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Breast cancer is thought to be largely preventable through dietary and lifestyle modifications. Diets high in vegetables and fruits have been associated with reduced risk of breast cancer in many epidemiologic studies. Minor components of diet such as micronutrients including vitamins may be involved in mediating these associations, but it is not known which micronutrients are involved or how they act. Such knowledge is critical to rationally design and to implement a preventive strategy against breast cancer. Folate, a B vitamin mostly found in vegetables and fruits, may be a protective micronutrient in diet. It is a crucial component in DNA methylation as well as DNA synthesis, both of which are important processes in etiology of breast cancer. Certain genes involved in these processes differ from on person to another. Therefore, a portion of the general population with inherited sub-optimal folate metabolism along with low folate intake may be at increased risk of developing breast cancer. We plan to use a multi-disciplinary approach to study both nutritional and genetic aspects of the disease. We will investigate: (1) whether dietary folate is a micronutrient that is protective against breast cancer; (2) whether a proportion of the population with inherited sub-optimal folate metabolism is at increased risk of breast cancer; (3) whether such inherited variability modifies the association of folate intake and risk of breast cancer; and (4) whether folate may interact with alcohol that interrupts folate metabolism in contributing to risk of breast cancer.

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Table of Contents

INTRODUCTION

Breast cancer is thought to be largely preventable through dietary and lifestyle modifications. Low levels of folate and related B vitamins both in diet and in circulating have been associated with risk of breast cancer in several prospective epidemiologic studies. Folate, a B vitamin mostly found in vegetables and fruits, is a crucial component in DNA methylation as well as DNA synthesis, both of which are important processes in etiology of breast cancer (Figure 1). Certain genes involved in these processes differ from on person to another. Therefore, a portion of the general population with inherited sub-optimal folate metabolism along with low folate intake may be at increased risk of developing breast cancer. We plan to utilize resource of the Long Island Breast Cancer Project, a large population-based case-control study, to study both nutritional and genetic aspects of the disease. We will investigate: (1) whether dietary folate is a micronutrient that is protective against breast cancer; (2) whether a proportion of the population with inherited sub-optimal folate metabolism is at increased risk of breast cancer; (3) whether such inherited variability modifies the association of folate intake and risk of breast cancer; and (4) whether folate may interact with alcohol that interrupts folate metabolism in contributing to risk of breast cancer. The significance of this research lies in its potential not only to clarify etiology of breast cancer but also to guide the prevention of breast cancer through dietary modification. Furthermore, because a high proportion of the general population may inherit sub-optimal folate metabolism, attributable risk associated with these genetic factors may be quite significant. Associations between such susceptibility and risk of breast cancer will provide an extremely valuable guide to preventive dietary and other lifestyle modifications in individuals and in the population at large.

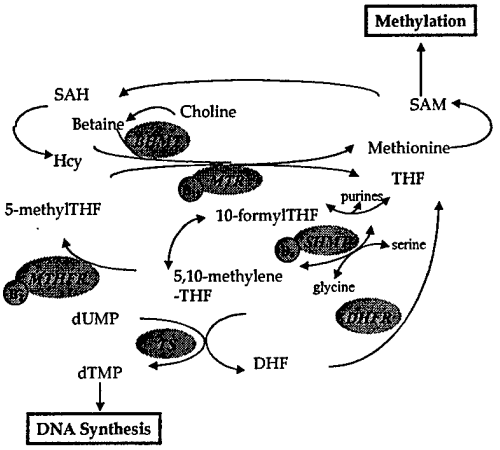


Figure 1. One-Carbon Metabolic Pathway

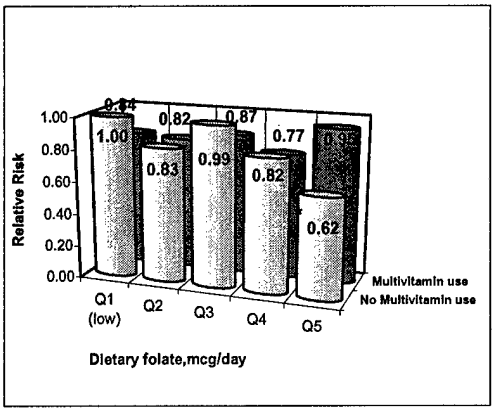
WORK ACCOMPLISHED

1) Associations of B vitamin intake with risk of breast cancer in the Long Island Breast Cancer Study Project.

We have computed dietary levels of B vitamins from the food frequency questionnaire (FFQ) of the LIBSCP. This instrument was self-administered and completed by 1,481 (98.2%) of cases and 1,518 (97.6%) control participants in an average of 36 minutes. Dietary intake values for one-carbon related micronutrients, folate (the bioactive ingredient is vitamin B_9 – folic acid), vitamins B_1 (thiamin), B2 (riboflavin), B_3 (niacin) and B_6 (pyridoxine), were calculated from the FFQ based on food items, serving sizes and consumption frequencies. We also examined total consumption for each B vitamin by summing dietary intake and supplemental sources of these micronutrients. Use of vitamin supplements was queried on the FFQ. We have built logistic regression models to assess risk of breast cancer associated B vitamins. We used unconditional logistic regression to estimate odds ratios (ORs) of BC with 95% confidence intervals (CIs). Significant inverse

associations between B vitamin intake and BC risk were observed among non-supplement users; the p for trend across the quintiles of intakes was 0.06 for dietary folate, 0.002 for vitamin B_1, 0.05 for vitamin B_2, and 0.03 for vitamin B_6. The greatest reduction in BC risk was observed among non-supplement users in the highest quintile of dietary folate intake (OR 0.62, 95%CI 0.41-0.93) as compared with non-supplement users in the lowest quintile of dietary folate intake (high-risk individuals) (Figure 2).

Figure 2. Relationship of folate intake and BC risk in the LIBCSP



2) MTHFR polymorphisms and risk of breast cancer in the Long Island Breast Cancer Study Project. MTHFR is at a critical metabolic branch point of one-carbon metabolism; it carries out the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which directs the folate pool towards remethylation of homocysteine to methionine, at the expense of thymidylate synthesis (Figure 1). A single nucleotide polymorphism of the *MTHFR* gene (*677C>T*) is associated with an alanine-to-valine substitution and is correlated with enzyme thermolability and reduced enzyme activity. Individuals with the *677TT* genotype tend to accumulate 5,10-methylene THF intracellularly at the expense of 5-methyl THF, while individuals with the 677*CC* or 677*CT* genotypes have predominantly 5-methyl THF intracellularly. Additionally, the *677TT* genotype has been shown to correlate with suboptimal folate status in terms of decreased folate and increased homocysteine levels in serum or plasma. A second common polymorphism in the C terminal regulatory domain of the gene, *MTHFR 1298A>C (gln>ala)*, has also been identified, but its function remains controversial.

The *MTHFR 677C>T* genotypes were ascertained from 1063 cases (70% of eligible cases) and 1104 controls (71% of eligible controls). To ensure that sub-samples are representative for the target population, we compared the distribution of risk factors for breast cancer among participants with ascertained genotypes to that of all eligible participants. Comparable results were obtained (data not shown). The genotype distribution was in agreement with Hardy-Weinberg Equilibrium in both cases (p = 0.30) and controls (p = 0.96). The *677T* allele frequency of 40% among the cases was higher than that of controls (37%). The *677T* variant allele was associated with increased breast cancer risk in a dose-dependent fashion (Table 3). Compared to individuals with the wild-type genotype of *677CC*, those with the *677TT* genotype had an age-adjusted OR of 1.34 (95% CI 1.04-1.73) (p, trend = 0.04). After adjusting for additional risk factors including family history of breast cancer in a first-degree relative, history of benign breast disease, education, and BMI at age 20, the dose-dependent relationship remained elevated with a multivariate-adjusted OR of 1.37 (95%CI 1.06-1.78) for *677TT* and a p-value for trend of 0.03.

The *MTHFR 1298A>C* polymorphism was ascertained from 1062 cases and 1103 controls (Table 3). The genotype distributions were in agreement with the Hardy-Weinberg Equilibrium (p=0.89 for cases; p=0.84 for controls). We observed an inverse association of the *1298C* allele and risk of breast cancer in a dose dependent fashion (p, trend = 0.03); the *1298CC* genotype conferred a significantly lower risk of breast cancer compared to the *1298AA* genotype (OR 0.73; 95%CI 0.53 – 1.00). This relationship was not modified by menopausal status or the stage of breast cancer.

A high degree of LD was observed between the *677C>T* and *1298A>C* polymorphisms (D'=-0.54, p<0.001). The negative sign of the D' indicates that the *677C-1298C* (or *677T-1298A*) alleles were linked. When combined genotypes were examined, individuals who are homozygous with risk alleles at both loci (*677TT-1298AA*) had significant significantly elevated risk of breast cancer (OR 1.77; 95%CI 1.28 – 2.50) compared to those who are homozygous with wild-type alleles (*677CC-1298AA*). Combined heterozygosity did not modify the disease risk; individuals who were heterozygous at both loci (*677CT-1298AC*) had similar risk as those with the *677CC-1298AA* genotype (OR 1.13, 95%CI 0.84 – 1.52) (Table 1).

We also examined the *MTHFR*-breast cancer association according to menopausal status (pre- *vs.* post-menopausal). Comparable results were observed in both groups for the *677C>T* polymorphism (data not shown). The inverse association of the *1298A>C* polymorphism with breast cancer risk was only present in post-menopausal women with a multivariate-adjusted OR of 0.65 (95%CI 0.44-0.96; p, trend = 0.02). The *MTHFR*-breast cancer associations did not differ significantly with respect to *in situ* and invasive cases.

