Research article

MTHFR C677T and postmenopausal breast cancer risk by intakes of one-carbon metabolism nutrients: a nested case-control study

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Abstract

Introduction The C677T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene has been hypothesized to increase breast cancer risk. However, results have been inconsistent, and few studies have reported the association by menopausal status or by intakes of nutrients participating in one-carbon metabolism. Our aims were to investigate whether MTHFR C677T was associated with postmenopausal breast cancer risk and whether this relation was modified by intakes of folate, methionine, vitamins B₂, B₆, and B₁₂, and alcohol.

Methods We studied 318 incident breast cancer cases and 647 age- and race-matched controls participating in a nested case-control study of postmenopausal women within the VITamins And Lifestyle (VITAL) cohort. Genotyping was conducted for MTHFR C677T and dietary and supplemental intakes were ascertained from a validated questionnaire. Adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated using unconditional logistic regression.

Results We observed a 62% increased risk of breast cancer among postmenopausal women with the TT genotype (OR = 1.62; 95% CI: 1.05 to 2.48). Women with a higher number of variant T alleles had higher risk of breast cancer (P for trend = 0.04). Evidence of effect-modification by intakes of some B vitamins was observed. The most pronounced MTHFR-breast cancer risks were observed among women with the lowest intakes of dietary folate (P for interaction = 0.02) and total (diet plus supplemental) vitamin B₆ (P for interaction = 0.01), with no significant increased risks among women with higher intakes.

Conclusions This study provides support that the MTHFR 677TT genotype is associated with a moderate increase in risk of postmenopausal breast cancer and that this risk may be attenuated with high intakes of some one-carbon associated nutrients.

Introduction

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate and methyl donor in DNA methylation. MTHFR is a critical enzyme in one-carbon metabolism, redirecting the pool of folate from DNA synthesis/repair to methylation. It is of interest because aberrations in DNA synthesis, repair, and methylation, have been implicated with cancer risk. The substitution of cytosine (C) with thymine (T) at nucleotide 677 in the MTHFR gene is a common polymorphism (C677T) and is correlated with increased thermolability and reduced MTHFR activity [1]. Homozygotes (677TT) have approximately 30% and heterozygotes (677CT) have approximately 65% the activity of homozygous wild-types (677CC), respectively [1,2].

The C677T polymorphism has been studied extensively, yet for breast cancer risk, three recent meta-analyses suggest that...
the association with the MTHFR C677T polymorphism has been largely inconsistent [3-5]. Several reviews suggest that for breast cancer, the relation may vary by menopausal status and also, highlight the importance of examining potential effect of risk modification by nutrients that contribute to or interrupt one-carbon metabolism [3,4]. However, few studies have reported estimates by menopausal status, and most have not examined additional nutrient effect modifiers other than folate or alcohol [3-5]. A further limitation among investigations examining dietary effect modifiers is that most have utilized diet recalled after cancer diagnosis which has the potential for recall bias.

We, therefore, investigated the relationship between the MTHFR C677T polymorphism and breast cancer risk among postmenopausal women in a nested case-control study within the VITamins And Lifestyle (VITAL) cohort. We also examined whether this relation was modified by prediagnostic intakes of nutrients involved in one-carbon metabolism (that is, folate, methionine, vitamins B2, B6, and B12, and alcohol). Previously, in the VITAL cohort, we observed a protective association between folate intakes and breast cancer risk [6].

Materials and methods

VITAL cohort

The VITAL cohort was principally designed to investigate supplement use and cancer risk. Details have been previously reported [7]. Briefly, men and women were eligible to join the VITAL cohort if they aged 50 and 76 years and living in the western area of Washington State covered by the Surveillance, Epidemiology, and End Results (SEER) cancer registry. A 24-page baseline questionnaire was sent to participants, using names from a commercial mailing list. Data collection occurred from October 2000 to December 2002. Among the 40,339 women who were eligible, 25.6% responded to the baseline questionnaire.

