B-Vitamin Intake, One-Carbon Metabolism, and Survival in a Population-Based Study of Women with Breast Cancer

Xinran Xu,[1] Marilie D. Gammon,[9] James G. Wetmur,[2,3] Patrick T. Bradshaw,[9] Susan L. Teitelbaum,[1] Alfred I. Neugut,[6,7] Regina M. Santella,[8] and Jia Chen[1,4,5]

Departments of [1]Community and Preventive Medicine, [2]Microbiology, [3]Genetics and Genomic Sciences, [4]Pediatrics, and [5]Oncological Science, Mount Sinai School of Medicine; Departments of [6]Epidemiology, [7]Medicine, and [8]Environmental Health Sciences, Columbia University, New York, New York; and [9]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Abstract

Breast cancer is the second leading cause of cancer mortality among women. Given its important role in DNA methylation and synthesis, one-carbon metabolism may affect breast cancer mortality. We used a population-based cohort of 1,508 women with breast cancer to investigate possible associations of dietary intake of B vitamins before diagnosis as well as nine polymorphisms of one-carbon metabolizing genes and subsequent survival. Women newly diagnosed with a first primary breast cancer in 1996 to 1997 were followed for vital status for an average of 5.6 years. Kaplan-Meier survival and Cox proportional hazard regression analyses were used to evaluate the association between dietary intakes of B vitamins (1,479 cases), genotypes (~1,065 cases), and all-cause as well as breast cancer–specific mortality. We found that higher dietary intake of vitamin B_1 and B_3 was associated with improved survival during the follow-up period (P_{trend} = 0.01 and 0.04, respectively). Compared with the major genotype, the *MTHFR 677 T* allele carriers have reduced all-cause mortality and breast cancer–specific mortality in a dominant model [hazard ratio (95% confidence interval): 0.69 (0.49-0.98) and 0.58 (0.38-0.89), respectively]. The *BHMT 742 A* allele was also associated with reduced all-cause mortality [hazard ratio, 0.70 (0.50-1.00)]. Estrogen receptor/progesterone receptor status modified the association between the *MTHFR C677T* polymorphism and survival (P = 0.05). The survival associations with one-carbon polymorphisms did not differ with the use of chemotherapy, although study power was limited for examining such effect modification. Our results indicate that one-carbon metabolism may be an important pathway that could be targeted to improve breast cancer survival. (Cancer Epidemiol Biomarkers Prev 2008;17(8):2109–16)

Introduction

The 5-year survival rate for breast cancer among U.S. women has increased from 75% during 1974 to 1976 to 85% during 1989 to 1995 (1, 2). Despite such marked improvement, breast cancer remains the leading cause of cancer mortality among women 20 to 59 years of age and the second leading cause of cancer mortality among all women (1). Disease-free survival after breast cancer treatment may be partially predicted by tumor size, hormone receptor status, and other clinical and pathologic factors (3-7). Although a number of lifestyle and host factors have been inconsistently or infrequently reported to affect disease-free or overall survival, only a few have been firmly established to adversely affect survival, including age, race, and obesity (8-10); few of these are factors that a patient can actively modify or that can help clinicians to tailor an effective treatment.

One-carbon metabolism may influence breast cancer mortality because of the critical role it plays in both DNA methylation and DNA synthesis (Fig. 1). An abnormal methylation profile, such as promoter-CpG island hypermethylation, is a common molecular defect in cancer cells (11, 12). Estrogen receptor (ER) and progesterone receptor (PR) status have been used widely as a prognostics markers as well as indicators for tailoring breast cancer treatment (endocrine therapy versus chemotherapy; ref. 13). Loss of function of these genes has been shown to predict poor prognosis of breast cancer (14). Promoter hypermethylation has been implicated as the underlying mechanism for silencing of these receptors (12, 15-17). Both dietary factors, such as folate and related B vitamins, and genetic variations of one-carbon metabolizing genes may modify the methylation profile of these prognostic genes, thus influencing breast cancer survival.

