High intakes of choline and betaine reduce breast cancer mortality in a population-based study

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ABSTRACT **Choline and betaine provide methyl groups for one-carbon metabolism. Humans obtain these nutrients from a wide range of foods. Betaine can also be synthesized endogenously from its precursor, choline. Although animal studies have implied a causal relationship between choline deficiency and carcinogenesis, the role of these two nutrients in human carcinogenesis and tumor progression is not well understood. We investigated the associations of dietary intakes of choline and betaine and breast cancer risk and mortality in the population-based Long Island Breast Cancer Study Project. Among the 1508 case-group women, 308 (20.2%) deaths occurred, among whom 164 (53.2%) died of breast cancer by December 31, 2005. There was an indication that a higher intake of free choline was associated with reduced risk of breast cancer (P_{trend}=0.04). Higher intakes of betaine, phosphocholine, and free choline were associated with reduced all-cause as well as breast cancer-specific mortality in a dose-dependent fashion. We also explored associations of polymorphisms of three key choline- and betaine-metabolizing genes and breast cancer mortality. The betaine-homocysteine methyltransferase gene (*BHMT*) rs3733890 polymorphism was associated with reduced breast cancer-specific mortality (hazard ratio, 0.64; 95% confidence interval, 0.42–0.97). Our study supports the important roles of choline and betaine in breast carcinogenesis. It suggests that high intake of these nutrients may be a promising strategy to prevent the development of breast cancer and to reduce its mortality.—Xu, X., Gammon, M. D., Zeisal, S. H., Bradshaw, P. T., Wetmur, J. G., Teitelbaum, S. L., Neugut, A. I., Santella, R. M., Chen, J. High intakes of choline and betaine reduce breast cancer mortality in a population-based study. *FASEB J.* 23, 4022–4028 (2009). www.fasebj.org**

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CHOLINE IS AN ESSENTIAL NUTRIENT for the structure and function of all cells; it plays central roles in choline-mediated one-carbon metabolism, structural integrity and signaling functions of cell membranes, and neurotransmitter synthesis (1). Although choline can be synthesized *de novo*, humans require additional choline from dietary sources (2). *N,N,N*-trimethylglycine (betaine) can be obtained from food or from choline metabolism in liver and kidney (3). These micronutrients provide methyl groups to form the universal methyl donor *S*-adenosylmethionine (SAM), which donates its labile methyl group in >80 biological methylation reactions, including the methylation of DNA, RNA, and protein.

Choline is used as a collective term for a group of compounds, including sphingomyelin, glycerophosphocholine, phosphatidylcholine, phosphocholine, and free (unesterified) choline. Although phosphatidylcholine and sphingomyelin are lipid soluble, other types of choline are water soluble and are absorbed through different pathways, which may result in different bioavailability and fate in the body. Cancers usually exhibit a significantly altered choline metabolite profile, which is characterized by an elevation of phosphocholine and total choline-containing compounds compared with that in normal tissues (4, 5). Although animal studies have implied a causal relationship between choline deficiency and carcinogenesis (6, 7), its role in human carcinogenesis is not clear.

Betaine is used in clinical practice to lower the homocysteine level, with a potential benefit for cardiovascular health (3). With respect to cancer, one study found a nonlinear inverse association between betaine intake and colorectal adenoma among women (8). Another study found no association between betaine intake and breast cancer risk among a group of premenopausal women (9).

Three key genes are involved in the choline-mediated one-carbon metabolic pathway. Phosphatidyleth-

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anolamine *N*-methyltransferase (*PEMT*) catalyzes the *de novo* synthesis of choline in the body by methylating phosphatidylethanolamine to form phosphatidylcholine using SAM as the methyl donor. Choline dehydrogenase (*CHDH*) catalyzes the oxidation of choline to betaine *via* a betaine-aldehyde intermediate. Betaine-homocysteine methyltransferase (*BHMT*) catalyzes the synthesis of methionine from betaine and homocysteine. There are five common coding polymorphisms in these genes, and we explored their association with breast cancer mortality.

Previously, we reported the associations between dietary total choline and betaine intake as well as functional polymorphisms in the choline-mediated one-carbon metabolic pathway and the risk of breast cancer in the population-based Long Island Breast Cancer Study Project (LIBCSP) (10). Recent release of updated food composition values from the U.S. Department of Agriculture (USDA Database for the Choline Content of Common Foods, Release Two, http://www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choln02.pdf) and correction of an overestimation of the choline content of coffee led us to revise our estimates of the effects of dietary total choline and betaine intake on risk of breast cancer. We also examined the effect of individual subtypes of choline on disease risk. More importantly, in this report, we explored the effects of these dietary components on breast cancer survival in the same population.

