	
Nondrinker	36 (12.3)	54 (9.9)		141 (18.5)	216 (16.3)	

Characteristics	Pre Menopausal		P Value	Post Menopausal		P Value
	Cases n = 296 Mean (SD)	Controls n = 549 Mean (SD)		Cases n = 767 Mean (SD)	Controls n = 1341 Mean (SD)	
Lifetime Drinker	257 (87.7)	490 (90.1)	0.29	621 (81.5)	1112 (83.7)	0.19

*Student t test for continuous characteristics

**Chi Square analysis for categorical characteristics

§Not Applicable for premenopausal women

Table 2

MTHFR C677T, *MTHFR* A1298C and *MTR* A2756G genotype and risk of breast cancer: The Western New York Exposures and Breast Cancer Study (WEB)

Genotype	Premenopausal*	
	N cases/controls	Adjusted OR (95% CI)
MTHFR C677T		
CC (ref)	120/222	1.00
CT	120/244	0.94 (0.67–1.30)
TT	34/65	0.95 (0.58–1.56)
CT/TT	154/309	0.93 (0.69–1.27)
MTHFR A1298C		
AA (ref)	119/248	1.00
AC	100/226	0.93 (0.66–1.29)
CC	29/49	1.15 (0.97–1.97)
AC/CC	129/275	0.97 (0.71–1.33)
MTR A2756G		
AA	206/358	1.00
AG	75/158	0.82 (0.59–1.16)
GG	11/30	0.70 (0.33–1.47)
AG/GG	86/188	0.81 (0.59–1.11)
	Postmenopausal**	
MTHFR C677T		
CC (ref)	309/566	1.00
CT	326/551	1.11 (0.91–1.36)
TT	85/154	1.03 (0.76–1.41)
CT/TT	411/705	1.10 (0.91–1.33)
MTHFR A1298C		
AA (ref)	324/594	1.00
AC	302/532	1.00 (0.82–1.22)
CC	54/132	0.73 (0.51–1.04)
AC/CC	356/666	0.94 (0.78–1.14)
MTR A2756G		
AA	472/826	1.00
AG	242/444	0.98 (0.80–1.20)
GG	43/59	1.35 (0.88–2.07)
AG/GG	285/503	1.02 (0.85–1.24)

*Adjusted for age, education, age at menarche, parity, age at first birth, history of benign breast disease and family history of breast cancer

** Adjusted for age, education, age at menarche, parity, age at first birth, history of benign breast disease, family history of breast cancer, age at menopause and BMI

Table 3

Risk of breast cancer by MTHFR C677T genotype and alcohol intake (lifetime total alcohol intake and lifetime drinks per drinking day): The Western New York Exposures and Breast Cancer Study (WEB)

Genotype	**CC**		**CT**		**TT**	
Premenopausal	**N Ca/Co**	**Adjusted OR (95% CI)**	**N Ca/Co**	**Adjusted OR (95% CI)**	**N Ca/Co**	**Adjusted OR (95% CI)**
§**Lifetime Total oz***						
Nondrinkers	20/25	1.00	14/27	0.71 (0.28–1.83)	6/7	1.14 (0.30–4.27)
Low drinkers	53/109	0.69 (0.33–1.45)	50/100	0.76 (0.36–1.61)	9/28	0.46 (0.16–1.35)
High drinkers	47/88	0.88 (0.42–1.87)	56/117	0.68 (0.33–1.41)	19/30	1.03 (0.42–2.51)
†**Lifetime Drinks Per Drinking Day****						
Nondrinkers	18/22	1.00	12/25	0.66 (0.24–1.81)	6/5	1.47 (0.35–6.16)
Low drinkers	47/100	0.67 (0.30–1.49)	51/98	0.72 (0.33–1.58)	13/30	0.58 (0.21–1.59)
High drinkers	52/94	0.91 (0.41–2.01)	54/117	0.72 (0.32–1.58)	15/28	0.98 (0.37–2.61)
Postmenopausal						
††**Lifetime Total oz***						
Nondrinkers	58/122	1.00	67/102	1.62 (1.02–2.58)	15/13	1.03 (0.48–2.19)
Low drinkers	130/222	1.46 (0.98–2.19)	135/225	1.49 (1.00–2.23)	28/68	1.05 (0.60–1.86)
High drinkers	121/222	1.27 (0.84–1.91)	124/224	1.35 (0.90–2.02)	42/55	1.92 (1.13–3.28)
‖**Lifetime Drinks Per Drinking Day****						
Nondrinkers	54/103	1.00	62/78	1.81 (1.10–2.98)	14/24	1. 09 (0.49–2.44)
Low drinkers	128/222	1.25 (0.82–1.91)	138/215	1.39 (0.91–2.12)	29/68	0.98 (0.55–1.75)
High drinkers	123/222	1.26 (0.82–1.94)	120/231	1.23 (0.80–1.89)	41/54	1.80 (1.03–3.14)

*Adjusted for age, history of benign breast disease and family history of breast cancer (BMI for postmenopausal women only)

**Further adjusted for lifetime total ounces of alcohol

[§] Low/High cutoff = 1001.58 oz.

[†] Low/High cutoff = 2.35 Drinks

[††] Low/High cutoff = 1161.84 oz

[||] Low/High cutoff = 1.91 Drinks