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Dietary choline and betaine intake, choline-metabolising genetic polymorphisms and breast cancer risk: a case–control study in China

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Abstract

Choline and betaine are essential nutrients involved in one-carbon metabolism and have been hypothesised to affect breast cancer risk. Functional polymorphisms in genes encoding choline-related one-carbon metabolism enzymes, including phosphatidylethanolamine *N*-methyltransferase (*PEMT*), choline dehydrogenase (*CHDH*) and betaine-homocysteine methyltransferase (*BHMT*), have important roles in choline metabolism and may thus interact with dietary choline and betaine intake to modify breast cancer risk. This study aimed to investigate the interactive effect of polymorphisms in *PEMT*, *BHMT* and *CHDH* genes with choline/betaine intake on breast cancer risk among Chinese women. This hospital-based case–control study consecutively recruited 570 cases with histologically confirmed breast cancer and 576 age-matched (5-year interval) controls. Choline and betaine intakes were assessed by a validated FFQ, and genotyping was conducted for *PEMT* rs7946, *CHDH* rs9001 and *BHMT* rs3733890. OR and 95 % CI were estimated using unconditional logistic regression. Compared with the highest quartile of choline intake, the lowest intake quartile showed a significant increased risk of breast cancer. The SNP *PEMT* rs7946, *CHDH* rs9001 and *BHMT* rs3733890 had no overall association with breast cancer, but a significant risk reduction was observed among postmenopausal women with AA genotype of *BHMT* rs3733890 (OR 0·49; 95 % CI 0·25, 0·98). Significant interactions were observed between choline intake and SNP *PEMT* rs7946 ($P_{interaction} = 0·029$) and *BHMT* rs3733890 ($P_{interaction} = 0·006$) in relation to breast cancer risk. Our results suggest that SNP *PEMT* rs7946 and *BHMT* rs3733890 may interact with choline intake on breast cancer risk.

Key words: *PEMT* gene: *BHMT* gene: *CHDH* gene: Choline: Breast cancer

Disturbances in one-carbon metabolism may potentially facilitate carcinogenesis by causing aberrant DNA methylation and DNA synthesis[1]. Choline and its metabolite betaine are important methyl nutrients involved in one-carbon metabolism[2]. A low status of choline and betaine could disturb the methyl pool and may thereby be related to carcinogenesis[3–6]. Some studies have assessed the relationship between choline and betaine intake and breast cancer risk, but the results remained inconsistent. The Nurses' Health Study found no protective effect of choline or betaine intake on breast cancer risk[7,8]. However, two case–control studies, the Long Island Breast Cancer Study Project (LIBCSP)[9] and our previous study conducted in China[10], indicated a significant reduction in breast cancer risk associated with a higher choline intake. Numerous factors could contribute to these inconsistent observations, including differences in study design and study populations, variations in the intake ranges and detail of dietary assessment. Genetic variations in the choline-related one-carbon metabolism enzymes may also partly contribute to the inconsistent results.

Choline metabolism in the one-carbon metabolic pathway includes three key enzymes. Phosphatidylethanolamine *N*-methyltransferase (PEMT) catalyses the *de novo* synthesis of phosphatidylcholine in the liver using the phosphatidylethanolamine

Abbreviations: *BHMT*, betaine-homocysteine methyltransferase; *CHDH*, choline dehydrogenase; *PEMT*, phosphatidylethanolamine *N*-methyltransferase.

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as a substrate. Choline dehydrogenase (CHDH) catalyses the oxidation of choline to betaine. Betaine-homocysteine methyltransferase (BHMT) catalyses the methylation of homocysteine, with betaine serving as a substrate. A SNP of *PEMT* rs7946 is associated with a *valine-to-methionine* substitution, and it results in a 30 % loss of enzyme function[11]. The SNP *CHDH* rs9001 has been shown to have a protective effect on susceptibility to choline deficiency[12]. The *BHMT* rs3733890 is a non-synonymous SNP resulting in the substitution of an *arginine-to-glutamine* residue at amino acid position 239, which is associated with altered affinity of BHMT to homocysteine[13]. The only report on the interactions between each of the three SNP and dietary choline/betaine intake with respect to breast cancer was from the LIBCSP[9], in which women with variant genotype of *PEMT* rs7946 and lower dietary betaine consumption had an elevated risk of breast cancer. However, dietary pattern and genetic background in Chinese women are different from their counterparts in Western countries. Furthermore, the associations between SNP of choline-metabolising genes and breast cancer risk, as well as gene–nutrient interactions, have not been explored among Chinese women.