Table 1. Odds ratios and 95% CI for *MTHFR* polymorphisms with risk of breast cancer in the Long Island Breast Cancer Study Project, 1996-1997.

Genotype	# cases (%)	# controls (%)	OR (95% CI)[1]	OR (95%CI)[2]
677C>T				
677CC	398 (37.4)	440 (39.9)	1.0 (ref)	1.0 (ref)
677CT	476 (44.8)	509 (46.1)	1.04 (0.87-1.26)	1.05 (0.87-1.27)
677TT	189 (17.8)	155 (14.0)	**1.34 (1.04-1.73)**	**1.37 (1.06-1.78)**
p, trend[3]			*0.04*	*0.03*
1298A>C				
1298AA	558 (52.5)	536 (48.6)	1.0 (ref)	1.0 (ref)
1298AC	417 (39.3)	457 (41.4)	0.88 (0.74-1.06)	0.87 (0.72-1.05)
1298CC	87 (8.2)	110 (10.0)	0.77 (0.56-1.04)	**0.73 (0.53-1.00)**
p,trend			*0.05*	*0.03*
Combined Genotypes				
677CC-1298AA	146 (13.8)	172 (15.6)	1.0 (ref)	1.0 (ref)
677CC-1298AC	188 (17.8)	198 (18.0)	1.16 (0.85-1.56)	1.10 (0.82-1.49)
677CC-1298CC	63 (6.0)	69 (6.3)	1.09 (0.72-1.64)	1.06 (0.70-1.59)
677CT-1298AA	251 (23.7)	259 (23.6)	1.17 (0.88-1.55)	1.13 (0.85-1.49)
677CT-1298AC	207 (19.6)	218 (19.8)	1.16 (0.86-1.56)	1.13 (0.84-1.52)
677CT-1298CC	17 (1.6)	32 (2.9)	0.65 (0.35-1.23)	0.63 (0.34-1.19)
677TT-1298AA	158 (14.9)	102 (9.3)	**1.85 (1.32-2.60)**	**1.77 (1.28-2.50)**
677TT-1298AC	22 (2.1)	41 (3.7)	0.66 (0.37-1.17)	0.62 (0.35-1.10)
677TT-1298CC	6 (0.6)	9 (0.8)	0.89 (0.31-2.59)	0.86 (0.30-2.48)

[1] Adjusted for age

[2]Adjusted for age, family history of breast cancer in first-degree relative, history of benign breast disease, educational attainment, BMI at age 20, and kcal per day.

3 p value for trend for categorical variables

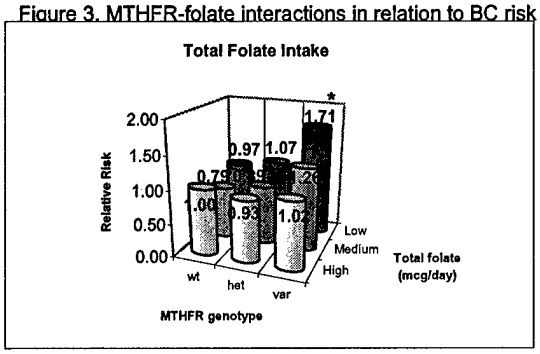
3) Diet - MTHFR interactions and risk of breast cancer in the Long Island Breast Cancer Study Project.

When the *MTHFR*-breast cancer relationship was examined according to supplement use, dose-dependent relations were only apparent among non-supplement users, with p values for trend of 0.005 and 0.02 for the *677C>T* and *1298A>C* polymorphisms, respectively. In this subgroup, the *677TT* genotype was associated with a 70% increase in breast cancer risk (95%CI 1.14-2.52) while the *1298CC* genotype was associated with a 38% reduction in risk (95%CI 0.38-1.01).

We examined interactions between *MTHFR* polymorphisms and folate intake in relation to breast cancer risk. With respect to the *677C>T* polymorphism, compared to low-risk individuals (*677CC* genotype with high folate intake), elevation of breast cancer risk was the most pronounced among *677TT* women who consumed the lowest levels of dietary folate (OR 1.83, 95%CI 1.13-2.96) or had the lowest total folate intake (OR 1.71, 95%CI 1.08-2.71), although the interactions for both models were not significant on a multiplicative scale (Figure 3). Similar associations were observed for every other B vitamin we examined; the *677TT* individuals had the highest ORs when their dietary consumption was in the lowest category of vitamins B_1, B_2, B_3 and B_6 at

2.06 (95%CI 1.25-3.40), 1.88 (95%CI 1.17-3.01), 2.05 (95%CI 1.25-3.38) and 2.36 (95%CI 1.47-3.88), respectively. No indication of effect modification by the *1298A>C* polymorphism was apparent in the study; the p for interaction was 0.84 for dietary folate and 0.94 for total folate.

Figure 3. MTHFR-folate interactions in relation to BC risk in the LIBCSP



4) Polymorphisms of TS and MTRR in relation to breast cancer in the Long Island Breast Cancer Study Project.

We have genotyped two additional folate-metabolizing genes, TS and MTRR (Figure 1). In a preliminary analysis, the TS 2R/2R genotype confers a non-significantly increased risk of breast cancer (OR 1.22, 95%CI 0.96 – 1.55). Such association was not modified by menopausal status, multivitamin use, alcohol intake, and tumor characteristics. There appear to be a gene-gene interactions between MTHFR and TS (p, interaction = 0.04). We are in the process of analyzing the relationship breast cancer risk and the MTRR polymorphism.

REPORTABLE OUTCOMES

We have presented the results of this study at the following meeting:

Chen J, Gammon MD, Chan W, Palomeque C, Kabat GC, Terry MB, Teitelbaum SL, Britton JA, Neugut AI, Santella RM. Folate Metabolism Modifies the Risk of Breast Cancer in the Long Island Breast Cancer Study Project. 91st Annual Meeting of American Association for Cancer Research, Washington DC, 2003.

Chen J, Gammon MD, Chan W, Kabat GC, Terry MB, Teitelbaum SL, Eng SM, Neugut AI, Santella RM. Intake and metabolism of b vitamins and risk of breast cancer in the Long Island Breast Cancer Study Project. Era of Hope Department of Defense Breast Cancer Research Program Meeting, Orlando, Florida, 2002

We have submitted the following manuscript for publication:

Chen J, Gammon MD, Chan W, Palomeque C, Wetmur JG, Kabat GC, Teitelbaum SL, Britton JA, Terry MB, Neugut AI, Santella RM. One-Carbon Metabolism, *MTHFR* polymorphisms and Risk of Breast Cancer. Cancer Research. Submitted.

Two additional manuscripts are in preparation.

CONCLUSIONS

- Dietary intakes of B vitamins are associated with modestly reduced risk of breast cancer among non-supplement users.
- Among non-multivitamin users, dietary intakes of B vitamins are protective against breast cancer in dose-dependent fashion.
- The MTHFR 677C>T variant allele is associated with significantly increased risk of breast cancer.

- Suboptimal folate metabolism (i.e. MTHFR polymorphism) increases the susceptibility to breast cancer, especially among those with insufficient folate intake; however, such enhanced risk may be reduced by increasing folate consumption.

APPENDICES

1) Abstract for the 91st Annual Meeting of American Association for Cancer Research, Washington DC, 2003.
2) Abstract for the Era of Hope Department of Defense Breast Cancer Research Program Meeting, Orlando, Florida, 2002
3) Manuscript submitted to the journal of Cancer Research/

Folate Metabolism Modifies the Risk of Breast Cancer in the Long Island Breast Cancer Study Project

Jia Chen, Marilie D. Gammon, Wendy Chan, Caroline Palomeque, Geoffrey C. Kabat, Mary Beth Terry, Susan L. Teitelbaum, Julie A. Britton, Alfred I. Neugut, Regina M. Santella

Breast cancer (BC) is thought to be largely preventable through dietary and lifestyle modifications. Insufficient intake of folate, in concert with consumption of alcohol which is a folate antagonist, has been associated with increased risk of BC in several large cohort studies. However, as micronutrients in diet are likely to be highly correlated, it is difficult to pinpoint folate as the responsible micronutrient. We investigated the effect of folate intake on risk of BC as well as effect modifications by a functional polymorphism of the folate-metabolizing gene, i.e. *methylenetetrahydrofolate reductase* (*MTHFR*) *677C->T*. The study utilizes the resources of the Long Island Breast Cancer Study Project (LIBSCP), a large population-based case-control study consisting of 1479 cases and 1522 controls. Total (dietary + supplemental) intake of folate was inversely related to BC risk (p, trend = 0.03). When stratified by multivitamin use, increased dietary folate consumption among women without use of supplements significantly reduced the BC risk; women in the highest quintile of dietary folate intake had an odds ratio (OR) of 0.63 [0.41, 0.99] compared to those with dietary folate intake in the lowest quintile. A similar association was apparent among women who did not consume any alcohol at the baseline (p, trend = 0.04); women in the highest quintile of dietary folate intake had an OR of 0.64 [0.42-0.98] compared to those with dietary folate intake in the lowest quintile. Among the 1063 cases and 1104 controls who donated blood specimens, the *MTHFR 677T* variant allele significantly increased risk of BC with multivariate-adjusted ORs of 1.05 [0.87, 1.27] and 1.37 [1.06, 1.78] for the *CT* and *TT* genotypes, respectively (p, trend = 0.03). These associations were more prominent among premenopausal women (OR 1.87 [1.17, 2.99] for the *TT* genotype, p, trend = 0.02); women who did not use multivitamin supplements (OR 1.70 [1.14, 2.52] for the *TT* genotype, p, trend = 0.005); and women who consumed alcohol (OR 2.02 [1.38, 2.96] for the *TT* genotype, p, trend = 0.002). We observed a significant interaction between the *MTHFR* polymorphism and dietary folate intake (p, interaction = 0.007); the *TT* homozygotes with low dietary folate intake (≤ 194 mg/day) had an OR of 1.82 [1.13, 2.95] compared to the *CC* homozygotes with high total folate intake (≥ 301 mg/day). A significant

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interaction between the *MTHFR* polymorphism and use of alcohol was also apparent (p, interaction = 0.01); the *TT* homozygotes who consumed alcohol had an OR of 1.51 [1.04, 2.20] compared to the *CC* homozygotes with no alcohol use. These observations suggest that folate is a key anticarcinogenic micronutrient in diet. Women with the *MTHFR 677TT* genotype, in particular those who consume alcohol and insufficient amounts of folate, appear to be more susceptible to BC.