Another reason one-carbon metabolism may influence breast cancer survival is that it is the direct target for several widely used anticancer drugs. Combination chemotherapy with cyclophosphamide, methotrexate, and 5-fluorouracil has been the treatment of choice for

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Requests for reprints: Jia Chen, Department of Community and Preventive Medicine, Box 1043, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029. Phone: 212-241-7519; Fax: 212-360-6965. E-mail: jia.chen@mssm.edu

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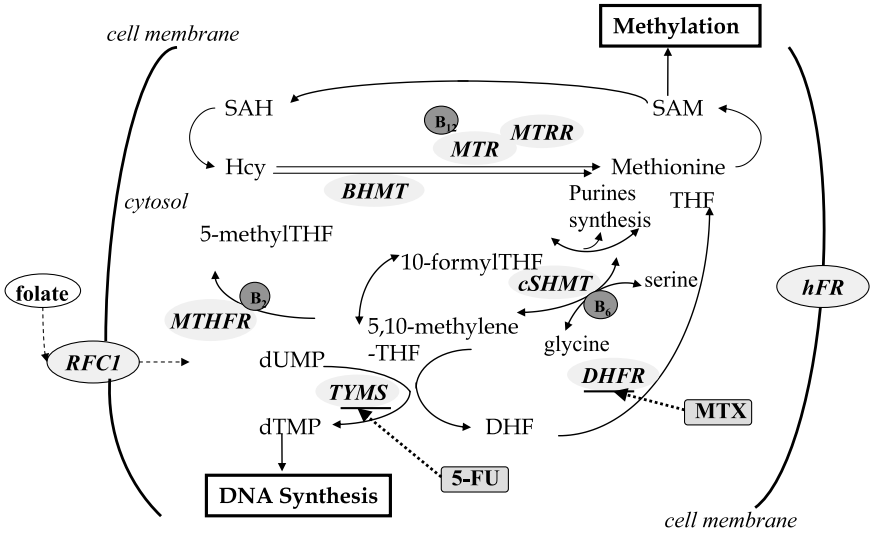

Figure 1. Schematic illustration of the one-carbon metabolism pathway. Key genes involved in one-carbon metabolism include methylenetetrahydrofolate reductase (*MTHFR*), thymidylate synthase (*TYMS*), methionine synthase (*MTR*), methionine synthase reductase (*MTRR*), cytosol serine hydroxymethyltransferase (*cSHMT*), dihydrofolate reductase (*DHFR*), and betaine-homocysteine methyltransferase (*BHMT*). Reduced folate carrier (*RFC1*) and human folate receptor (*hFR*) transport the dietary polyglutamyl folate (the predominant form of folate in diet) in intestinal absorption. B vitamins are cofactors of one-carbon enzymes as shown. Chemotherapy drug 5-fluorouracil (*5-FU*) targets TYMS and methotrexate targets DHFR to block DNA synthesis. *Hcy,* homocysteine; *SAM, S*-adenosylmethionine; *SAH,* adenosylhomocysteine; *THF,* tetrahydrofolate; *DHF,* dihydrofolate.

the majority of patients with stage II, node-negative breast cancer, as clinical trials have shown its efficacy and long-term safety (18). 5-Fluorouracil targets the enzyme thymidylate synthase (TYMS), whereas methotrexate targets dihydrofolate reductase (DHFR), both involved in one-carbon metabolism (Fig. 1). These drugs block DNA synthesis and ultimately cell replication. Genetic variations of these enzymes may have functional consequences and ultimately affect the efficacy of the cyclophosphamide, methotrexate, and 5-fluorouracil treatment modality.

In humans, folate plays the fundamental role of providing methyl groups for intracellular methylation reactions and *de novo* deoxynucleoside synthesis. Folate deficiency is associated with genomic instability and could lead to abnormal DNA methylation status (19). Recently, studies on colorectal cancer suggested that folate may play a dual role in tumor progression (20, 21). Whereas folate is in general antineoplastic before the tumor foci are established, it may enhance tumor proliferation after the tumor is established. Thus, the effect of one-carbon metabolism on breast cancer survival may be complicated. Using the population-based data from the Long Island Breast Cancer Study Project, we investigated the associations of dietary intakes of B vitamins before diagnosis as well as functional polymorphisms in one-carbon metabolism and breast cancer survival.

Materials and Methods

Study Population. We used data from the follow-up study of the Long Island Breast Cancer Study Project, a population-based study that includes women newly diagnosed with a first primary breast cancer who participated in the original case-control study (22) and were subsequently reinterviewed about 5 y later and followed for vital status (23). The study protocol was approved by the institutional review boards of the collaborating institutions.

Women considered eligible for the follow-up study included all case participants of the parent case-control study, which includes 1,508 women with a first primary *in situ* or invasive breast cancer who were newly diagnosed between August 1, 1996, and July 31, 1997, and were residents of Nassau or Suffolk counties, Long Island, New York, at the time of diagnosis. Cases were identified through review of pathology/cytology records of 33 collaborating institutions and were contacted by letter and telephone after obtaining physician permission.