This hospital-based case–control study, therefore, was conducted to investigate whether choline/betaine intake interact with polymorphisms in the *PEMT*, *BHMT* and *CHDH* gene in the development of breast cancer among Chinese women.

Methods

Study population

A detailed description of the ongoing case–control study has been published elsewhere[10]. In brief, potential cases were recruited in two hospitals in Guangzhou, China, between 1 December 2011 and 30 September 2014. Eligible cases were patients aged 25–70 years with histologically confirmed breast cancer diagnosed no more than 3 months before the interview, and who were Guangdong natives or those who have lived in Guangdong for at least 5 years. Women were excluded if they simultaneously had a history of any other cancers. All cases were confirmed by the physician and medical records. In total, 613 eligible cases were identified, of whom 570 were successfully interviewed (93·0 %).

Controls who were cancer-free were selected from the departments of Ear-Nose-Throat, Ophthalmology, Plastic and Reconstructive Surgery and Vascular Surgery in the same hospitals during the same period as the cases. They were frequency-matched to cases on the basis of age, with a 5-year interval. A total of 576 controls out of 613 eligible controls completed in-person interviews (94·0 %).

All participants were informed of the requirements of the study and signed a written consent form. The study was approved by the Ethical Committee of School of Public Health, Sun Yat-sen University.

Data collection

Trained interviewers administered face-to-face interviews to all participants via a standardised questionnaire, which included questions on socio-demographic factors, anthropometric factors, lifestyle behaviours, reproductive information and family history of cancer. We also recorded relevant medical information and medical diagnosis by reviewing hospital medical records. More specifically, in the present study, regular smoking was defined as smoking at least 1 cigarette/d for >6 consecutive months. Passive smoking was defined as non-smokers who reported being exposed to the smoke exhaled by smokers at least 15 min/d in a week. Regular drinking was defined as drinking alcohol at least once per week over the past year. Menopausal status was defined as having cessation of menstrual period for at least 12 months since the last menstruation. Women were considered as premenopausal if they were currently menstruating, or if they had ceased menstruation because of hysterectomy and were younger than 50 years. Women who reported menstrual cessation or underwent bilateral oophorectomy and who were older than 50 years were defined as postmenopausal. BMI was calculated by dividing weight (kg) by height squared (m^2). Body weight and height were directly measured by nurses on the 1st day after admission. Data on current occupational activity were obtained by asking each participant on their employment status and the level of physical activity done at work (non-working, sedentary, standing, manual, heavy manual). Information on frequency and duration of household activities (mopping, cooking and so on) and recreational activities (walking, jogging, climbing, running, playing table tennis and so on) during the past year were collected. A metabolic equivalent (MET) value was assigned to each reported activity based on the Compendium[14,15]. MET hours per week (how many days per week × how many hours per day × MET for a specific activity) were calculated for household and recreational activities.

Dietary assessment

A validated 81-item FFQ was used for assessing dietary information during the previous year before diagnosis for cases or before the time of interview for controls. Each subject was asked how frequently, on average, they consumed each type of food over the previous year, and detailed alterations in diets were further asked for those who changed their diet habits during the preceding 5 years. Pictures about different portion size of foods were used to help participants quantify the amount of food intake. Choline and betaine intake per day was calculated from FFQ based on frequency of food consumption, food items and serving sizes. Nutrient values in foods were obtained from the Chinese Food Composition Table[16]. The validity and reliability of FFQ have been described elsewhere[17]. The correlation coefficients between FFQ and 18-d dietary records were 0·34 for choline and 0·26 for betaine. The correlation coefficients between the two FFQ of choline and betaine were 0·59 and 0·44 correspondingly.

Genotype of polymorphisms

Approximately 5 ml of fasting blood sample of each participant was obtained on the 2nd day after patients were admitted to the hospital. Blood samples were fractioned into plasma, buffy coat and red cells, and were stored at −80°C in a continuously alarmed and monitored refrigerator until needed for the analysis. Blood samples were available for all participants.