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INTAKE AND METABOLISM OF B VITAMINS AND RSIK OF BREAST CANCER IN THE LONG ISLAND BREAST CANCER STUDY PROJECT

Jia Chen, Marilie D. Gammon, Geoffrey C. Kabat, Mary Beth Terry, Wendy Chan, Alfred I. Neugut, Regina M. Santella

Department of Community and Preventive Medicine, Mount Sinai School of Medicine [J.C., W.C]; Department of Epidemiology, University of North Carolina at Chapel Hill [M.D.G]; Department of Epidemiology, Columbia University [G.C.K., M.B.T, A.I.N, R.M.S]

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ABSTRACT: Begin single-space abstract here. Abstract MUST NOT EXCEED ONE PAGE. Abstracts will appear in the proceedings EXACTLY as submitted.
Breast cancer is thought to be largely preventable through dietary and lifestyle modifications. Micronutrients in diet such as vitamins may modify the risk of breast cancer. Insufficient levels of folate (Vitamin B9) and other B vitamins have been associated with increased risk of breast cancer in several epidemiologic studies. A functional polymorphism in a folate-metabolizing gene, methylenetetrahydrofolate reductase (MTHFR) 677C->T, influences the distribution and bioavailability of these nutrients in the body, thus may modify the risk of breast cancer associated with these nutrients. We are investigating the association of dietary intake of B vitamins in relation to breast cancer risk in the Long Island Breast Cancer Study Project, a large population-based case-control study consisting 1508 cases and 1555 controls. More specifically, we will report our findings on risk of breast cancer associated with dietary intake of folate as well as vitamins B1, B2, B3, B6 and B12. In addition, we will report the association of the MTHFR 677C->T polymorphism and risk of breast cancer and its interactions with dietary B vitamins in relation to breast cancer risk.

THE U.S. ARMY MEDICAL RESEARCH MATERIEL COMMAND UNDER DAMD17-XX-X-XXXX SUPPORTED THIS WORK.

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One-Carbon Metabolism, *MTHFR* polymorphisms and Risk of Breast Cancer[1]

Jia Chen[2], Marilie D. Gammon, Wendy Chan, Caroline Palomeque, James G. Wetmur, Geoffrey C. Kabat, Susan L. Teitelbaum, Julie A. Britton, Mary Beth Terry, Alfred I. Neugut, Regina M. Santella

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Running title: Folate, *MTHFR* and breast cancer

Key words: folate, one-carbon, *MTHFR*, breast cancer, gene-environment

ABSTRACT

Accumulating evidence from epidemiologic studies suggests that risk of breast cancer is reduced in relation to increased consumption of folate and related B vitamins. We investigated independent and joint effects of B vitamin intake as well as two polymorphisms of a key one-carbon-metabolizing gene, i.e. *methylenetetrahydrofolate reductase* (*MTHFR*) *677C>T* and *1298A>C*, on breast cancer risk. The study utilizes the resources of a population-based case-control study, which includes 1,481 cases and 1,518 controls. Significant inverse associations between B vitamin intake and breast cancer risk were observed among non-supplement users. The greatest reduction in of breast cancer risk was observed among non-supplement users in the highest quintile of dietary folate intake [odds ratio (OR) 0.61, 95%Confidence Interval (CI) 0.41-0.93] as compared with non-supplement users in the lowest quintile of dietary folate intake (high-risk individuals). The *MTHFR 677T* variant allele was associated with increased risk of breast cancer (p, trend = 0.03) with a multivariate-adjusted OR of 1.37 (95%CI1.06- 1.78) for the *677TT* genotype. The *1298C* variant allele was inversely associated with breast cancer risk (p, trend = 0.03), and was likely due to the linkage of this allele to the low risk allele of *677C*. The *MTHFR*-breast cancer associations were more prominent among women who did not use multivitamin supplements. Compared to *677CC* individuals with high folate intake, elevation of breast cancer risk was most pronounced among *677TT* women who consumed the lowest levels of dietary folate (OR 1.83, 95%CI 1.13-2.96) or total folate intake (OR 1.71, 95%CI 1.08-2.71). From a public heath perspective, it is important to identify risk factors, such as low B vitamin consumption, that may guide an effective prevention strategy against the disease.

INTRODUCTION

There is considerable interest in identifying risk factors associated with breast cancer that can be modified to reduce the risk of the disease. Accumulating evidence from epidemiologic studies suggests a protective role of folate and related B vitamins against breast cancer, especially among alcohol users. Four large prospective epidemiologic studies on these associations have been published[1-4]; three found that adequate folate intake may reduce the risk of breast cancer. In the large Nurses' Health Study[1], a significant reduction in risk associated with total folate as well as folate from multivitamin supplements was observed among women with daily consumption of ≥15g of alcohol, a known folate antagonist. Similar results have also been observed on dietary folate in the Canadian National Breast Screening Study[2] and the Iowa Women's Health Study[3]. However, in a recent study on 1303 postmenopausal breast cancer cases in the American Cancer Society Cancer Prevention Study II Nutrition cohort (N=66,561), no effect of folate on risk of breast cancer was apparent[4]. In addition to these four prospective studies focusing on dietary folate intake, two other prospective studies on biological methyl levels also suggest that higher plasma B vitamin levels are associated with lower risk of breast cancer[5,6]. Most of these findings corroborate evidence from case-control studies conducted in the US[7,8], Italy[9,10] and China[11].

Breast cancer is a manifestation of abnormal genetic as well as epigenetic changes. Interruption of one-carbon metabolism may be important in breast cancer etiology as it facilitates the cross-talk between genetic and epigenetic processes by playing critical roles in both DNA methylation and DNA synthesis (Figure 1). One-carbon metabolism is a network of interrelated biological reactions that provides essential cofactors for the production of S-adenosylmethionine (SAM), the primary methyl donor for methylation, as well as the methyl group in methylation of dUMP to dTMP for DNA synthesis (reviewed by Choi and Mason[12]). A low methyl supply induces DNA global hypomethylation[13] as well as deficient methylation of dUMP to dTMP leading to uracil misincorporation[14]. Folate deficiency results in interruption of

DNA repair capability,[15] which may lead to DNA strand breaks, enhanced mutagenesis and apoptosis.

Folate (as well as methionine and choline) is the major source of methyl groups from foods [16]; dietary folate depletion alone is a sufficient perturbing force to diminish the methyl pool[17]. Other B vitamins, such as vitamins B_2, B_6 and B_{12}, are also key cofactors for one-carbon metabolism that involves a constellation of genes, such as *methylenetetrahydrofolate reductase* (*MTHFR*). MTHFR is at a critical metabolic branch point of one-carbon metabolism; it carries out the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which directs the folate pool towards remethylation of homocysteine to methionine, at the expense of thymidylate synthesis (Figure 1). A single nucleotide polymorphism of the *MTHFR* gene (*677C>T*) is associated with an alanine-to-valine substitution and is correlated with enzyme thermolability and reduced enzyme activity[18]. Individuals with the *677TT* genotype tend to accumulate 5,10-methylene THF intracellularly at the expense of 5-methyl THF, while individuals with the 677*CC* or 677*CT* genotypes have predominantly 5-methyl THF intracellularly[19]. Additionally, the *677TT* genotype has been shown to correlate with suboptimal folate status in terms of decreased folate and increased homocysteine levels in serum or plasma[20]. A second common polymorphism in the C terminal regulatory domain of the gene, *MTHFR 1298A>C (gln>ala)*, has also been identified[21], but its function remains controversial.

Despite ample epidemiologic evidence and strong biological plausibility, few studies have examined whether functional polymorphisms in one-carbon metabolizing genes modify the risk of breast cancer associated with dietary intake of folate and other methyl-related nutrients. The only report on folate-gene interactions comes from the Shanghai Breast Cancer Study conducted in China[22], in which the *MTHFR 677C>T* polymorphism was not an independent predictor of breast cancer risk, whilst individuals with the *677TT* genotype had elevated risk of breast cancer when dietary folate consumption was low. Since the dietary pattern in Chinese women tends to be different from their counterparts in western countries, it is not clear whether

these findings would be reproduced in the US population. We utilized the resources of the Long Island Breast Cancer Study Project, a US-population-based study, to examine the independent and joint effects of B vitamin intake and related metabolizing genes on risk of breast cancer.