As previously reported (22), at the time of the first primary breast cancer diagnosis, the mean age was 58.8 y (range, 25.1-98.1 y); 94% were White, 4% were African American, and 2% were other; 235 (15.6%) had carcinoma *in situ* and 1,273 (84.4%) had invasive tumor; 472 (31.3%) women were premenopausal and 1,006 (66.7%) were postmenopausal; and 583 (58.9%) were ER^+/PR^+, 143 (14.4%) were ER^+/PR^-, 52 (5.3%) were ER^-/PR^+, and 212 (21.4%) were ER^-/PR^-.

Exposure Assessment. Exposure data were obtained as part of the (*a*) case-control (baseline) interview, (*b*) follow-up interview, and (*c*) medical record abstraction, as described below. For our analyses, dietary intake data and most of the potential confounders and effect modifiers were derived from the baseline interview. Complete course of treatment for the primary breast cancer was obtained during the follow-up interview and through medical record abstraction.

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Case-Control Data. The baseline questionnaire was administered by a trained interviewer ~3 mo after diagnosis (average time was 96 d) and elicited information on reproductive/menstrual history, cigarette smoking, alcohol, body size, physical activity, and medical history.[10] Dietary intake in the year before the interview (which primarily reflects prediagnostic intake) was assessed using a self-administered modified Block Food Frequency Questionnaire that was completed at the time of the baseline interview (24). The frequency and portion sizes data were translated to daily intakes of nutrients from both dietary and supplement sources using the National Cancer Institute DietSys version 3. Habitual use of multivitamin supplements was also obtained from the Food Frequency Questionnaire. The questions included multivitamin use over the past 10 to 15 y before the interview, type of multivitamin, and dosage. Details have previously been described (24-26). Total folate was calculated by using the estimated dietary folate equivalent conversion factor of 1.7 for folic acid from supplement (27, 28): total folate = dietary folate + 1.7 × supplement folate. In this study, we examined the B vitamins (folate, B_1, B_2, B_3, B_6, and B_{12}) and two cofactors involved in methyl transfer in the one-carbon metabolism pathway (i.e., methionine and betaine). Spearman coefficients ranged from a low of 0.41 between total folate and vitamin B3 to a high of 0.90 between dietary folate and vitamin B1 (29). Energy intake was also estimated using data collected from the Food Frequency Questionnaire. The dietary intake information was available for 1,479 cases in our analysis.

Follow-up Data. In 2002 to 2004, case participants, or their proxy (close relative or friend) were recontacted first by mail, and then by telephone, to invite them to participate in the follow-up study (23, 30). Follow-up data used in this study were the information for tumor characteristics (tumor size and nodal status) and treatment information. Of the 1,098 case women for whom we have data from the follow-up questionnaire, 784 (71.4%) completed the full interview themselves; 165 (15.0%) respondents completed the critical interview, which was an abbreviated questionnaire that focused on collecting treatment data only; and 84 (7.7%) proxy interviews were conducted. Among the 1,022 case women who provided information on chemotherapy at the time of the follow-up interview, 423 (41.4%) reported receiving chemotherapy as part of their first course of treatment for their primary breast cancer diagnosis.

Medical Record Data. For cases who signed medical record release forms, medical records were abstracted twice: at baseline (as part of the case-control study) and again during the follow-up study. At baseline, information was obtained on disease stage (*in situ*/invasive), tumor size, ER/PR status (assessed by immunohistochemistry; n = 990), and initial course of treatment for their breast cancer. More than three quarters of the baseline case interviews occurred before the initiation of chemotherapy (22). Additional treatment information was obtained by reabstracting medical records as part of the follow-up study. Among the subset of women for whom we had complete course of treatment data from the medical record (n = 499), we compared their self-reported responses at the follow-up interview with the medical record data using the κ coefficient; concordance between the two sources was high (κ = 0.97 for radiation therapy, κ = 0.96 for chemotherapy, and κ = 0.92 for hormone therapy; ref. 23).