Selection of cases and controls

Breast cancer cases were identified by linkage to the SEER cancer registry. From baseline to December 30, 2003, 514 breast cancer patients were identified. To form the nested case-control dataset, we excluded women who reported a history of breast cancer (n = 48), had rare breast histologies (that is, sarcoma, phyllodes, or lymphoma, n = 4), did not provide a buccal cell sample, and 185 were excluded because the breast-cancer risk-factor page of the baseline questionnaire was not completed. Two controls for each case were randomly selected from the remaining 24,113 possible (pre- and postmenopausal) controls by frequency matching on age at baseline (in five-year intervals) and race resulting in 668 controls. We also ensured that the follow-up times of the controls (time from baseline to death, a move out of area, or December 31, 2003) were greater than or equal to the follow-up times of the cases (time from baseline to breast cancer diagnosis). Information on deaths and moves out of the area were obtained by linking the VITAL cohort to the Washington State death files and the National Change of Address system. For this current analysis, we excluded 19 controls who were not postmenopausal and two controls whose samples failed to be genotyped for MTHFR, leaving 647 postmenopausal controls. This study was approved by the Fred Hutchinson Cancer Research Center Institutional Review Board (IRB), Seattle, Washington. Voluntary return of the questionnaire was considered implied consent.

Genotyping

DNA was obtained from buccal cells collected from cytobrushes. Women who completed the VITAL baseline questionnaire were mailed a DNA kit containing three sterile cytobrushes, detailed instructions with pictures for use of kit, and a consent form. Genotyping was done by InterGenetics Incorporated (Oklahoma City, OK, USA). Genotyping of MTHFR C677T was determined using a microsphere-based, allele-specific primer extension (ASPE) assay followed by analysis on the Luminex 100 flow cytometer (Luminex, Austin, TX, USA) as previously described [8]. DNA was amplified by multiplex PCR using HotStar Taq DNA polymerase (Qiagen Inc., Valencia, CA, USA). To ensure quality control, cases and controls were mixed on genotyping plates, and genotyping was performed with blinding to case-control status. To ensure there was no signal in wells without DNA added, each plate had at least three buffer blanks that were carried through the entire PCR/ASPE/Luminex process. Moreover, at least 5% of the samples were randomly selected and genotyped in duplicate with a concordance rate >99%.

Effect modifiers and covariates

Information on diet, medical history, reproductive history, lifestyle factors, personal characteristics, and family history of breast cancer was collected at baseline using a 24-page self-administered questionnaire. Women were considered postmenopausal if they had a natural menopause with no periods in the year before baseline, had ever used postmenopausal hormones (PMH), had bilateral oophorectomy, or were 60
years or older at baseline. Because women with a hysterectomy without bilateral oophorectomy cannot report on menopause, they were considered to be postmenopausal if they had ever used hormone therapy or were 55 years or older at baseline.

Intakes of folate, methionine, vitamins B2, B6, and B12, multivitamins and alcohol reported on the baseline questionnaire were examined as potential effect modifiers of the MTHFR-breast cancer relationship. Intakes from diet over the past year were determined from a semi-quantitative food frequency questionnaire (FFQ), adapted from the Women’s Health Initiative and other studies [9-11]. Participants also reported intakes of multivitamins and individual vitamin supplements, taken singly or as mixtures (for example, stress/B-complex, antioxidant mixtures), including questions on years of use in the 10 years before baseline, days per week of use and dose per day. The validity and reliability of supplement reporting in this cohort has been previously examined [12].

We analyzed B vitamins from diet, supplement and diet plus supplement (total) sources. Vitamin B2 was not asked as an individual supplement, so only B2 as a multivitamin was considered, and therefore supplemental B2 was not considered alone. Methionine was determined from diet only. Average daily intake of dietary nutrients was calculated from the FFQ by multiplying the adjusted serving frequency (calculated as frequency times portion size) of each food/beverage item by its nutrient content and summing the nutrient contributions of all foods or beverages. The nutrient database used, the Minnesota Nutrient Data System for Research (University of Minnesota’s Nutrition Coordinating Center, Minneapolis, MN, USA) [13], took into account the U.S. mandated folic acid fortification of grain products. Total alcohol intakes were calculated from all reported past-year consumption of red wine, white wine, beer, and liquor/mixed drinks.

Intakes of supplemental folate, other micronutrients, and multivitamins were averaged over 10 years, as previously described [6]. We needed a conversion factor [14] for the calculation of total folate because synthetic folate (folic acid) is more bioavailable than naturally occurring folate (polyglutamates). Thus, total folate, expressed in dietary folate equivalents (DFE), was obtained by first multiplying synthetic folate (supplements and fortified in foods) by a conversion factor of 1.7 and then, adding intakes of natural food folate (μg). To summarize intakes of micronutrients that act as cofactors in the one-carbon pathway, we generated a one-carbon nutrient score by summing the z-scores of total folate, dietary methionine, and total vitamins B2, B6, B12 and then, dividing this score into high (upper median) and low (lower median) for categorical analyses. Women were excluded from the dietary and total (diet plus supplement) nutrient analyses if they did not complete all pages of the FFQ or if their reported total energy intake was <600 or >4,000 kcal. Breast cancer risk factors and demographic variables were reported on the baseline questionnaire [6].