MATERIALS AND METHODS

Study population

We used the resources of the parent case-control and the follow-up study of the LIBCSP, a population-based study. The study participants included women newly diagnosed with a first primary breast cancer in 1996–1997 who participated in the original case-control study (n=1508 cases) and were subsequently reinterviewed ~5–6 yr later to ascertain details of their complete treatment regimen for the primary breast cancer diagnosis and followed through 2005 for vital status. The population-based controls included women who participated in the case-control study (n=1556) without a previous history of breast cancer who were identified using random digit dialing techniques (for women <65 yr) and Health Care Finance rosters (for women >65 yr) and were frequency matched to the expected distribution of case-group women. Details of the case-control and follow-up studies have been described in detail previously (11–13). The study protocol was approved by the institutional review boards of the collaborating institutions.

Outcome data

The National Death Index was used to ascertain all-cause and breast cancer-specific mortality through the end of 2005. International Classification of Diseases codes 174.9 and C-50.9 listed as a primary or secondary code on the death certificate were used to assess the breast cancer-specific death. Among the 1508 women diagnosed with breast cancer in 1996–1997, 308 (20.2%) deaths occurred, among which 164 (53.2%) were attributable to breast cancer, by December 31, 2005. The mean follow-up time was 8.0 yr (range, 0.3–9.4 yr). Cardiovascular disease was the second most common cause of death, accounting for 21.3% of all deaths. For the assessment of breast cancer-specific death, we evaluated breast cancer listed in medical records as being the primary or underlying cause of death separately. Breast cancer as both the primary and underlying cause of death yielded similar results; therefore, we report only the results for breast cancer as the underlying cause of death.

Exposure data

Participant information used in this study was obtained as part of the case-control (baseline) interview (including demographic characteristics and most of the confounder data and donated biospecimens), follow-up interview (self-reported treatment data for the primary breast cancer were available for a total of 1098 cases), and medical record abstraction (complete course of treatment and tumor characteristics for the primary breast cancer).

Dietary data

At the time of the case-control interview, administered shortly after diagnosis, 98% of participants also completed a modified Block food frequency questionnaire (FFQ), which assessed intake of >100 food items in the year before the interview (14). The frequency and portion-size data were translated to daily intakes of nutrients from both dietary and supplement sources using the National Cancer Institute's DietSys version 3 for folate (National Cancer Institute, Rockville, MD, USA). A protocol described previously (2) and the USDA database were used to assess intakes of choline and betaine. Habitual use of multivitamin supplements was also obtained from the FFQ.

Genotyping

Blood samples collected as part of the case-control study were available for 1065 case-group women (70.6% of case-group participants) and 1109 control-group women (71.3% of control-group participants). DNA was isolated from blood specimens using the methods described previously (12). Genotyping of the genetic polymorphisms was performed using the protocol described previously (10).

Statistical analysis

Unconditional logistic regression was used to estimate odds ratios and corresponding 95% confidence intervals (CIs) for the association between the dietary nutrient intake as well as genotypes and risk of developing breast cancer (15, 16). Dietary nutrient intakes were categorized on the basis of the distribution among the control-group women. Age at reference (date of diagnosis for case women and date of identification for control women) and energy intake were included in all models as continuous variables. Tests for trend were undertaken by treating each categorized variable as a continuous term and entering the variable into a logistic regression model.

Survival analysis was performed only among case-group women in the study population. Kaplan-Meier and log-rank tests were used to examine crude associations between dietary intake as well as genotypes and survival (17). The Cox proportional hazard regression model (17) was used to estimate the hazard ratio (HR) and 95% CI for all-cause and

breast cancer-specific mortality, with adjustments made for age at diagnosis and energy intake. To increase statistical power, heterozygous and variant homozygous genotypes were combined as a single risk group.

Potential confounders were evaluated for age-adjusted models, including menopausal status (pre-/postmenopausal), family history of breast cancer in a first-degree relative, cancer type (*in situ*/invasive), active/passive cigarette smoking, body mass index at diagnosis, average lifetime alcohol intake (g/d), education, income, tumor size, and radiation treatment and chemotherapy undergone for the original breast cancer diagnosis. If addition of a covariate to the logistic regression model changed the effect estimate by ≥10%, the covariate was considered a confounder (15). None of the covariates tested met this criterion; thus, only the results of the age-adjusted model are presented.

All statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Using the latest food composition data, we estimated the mean intakes of betaine and choline in our study population (**Table 1**). These values are lower than the ones we estimated (10) using the previous data release of the choline content in common food. Approximately 40% of choline intake came from phosphatidylcholine, ~30% from free choline, and 22% from glycerophosphocholine. Table 1 shows the top food sources (percentage contribution to total intake) of betaine and choline among control participants within the LIBCSP population. Cooked spinach contributed more than one-quarter (25.8%) of the total betaine intake, whereas the other main sources of betaine were grain products. Eggs and milk were the main sources of choline in our study population. Eggs contributed more than one-quarter of sphingomyelin (25.5%) and of phosphatidylcholine (38.1%). Milk products contributed more than half of the glycerophosphocholine and more than one-third of the phosphocholine.

When mean dietary intakes of these nutrients were compared between case- and control-group women, we found that the intakes of total choline, phosphatidylcholine, and free choline were lower among case-group women than among control-group women (P=0.04, 0.03, and 0.04, respectively). The estimates of effects on breast cancer risk by dietary intakes of choline and betaine are shown in **Table 2**. Only free choline showed

TABLE 1. *Top 5 contributors of food sources (percentage contribution to total intake) of betaine, choline, and choline-containing compounds among controls within the LIBCSP*

Nutrient	Mean intake (mg/d)	Food source	Proportion (%)
Betaine	126.8	Cooked spinach	25.8
		Spaghetti	12.4
		Dark bread	11.5
		White bread	11.3
		Bran cereal	9.4
Total choline	187.8	Eggs	16.5
		Skim milk	9.8
		2% milk	6.2
		Spaghetti	4.1
		Coffee	3.7
Sphingomyelin	7.4	Eggs	25.5
		Skim milk	10.4
		Chicken	7.4
		2% milk	7.4
		Cheese	4.8
Glycerophosphocholine	40.7	Skim milk	26.8
		2% milk	15.7
		Whole milk	7.1
		Low-fat yogurt	6.3
		Coffee	4.6
Phosphatidylcholine	75.2	Eggs	38.1
		Fish	3.1
		Hamburger	3.0
		Orange juice	2.8
		Chicken	2.8
Phosphocholine	10.1	Skim milk	19.7
		2% milk	9.3
		Salad	8.7
		Broccoli	8.4
		Whole milk	6.8
Free choline	51.7	Coffee	9.8
		Spaghetti	7.4
		Skim milk	7.3
		2% milk	5.5
		Salad	5.0

TABLE 2. *Odds ratios (ORs) and 95% CIs for the associations of daily intake of betaine and choline subtypes with risk of developing breast cancer in the LIBCSP, 1996–1997*