Genomic DNA samples were extracted from buffy coat using a TIANamp Genomic DNA Kit (TianGen Biotech Co., Ltd) according to the manufacturer's instruction. Genotyping of *PEMT* rs7946, *BHMT* rs3733890 and *CHDH* rs9001 was performed by Genesky Bio-Tech Co., Ltd using an improved multiplex ligation detection reaction technique. The alleles of each SNP were discriminated by different fluorescent labels of allele-specific oligonucleotide probe pairs. Different SNP were further distinguished by extended lengths at the 3' end. Genotyping of each sample was analysed by the GeneMapper 4.1 software (Applied Biosystems). The laboratory staff was blinded to the case–control status of the samples. We interspersed fifty-five random duplicate samples (4·8 % of the total) as quality control samples. The concordance rate was 100, 100 and 98 % for *PEMT* rs7946, *CHDH* rs9001 and *BHMT* rs3733890, respectively. All of three selected SNP in this study had possible functional significance located in exons and with a minor allele frequency (MAF) >5 % among Chinese population.

Statistical analysis

Differences in baseline characteristics of breast cancer cases and controls were examined using Student's *t* test for continuous variables and χ^2 test for discrete variables. The χ^2 test was used to detect whether genotype distribution was in agreement with Hardy–Weinberg equilibrium among controls. Dietary choline and betaine intake were adjusted for total energy intake by the regression residual method[18] and then categorised into quartiles based on the distribution among the control subjects. OR and 95 % CI for breast cancer risk in relation to dietary nutrition intake and gene polymorphisms were determined using unconditional logistic regression. OR were adjusted for several potential confounders, which were selected based on comparison of baseline characteristics between cases and controls. The following variables were adjusted in multivariate models: education (primary school or below, junior high school, senior high school/secondary technical school, college or above), income (<2000, 2001–5000, 5001–8000, >8000 yuan/month), age at menopause (≤46, >46–49, >49–52, >52–55, >55 years), first-degree relative with cancer (yes/no), regular drinking (yes/no) and passive smoking (yes/no). Tests for trend were undertaken by entering categorised variable as continuous in the regression model.

Stratified analyses by menopausal status (premenopausal or postmenopausal) were performed. Potential gene–environment interactions were evaluated by adding the multiplicative interaction terms (genotype × dietary intake) to the final models as indicator variables. Median intake of choline and betaine were calculated based on controls. Women with homozygous wild-type genotype and dietary nutrient intake above the medians were selected as the reference. Because the socio-economic factors were not well comparable between cases and controls, stratified analyses by income level (≤5000 yuan/month and >5000 yuan/month) and educational level (junior high school or below, senior high school or secondary technical school or above) were also performed. In this study, significance was defined as $P < 0{\cdot}05$, and all statistical tests were two-tailed. Statistical analyses were conducted using the SPSS software (version 20.0).

Results

A comparison of baseline characteristics between cases and controls is displayed in Table 1. Compared with controls, cases were, in general, older at menopause and less highly educated. They were also more likely to have a lower household income, to be exposed to passive smoking, to be regular drinkers and to have a history of a first-degree relative with cancer. Case and control groups were similar in marital status, occupation, BMI, occupational activity, smoking status, age at menarche, parity, age at first live birth, percentage of adopting breast-feeding, menopausal status and use of oral contraceptive.

As shown in Table 2, lower choline intake was associated with an increased risk of breast cancer after controlling for the potential confounders. The multivariate OR of breast cancer comparing women in the lowest quartile of choline intake with those in the highest quartile was 2·01 (95 % CI 1·43, 2·82). Dietary betaine showed no significant association with breast cancer risk, and OR for bottom *v.* top quartile of betaine was 1·00 (95 % CI 0·72, 1·39). The association between choline intake and breast cancer risk was not significantly different between premenopausal and postmenopausal women (data not shown).

The frequencies of *PEMT* rs7946, *CHDH* rs9001 and *BHMT* rs3733890 and the associations of these SNP with breast cancer risk are presented in Table 3. All genotype distributions were in accordance with Hardy–Weinberg equilibrium among controls, except for the *PEMT* rs7946 polymorphism ($P = 0{\cdot}036$). The frequencies of the minor alleles were 16·7, 34·8 and 36·0 % for *PEMT* rs7946, *CHDH* rs9001 and *BHMT* rs3733890 among controls and 17·4, 33·9 and 34·8 % among cases. None of the three SNP was associated with breast cancer risk in our study, but a significant reduced risk was observed among postmenopausal women who carry homozygous variant AA genotype of *BHMT* rs3733890 (OR 0·49; 95 % CI 0·25, 0·98) (Table 4).