Statistical analyses
We compared baseline characteristics of cases and controls using Wilcoxon signed-rank tests (for continuous variables) or Chi-square tests (for dichotomous variables). Observed genotype frequencies were in Hardy-Weinberg equilibrium (P > 0.05). Odds ratios (OR) for breast cancer risk and 95% confidence intervals (CI) were calculated using unconditional logistic regression adjusting for the matching factors of age at baseline (50 to 54 years, 55 to 59, 60 to 64, 65 to 69, 70 to 76) and race (white, other). For analyses of the MTHFR-breast cancer association, we assigned the wild-type genotype MTHFR 677CC as the reference group. Linear trend was calculated by modeling the MTHFR genotype (CC, CT, and TT) as one term, ordinarily.

Intakes of folate, methionine, vitamins B2, B6, and B12, multivitamins, and alcohol were examined as potential effect modifiers of the MTHFR-breast cancer relationship. We dichotomized dietary and total intakes of the B vitamins and methionine by median intakes. Multivitamin intake was divided into some and no intake over the 10 years prior to baseline, and B vitamin consumption from supplements (that is, individual vitamins plus multivitamins) were dichotomized by levels at or above the dose obtained by 1 year daily use of a standard (Centrum®) multivitamin versus below that level. Based on previous literature on breast cancer risk [15], and the distribution of alcohol consumption among VITAL women, alcohol was dichotomized into <10 g/d and ≥ 10 g/d intakes (that is, approximately one drink/day). Categorizations of all nutrients were based according to the distribution of intakes among controls. For our examination of effect modification, odds ratios were adjusted for the matching factors of age and race in addition to following standard breast cancer risk factors, as described in the footnote of the last table. To best illustrate the joint effects, we cross-classified women by their MTHFR genotype and the dichotomous effect modifier and used a single reference group for the odds ratios. The group hypothesized to have the lowest risk was selected as the reference group. Tests for interaction were performed by entering the product term of the ordinal MTHFR variable and dichotomous effect modifier in a multivariable-adjusted model and using Wald statistic to obtain a P-value. All statistical analyses were performed using SAS, version 9.1, (SAS Institute Inc., Cary, NC, USA). P values < 0.05 were considered statistically significant and all statistical tests were two-sided.

Results
Participants were on average 64 years of age and mostly Caucasian (Table 1). As expected, cases were significantly more likely than controls to have breast cancer risk factors such as having a prior breast biopsy, having fewer births, using PMH, and drinking more alcohol. Additionally, although not statis-
cally significant, more cases than controls had a first-degree family history, young age at menarche, and were nulliparous. The majority of breast tumors were invasive (n = 253). Among controls, the frequencies of MTHFR genotypes were: 677CC (46.5%), 677CT (43.9%), and 677TT (9.6%).

Postmenopausal women with the MTHFR 677TT genotype had significantly higher risk of breast cancer (OR = 1.62; 95% CI: 1.05 to 2.48) than 677CC individuals (Table 2). The test for increasing breast cancer risk with increasing number of variant T alleles was significant (P for trend = 0.04), although there was no clear excess risk for the heterozygous MTHFR 677CT genotype (OR = 1.08; 95% CI: 0.81 to 1.43) (Table 2). We observed an increased breast cancer risk with MTHFR 677TT even after restricting to invasive breast cancer cases (RR = 1.65; 95% CI: 1.03 to 2.63) and when restricting to Caucasians (RR = 1.59; 95% CI: 1.03 to 2.46 for 302 cases) (data not shown).