Category	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P_{trend}
Betaine						
Amount (mg/d)	<61.11	61.11–89.95	89.95–123.95	123.95–179.71	>179.71	
Case group (*n*)	314	288	287	311	279	
Control group (*n*)	304	304	305	305	304	
OR and 95% CI	1.00 (ref.)	0.94 (0.75–1.19)	0.95 (0.75–1.21)	1.04 (0.81–1.33)	0.96 (0.73–1.25)	0.93
Total choline						
Amount (mg/d)	<122.7	122.7–160.0	160.0–196.9	196.9–247.2	>247.2	
Case group (*n*)	332	298	299	267	283	
Control group (*n*)	305	303	306	304	304	
OR and 95% CI	1.00 (ref.)	0.91 (0.72–1.15)	0.89 (0.69–1.15)	0.79 (0.59–1.06)	0.85 (0.61–1.18)	0.25
Sphingomyelin						
Amount (mg/d)	<3.8	3.8–5.6	5.6–7.6	7.6–10.5	>10.5	
Case group (*n*)	318	320	270	286	285	
Control group (*n*)	305	304	305	303	305	
OR and 95% CI	1.00 (ref.)	1.04 (0.83–1.31)	0.88 (0.69–1.12)	0.95 (0.74–1.23)	0.97 (0.73–1.29)	0.59
Glycerophosphocholine						
Amount (mg/d)	<21.4	21.4–31.4	31.4–41.7	41.7–58.3	>58.2	
Case group (*n*)	297	319	272	305	286	
Control group (*n*)	306	303	305	303	305	
OR and 95% CI	1.00 (ref.)	1.13 (0.89–1.42)	0.96 (0.75–1.22)	1.09 (0.84–1.41)	1.05 (0.80–1.40)	0.86
Phosphatidylcholine						
Amount (mg/d)	<39.8	39.8–56.5	56.5–75.3	75.3–102.1	>102.1	
Case group (*n*)	309	313	306	266	285	
Control group (*n*)	305	304	305	304	305	
OR and 95% CI	1.00 (ref.)	1.06 (0.84–1.34)	1.02 (0.80–1.31)	0.90 (0.70–1.16)	0.98 (0.74–1.29)	0.48
Phosphocholine						
Amount (mg/d)	<5.9	5.9–8.3	8.3–10.6	10.6–14.0	>14.0	
Case group (*n*)	331	281	280	313	271	
Control group (*n*)	304	305	305	303	305	
OR and 95% CI	1.00 (ref.)	0.87 (0.69–1.10)	0.86 (0.67–1.09)	0.97 (0.75–1.25)	0.86 (0.64–1.14)	0.57
Free Choline						
Amount (mg/d)	<35.1	35.1–44.9	44.9–55.5	55.5–68.3	>68.3	
Case group (*n*)	338	293	320	257	271	
Control group (*n*)	304	304	305	304	305	
OR and 95% CI	1.00 (ref.)	0.84 (0.67–1.06)	0.89 (0.69–1.15)	0.71 (0.53–0.94)	0.74 (0.53–1.02)	0.04

an inverse dose-response relationship with breast cancer risk (P_{trend}=0.04). Intakes of betaine and other choline subtypes were not associated with risk of developing breast cancer in the regression models.

Table 3 shows the association between nutrient intakes and survival (all-cause mortality and breast cancer-specific mortality) among the cohort of case women in the LIBCSP. Higher betaine, phosphocholine, and free choline intakes were associated with reduced all-cause as well as breast cancer-specific mortality in a dose-dependent fashion. Women in the highest betaine intake group had a 35% decreased risk of dying from breast cancer (HR, 0.65; 95% CI, 0.41–1.00). Although high intake of phosphocholine reduced breast cancer mortality by ~40% (HR, 0.61; 95% CI, 0.38–0.99), a close to 50% reduction (HR, 0.54; 95% CI, 0.32–0.93) was observed among women with a high free choline diet.

We examined five putatively functional genetic variations involved in choline-mediated one-carbon metabolism [*PEMT*+5645T>C (rs7946), *PEMT*−744G>C (rs12325817), *CHDH*+432 G>T (rs12676), *CHDH*+318 A>C (rs9001), and *BHMT*+742G>A (rs3733890)]. The *BHMT* variant allele (A allele) carriers had a 36% lower risk of dying from breast cancer than those with the *BHMT* GG genotype (HR, 0.64; 95% CI, 0.42–0.97). However, this SNP was not associated with all-cause mortality (HR, 0.81; 95% CI, 0.62–1.07). The other four SNPs examined were not associated with either breast cancer-specific or all-cause mortality (data not shown).

DISCUSSION

We examined the dietary intake of choline and betaine in relation to the risk of development of breast cancer as well as mortality after diagnosis in a population-based study. We found that higher intakes of betaine, phosphocholine, and free choline were associated with reduced all-cause as well as breast cancer-specific mortality. To the best of our knowledge, this is the first study on the effect of choline and betaine intakes on cancer survival. The population-based study design, in which case participants encompassed a broad range of ages and were drawn from a defined geographic area, yields results that are more generalizable than a series

TABLE 3. *HRs and 95% CIs for the associations of daily intake of choline and betaine with all-cause and breast cancer-specific mortality among the cohort of case women diagnosed with breast cancer in 1996–1997 and followed through 2005, LIBCSP*