SUBJECTS AND METHODS

Subjects: The Long Island Breast Cancer Study Project was designed to determine whether the risk of breast cancer is associated with PAH-DNA adducts and organochlorine compounds. A detailed description of the study has been published elsewhere[23]. In brief, cases were women newly diagnosed with a primary *in situ* or invasive breast cancer between August 1, 1996, and July 31, 1997, and who were residents of Long Island (Nassau and Suffolk counties) in New York at the time of their diagnosis. Among a total of 2,030 eligible cases, we were able to obtain physician's permission for 1,837 cases (90.5%). Physician refusal was commonly due to illness of the patient. Control women were a sample of current residents of Nassau and Suffolk counties who spoke English and who were frequency matched to the expected age distribution of case women by five-year age groups. Potentially eligible control women were identified by Waksberg's method of random digit dialing (RDD)[24] for those under 65 years of age, and by Health Care Finance Administration (HCFA) rosters for those 65 years of age and older. The response rate to the RDD telephone screener was 77.9%, which is applicable only to the control respondents who are under age 65 years (and comprise 57.9% of the control group). 1508 cases (82.1%) and 1556 controls (62.7%) completed the in-home interview.

Dietary Assessment: A modification of the Block food frequency questionnaire (FFQ)[25,26], which has been previously validated,[25,27] was used to assess dietary intake in the year prior to the interview. This instrument was self-administered and completed by 1,481 (98.2%) of cases and 1,518 (97.6%) control participants in an average of 36 minutes. Response for this component[23] did not appear to vary with the age of the respondent. Dietary

intake values for one-carbon related micronutrients, folate (the bioactive ingredient is vitamin B_9 – folic acid), vitamins B_1 (thiamin), B2 (riboflavin), B_3 (niacin) and B_6 (pyridoxine), were calculated from the FFQ based on food items, serving sizes and consumption frequencies. We also examined total consumption for each B vitamin by summing dietary intake and supplemental sources of these micronutrients. Use of vitamin supplements was queried on the FFQ. Conversion of FFQ data to daily intakes of B vitamins was carried out using the National Cancer Institute's DietSys, version 3.

Genotyping Methods: We obtained a 40ml blood specimen from 1,102 (73.1%) cases and 1,141 (73.3%) control subjects. DNA was isolated utilizing methods previously described[28]. Genotypes of the *MTHFR 677C>T* and *1298A->C* polymorphisms were ascertained by previously published methods[29]. About an additional 10% of the study population were included as quality control samples; the rate of concordance was 98% and 99% for the *MTHFR 677C>T and 1298A>C* polymorphisms, respectively. All laboratory personnel were blinded to the case-control as well as quality control status of the specimens.

Other Study Variables. Information on other key covariates considered as potential confounders and/or effect modifiers was obtained during the structured, interviewer-administered, in-person, two-hour main questionnaire. The distribution of risk factors for breast cancer from the main study population (1508 cases and 1556 controls who completed the main questionnaire) has been published in detail elsewhere[23]. Similar distributions were observed among the subset of the 1,481 of cases and 1,518 control participants who also completed the FFQ[30]. Distribution of risk factors for breast cancer as well as B vitamin intake from the sub-population from which we were able to ascertain the *MTHFR* genotype (data not shown) were comparable to those identified and reported for the full study population[23].

Statistical Method: Unconditional logistic regression analysis was conducted to estimate odds ratios (OR) and 95% confidence intervals (95%CI) for associations of individual B vitamins and *MTHFR* genotype with breast cancer risk. Age at reference date (defined as date

of diagnosis for cases and date of identification for controls, and categorized as: <44, 45-54, 55-64, 65-74, 75+ years) was included in all models. Univariate analyses were performed to compare distributions of covariates and/or confounding variables among cases and controls. Variables that were independently related to disease risk were included as adjustment terms for multivariate analyses. They included: family history of breast cancer in a first-degree relative (yes/no), history of benign breast disease (yes/no), education (<high school, high school graduate, some college, college graduate, post- college), and BMI at age 20 (≤ 18, >18-19, >19-21, >21-22, >22 kg/m^2). We also included several established risk factors in the multivariate analyses even though they were not significantly associated with breast cancer risk in our study population. They included: menopausal status (pre/post-menopausal), age at menarche (≤ 11, >11-12, >12-13, >13-14, >14 years), age at menopause (≤ 45, >45-48, >48-50, >50-53, >53 years), and energy intake (≤ 902, >902-1147, >1147-1399, >1399-1745, >1745 kcal/day). These covariates were included in models as indicator variables. Although age-adjusted and multivariate-adjusted analyses yielded similar results, only those from multivariate analyses were presented.

We calculated the risk of breast cancer for intake of B vitamins from dietary sources alone as well as for combined intake from diet and supplements. The B vitamins that were explored included folate, vitamins B_1, B_2, B_3, and B_6. Intakes of these nutrients were categorized into quantiles based upon the distribution among the controls; those in the lowest quantile were considered as the referent category. For *MTHFR*, subjects were grouped according to the genotype; individuals with the homozygous wild-type genotype (i.e. *677CC* and *1298AA*) were considered as the referent group. Stratified analyses were performed by multivitamin use (any or none), menopausal status (pre or post), and breast cancer type (invasive or *in situ*). Tests for trend were performed by treating each categorized variable as a continuous term and entering the variable into a logistic regression model. To test the degree of

correlation of between B vitamin subtypes, Spearman correlation coefficients were analyzed based on deciles of intake for each individual B vitamin.

Log likelihood tests (LRT) were performed to evaluate effect modification on a multiplicative scale. The likelihood ratio statistic was calculated by comparing the difference of the log likelihood value for a model with a cross-product term for two main effect variables to the log likelihood value for a model without the cross-product term. For example, to assess the folate-*MTHFR* interaction, folate intake (low, medium, and high) and the *MTHFR 677C>T* genotype (*677CC, 677CT, 677TT*) were categorized into tertiles; cross-product terms were created using these categories and were included in the model as indicator variables. Odds ratios were then calculated to compare variable combinations with the lowest-risk referent category in unconditional logistic regression analysis.

Linkage disequilibrium (LD) between the *MTHFR 677C>T* and *1298A>C* polymorphisms was calculated as D' which ranges from 0 (no LD) to 1 or -1 (complete LD)[31]. The EH linkage utility program[32] was used to determine chi-square statistics and p values for tests of allelic association between polymorphic markers. All statistical analysis was performed using SAS Version 8.0.

RESULTS

B vitamins and breast cancer risk

Table 1 reports the risk of breast cancer in relation to intake of B vitamins from food sources only, as well as total folate from food and supplements. The focus of the analyses was on folate because of its central role in transporting the methyl moiety in one-carbon metabolism. We found no association of dietary folate or total folate with risk of breast cancer. Vitamins B_2 and B_6 are directly involved in one-carbon metabolism as cofactors. Vitamins B_1 and B_3, on the other hand, participate in energy production and are not directly involved in the one-carbon pathway. Information on another key one-carbon related vitamin, B_{12}, was not available for this

population. Overall, slight reductions of breast cancer risk (OR < 1) were observed among people with increased consumption of these B vitamins, with the strongest effect seen for vitamin B_1, for which significantly reduced breast cancer risk was observed in the highest three quintiles of consumption (p, trend = 0.02). Given that B vitamins from food sources overlapped, we examined the degree of correlation between B vitamin subtypes. Spearman coefficients ranged from a low of 0.41 between total folate and vitamin B_3 to a high of 0.90 between dietary folate and vitamin B_1.

In our study population, about 50% of the participants were multivitamin supplement users, and 97% of women in the highest quintile of total folate intake were supplement users. Use of multivitamin supplements was not associated with breast cancer risk in the LIBSCP[30]. Similar findings were also observed in this subset of the population. Associations between dietary B vitamins and breast cancer risk presented in Table 1 did not change after including multivitamin use (yes, no) in the multivariate models. We performed stratified analyses with respect to multivitamin use (Table 2). For every B vitamin we examined, stronger inverse B vitamin – breast cancer associations were observed among non-supplement users compared to the users; the p for trend across the quintiles of intakes was 0.06 for dietary folate, 0.002 for vitamin B_1, 0.05 for vitamin B_2 and B_3, and 0.03 for vitamin B_6 among non-supplement users. Compared to non-supplemental users in the lowest quintile of B vitamin intakes, non-supplemental users in the highest quintile of folate, B_1, B_2 and B_3 had significantly lower risks of breast cancer with ORs of 0.61 (95%CI 0.41-0.93), 0.45 (95%CI 0.28-0.74), 0.62 (95%CI 0.39-0.99), and 0.57 (95%CI 0.35-0.94), respectively. A reduced but non-significant reduction (OR 0.70, 95%CI 0.45-1.09) was also observed for B_6. The risk reduction was not apparent among supplemental users. Figure 2 illustrates joint effects of dietary folate and supplement use on breast cancer risk. Compared to high-risk individuals (lowest quintile of dietary folate intake and no supplemental use), the greatest reduction of breast cancer risk (OR 0.61, 95%CI 0.41-0.93) was observed among individuals with the highest intake of dietary folate but who do not use

multivitamin supplements (p, interaction = 0.04). Controlling for uses of hormone replacement therapy and birth control pills did not significantly change the results reported in Table 2 and Figure 2. The associations of B vitamin intake and breast cancer risk did not substantially vary with menopausal status (pre- *vs.* postmenopausal) or disease type (invasive *vs. in situ*).