Table 1

Characteristics of postmenopausal breast cancer cases and controls, VITAL study *

|                        | Cases (n = 318) | Controls (n = 647) | P value † |
|------------------------|----------------|-------------------|-----------|
| Demographics           |                |                   |           |
| Age, years (mean ± SD) | 64.4 ± 6.88    | 64.2 ± 6.86       | 0.67      |
| White, %               | 95.0           | 95.1              | 0.95      |
| Family history/breast-related procedures |                |                   |           |
| Mother or sister with breast cancer,% | 18.6 | 15.6 | 0.25 |
| Mammography in past 2 years,% | 93.1 | 93.7 | 0.73 |
| Prior breast biopsy,%  | 28.0           | 21.8              | 0.03      |
| Reproductive factors   |                |                   |           |
| Early age at menarche,% <12 years | 20.1 | 18.1 | 0.44 |
| Nulliparous, %         | 12.9           | 9.74              | 0.14      |
| Age at first birth, years (mean ± SD) ‡ | 24.1 ± 4.85 | 23.3 ± 4.39 | 0.05 |
| Parity, number of births (mean ± SD) ‡ | 2.60 ± 1.09 | 2.86 ± 1.21 | <0.01 |
| Age at menopause, years (mean ± SD) | 47.9 ± 5.37 | 47.4 ± 5.84 | 0.37 |
| Ever use of estrogen plus progestin postmenopausal hormones, % | 48.1 | 35.2 | <0.01 |
| Lifestyle/anthropomorphic factors |                |                   |           |
| Height, in (mean ± SD) | 64.9 ± 2.65    | 64.8 ± 2.61       | 0.36      |
| Baseline BMI, kg/m² (mean ± SD) | 26.9 ± 5.63 | 27.2 ± 5.61 | 0.21 |
| Total physical activity, MET-hour/week (mean ± SD) | 9.44 ± 11.9 | 10.1 ± 13.9 | 0.90 |
| Dietary factors        |                |                   |           |
| Total folate, DFE/day (mean ± SD) § | 819 ± 420 | 856 ± 419 | 0.15 |
| Dietary methionine, g/day (mean ± SD) | 1.43 ± 0.57 | 1.48 ± 0.61 | 0.32 |
| Total B₂, mg/day (mean ± SD) § | 2.53 ± 1.21 | 2.64 ± 1.28 | 0.16 |
| Total B₆, mg/day (mean ± SD) § | 10.1 ± 30.3 | 7.97 ± 15.4 | 0.38 |
| Total B₁₂, µg/day (mean ± SD) § | 22.5 ± 40.2 | 18.9 ± 29.3 | 0.57 |
| Alcohol, g/day (mean ± SD) | 5.96 ± 8.91 | 4.76 ± 9.76 | 0.03 |
| Multivitamin use, days/week over 10 years (mean ± SD) | 3.29 ± 2.90 | 3.50 ± 2.93 | 0.25 |

* Cases and controls were originally 2:1 matched on age and race; the number of cases and controls are not exactly 2:1 because women who were premenopausal or who did not have MTHFR genotype data were excluded.
† Wilcoxon rank-sum test for continuous variables and chi-square test for dichotomous variables
‡Among parous women only
§Intake from dietary plus supplemental sources
intakes of total folate and vitamins B2, B6, and B12 (range of diet or supplements alone. The similarity of these results defined by low intake of nutrients from total sources than from Furthermore, odds ratios appeared stronger among groups defined by low intake of nutrients from total sources than from diet or supplements alone. The similarity of these results across nutrients is due, in part, to the high correlation between intakes of total folate and vitamins B2, B6, and B12 (range of correlations between nutrients \( r \): 0.60 to 0.79). However, only two of these results showed a statistically significant interaction: the risk of breast cancer among women with the MTHFR 677TT genotype was significantly higher among women with low intakes of dietary folate \( P \) for interaction = 0.02) and total B6 \( P \) for interaction = 0.01) than those with higher intakes. Results for total vitamin B6 were particularly striking. Among women with high B6 intake, there was no MTHFR-breast cancer association. However, among women with low B6 intake, the TT genotype was associated with an approximate four-fold risk \( OR = 4.03/1.11 \). Alcohol intake did not appear to modify the exposure-disease association \( P \) for interaction = 0.22), but few VITAL women drank ≥ 10 g/d of alcohol.

### Discussion

In this moderate-sized, nested case-control study, we observed a 62% increase risk of breast cancer among postmenopausal women with the TT genotype. The most pronounced risks were observed among individuals with the TT genotype and lowest intakes of folate and vitamin B6.