Table 5 presents the joint effect of the genetic polymorphisms and dietary choline and betaine on breast cancer risk. Notably, significant interactions were observed for choline intake with SNP *PEMT* rs7946 ($P_{interaction} = 0{\cdot}029$) and *BHMT* rs3733890 ($P_{interaction} = 0{\cdot}006$). Compared with women with the *PEMT* rs7946 GG genotype and choline intake >154 mg/d, those having the *PEMT* rs7946 GG genotype and choline intake <154 mg/d had the greatest increased risk of breast cancer (OR 1·83; 95 % CI 1·36, 2·45). Similarly, a substantial increased risk of breast cancer was seen for women with *BHMT* rs3733890 GG genotype in the presence of lower choline intake in comparison with those with GG genotype in the presence of higher choline intake (OR 2·48; 95 % CI 1·70, 3·63). Betaine intake did not significantly interact with any of the three SNP. The associations of choline intake, betaine intake and the three studied SNP in relation to breast cancer risk did not differ significantly stratified by socio-economic status (income level and education level) (data not shown).

Discussion

To our knowledge, this is the first study to examine associations of choline-related one-carbon metabolism genes with breast cancer in Chinese populations. The present study confirmed

Table 1. Socio-demographic characteristics and selected risk factors of breast cancer in the study population (Numbers and percentages; mean values and standard deviations)

	Cases (*n* 570)		Controls (*n* 576)		
	n	%	*n*	%	*P**
Age (years)					0·359
Mean		47·54		48·05	
SD		9·29		9·44	
Marital status					0·238
Married	537	94·2	532	92·4	
Unmarried/divorced/widowed	33	5·8	44	7·6	
Educational level					0·037
Primary school or below	144	25·3	134	23·3	
Junior high school	168	29·5	138	24·0	
Senior high school/secondary technical school	138	24·2	147	25·5	
College or above	120	21·1	157	27·3	
Occupation					0·054
Administrator/other white-collar worker	121	21·2	147	25·5	
Blue-collar worker	150	26·3	167	29·0	
Farmer/other	299	52·5	262	45·5	
Income (yuan/month)					<0·001
<2000	60	10·5	35	6·1	
2001–5000	171	30·0	141	24·5	
5001–8000	204	35·8	214	37·2	
>8001	135	23·7	186	32·3	
BMI (kg/m^2)					0·053
Mean		23·05		22·69	
SD		3·28		3·18	
Regular smoker	6	1·1	5	0·9	0·772
Passive smoking	422	74·0	271	47·2	<0·001
Regular drinker	48	8·4	29	5·0	0·025
Household and recreational activities (MET-h/week)					0·655
Mean		40·17		40·77	
SD		22·72		22·55	
Occupational activity					
Non-working	164	28·8	145	25·2	0·074
Sedentary	240	42·1	234	40·6	
Standing	91	16·0	122	21·2	
Manual	41	7·2	51	8·9	
Heavy manual	34	6·0	24	4·2	
Menopausal status					0·456
Premenopausal	380	66·7	371	64·4	
Postmenopausal	190	33·3	205	35·6	
Age at menarche (years)					0·420
Mean		14·60		14·50	
SD		1·91		1·77	
Age at menopause (years)†					0·026
Mean		49·72		48·79	
SD		4·31		3·99	
Age at first live birth (years)‡					0·914
Mean		25·60		25·58	
SD		3·59		3·26	
Parity					
0	26	4·6	35	6·1	0·123
1–2	424	74·4	444	77·4	
≥3	120	21·1	97	16·6	
Breast-feeding	471	82·6	477	83·1	0·833
Multivitamin use	55	9·6	66	11·5	0·337
First-degree relative with cancer	107	18·8	70	12·2	0·002
Ever used an oral contraceptive	40	7·0	28	4·9	0·134

MET, metabolic equivalent.
* Continuous variables were analysed by *t* tests. Categorical variables were analysed by χ^2 tests.
† Among menopausal women.
‡ Among women who have had a live birth.

that dietary choline intake was inversely associated with breast cancer risk. None of the three SNP of choline-metabolising gene was associated with breast cancer risk among Chinese women. However, women with low choline intake and wild genotype of *PEMT* rs7946 or *BHMT* rs3733890 had a substantially increased risk of breast cancer.