Nutrient	Low	Medium	High	P_{trend}
Betaine				
Range (mg/d)	<79	79–138	>138	
All HR and 95% CI	1.00 (ref.)	0.76 (0.58–1.01)	0.64 (0.46–0.89)	0.01
BC HR and 95% CI	1.00 (ref.)	0.65 (0.43–0.97)	0.65 (0.41–1.00)	0.05
Total choline				
Range (mg/d)	<142	142–205	>205	
All HR and 95% CI	1.00 (ref.)	0.79 (0.58–1.08)	1.04 (0.71–1.52)	0.92
BC HR and 95% CI	1.00 (ref.)	0.79 (0.52–1.19)	0.72 (0.43–1.22)	0.22
Sphingomyelin				
Range (mg/d)	<4.9	4.9–8.3	>8.3	
All HR and 95% CI	1.00 (ref.)	1.09 (0.81–1.46)	1.26 (0.90–1.77)	0.19
BC HR and 95% CI	1.00 (ref.)	0.93 (0.63–1.40)	0.94 (0.59–1.49)	0.79
Glycerophosphocholine				
Range (mg/d)	<27.6	27.6–46.5	>46.5	
All HR and 95% CI	1.00 (ref.)	1.21 (0.90–1.62)	1.13 (0.80–1.59)	0.48
BC HR and 95% CI	1.00 (ref.)	1.15 (0.77–1.69)	0.69 (0.43–1.12)	0.14
Phosphatidylcholine				
Range (mg/d)	<79.3	79.3–80.2	>80.2	
All HR and 95% CI	1.00 (ref.)	0.94 (0.69–1.27)	1.23 (0.88–1.70)	0.21
BC HR and 95% CI	1.00 (ref.)	0.95 (0.62–1.45)	1.17 (0.74–1.84)	0.48
Phosphocholine				
Range (mg/d)	<7.3	7.3–11.4	>11.4	
All HR and 95% CI	1.00 (ref.)	0.87 (0.65–1.15)	0.72 (0.51–1.02)	0.06
BC HR and 95% CI	1.00 (ref.)	0.86 (0.58–1.27)	0.61 (0.38–0.99)	0.04
Free choline				
Range (mg/d)	<40.5	40.5–57.4	>57.4	
All HR and 95% CI	1.00 (ref.)	0.76 (0.57–1.02)	0.60 (0.41–0.89)	0.01
BC HR and 95% CI	1.00 (ref.)	0.88 (0.59–1.32)	0.54 (0.32–0.93)	0.03

All HR, HR for all-cause mortality; BC HR, HR for breast cancer-specific mortality.

of cases from a narrow age range or from a single institution.

Limited data are available on choline and betaine intake in relation to disease at the population level, which may be due in part to the lack until recently of an available food composition database for choline and betaine (2). Adequate intakes of choline have been estimated to be ~550 mg/d for men and ~425 mg/d for women, although the general intake level of choline of the U.S. population is not well known (18). In March 2004, a choline database (USDA Database for the Choline Content of Common Foods, Release One), which provides researchers and consumers with the means to estimate choline intake from common foods, was developed. In January 2008, an updated report (USDA Database for the Choline Content of Common Foods, Release Two) was published. In the new release, the betaine contents of foods made from grains, such as cereal, bread, and pasta, and of seafood and spinach were corrected and are significantly lower than those reported previously (http://www.ars.usda.gov/nutrientdata). Because these are commonly consumed foods, these differences might change previous conclusions about the probable associations between dietary intake of these nutrients and disease risk. The estimated mean choline intake from all choline-containing compounds in the diet (total choline) using the revised database was lower than our previous estimate (187.8 *vs.* 325.8 mg/d for total choline; 126.8 *vs.*138.3 mg/d for betaine) (10). The rank order of the major food sources was also altered (Table 1). Furthermore, when we recalculated the risk estimates associated with total choline intake, the association was attenuated, but the dose-response trend was similar to our previous report (10).

Several recent epidemiological studies examined choline and betaine intake at the population level. One is the Nurses' Health Study, in which the median intakes of total choline and of betaine were estimated to be 323 and 189 mg/d, respectively (8, 19). The other is the European Prospective Investigation into Cancer and Nutrition (EPIC) study, in which the mean choline intake was 300 mg/d, and mean betaine intake was 214 mg/d (20). In the Framingham Offspring Study, the estimated mean choline and betaine intakes were 313 and 208 mg/d, respectively. Compared with these reports, the estimated choline and betaine intakes were lower in our study population (Table 1). Several factors may account for this difference. As discussed above, our estimates of the nutrient intake were based on the most updated database, which corrected the overestimation in the previous database. Second, the coverage of food items in the FFQs differed among studies. However, the FFQ administrated in our study included most food items that are major sources of choline and betaine. The major contributors for these nutrients (Table 1) are comparable with those in other studies.