Results from 26 case-control studies [4,16-40] investigating MTHFR C677T and breast cancer risk have been inconsistent. Our results are consistent with two [18,19] of nine studies [17-20,22,26,27,29,31] reporting separate estimates for postmenopausal women. Ericson et al observed a significant 34% increase in breast cancer risk among postmenopausal women in Sweden with CT and TT genotypes compared to wild-type in a nested case-control study of the Malmo Diet and Cancer cohort [19]. Suzuki reported a significant 83% increased breast cancer risk among postmenopausal Japanese women with the TT genotype compared to wild-type [18]. Among 10 studies reporting estimates for premenopausal women [16-19,22,26,27,29,31], three have reported significant positive associations, ranging from a 64% to a 2.8-fold increased risk among CT and/or TT individuals [16,17,30]. Two studies [23,32] observed statistically significant increased risks among pre- and postmenopausal women combined. Other investigations have not reported significant associations. Differences in results may be due to variation between populations with regards to prevalence of polymorphisms in genes related to one-carbon metabolism, intakes of nutrients, and/or risk factors for breast cancer. Several studies reporting no association had <150 breast cancer cases [4,24,25,33,34,36,37,39], and thus, may have been too small to detect an association. Our study tended to have a larger population of postmenopausal breast cancer patients, and thus, may have had more power to detect an effect.

Eight studies, to-date, have examined interactions between MTHFR C677T and nutrients, including alcohol [17-23,25]. Among these, only a case-control study of Brazilian women (458 age-matched pairs) observed statistically significant gene-diet interactions [22]. However, the folate results were opposite than expected: a significantly reduced risk of breast cancer was observed among TT and CT individuals with the lowest intakes of dietary folate. Major limitations were that diet was collected after breast cancer diagnosis and recall of diet was poor. While the tests of interaction were not significant, three studies observed increased MTHFR-breast cancer risks with low folate intakes which are in line with our results [18,21,23].

Our results are biologically plausible. The TT genotype is associated with approximately 65% less activity than wild-type [1,2]. Reduced MTHFR activity among 677TT individuals may increase cancer risk by leading to lowered availability of 5-methyltetrahydrofolate and subsequently, impaired DNA methylation. DNA methylation plays a critical role in gene expression and the maintenance of genomic stability [41,42], and dysregulation of methylation patterns have been implicated with carcinogenesis [43,44]. Furthermore, our results suggest a more pronounced risk of breast cancer among 677TT individuals when intakes of nutrients associated with one-carbon metabolism are comparatively low. This finding is consistent with multiple studies showing increases in risk of colorectal adenomas or cancer under a low one-carbon status [45,46]. Furthermore, our results are supported by other reports suggesting that when folate levels are low, the 677TT genotype is associated with higher levels of homocysteine, lower levels of methylated folate, and reductions in genomic DNA methylation [47,48]. Our previous study in VITAL and other reports suggest that folate intake lowers breast cancer risk [6]. Reasons for not being able to replicate the earlier VITAL cohort findings

| Odds ratios (OR) for postmenopausal breast cancer by MTHFR C677T genotype, VITAL study |
|---------------------------------|-----------------|-----------------|
| Cases, n | Controls, n | OR (95% CI) |
| CC | 133 | 301 | 1.00 |
| CT | 139 | 284 | 1.08 (0.81 to 1.43) |
| TT | 46 | 62 | 1.62 (1.05 to 2.48) |

* Adjusted for age in five year-age categories and race (white, other).
† P for trend calculated by modelling the MTHFR genotypes CC, CT, and TT as one term, ordinarily.

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Table 3  

Joint association of \textit{MTHFR} C677T genotype and nutrient intakes on postmenopausal breast cancer risk, VITAL study *,†

| MTHFR C677T genotype | Cases/controls | Multivariate OR (95% CI) | Cases/controls | Multivariate OR (95% CI) | Cases/controls | Multivariate OR (95% CI) | P for trend ‡ | P for interaction § |
|----------------------|----------------|--------------------------|----------------|--------------------------|----------------|--------------------------|----------------|---------------------|
| CC                   | 92/218         | 1.00                     | 108/213        | 1.20 (0.82-1.75)         | 29/51          | 1.46 (0.83-2.56)         | 0.24           |                     |
| CT                   | 41/83          | 1.24 (0.75-2.03)         | 31/71          | 0.90 (0.52-1.59)         | 17/11          | 6.24 (2.37-16.4)         | 0.02           | 0.27                |

**Multivitamin use**

- Some: 92/218, OR 1.00 (95% CI 1.00-1.20), P for trend 0.24
- None: 41/83, OR 1.24 (0.75-2.03), P for trend 0.02