MTHFR polymorphisms and breast cancer risk

The *MTHFR 677C>T* genotypes were ascertained from 1063 cases (70% of eligible cases) and 1104 controls (71% of eligible controls). To ensure that sub-samples are representative for the target population, we compared the distribution of risk factors for breast cancer among participants with ascertained genotypes to that of all eligible participants. Comparable results were obtained (data not shown). The genotype distribution was in agreement with Hardy-Weinberg Equilibrium in both cases (p = 0.30) and controls (p = 0.96). The *677T* allele frequency of 40% among the cases was higher than that of controls (37%). The *677T* variant allele was associated with increased breast cancer risk in a dose-dependent fashion (Table 3). Compared to individuals with the wild-type genotype of *677CC*, those with the *677TT* genotype had an age-adjusted OR of 1.34 (95% CI 1.04-1.73) (p, trend = 0.04). After adjusting for additional risk factors including family history of breast cancer in a first-degree relative, history of benign breast disease, education, and BMI at age 20, the dose-dependent relationship remained elevated with a multivariate-adjusted OR of 1.37 (95%CI 1.06-1.78) for *677TT* and a p-value for trend of 0.03.

The *MTHFR 1298A>C* polymorphism was ascertained from 1062 cases and 1103 controls (Table 3). The genotype distributions were in agreement with the Hardy-Weinberg Equilibrium (p=0.89 for cases; p=0.84 for controls). We observed an inverse association of the *1298C* allele and risk of breast cancer in a dose dependent fashion (p, trend = 0.03); the *1298CC* genotype conferred a significantly lower risk of breast cancer compared to the *1298AA*

genotype (OR 0.73; 95%CI 0.53 – 1.00). This relationship was not modified by menopausal status or the stage of breast cancer.

A high degree of LD was observed between the *677C>T* and *1298A>C* polymorphisms (D'=-0.54, p<0.001). The negative sign of the D' indicates that the *677C-1298C* (or *677T-1298A*) alleles were linked. When combined genotypes were examined, individuals who are homozygous with risk alleles at both loci (*677TT-1298AA*) had significant significantly elevated risk of breast cancer (OR 1.77; 95%CI 1.28 – 2.50) compared to those who are homozygous with wild-type alleles (*677CC-1298AA*). Combined heterozygosity did not modify the disease risk; individuals who were heterozygous at both loci (*677CT-1298AC*) had similar risk as those with the *677CC-1298AA* genotype (OR 1.13, 95%CI 0.84 – 1.52) (Table 3).

We also examined the *MTHFR*-breast cancer association according to menopausal status (pre- *vs.* post-menopausal). Comparable results were observed in both groups for the *677C>T* polymorphism (data not shown). The inverse association of the *1298A>C* polymorphism with breast cancer risk was only present in post-menopausal women with a multivariate-adjusted OR of 0.65 (95%CI 0.44-0.96; p, trend = 0.02). The *MTHFR*-breast cancer associations did not differ significantly with respect to *in situ* and invasive cases.

Gene-Environment Interactions in breast cancer

When the *MTHFR*-breast cancer relationship was examined according to supplement use, dose-dependent relations were only apparent among non-supplement users (Table 4), with p values for trend of 0.005 and 0.02 for the *677C>T* and *1298A>C* polymorphisms, respectively. In this subgroup, the *677TT* genotype was associated with a 70% increase in breast cancer risk (95%CI 1.14-2.52) while the *1298CC* genotype was associated with a 38% reduction in risk (95%CI 0.38-1.01).

We examined interactions between *MTHFR* polymorphisms and folate intake in relation to breast cancer risk. With respect to the *677C>T* polymorphism, compared to low-risk

individuals (*677CC* genotype with high folate intake), elevation of breast cancer risk was the most pronounced among *677TT* women who consumed the lowest levels of dietary folate (OR 1.83, 95%CI 1.13-2.96) or had the lowest total folate intake (OR 1.71, 95%CI 1.08-2.71), although the interactions for both models were not significant on a multiplicative scale (Figure 3). Similar associations were observed for every other B vitamin we examined; the *677TT* individuals had the highest ORs when their dietary consumption was in the lowest category of vitamins B_1, B_2, B_3 and B_6 at 2.06 (95%CI 1.25-3.40), 1.88 (95%CI 1.17-3.01), 2.05 (95%CI 1.25-3.38) and 2.36 (95%CI 1.47-3.88), respectively. No indication of effect modification by the *1298A>C* polymorphism was apparent in the study; the p for interaction was 0.84 for dietary folate and 0.94 for total folate.

DISCUSSION

Low consumption of folate and related B vitamins has been implicated as one of the few modifiable risk factors associated with breast cancer[1-3]. Findings from our study lend additional support to the concept that folate as well as other B vitamins may have anti-carcinogenic properties against breast cancer, especially among individuals who do not use multivitamin supplements. The study population was recruited between 1996-1997, a period just prior to FDA-mandated folate fortification in the US food supply starting in January of 1998. Nevertheless, our population-based study consists of women with rather healthy dietary habits with respect to B vitamin intake. For example, the median intakes of total and dietary folate were 433 µg/day and 242 µg/day, respectively, both of which are higher than the Recommended Dietary Allowances (RDA) of 180 µg/day for non-pregnant and non-nursing women aged 15 years and older. Only 18% of the women in our study population fell below the RDA. The relatively sufficient intake of folate and one-carbon related B vitamins may explain the lack of overall associations between these micronutrients and breast cancer risk. It is interesting to see that compared to high-risk individuals (non-supplement users with lowest

quintile of dietary folate intake), women in the highest quintile of dietary folate intake who do not use supplements had even lower risk of breast cancer (OR 0.61, 95%CI 0.41-0.93) than those with comparable dietary folate intake but who also used supplements at the same time (OR 0.95, 95%CI 0.67-1.33), although the confidence intervals were overlapping. It is very unlikely that supplement use can abolish the reduction in risk associated with dietary folate; however, it is possible that those non-supplement users who are in the highest category of dietary folate intake may belong to a unique subgroup of women who have other healthy lifestyles that have not been identified or controlled for in this analysis. Although supplement users in our study have higher mean dietary folate intake overall (consistent with the notion that supplement users are more health conscious and have a healthier lifestyle[33,34]), the mean dietary folate consumption was actually lower in supplement users in the highest quintile of dietary folate intake. This finding seems to suggest that folate from food sources may have stronger anti-carcinogenic effects than the synthetic folate found in supplements. Alternatively, it has been reported that supplement users may be less healthy in terms of increased use of prescription drugs as well as increased number of health visits in the previous year among the elderly population[35]. Nevertheless, interpretation of this surprising finding is speculative and warrants caution.

The goal of the study was to examine whether the folate-breast cancer association is modified by polymorphisms of the folate-metabolizing gene, *MTHFR*, in the hope of clarifying how folate may be protective against breast cancer. We observed an increased susceptibility of breast cancer among women with the *MTHFR 677TT* genotype. This result corroborated the findings from the Nurses' Health Study[36] that low plasma folate levels conferred higher risk of breast cancer. We also observed an elevated risk of breast cancer in *677TT* individuals that was even stronger if the consumption of dietary or total folate was low. One possible mechanism is that low folate intake as well as slow metabolism associated with the *MTHFR* polymorphism result in a suboptimal methyl supply inside the body and in turn, increased breast

cancer risk through an epigenetic process such as aberrant DNA methylation. As in many neoplasia, the hallmark feature of global hypomethylation and region-specific hypermethylation is present in breast cancer. In a study by Soares et al.[37] on 136 breast cancer cases, DNA methylation of breast tumors was significantly less than that of adjacent as well as normal parenchyma. A statistically significant correlation was found between global hypomethylation and the disease stage, tumor size, and the histological grade of the tumor. Subjects with the *MTHFR 677TT* genotype have been shown to possess a lower degree of genomic DNA methylation in peripheral lymphocytes compared with the wild-type *677CC* subjects; an inverse correlation between red blood cell folate and DNA methylation status was also apparent[38]. A follow-up analysis using a new quantitative method also showed that genomic DNA methylation in peripheral blood mononuclear cells directly correlated with folate status and inversely correlated with plasma homocysteine levels; and when analyzed according to folate status, only the *677TT* subjects with low levels of folate accounted for the diminished DNA methylation[39]. In another recent study[40] on 233 cancer patients (with colorectal, breast, and lung tumors), carriers of the *MTHFR 677T* allele showed a lower level of methylation in the genome (p=0.002) and tumors (p=0.047). Additionally, tumors from patients with a variant genotype of another one-carbon-metabolizing gene, *methionine synthase*, showed promoter hypermethylation in a large panel of tumor suppressor genes including $p16^{INK4A}$ and *BRCA1*, both of which are important in mammary tumorigenesis[40].

There are several reports on the association of the *MTHFR 677C>T* polymorphism and breast cancer risk [22,41-45]; most were clinic-based studies that had limited sample sizes and were restricted to specific ethnic (e.g. Jewish[41]) or clinical characteristics (e.g. < 40 years of age with bilateral breast cancer[42]); results from these studies were variable. The only population-based results come from the Shanghai Breast Cancer Study which consisted of women 25-64 years of age in which multivitamin use was low[22]. In this Chinese population, *MTHFR* polymorphism was not an independent predictor of breast cancer risk. However, the *MTHFR 677C>T*

polymorphism significantly modified the risk of breast cancer associated with dietary folate consumption[22], a finding that is consistent with our current study. These findings add additional support to the notion that dietary folate may be protective against breast cancer.