This study found no association between *PEMT* rs7946, *CHDH* rs9001 and *BHMT* rs3733890 and breast cancer risk.

Table 2. Risks for breast cancer according to quartiles of the energy-adjusted daily nutrient intake (Odds ratios and 95 % confidence intervals)

Nutrient intakes	No. of cases/controls	Crude OR	95 % CI	Adjusted OR*	95 % CI
Total choline (mg/d)					
≥190·64	107/144	1·00		1·00	
154·18–190·64	109/144	1·02	0·72, 1·45	1·04	0·73, 1·49
121·93–154·18	130/144	1·22	0·86, 1·72	1·17	0·82, 1·66
<121·93	224/144	2·09	1·51, 2·90	2·01	1·43, 2·82
P_{trend}		<0·001		<0·001	
Betaine (mg/d)					
≥281·03	143/144	1·00		1·00	
192·83–281·03	142/144	0·99	0·72, 1·38	0·99	0·71, 1·39
126·71–192·83	135/144	0·94	0·68, 1·31	0·96	0·68, 1·34
<126·71	150/144	1·05	0·76, 1·45	1·00	0·72, 1·39
P_{trend}		0·853		0·942	

* OR adjusted for education, income, age at menopause, first-degree relative with cancer, regular drinking and passive smoking.

Table 3. Association between genetic polymorphisms in choline-metabolising genes and breast cancer risk (Numbers and percentages; odds ratios and 95 % confidence intervals)

	Cases		Controls					
	n	%	*n*	%	Crude OR	95 % CI	Adjusted OR*	95 % CI
PEMT rs7946								
GG	394	69·1	407	70·7	1·00		1·00	
GA	154	27·0	146	25·4	1·09	0·84, 1·42	1·10	0·84, 1·44
AA	22	3·9	23	4·0	0·99	0·54, 1·80	0·97	0·52, 1·78
P_{trend}					0·666		0·667	
GA + AA	176	30·9	169	29·1	1·08	0·84, 1·39	1·07	0·83, 1·38
A allele frequency	0·174		0·167					
CHDH rs9001								
TT	242	42·5	249	43·2	1·00		1·00	
TG	270	47·4	253	43·9	1·09	0·86, 1·41	1·01	0·78, 1·30
GG	58	10·2	74	12·8	0·81	0·55, 1·19	0·79	0·53, 1·18
P_{trend}					0·630		0·400	
TG + GG	328	57·5	327	56·8	1·03	0·82, 1·30	0·97	0·77, 1·23
G allele frequency	0·339		0·348					
BHMT rs3733890								
GG	246	43·2	242	42·0	1·00		1·00	
GA	251	44·0	253	43·9	0·98	0·77, 1·25	1·00	0·78, 1·30
AA	73	12·8	81	14·1	0·89	0·62, 1·27	0·90	0·62, 1·31
P_{trend}					0·556		0·676	
GA + AA	324	56·8	334	58·0	0·95	0·76, 1·21	0·97	0·76, 1·23
A allele frequency	0·348		0·360					

PEMT, phosphatidylethanolamine *N*-methyltransferase; *CHDH*, choline dehydrogenase; *BHMT*, betaine-homocysteine methyltransferase.
* OR adjusted for education, income, age at menopause, first-degree relative with cancer, regular drinking and passive smoking.

This result was consistent with the LIBCSP study, the only study examining the genetic polymorphisms in choline-metabolising genes and breast cancer risk[9]. Some studies examined the relationship between SNP *BHMT* rs3733890 and cancer risk at other sites, and controversial results have been reported. Variant A allele of *BHMT* rs3733890 has been observed to be associated with a reduced risk of uterine cervical carcinoma[19] and colorectal adenoma among persons with high methyl status[20], whereas AA genotype was associated with an increased risk of colorectal cancer[21]. No association was observed for variant AA genotype with either colorectal cancer[22] or ovarian cancer[23], which is in line with our results.