**Folate**

- Dietary folate (μg/day)
  - ≥ 224 (median, high): 79/128, OR 1.00 (95% CI 0.85-1.30), P for trend 0.86
  - <224: 43/139, OR 0.41 (0.25-0.67), P for trend <0.01

- Supplemental folate (μg/day) #
  - ≥ 400: 48/129, OR 1.00 (95% CI 0.85-1.20), P for trend <0.01
  - <400: 85/172, OR 1.32 (0.83-2.00), P for trend <0.01

- Total folate (DFE/day)
  - ≥ 850 (median, high): 54/132, OR 1.00 (95% CI 0.79-1.26), P for trend <0.01
  - <850: 68/134, OR 1.32 (0.83-2.09), P for trend <0.01

**Methionine**

- Dietary methionine (g/day)
  - ≥ 1.38 (median, high): 60/133, OR 1.00 (95% CI 0.91-1.44), P for trend 0.29
  - <1.38: 62/134, OR 1.07 (0.65-1.77), P for trend 0.04

**Vitamin B₂**

- Dietary vitamin B₂ (mg/day)
  - ≥ 1.81 (median, high): 63/127, OR 1.00 (95% CI 0.92-1.20), P for trend 0.72
  - <1.81: 59/140, OR 0.85 (0.52-1.37), P for trend <0.01

- Total vitamin B₂ (mg/day)
  - ≥ 2.70 (median, high): 59/125, OR 1.00 (95% CI 0.94-1.29), P for trend 0.96
  - <2.70: 63/142, OR 1.02 (0.64-1.63), P for trend 0.02

**Vitamin B₆**

- Dietary vitamin B₆ (mg/day)
  - ≥ 1.61 (median, high): 59/129, OR 1.00 (95% CI 0.93-1.20), P for trend 0.97
  - <1.61: 63/138, OR 1.08 (0.66-1.77), P for trend <0.01

- Supplemental vitamin B₆ (mg/day) #
  - ≥ 2.00: 49/108, OR 1.00 (95% CI 1.17-2.00), P for trend 0.09
  - <2.00: 84/192, OR 0.87 (0.57-1.34), P for trend <0.01

- Total vitamin B₆ (mg/day)
  - ≥ 3.32 (median, high): 57/132, OR 1.00 (95% CI 1.11-2.00), P for trend <0.01
  - <3.32: 65/135, OR 1.11 (0.70-1.76), P for trend 0.01

**Vitamin B₁₂**

- Dietary vitamin B₁₂ (μg/day)
  - ≥ 5.30 (median, high): 52/131, OR 1.00 (95% CI 1.13-2.00), P for trend 0.15
  - <5.30: 70/136, OR 1.38 (0.85-2.22), P for trend 0.10
may be that this nested case-control study had approximately three-years shorter follow-up and fewer cases (334 versus 743 cases in the cohort study publication). Vitamin B₆ has an important role in one-carbon metabolism in that it acts as a cofactor for methionine synthesis and is a coenzyme of serine hydroxymethyltransferase, which is involved in nucleotide synthesis. We did not observe an overall statistically significant association between B₆ intake and breast cancer risk, previously [6]. However, it is possible that intakes below a certain threshold may make 677TT individuals more susceptible.

Our study has some limitations. First, we did not have data on other MTHFR polymorphisms, such as A1298C and G1793A. However, data suggest that C677T is the major genetic determinant of MTHFR activity [49]. Second, the high correlation between nutrient intakes (r ≥ 0.60) made it difficult to separately examine effect modification of individual nutrients. Lastly, nutrient and supplement intakes were based on self-report; however, because this information was collected prior to diagnosis, any misclassification would have been non-differential and would most likely have attenuated the associations. Another source of measurement error, apart from inaccuracies in self-report, is that the folate content of food changed over time. Food manufacturers began fortifying grain products starting in 1996 to 1998 in response to U.S. governmental regulations, a few years before the VITAL baseline questionnaire (2000 to 2002). Thus, the high levels of intake in this population represent the post-fortification period, while intake earlier during the pre-fortification period may be more predictive of breast cancer risk. Since low folate intake is of most interest in MTHFR effect modification, use of post-fortification folate values may have weakened our ability to detect effect modification by low folate intakes.