It is worth pointing out that the main effect of the *MTHFR 677C>T* polymorphism on breast cancer risk is different from its effect on colorectal cancer. Although high folate status reduced the risk of both cancers, the *MTHFR 677TT* genotype was associated with a decreased risk of colorectal cancer[46,47] and increased risk of breast cancer. In the meantime, interactions between folate and *MTHFR* were similar in both diseases; the highest risk was observed among *677TT* individuals with low folate intake[46,47]. Because the MTHFR is situated at the critical junction of one-carbon metabolism balancing DNA methylation and synthesis (Figure 1), reduced MTHFR activity conferred by the *677C->T* polymorphism may tilt the balance in favor of the DNA synthesis pathway at the expense of methyl supply (i.e. SAM) for methylation reactions. The opposite effects of this polymorphism seem to suggest that colon and breast cancer may have different underlying etiologic pathways. This hypothesis needs to be investigated in mechanistic studies using cell lines or animal models.

Functionality of the *MTHFR 1298A>C* polymorphism has not been well established. Individuals with combined heterozygosity for *677CT-1298AC* showed reduced enzyme activities, elevated plasma Hcy and decreased plasma folate, similar to those with the *677TT* genotype[21]; however, these findings were not entirely reproducible in other studies[29,48]. Our results confirmed that the *two MTHFR* polymorphisms were in strong LD. The apparent reduced breast cancer risk associated with *1298CC* individuals may be attributed to the fact that the *1298C* allele was highly linked with the *677C*, the low risk allele[49]. The absence of elevated risk in individuals with compound heterozygous genotype (i.e. *677CT-1298AC*) indicated that the *1298A>C* polymorphism might have limited functionality.

The major strength of this study lies in its population-based study design in which cases encompassed a broad age range and were drawn from a population-based sample. Thus,

results of this study may be more generalizable than a series of cases from a narrow age range or from a single institution. In addition, the relatively large sample size allows multiple risk factors to be taken into consideration in studying associations, with the ability to conduct stratified analyses and adjustment in multivariate models. On the other hand, besides recall bias associated with all case-control studies, the major limitation is the lack of measurement of biological folate status (folate in plasma or red blood cells). We did not measure biological folate levels for the Long Island Breast Cancer Study Project because of the case-control design of the study; biological samples were collected after disease diagnosis so the biological folate levels may have been influenced by the onset, development, or even treatment of the disease.

In summary, this population-based study adds to the increasing evidence that risk of breast cancer is reduced in relation to intake of dietary folate and related B vitamins, especially among non-supplement users. Further, it appears that suboptimal folate metabolism increases the susceptibility to breast cancer, especially among those with insufficient folate intake; however, such enhanced risk may be reduced by increasing folate consumption. Although several risk factors such as family and reproductive history have been associated with breast cancer, few modifiable factors have been identified to reduce the disease risk. From a public heath perspective, it is important to identify such risk factors, such as B vitamin consumption, that may guide an effective prevention strategy against the disease.

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Figure Legend:

Figure 1: Schematic illustration of one-carbon metabolism noting the cross-talk between genetic (DNA synthesis) and epigenetic (methylation) processes. Key genes involved in one-carbon metabolism include *methylenetetrahydrofolate reductase* (*MTHFR*), *thymidylate synthase* (*TS*), *methionine synthase* (*MTR*), *methionine synthase reductase* (*MTRR*), *serine hydroxymethyltransferase* (*SHMT*), and *dihydrofolate reductase* (*DHFR*), and *betaine-homocysteine methyltransferase* (*BHMT*). Vitamins B_2, B_6, and B_{12} are cofactors in the pathway. *MTHFR* is at a critical metabolic branch point of the metabolic pathway, carrying out the irreversible conversion of 5,10-methylenetetrahydrofolate (5,10-methyleneTHF to 5-methylTHF, which directs the folate pool toward remethylation of homocysteine (Hcy) to methionine at the expense of thymidylate synthesis. Other abbreviations in the diagram include: S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH).

Figure 2. Relationship between dietary folate intake and risk of breast cancer with respect to multivitamin use in the Long Island Breast Cancer Study Project, 1996-1997. The ORs were adjusted for age, family history of breast cancer in first-degree relatives, history of benign breast disease, education, BMI at age 20, and daily caloric intake. p value for interaction was 0.04. * denotes $p<0.05$.

Figure 3. Interactions of the *MTHFR 677C>T* polymorphism with dietary folate (A) and total folate (B) intake in the Long Island Breast Cancer Study Project, 1996-1997. The ORs were adjusted for age, family history of breast cancer in first-degree relatives, history of benign breast disease, education, BMI at age 20, and daily caloric intake. p values for interaction were 0.42 for dietary folate and 0.16 for total folate.

* denotes $p<0.05$.

Table 1. Multivariate-adjusted ORs and 95% CI for associations of daily intake of B vitamins with risk of breast cancer in the Long Island Breast Cancer Study Project, 1996-1997.

	Quintiles of Dietary Intake					
Nutrient Type	**Q1 (lowest intake)**	**Q2**	**Q3**	**Q4**	**Q5**	**p, trend[2]**
Dietary Folate						
Range (µg/day)	<=159	>159-216	>216-279	>279-356	>356	
Cases/Controls	314/296	276/297	308/296	265/297	263/296	
OR (95% CI)[1]	1.00 (Referent)	0.89 (0.70-1.13)	1.00 (0.78-1.29)	0.85 (0.65-1.11)	0.85 (0.64-1.14)	0.29
Total Folate (diet + supplements)						
Range (µg/day)	<=208	>208 - 330	>330 - 561	>561 - 722	>722	
Cases/Controls	309/300	305/294	256/295	280/299	276/294	
OR (95% CI)	1.00 (Referent)	1.03 (0.81-1.31)	0.85 (0.67-1.08)	0.93 (0.73-1.18)	0.95 (0.74-1.22)	0.43
Vitamin B_1(Thiamin)						
Range (mg/day)	<=0.72	>0.72-0.95	>0.95-1.16	>1.16-1.48	>1.48	
Cases/Controls	344/307	300/290	259/292	273/303	250/290	
OR (95% CI)	1.00 (Referent)	0.87 (0.67-1.13)	**0.74 (0.56-0.99)**	**0.72 (0.53-0.98)**	**0.69 (0.49-0.96)**	0.02
Vitamin B_2 (Riboflavin)						
Range (mg/day)	<=0.95	>0.95-1.30	>1.30-1.62	>1.62-2.12	>2.12	
Cases/Controls	331/293	296/302	265/295	271/296	263/296	
OR (95% CI)	1.00 (Referent)	0.85 (0.66-1.09)	0.78 (0.59-1.02)	0.80 (0.59-1.07)	0.76 (0.55-1.04)	0.13
Vitamin B_3 (Niacin)						
Range (mg/day)	<=9.8	>9.8-12.9	>12.9-15.7	>15.7-19.9	>19.9	
Cases/Controls	337/296	292/299	252/293	287/299	258/295	
OR (95% CI)*	1.00 (Referent)	0.85 (0.65-1.10)	**0.75 (0.56-1.00)**	0.85 (0.63-1.16)	0.78 (0.56-1.09)	0.24
Vitamin B_6 (Pyridoxin)						
Range (mg/day)	<=0.84	>0.84-1.15	>1.15-1.42	>1.42-1.84	>1.84	
Cases/Controls	309/300	321/297	269/295	275/300	252/290	
OR (95% CI)	1.0 (Referent)	1.09 (0.85-1.40)	0.92 (0.70-1.20)	0.91 (0.68-1.21)	0.87 (0.64-1.18)	0.17

[1]Adjusted for age, family history of breast cancer in first-degree relative, history of benign breast disease, educational attainment, BMI at age 20,and kilocalories per day.

[2] p value for trend for categorical variables

Table 2. Multivariate-adjusted ORs and 95% CI stratified by supplement use for associations of daily intake of B vitamins with risk of breast cancer in the Long Island Breast Cancer Study Project, 1996-1997.