Our results revealed that *BHMT* rs3733890 SNP was associated with a 51 % decreased risk of breast cancer only among postmenopausal women. BHMT, a zinc metalloenzyme that is expressed in the kidney cortex and in liver hepatocytes, catalyses the conversion of homocysteine to methionine. Although methylation of homocysteine catalysed by BHMT only occurs in certain organs, animal studies have shown that this pathway is equally important as another parallel pathway that uses 5-methyltetrahydrofolate as the methyl donor[24]. *BHMT* rs3733890 SNP has been found to produce an enzyme with higher affinity to homocysteine than the wild genotype[13], and thus mutant enzyme promotes a higher rate of the homocysteine remethylation. Supporting this finding, the variant AA genotype has been found to be associated with lower plasma homocysteine concentrations than wild GG carrier in two previous studies[25,26]. The disturbance of homocysteine removal may contribute to DNA hypomethylation[27], which is

Table 4. Association between genetic polymorphisms in choline-metabolising genes and breast cancer risk according to menopausal status
(Odds ratios and 95 % confidence intervals)

	Premenopausal women (*n* 751)			Postmenopausal women (*n* 395)		
	No. of cases/controls	Adjusted OR*	95 % CI	No. of cases/controls	Adjusted OR*	95 % CI
PEMT rs7946						
GG	275/271	1·00		119/136	1·00	
GA	93/84	1·10	0·78, 1·57	61/62	1·11	0·72, 1·72
AA	12/16	0·63	0·28, 1·39	10/7	1·70	0·62, 4·63
P_{trend}		0·744			0·330	
GA + AA	105/100	1·02	0·73, 1·43	71/69	1·18	0·77, 1·79
CHDH rs9001						
TT	156/151	1·00		86/98	1·00	
TG	190/174	0·95	0·69, 1·31	80/79	1·14	0·74, 1·75
GG	34/46	0·72	0·43, 1·21	24/28	0·93	0·50, 1·74
P_{trend}		0·286			0·951	
TG + GG	224/220	0·91	0·67, 1·23	104/107	1·09	0·73, 1·62
BHMT rs3733890						
GG	162/159	1·00		84/83	1·00	
GA	160/162	1·02	0·74, 1·41	91/91	0·99	0·65, 1·51
AA	58/50	1·16	0·74, 1·83	15/31	0·49	0·25, 0·98
P_{trend}		0·572			0·119	
GA + AA	218/212	1·04	0·77, 1·41	106/122	0·86	0·57, 1·28

PEMT, phosphatidylethanolamine *N*-methyltransferase; *CHDH*, choline dehydrogenase; *BHMT*, betaine-homocysteine methyltransferase.
* OR adjusted for education, income, age at menopause, first-degree relative with cancer, regular drinking and passive smoking.

Table 5. Interactions of the genetic polymorphisms in choline-metabolising genes and energy-adjusted nutrient intakes in relation to breast cancer risk
(Odds ratios and 95 % confidence intervals)

		Choline ≥ 154 mg/d/betaine ≥ 193 mg/d*			Choline < 154 mg/d/betaine < 193 mg/d*			
	Genotype	No. of cases/controls	OR†	95 % CI	No. of cases/controls	OR†	95 % CI	$P_{interaction}$
Choline intake								
PEMT rs7946	GG	139/212	1·00		255/195	1·83	1·36, 2·45	0·029
	GA	70/69	1·48	0·99, 2·22	84/77	1·58	1·07, 2·32	
	AA	7/6	1·48	0·48, 4·60	17/15	1·29	0·62, 2·70	
CHDH rs9001	TT	95/133	1·00		147/116	1·62	1·12, 2·34	0·377
	TG	99/125	1·00	0·69, 1·47	171/128	1·64	1·14, 2·34	
	GG	22/29	1·02	0·55, 1·91	36/45	1·04	0·62, 1·75	
BHMT rs3733890	GG	81/137	1·00		165/105	2·48	1·70, 3·63	0·006
	GA	111/118	1·59	1·08, 2·33	140/135	1·68	1·16, 2·44	
	AA	24/32	1·30	0·71, 2·38	49/49	1·62	0·99, 2·65	
Betaine intake								
PEMT rs7946	GG	193/211	1·00		201/196	1·09	0·82, 1·44	0·093
	GA	79/67	1·27	0·86, 1·87	75/79	1·02	0·70, 1·49	
	AA	13/9	1·52	0·63, 3·69	9/14	0·65	0·27, 1·57	
CHDH rs9001	TT	122/121	1·00		120/128	0·88	0·61, 1·26	0·940
	TG	132/135	0·88	0·62, 1·25	138/118	1·05	0·73, 1·50	
	GG	31/31	0·95	0·54, 1·68	27/43	0·60	0·35, 1·05	
BHMT rs3733890	GG	125/121	1·00		121/121	0·93	0·64, 1·33	0·464
	GA	123/119	1·00	0·69, 1·43	128/134	0·92	0·64, 1·31	
	AA	37/47	0·77	0·46, 1·28	36/34	1·01	0·59, 1·73	