Our study adds to the literature by being the first study, to our knowledge, examining effect modification of MTHFR by multiple nutrients involved in the one-carbon metabolism pathway; data on supplement use also allowed for analysis by nutrient type (diet, supplement, and total). This study also adds to current knowledge regarding the association of MTHFR among postmenopausal women. An advantage of this study’s prospective design is that it avoids recall bias for the recall of nutrients. An additional strength is that we had a broad range of information on possible confounders for adjustment. Also, selection bias is highly unlikely, because this was a prospective study and participants would not have known their future breast cancer status when deciding to give buccal cells or complete the breast cancer risk questions.

### Conclusions

In summary, this study provides support that the MTHFR 677TT genotype is associated with a moderately increased risk of postmenopausal breast cancer and with a substantial increase among women with low intakes of folate and vitamin B₆.

### Table 3 (Continued)

| Supplementation vitamin B₁₂ (μg/day) | Median intake (based on distribution of nutrient intake from controls) |
|-------------------------------------|---------------------------------|
| <6.00                              | 56/120 (1.00)                   |
| ≥6.00                              | 69/109 (1.30)                   |
| Total vitamin B₁₂ (μg/day)         |                                |
| <11.4 (median, high)               | 60/130 (1.00)                   |
| ≥11.4                              | 69/131 (1.17)                   |
| MTHFR genotype and nutrient intakes on postmenopausal breast cancer risk, VITAL study *,† |
| Low                                | 65/138 (1.12)                   |
| <10.0 (low)                        | 61/127 (1.09)                   |
| ≥10.0                              | 65/127 (1.15)                   |
| Total alcohol (g/d)                |                                |
| <10.0 (low)                        | 100/254 (1.00)                  |
| ≥10.0                              | 105/229 (1.15)                  |
| Median intakes, based on distribution of nutrient intake from controls |
| Intakes of all supplements over 10 years; cutpoints represent amount in a 10 year daily use of standard multivitamin or greater |
| Micronutrient score **              |                                |
| High (median)                      | 57/129 (1.00)                   |
| Low                                | 65/138 (1.12)                   |
| ** Micronutrient score computed by summing the z-scores of folate, methionine, and vitamins B₂, B₆, B₁₂ and then, dividing this sum into high (upper median) and low (lower median) groups.

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*All analyses adjusted for the following: age (50-54 years, 55-59, 60-64, 65-69, 70-76), race (white, other), family history of breast cancer (no, 1 affected mother or sister, ≥2 affected mother or sister(s)), mammography within two years preceding baseline (no, yes), history of breast biopsy (no, yes), age at menarche (≤11 years, 12-13, ≥14), age at first birth (nulliparous, ≤19 years, 20-24, 25-34, >35), age at menopause (≤44 years, 45-49, ≥50), years of combined estrogen and progestin postmenopausal hormones (PMH, never or <1 years, 1-4.5, 5-9, ≥10), height (<62 in, 62-<65, 65-<68, ≥68), body mass index (BMI) (<25 kg/m², 25≤<30, ≥30), total physical activity (none, 1-3 tertiles of MET-hours/week), total energy intake (kcal/day), and for non-alcohol exposures, past-year alcohol intake (<1.5 g/day, ≥1.5-4.9, 5.0-9.9, ≥10). Energy was added to the models for all dietary and total nutrient exposures.

† Case/control numbers do not add up to total due to missing data on nutrient intakes

‡ P for trend testing trend for MTHFR genotypes by strata of nutrient/alcohol intakes and calculated by modelling the MTHFR genotypes (CC, CT, and TT) as one term, ordinarily

§ P for interaction testing whether the association between MTHFR and breast cancer varies by nutrient/alcohol intake

# Intakes of all supplements over 10 years; cutpoints represent amount in a 10 year daily use of standard multivitamin or greater

Available online http://breast-cancer-research.com/content/11/6/R91
B6. From a public health standpoint, these results are of interest in that they suggest that the increased risk associated with the TT genotype may be attenuated with intakes of some one-carbon-associated nutrients.

Competing interests
ERJ is a salaried employee of and holds stock options in InterGenetics Incorporated, but is not a major shareholder. A grant from InterGenetics Incorporated provided partial support for genotyping analyses. The other authors declare that they have no competing interests.

Authors’ contributions
SSM planned the data analysis, carried out all statistical analyses, interpreted the results, and drafted the manuscript. CMU contributed to the interpretation of the data and revised the manuscript for important intellectual content. ERI directed the DNA isolation and the acquisition and finalization of the genotyping studies and revised the manuscript. EW conceived of the VITAL study, obtained funding, participated in the study’s design and coordination, contributed to the interpretation of results, and revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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