Blood Sample Collection and Genotyping. Blood samples were collected from 73% of the 1,508 cases at the time of the baseline interview by trained field staff (22). DNA was isolated from blood specimens using the methods previously described (25); DNA was available from 1,065 individuals for genotyping. Using methods described elsewhere (29, 31), genotyping was conducted on nine polymorphisms in the one-carbon metabolism pathway [i.e., *MTHFR C677T* (rs1801133) and *A1298C* (rs1801131); *TYMS* 5′-untranslated region tandem repeat; *DHFR* 19-bp deletion; *MTR A2756* (rs1805087); *MTRR A66G* (rs1801394); *BHMT G742A* (rs3733890); *RFC1 A80G* (rs1051266); and *cSHMT C1420T* (rs1979277)]. The mean call rate was 96%; the main reason for missing genotypes was insufficient DNA. About 10% of the study population samples were randomly duplicated as quality control samples; the concordance rate was >98% for all polymorphisms in this study. All laboratory personnel were blinded to the outcome status of the breast cancer cases as well as quality control status of the specimens. Characteristics were comparable between cases with blood and those with genotype information available.

Study Outcome. The National Death Index was used to ascertain all-cause and breast cancer–specific mortality. Among the 1,508 women diagnosed with breast cancer in 1996 to 1997, 198 (13.1%) deaths occurred by December 31, 2002. The mean follow-up time was 5.6 y (range, 0.2-7.4 y).

Based on International Classification of Diseases codes 174.9 and C-50.9 listed as a primary or secondary code on the death certificate, 124 (62.6%) of these 198 deaths were due to breast cancer. When restricted to the cohort from which DNA was available for genotype analysis (n = 1,065), a total of 131 (12.3%) deaths were observed; 84 (64.1%) of these were due to breast cancer.

Statistical Analysis. The Kaplan-Meier and log-rank tests were used to examine the crude association between dietary intake or genotypes and survival (32). The Cox proportional hazard regression (32) was used to estimate the hazard ratio (HR) and 95% confidence interval (95% CI) for all-cause and breast cancer–specific mortality, with adjustments made for age at diagnosis (continuous) and energy intake (quintiles). Nutrient intakes in the year before the interview were categorized based on the distributions observed among all cases regardless of outcome status; results based on tertiles are shown. Tests of trend were conducted by coding the variables ordinarily in the model. To increase statistical power, heterozygous and variant homozygous genotypes were combined as a single risk group.

Confounding was evaluated using the methods described by Rothman and Greenland (33) starting with a full multivariate model and using backward elimination. Factors considered as potential confounders included menopausal status (premenopausal/postmenopausal), family history of breast cancer in a first-degree relative, cancer type (*in situ*/invasive), active/passive

[10] http://www.epi.grants.cancer.gov/LIBCSP/projects/Questionnaire.html

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Table 1. Age- and energy-adjusted HRs and 95% CIs for the association between B vitamin intakes and all-cause as well as breast cancer–specific mortality

Nutrient*	Low	Medium	High	P_{trend}
Dietary folate				
Range (μg/d)	<194.1	194.1-300.8	>300.8	
HR (95% CI) †	1.00 (Reference)	0.82 (0.57-1.17)	0.79 (0.52-1.12)	0.28
HR (95% CI) ‡	1.00 (Reference)	0.88 (0.55-1.39)	0.81 (0.47-1.39)	0.44
Total folate (diet + supplements)				
Range (μg/d)	<291.0	291.0-869.0	>869.0	
HR (95% CI) †	1.00 (Reference)	0.82 (0.57-1.18)	0.97 (0.69-1.36)	0.85
HR (95% CI) ‡	1.00 (Reference)	0.80 (0.49-1.31)	1.24 (0.81-1.90)	0.27
Vitamin B_1 (thiamin)				
Range (mg/d)	<0.87	0.87-1.25	>1.25	
HR (95% CI) †	1.00 (Reference)	0.72 (0.49-1.04)	0.54 (0.38-0.88)	0.01
HR (95% CI) ‡	1.00 (Reference)	0.71 (0.44-1.12)	0.44 (0.24-0.81)	0.01
Vitamin B_2 (riboflavin)				
Range (mg/d)	<1.17	1.17-1.75	>1.75	
HR (95% CI) †	1.00 (Reference)	0.75 (0.51-1.09)	0.92 (0.58-1.44)	0.67
HR (95% CI) ‡	1.00 (Reference)	0.86 (0.54-1.38)	0.72 (0.41-1.29)	0.27
Vitamin B_3 (niacin)				
Range (mg/d)	<11.9	11.9-16.7	>16.7	
HR (95% CI) †	1.00 (Reference)	0.68 (0.47-0.99)	0.61 (0.38-0.98)	0.04
HR (95% CI) ‡	1.00 (Reference)	0.76 (0.47-1.21)	0.61 (0.34-1.09)	0.09
Vitamin B_6 (pyridoxine)				
Range (mg/d)	<1.05	1.05-1.54	>1.54	
HR (95% CI) †	1.00 (Reference)	0.99 (0.68-1.43)	0.95 (0.61-1.48)	0.82
HR (95% CI) ‡	1.00 (Reference)	0.95 (0.60-1.51)	0.77 (0.44-1.36)	0.37
Vitamin B_{12} (cobalamin)				
Range (μg/d)	<3.05	3.05-5.12	>5.12	
HR (95% CI) †	1.00 (Reference)	0.96 (0.67-1.40)	1.20 (0.80-1.81)	0.38
HR (95% CI) ‡	1.00 (Reference)	0.93 (0.58-1.49)	1.10 (0.65-1.85)	0.71
Methionine				
Range (g/d)	<0.79	0.79-1.14	>1.14	
HR (95% CI) †	1.00 (Reference)	0.84 (0.59-1.21)	0.70 (0.44-1.13)	0.14
HR (95% CI) ‡	1.00 (Reference)	0.93 (0.59-1.49)	0.70 (0.39-1.28)	0.25
Betaine				
Range (mg/d)	<86.5	86.5-149.6	>149.6	
HR (95% CI) †	1.00 (Reference)	0.87 (0.61-1.25)	0.81 (0.54-1.20)	0.28
HR (95% CI) ‡	1.00 (Reference)	0.63 (0.40-1.02)	0.72 (0.44-1.17)	0.19