	Quintiles of Dietary Intake					
Nutrient Type	**Q1 (low)**	**Q2**	**Q3**	**Q4**	**Q5**	**p, trend[2]**
Folate						
			Supplement use = No			
Cases/Controls	189/164	142/154	164/148	125/134	93/133	
OR (95% CI)[1]	1.0 (ref)	0.82 (0.59-1.16)	0.98 (0.69-1.38)	0.83 (0.57-1.23)	**0.61 (0.41-0.93)**	0.06
			Supplement use = Yes			
Cases/Controls	125/132	134/143	144/148	140/163	170/163	
OR (95% CI)	1.0 (ref)	0.99 (0.69-1.42)	1.04 (0.71-1.51)	0.91 (0.62-1.35)	1.11 (0.73-1.67)	0.75
Vitamin B_1						
			Supplement use = No			
Cases/Controls	193/157	163/150	125/137	133/148	99/141	
OR (95% CI)	1.0 (ref)	0.81 (0.56-1.16)	**0.66 (0.44-1.00)**	**0.62 (0.40-0.97)**	**0.45 (0.28-0.74)**	**0.002**
			Supplement use = Yes			
Cases/Controls	151/150	137/140	134/155	140/155	151/149	
OR (95% CI)	1.0 (ref)	0.95 (0.66-1.37)	0.83 (0.55-1.24)	0.85 (0.55-1.31)	0.96 (0.61-1.53)	0.81
Vitamin B_2						
			Supplement use = No			
Cases/Controls	186/157	161/154	135/139	118/143	113/140	
OR (95% CI)	1.00 (ref)	0.81 (0.57-1.15)	0.77 (0.52-1.14)	0.69 (0.45-1.04)	**0.62 (0.39-0.99)**	**0.05**
			Supplement use = Yes			
Cases/Controls	145/136	135/148	130/156	153/153	150/156	
OR (95% CI)	1.0 (ref)	0.82 (0.57-1.19)	0.74 (0.49-1.10)	0.88 (0.57-1.34)	0.81 (0.51-1.28)	0.60
Vitamin B_3						
			Supplement use = No			
Cases/Controls	187/147	150/152	121/138	144/153	111/143	
OR (95% CI)	1.0 (ref)	0.73 (0.50-1.06)	**0.65 (0.43-0.99)**	0.69 (0.44-1.08)	**0.57 (0.35-0.94)**	**0.05**
			Supplement use = Yes			
Cases/Controls	150/149	142/147	131/155	143/146	147/152	
OR (95% CI)	1.0 (ref)	0.97 (0.68-1.40)	0.84 (0.57-1.25)	0.99 (0.65-1.51)	0.98 (0.61-1.57)	1.00
Vitamin B_6						
			Supplement use = No			
Cases/Controls	172/153	184/159	131/142	119/146	107/133	
OR (95% CI)	1.0 (ref)	1.08 (0.76-1.52)	0.81 (0.55-1.20)	0.73 (0.48-1.10)	0.70 (0.45-1.09)	**0.03**
			Supplement use = Yes			
Cases/Controls	137/147	137/138	138/153	156/154	145/157	
OR (95% CI)	1.0 (ref)	1.11 (0.78-1.60)	0.98 (0.67-1.44)	1.11 (0.75-1.65)	0.99 (0.65-1.52)	0.96

[1]Adjusted for age, family history of breast cancer in first-degree relative, history of benign breast disease, educational attainment, BMI at age 20,and kilocalories per day.

[2] p value for trend for categorical variables

Table 3. Odds ratios and 95% CI for *MTHFR* polymorphisms with risk of breast cancer in the Long Island Breast Cancer Study Project, 1996-1997.

Genotype	# cases (%)	# controls (%)	OR (95% CI)[1]	OR (95%CI)[2]
677C>T				
677CC	398 (37.4)	440 (39.9)	1.0 (ref)	1.0 (ref)
677CT	476 (44.8)	509 (46.1)	1.04 (0.87-1.26)	1.05 (0.87-1.27)
677TT	189 (17.8)	155 (14.0)	**1.34 (1.04-1.73)**	**1.37 (1.06-1.78)**
p, trend[3]			*0.04*	*0.03*
1298A>C				
1298AA	558 (52.5)	536 (48.6)	1.0 (ref)	1.0 (ref)
1298AC	417 (39.3)	457 (41.4)	0.88 (0.74-1.06)	0.87 (0.72-1.05)
1298CC	87 (8.2)	110 (10.0)	0.77 (0.56-1.04)	**0.73 (0.53-1.00)**
p,trend			*0.05*	*0.03*
Combined Genotypes				
677CC-1298AA	146 (13.8)	172 (15.6)	1.0 (ref)	1.0 (ref)
677CC-1298AC	188 (17.8)	198 (18.0)	1.16 (0.85-1.56)	1.10 (0.82-1.49)
677CC-1298CC	63 (6.0)	69 (6.3)	1.09 (0.72-1.64)	1.06 (0.70-1.59)
677CT-1298AA	251 (23.7)	259 (23.6)	1.17 (0.88-1.55)	1.13 (0.85-1.49)
677CT-1298AC	207 (19.6)	218 (19.8)	1.16 (0.86-1.56)	1.13 (0.84-1.52)
677CT-1298CC	17 (1.6)	32 (2.9)	0.65 (0.35-1.23)	0.63 (0.34-1.19)
677TT-1298AA	158 (14.9)	102 (9.3)	**1.85 (1.32-2.60)**	**1.77 (1.28-2.50)**
677TT-1298AC	22 (2.1)	41 (3.7)	0.66 (0.37-1.17)	0.62 (0.35-1.10)
677TT-1298CC	6 (0.6)	9 (0.8)	0.89 (0.31-2.59)	0.86 (0.30-2.48)

[1] Adjusted for age

[2]Adjusted for age, family history of breast cancer in first-degree relative, history of benign breast disease, educational attainment, BMI at age 20, and kcal per day.

3 p value for trend for categorical variables

Table 4. Odds ratios and 95% CI for *MTHFR* polymorphisms with risk of breast cancer stratified by supplement use in the Long Island Breast Cancer Study Project, 1996-1997.

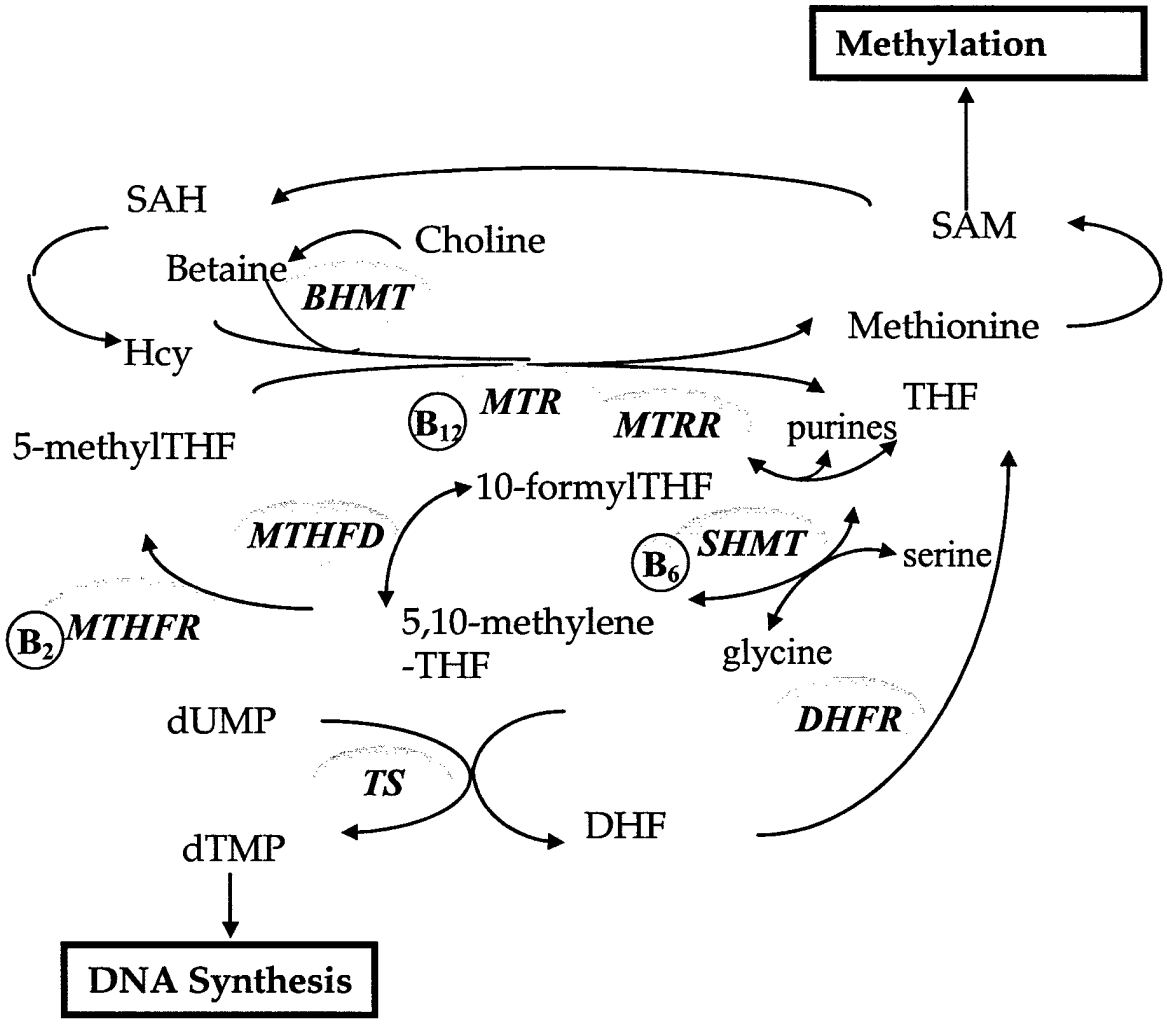
Genotype	# cases (%)	# controls (%)	OR (95% CI)[1]	OR (95%CI)[2]
677C>T				
			Supplement use= Yes	
677CC	216 (40.2)	221 (38.3)	1.0 (ref)	1.0 (ref)
677CT	222 (41.3)	271 (47.0)	0.84 (0.65-1.09)	1.18 (0.82-1.68)
677TT	99 (18.4)	85 (14.7)	1.20 (0.85-1.70)	0.83 (0.64-1.09)
p, trend			*0.68*	*0.74*
			Supplement use= No	
677CC	174 (34.1)	213 (41.7)	1.0 (ref)	1.0 (ref)
677CT	247 (48.4)	230 (45.0)	**1.33 (1.02-1.75)**	**1.36 (1.03-1.81)**
677TT	89 (17.5)	68 (13.3)	**1.55 (1.07-2.27)**	**1.70 (1.14-2.52)**
p, trend			*0.007*	*0.005*
1298A>C				
			Supplement use= Yes	
1298AA	275 (51.3)	282 (49.1)	1.00 (referent)	1.00 (referent)
1298AC	214 (39.9)	235 (40.9)	0.94 (0.73-1.20)	0.93 (0.72-1.20)
1298CC	47 (8.8)	57 (9.9)	0.81 (0.53-1.24)	0.83 (0.54-1.29)
p, trend			*0.34*	*0.38*
			Supplement use= No	
1298AA	274 (53.7)	249 (48.4)	1.00 (referent)	1.00 (referent)
1298AC	198 (38.8)	214 (41.6)	0.85 (0.66-1.11)	0.80 (0.61-1.05)
1298CC	38 (7.5)	51 (9.9)	0.71 (0.45-1.12)	0.62 (0.38-1.01)
p, trend			*0.09*	*0.02*

[1] Adjusted for age

[2]Adjusted for age, family history of breast cancer in first-degree relative, history of benign breast disease, educational attainment, BMI at age 20, and kcal per day.