Results from the Nurses' Health Study showed that higher choline intake was associated with an elevated risk of colorectal adenoma (8) and the positive association persisted after adjustment for multiple dietary factors and when different sizes and sites of the adenoma were assessed. In our study, we observed an inverse association between free choline intake and breast cancer risk. The different relation between choline intake and disease outcome suggests different etiologies of breast cancer and colorectal adenoma. On the other hand, free choline is unesterified, thus the bioavailability and metabolic fate in the body could differ from those for other subtypes of these compounds. However, our findings are consistent with results from animal studies, in which low dietary choline intake has long been recognized as a risk factor for liver (21, 22) and mammary cancer (23) in rodents, but few data are available for humans. Aberrant DNA methylation may be the underlying mechanism, as rats with a choline-deficient diet have hypomethylation of CpG sites (7); however, there are multiple other plausible mechanisms for the effect of choline on breast cancer risk (6). One interesting point is that coffee is the number one contributor of free choline in our study. There are studies showing an association between increased coffee consumption and reduced risk of breast cancer (24, 25). A meta-analysis (26) based on 9 cohort and 9 case-control studies assessing the association between coffee consumption and breast cancer risk showed a borderline significant influence of the highest coffee consumption [relative risk (RR), 0.95; 95% CI, 0.90–1.00] or an increment of 2 cups/d of coffee consumption (RR, 0.98; 95% CI, 0.96–1.00) on the risk of breast cancer. However, free choline (unesterified choline) is only a minor component of all the choline-containing molecules in the diet. Most choline moiety is taken as phosphatidylcholine and phosphocholine, and free choline is usually phosphorylated when it enters cells. Thus, coffee may be a good source of free choline but contributes only ~4% of total choline intake. The indication that higher free choline intake is associated with lower risk observed in this study needs to be confirmed in future well-designed studies.

We also found that betaine, phosphocholine, and free choline intakes were associated with reduced mortality after cancer diagnosis. These three choline-containing compounds are the water-soluble forms of choline in foods, whereas the other two forms, phosphatidylcholine and sphingomyelin, are fat soluble. The relationship between choline intake and cancer survival is not well known. There is evidence from animal studies showing that high choline intake resulted in prolonged cancer survival. One study showed that a diet supplemented with moderate quantities of methionine and choline resulted in enhanced survival of spontaneously leukemic AK mice, in comparison with animals fed with the same diet without the supplements (27). Another recent report on rats showed that the tumor growth rate was inversely related to choline content in the prenatal diet, resulting in 50% longer survival when prenatally choline-supplemented rats are compared with prenatally choline-deficient rats (28). The study also suggested that an epigenetic mechanism may underlie the altered molecular phenotype and tumor growth. Given the important role that choline and betaine play in methylation, suboptimal intake of these nutrients could disturb the methyl pool in the cells. A disrupted methyl supply may lead to alteration of the epigenome, *i.e.*, DNA methylation (29), and DNA methylation has been implicated in the silencing of many breast cancer genes (30–33).

In our analysis, the HR estimate for breast cancer-specific mortality is nearly identical to the estimate for all-cause mortality. Most deaths (52.8%) in our cohort of survivors were due to breast cancer. Choline may lower all-cause mortality because increased dietary intake lowers homocysteine, a risk factor for cardiovascular disease (19), which was the second most common reason for death in our cohort of breast cancer patients.

We found that the *BHMT* rs3733890 polymorphism was associated with breast cancer-specific survival in our study population. BHMT may play a critical role in the remethylation of homocysteine when the folate-dependent pathway is compromised by either genetic or dietary factors (34). This G>A polymorphism is a missense mutation resulting in an Arg>Gln amino acid change. To the best of our knowledge, few functional studies on this genetic variation are available. The protein product of the gene variant did not differ in either catalytic activity or betaine binding compared with the enzyme, which did not contain the polymorphism (35, 36). Furthermore, this polymorphism has been reported to be associated with certain disease outcomes, *i.e.*, to be protective against the risk of cardiovascular disease (35). It is not known whether the homocysteine level correlates with breast cancer prognosis, and further investigation is warranted. We found previously that two SNPs, *PEMT* (rs12325817) and *CHDH* (rs12676), were associated with breast cancer risk (10). However, as we report here, they were not associated with survival.

In summary, our study suggests the important roles of choline and betaine in breast carcinogenesis. These nutrients may be a promising strategy to either prevent the development of breast cancer or to help reduce breast cancer mortality once the cancer is diagnosed. FJ

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