* Intake of B vitamins was tertiled for analysis.
† All-cause mortality.
‡ Breast cancer–specific mortality.

cigarette smoking, body mass index at diagnosis, average lifetime alcohol intake (grams per day), education, income, tumor size, and radiation treatment and chemotherapy undergone for the original breast cancer diagnosis. If eliminating a covariate from the full Cox regression model changed the effect estimate by 10% or more, the covariate was considered a confounder and kept in the model (33). Otherwise, that covariate was dropped from the multivariate model. None of the covariates tested met such criterion; thus, only results adjusted for age and energy intake are presented.

Effect modification on the multiplicative scale was evaluated using the log likelihood ratio test to compare Cox models with and without the interaction term as a cross-product term of genotype and effect modifier. Factors considered as potential effect modifiers of the genotype-mortality association include menopausal status (premenopausal/postmenopausal), cancer type (*in situ*/invasive), ER/PR status [cases were categorized into two groups: ER and PR both positive (ER^+/PR^+) versus all others (ER^+/PR^-, ER^-/PR^+, ER^-/PR^-); when he latter receptor types were analyzed individually, their respective HRs were similar] and chemotherapy. *P* values for the interaction were evaluated for the potential modification on gene effect by factors examined.

Unconditional logistic regression was used to explore the relationship of one-carbon genotype and hormone receptor (ER/PR) status. Genotypes were used as exploratory variables in the model. ER/PR status (either both positive versus all others) was treated as outcome in the logistic regression model and the odds ratio and 95% CI were estimated by modeling the probability of the case tumor being ER/PR positive.

All statistical analyses were done using SAS statistical software version 9.1 (SAS Institute).

Results

The associations between dietary intake of B vitamins in the year before the baseline interview and subsequent all-cause as well as breast cancer–specific mortality are summarized in Table 1. Intakes of vitamin B_1 and B_3 were inversely associated with all-cause mortality in this population-based cohort of women with breast cancer. Compared with the low intake group, cases in the high intake group had a 46% (HR, 0.54; 95% CI, 0.34-0.88) and 39% (HR, 0.61; 95% CI, 0.38-0.98) lower risk of death for B_1 and B_3, respectively. Trend tests for the associations were significant (P_{trend} = 0.01 for B_1 and

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Table 2. Age-adjusted HRs and 95% CIs for the associations of polymorphisms of the one-carbon metabolizing genes and all-cause as well as breast cancer–specific mortality