3 p value for trend for categorical variables

Figure 1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MTHFD
B6
SHMT
serine
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-THF
glycine
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DHFR
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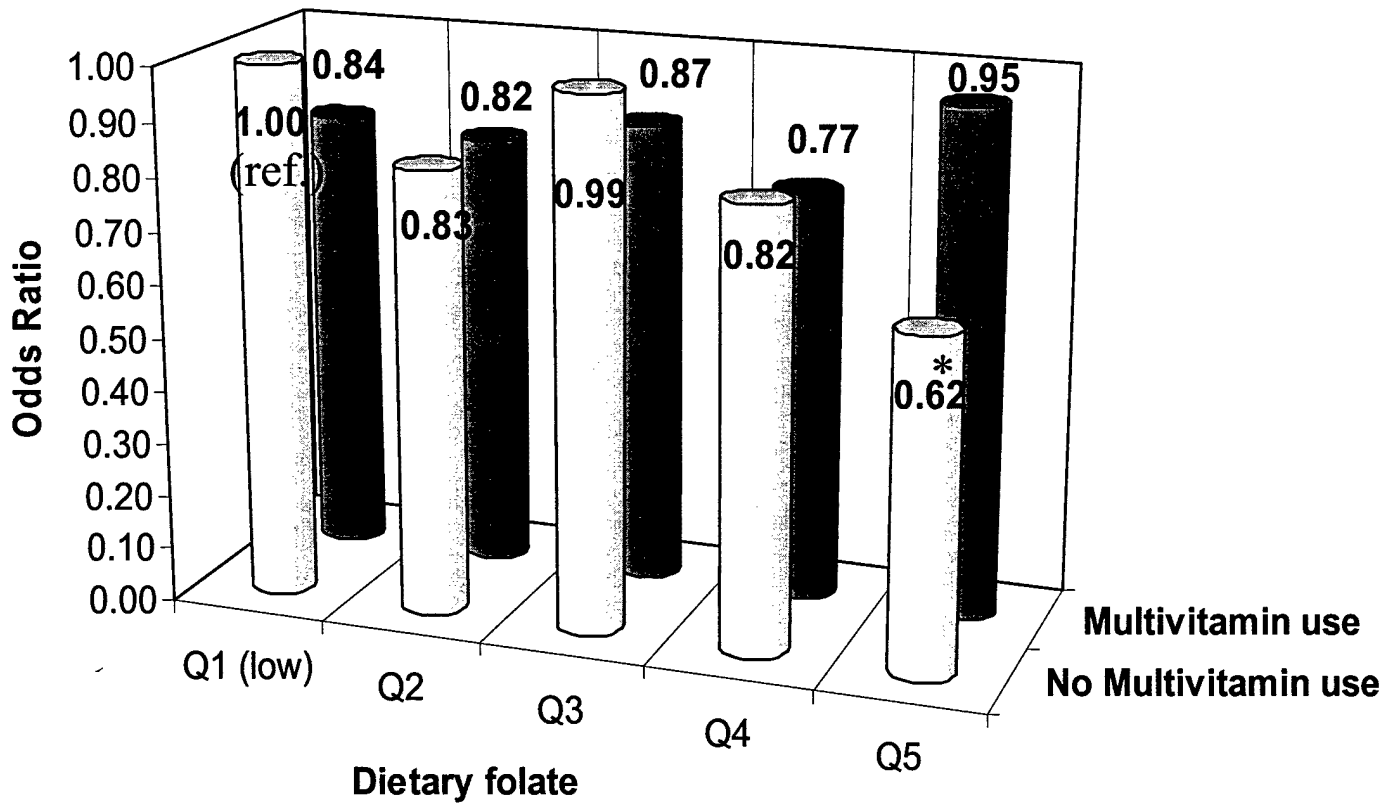
DHF
dTMP
DNA Synthesis

Figure 2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0.80
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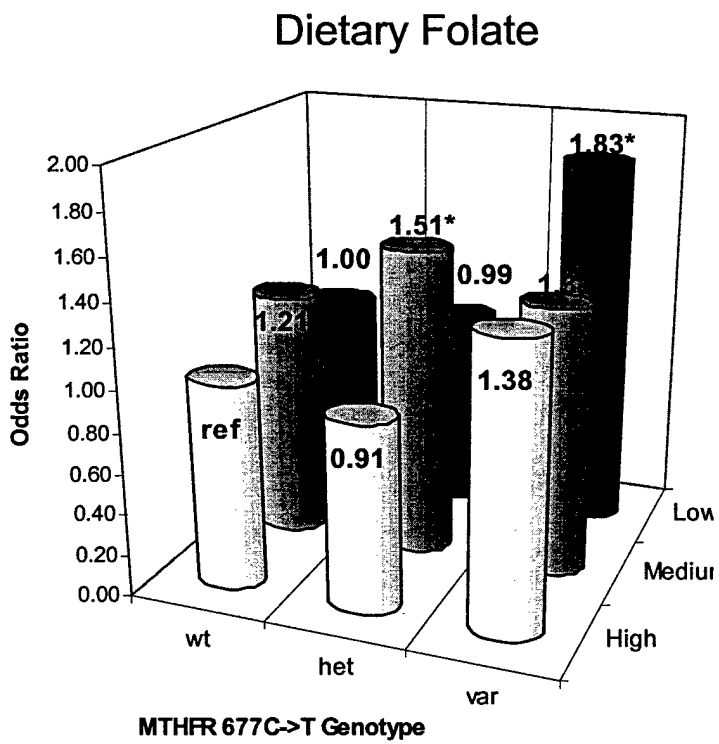
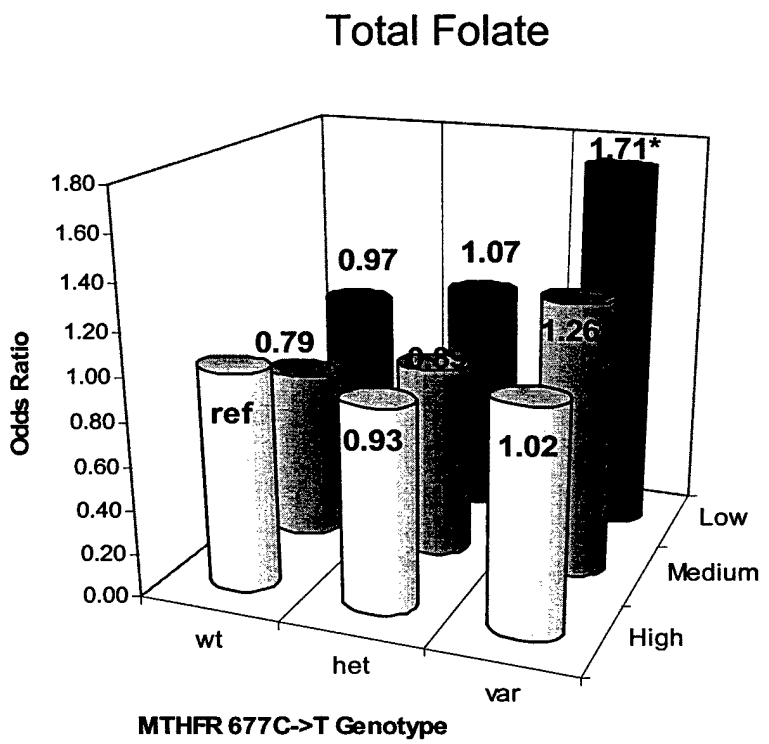
Odds Ratio
1.00
(ref.)
0.84
0.83
0.82
0.99
0.87
0.82
0.77
0.62
*
0.95
Q1 (low)
Q2
Q3
Q4
Q5
Dietary folate
Multivitamin use
No Multivitamin use

Figure 3


Dietary Folate
Odds Ratio
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
ref
1.21
1.00
0.91
1.51*
0.99
1.38
1.83*
wt
het
var
Low
Mediu
High
MTHFR 677C->T Genotype


Total Folate
Odds Ratio
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
ref
0.79
0.97
0.93
1.07
1.02
1.71*
1.26
wt
het
var
Low
Medium
High
MTHFR 677C->T Genotype