Gene	Genotype	All-cause mortality			Breast cancer-specific mortality		
		No. death	No. censored (%)	HR* (95% CI)	No. death	No. censored (%)	HR* (95% CI)
MTHFR (C677T)	CC	60	338 (84.9)	1.00 (Reference)	42	356 (89.5)	1.00 (Reference)
	CT/TT	71	594 (89.3)	0.69 (0.49-0.98)	42	623 (93.7)	0.58 (0.38-0.89)
MTHFR (A1298C)	CC	69	489 (87.6)	1.00 (Reference)	41	517 (92.7)	1.00 (Reference)
	AC/AA	61	443 (87.9)	1.01 (0.71-1.42)	43	461 (91.5)	1.16 (0.76-1.78)
TSTR (5′-UTR)	3R/3R	43	268 (86.2)	1.00 (Reference)	26	285 (91.6)	1.00 (Reference)
	3R/2R/2R/2R	85	657 (88.5)	0.80 (0.56-1.16)	57	685 (92.5)	0.91 (0.57-1.45)
DHFR (19bp del)	+/+	46	288 (86.2)	1.00 (Reference)	31	303 (90.7)	1.00 (Reference)
	+/−/−/−	84	645 (88.5)	0.85 (0.60-1.22)	52	677 (92.9)	0.76 (0.49-1.18)
MTR (A2756G)	GG	96	609 (86.4)	1.00 (Reference)	58	647 (91.8)	1.00 (Reference)
	AG/AA	34	315 (90.3)	0.70 (0.47-1.03)	25	324 (92.8)	0.85 (0.53-1.35)
MTRR (A66G)	GG	42	237 (85.0)	1.00 (Reference)	26	253 (90.7)	1.00 (Reference)
	AG/AA	88	691 (88.7)	0.75 (0.52-1.08)	57	722 (92.7)	0.77 (0.49-1.23)
BHMT (G742A)	GG	74	436 (85.5)	1.00 (Reference)	47	463 (90.8)	1.00 (Reference)
	AG/AA	56	495 (89.8)	0.70 (0.50-1.00)	36	515 (93.5)	0.70 (0.45-1.08)
RFC1 (A80G)	GG	34	253 (88.2)	1.00 (Reference)	19	268 (93.4)	1.00 (Reference)
	AG/AA	97	682 (87.5)	1.02 (0.69-1.51)	65	714 (91.7)	1.25 (0.75-2.08)
cSHMT (C1420T)	CC	70	438 (86.2)	1.00 (Reference)	47	461 (90.8)	1.00 (Reference)
	CT/TT	60	493 (89.2)	0.73 (0.52-1.03)	36	517 (93.5)	0.69 (0.44-1.06)

NOTE: Hazards ratio were adjusted for age (continuous).

P_{trend} = 0.04 for B_3). Although beneficial effects were observed for other one carbon–related B vitamins (except for B_{12}), where all the HRs were <1, the reductions were not statistically significant. No association was observed for intakes of methionine or betaine and all-cause mortality. When we examined the association with breast cancer–specific mortality, similar HRs were observed (Table 1); however, the association for vitamin B_3 did not reach significance (P_{trend} = 0.09).

Table 2 summarizes the association between one-carbon polymorphisms and all-cause as well as breast cancer–specific mortality. The variant alleles of two polymorphisms, *MTHFR C677T* and *BHMT G742A*, were significantly associated with better survival. The *MTHFR677 T* allele carriers had 31% lower risk of death than patients with the *MTHFR677* CC genotype (HR, 0.69; 95% CI, 0.49-0.98). The *BHMT 742 A* allele carriers had 30% lower risk of death than those with the *BHMT* GG genotype (HR, 0.70; 95% CI, 0.50-1.00). Two other single-nucleotide polymorphisms, *MTR2756 G* and *cSHMT1420 T* alleles, were associated with better survival with borderline significance. When we examined breast cancer–specific mortality, similar results were observed (Table 2); however, the association with *BHMT* did not reach statistical significance (P = 0.11).

To explore whether two single-nucleotide polymorphisms, *MTHFR C677T* and *BHMT G742A*, influence breast cancer survival via the hormone receptor (ER/PR) status of the tumor, we examined the associations between genotypes and ER/PR status (Table 3). Tumors of cases with the *MTHFR677 TT* genotype had an ~60% higher chance to be ER/PR positive compared with tumors of individuals with the CC genotype. Tumors of the *T* allele carriers had an ~28% higher chance to be ER/PR positive. There was no relationship between the *BHMT* polymorphism and ER/PR status in this population. Although no main effects on mortality were observed for the other seven polymorphisms investigated in the study, we also examined the associations between genotypes and ER/PR status but no association was found (data not shown).

We also examined whether the polymorphism-survival relationship differed by hormone receptor (ER/PR) status. Effect modification by ER/PR status was observed with respect to the *MTHFR C677T* polymorphism and all-cause mortality ($P_{interaction}$ = 0.05). The *T* allele was associated with better survival with borderline significance in all-cause mortality only among the grouped cases with ER^+/PR^-, ER^-/PR^+, and ER^-/PR^- status (HR, 0.61; 95% CI, 0.36-1.01). The associations between

Table 3. Relation of one-carbon genotype to ER/PR status

Gene	Genotype	ER/PR status*		OR (95% CI)	P_{trend}
		Positive (%)	Negative (%)		
MTHFR (C677T)	CC	145 (34.6)	107 (40.2)	1.00 (Reference)	0.05
	CT	195 (46.5)	123 (46.2)	1.17 (0.84-1.64)	
	TT	79 (18.9)	36 (13.5)	1.63 (1.02-2.60)	
	CT/TT	274 (75.4)	159 (59.8)	1.28 (0.93-1.75)	
BHMT (G742A)	GG	192 (45.9)	128 (47.9)	1.00 (Reference)	0.89
	GA	183 (43.8)	108 (40.5)	1.13 (0.82-1.57)	
	AA	43 (10.3)	31 (11.6)	0.92 (0.55-1.54)	
	GA/AA	226 (54.1)	139 (52.1)	1.09 (0.80-1.48)	

Abbreviation: OR, odds ratio.
*ER/PR status: positive represents for ER^+PR^+ cases and negative represents for ER^+/PR^-, ER^-/PR^+, and ER^-/PR^- cases.

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the other eight polymorphisms and survival did not differ with respect to hormone receptor status (data not shown).

To explore the potential modifying effect of one-carbon gene polymorphisms on chemotherapy response in relation to breast cancer survival, we stratified the cases by whether they received chemotherapy or not. About 800 cases with both genotype and chemotherapy treatment information were include in this analysis. Associations of one-carbon metabolism polymorphisms and overall survival did not differ by chemotherapy status (data not shown).

Discussion

Using data from a population-based cohort of breast cancer cases, we found inverse associations between several micronutrients and genotypes in the one-carbon metabolism and all-cause mortality. To the best of our knowledge, our study is the first to systematically evaluate prediagnostic intake of B vitamins involved in the one-carbon metabolism pathway in relation to breast cancer survival. This study is based on a strong biological rationale because one-carbon metabolism not only involves in regulation of prognosis-predictive genes in breast cancer but also is the major target for treatment of the disease (Fig. 1). Considering the high prevalence of these polymorphisms in the general population, results from the study can help us to identify factors that may influence disease outcomes.

B vitamins (B_1, thiamin; B_2, riboflavin; B_3, niacin; B_6, pyridoxine; B_9, folate; B_{12}, cobalamin) play important roles in cell metabolism, and some of them are cofactors involved in the one-carbon pathway. In a previous report (29), we found that increased dietary intakes of B vitamins were associated with reduced risk of developing breast cancer. Herein, we reported a beneficial effect of B vitamins, B_1 and B_3 in particular, on survival in the same population of breast cancer cases. These findings imply that a healthy diet reduces a woman's risk of developing breast cancer; however, should a breast cancer occur, the tumor would also display characteristics associated with a more favorable prognosis.

In this study, we found two genetic polymorphisms, *MTHFR C677T* and *BHMT G742A*, which were inversely associated with all-cause mortality. MTHFR catalyzes an irreversible reaction and it is the rate-limiting step in folate metabolism. Changes in MTHFR activity may tilt the balance of one-carbon metabolism in favor of DNA synthesis at the expense of methyl supply (i.e., *S*-adenosylmethionine) for methylation reactions. The *C677T* polymorphism of *MTHFR* results in an alanine to valine substitution and has been correlated with enzyme thermolability and reduced enzyme activity (34). Lower MTHFR activity has been shown to decrease DNA methylation in animal experiments (35). DNA methylation has been implicated in the silencing of *ER*, a prognosis-predictive gene (12, 15-17). Consistent with this reasoning, the *TT* genotype was associated with ER^+/PR^+ tumors in our study population (Table 3), leading to improved survival. This finding is based on a small sample size, and replication is warranted.

Although not directly involved in folate metabolism, BHMT is involved in the metabolism of homocysteine. BHMT may play a critical role in the remethylation of homocysteine when the folate-dependent pathway is compromised by either genetic or dietary factors (36). The *BHMT G742A* polymorphism was associated with overall survival in our study population; it is unknown whether homocysteine level correlates with breast cancer prognosis in our study population.

Many studies have been conducted to examine the effects of one-carbon metabolism polymorphisms on breast cancer risk, but reports of effects on subsequent survival are relatively sparse. There are two other studies that examined the effect of *MTHFR* genotypes on breast cancer survival. Results from the Shanghai Breast Cancer study showed that *MTHFR* genotypes were not associated with all-cause mortality, but the *677TT* genotype was associated with poor survival among those with late-stage disease (37). In our study, we observed a beneficial effect of the *MTHFR677 T* allele on all-cause mortality. Difference among these reports could be due to the difference in study populations, given that our population is overwhelmingly Caucasian, whereas the Shanghai Breast Cancer Study is restricted to Chinese women. Factors that may influence the *MTHFR*-survival relationship, such as treatment modality, may also be different in these two populations. Results from a small cohort reported by Martin et al. (38) showed that the *MTHFR1298 C* allele was associated with worse survival compared with the AA genotype; furthermore, this effect was stronger in ER^- patients. This latter study is based on a cohort containing a mixture of Caucasian and African-American women and the sample size was relatively small ($\sim$250).

We did not observe any survival differences by genotype stratified by chemotherapy. Although our sample size is much larger than previous studies, statistical power is still limited in stratified analyses. In addition, because the Long Island Breast Cancer Study Project is a population-based study, the breast cancer patients were treated in multiple institutions with non-standardized protocols. The majority of women in our study received adjuvant chemotherapy as part of their regular treatment for breast cancer. Specific information such as chemotherapy dose and duration was not available for all our study subjects, which limited our ability to examine the potential interaction between one-carbon metabolism and chemotherapy. Investigation of such relationships in the context of a larger population of breast cancer patients with more complete information on treatment modality is warranted because it is plausible that these factors could potentially be used to tailor treatment and ultimately improve survival.

In our study, we did not find any substantial differences in terms of associations of one-carbon metabolism with all-cause mortality and breast cancer–specific mortality. Vitamin B_3 intake and *BHMT* (*G742A*) polymorphism were associated with all-cause mortality but did not reach statistical significance for breast cancer–specific mortality. Given that the point estimates are similar, the wide confidence interval could be a result of limited study outcome ($\sim$130 death). Results on breast cancer–specific mortality may help us better understand the role of one-carbon metabolism in breast cancer progression and to develop more efficient treatments for this disease. Accurate assessment on cause of death is crucial in this type of investigation (39-41). The reliability

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of the cause of death listed on the death certificate, particularly when looking at a specific cancer site, may be questionable (e.g., sometimes a metastatic site may be recorded as the cause of death; refs. 42, 43). Thus, our findings based on all-cause mortality may be more valid. In addition, estimation of overall survival (with death as the end point regardless of the specific cause) is of public health significance and provides us with better power for detecting associations (44).

We used the dietary information collected at the baseline interview, which reflects intake patterns 1 y before the interview including the months just before and at diagnosis. Recall of this information was ascertained before the study outcome (i.e., death); thus, any possible misclassification is likely to be nondifferential. However, it is possible that patients change their lifestyle after a diagnosis of cancer (45, 46). Consequently, our results should be interpreted with caution. Studies have indicated that after breast cancer diagnosis, women are motivated to change their diet, but this is observed primarily in younger women (47, 48); our study population, on the other hand, is primarily composed of postmenopausal women. Interestingly, a substantial percentage (50%) of cancer survivors has been reported to continue to engage in health-risk behaviors (45). As discussed in a recent review, supplement use among breast cancer patients is high and frequently increases after diagnosis (49). We were unable to identify studies that specifically address the issue of whether breast cancer survivors change their dietary intake of foods containing B vitamins after diagnosis. If women in our study continued to follow their prediagnostic diets after their breast cancer diagnosis, then the implications of our results are that women in the general population should be encouraged to consume more B vitamins.

One potential issue to consider in interpreting our results is whether our analyses should have considered the potential confounding effects of tumor stage (or its surrogates tumor size and first course of treatment). However, because tumor stage is more likely a causal intermediate (e.g., the biological link between the exposure with the outcome), it would be epidemiologically inappropriate to include tumor stage in the model. However, in the subanalyses, we did consider the potential confounding effects of tumor size and first course of treatment (as surrogates for tumor stage, which was not reliably recorded on the medical record for all cases) and found that our results remain unchanged.

A potential limitation of our study is that we focused solely on the dominant model in our genetic association analyses. Consideration of other models would be of interest, but because our study power was constrained by the number of deaths (about 130) in our cohort of breast cancer cases, the results would be unstable and perhaps misleading. Another limitation of our study is its limited power for testing potential gene-gene and gene-diet interactions. Thus, we did not investigate these interactions in our analysis.

In summary, results from our population-based study suggest that in addition to its role in breast cancer etiology, one-carbon metabolism may be an important pathway that can be targeted to improve breast cancer survival among women with breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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