PEMT, phosphatidylethanolamine *N*-methyltransferase; *CHDH*, choline dehydrogenase; *BHMT*, betaine-homocysteine methyltransferase.
* Median intake among controls.
† OR adjusted for education, income, age at menopause, first-degree relative with cancer, regular drinking and passive smoking.

considered important in carcinogenesis. In addition, carriers of the variant alleles of *BHMT* rs3733890 have been reported to have favourable health profiles. Follow-up study of LIBCSP found that individuals with *BHMT* rs3733890 AA genotype have a 36 % lower risk of dying from breast cancer than the wild GG genotype[28]. The minor A allele of the *BHMT* rs3733890 has also been shown to protect against CVD[29], spina bifida[30], orofacial clefts[31], uterine cervical carcinoma[19] and maternal Down syndrome risk[32–34]. There is some evidence that cloned human *BHMT* gene contains several consensus sites for steroid hormone receptors, including oestrogens[35]. The null association between the *BHMT* rs3733890 and breast cancer risk among premenopausal women may be explained by the confounding effect from oestrogen.

The present study showed that women with low choline intake and wild genotype of *BHMT* rs3733890 had a substantially increased risk of breast cancer. Choline, similar to folate, is essential in the formation of *S*-adenosylmethionine, the chief methyl donor for DNA methylation reactions. Aberrant DNA methylation and impaired DNA repair because of choline deficiency were thought to be involved in carcinogenesis[4]. Animal studies have shown that a choline-deficient diet itself can induce liver cancer without using any carcinogens[36]. Results from LIBSCP and our previous two-stage case–control study also support the protective role of higher choline intake in breast cancer[9,10]. Furthermore, our result suggested that wild GG genotype is particularly deleterious when intake of choline is comparatively low, with a 2·48-fold increased risk of breast cancer observed. This result is biologically plausible. In agreement with our finding that variant AA genotype of *BHMT* rs3733890 protected against breast cancer among postmenopausal women, the increased homocysteine remethylation efficacy of mutant enzyme may mitigate the adverse effect of low choline intake, whereas such an effect is unavailable for the enzyme produced by wild GG genotype. Besides, gene–environment interaction has been found in an animal study in which dietary choline induced BHMT expression when choline was added to a methionine-deficient diet[35].

We also noted a significant interaction between choline intake and *PEMT* rs7946 polymorphism in the present study. Women who had the wild GG genotype with low choline intake have a substantially increased breast cancer risk. Choline is derived not only from the diet but also from *de novo* synthesis of phosphatidylcholine using *S*-adenosylmethionine catalysed by PEMT. *PEMT* rs7946, a missense mutation in exon 8 of the gene, has been reported to result in diminished enzyme activity[11] but was not associated with susceptibility to choline deficiency[12]. However, this interaction should be interpreted with caution because the genotype distribution of *PEMT* rs7946 was not in accordance with Hardy–Weinberg equilibrium among controls ($P=0·036$). It is worth noting that *de novo* synthesis of choline through *S*-adenosylmethionine-dependent transmethylation would be a futile cycle in the presence of low dietary choline[35]. The *PEMT* gene expression is induced by oestrogen, which is mainly present in premenopausal women, and these women make some of their own needed choline. It was reported that premenopausal women are relatively resistant to choline deficiency compared with postmenopausal women and men[37]. However, in the present study, the effects of diet and *PEMT* rs7946 on breast cancer risk are not different in premenopausal and postmenopausal women. In addition, a study indicated that neural tube defect cases were more likely to have the wild GG genotype of *PEMT* rs7946 than controls[38]. All of the above explanations on the gene–environment associations are highly putative, and further studies with larger sample size are needed to clarify the mechanisms of these interactions.

We did not observe a significant interaction between *CHDH* rs9001 and choline, although *CHDH* rs9001 had a protective effect on susceptibility to choline deficiency[12]. The SNP *CHDH* rs9001 is less studied, and its biological function needs to be determined in future studies.

The strengths of our study include standardised specimen and data collection, and extensive collections of multiple risk factors. Some limitations of the present study warrant consideration. This study was a hospital-based case–control study, and thus selection bias may have affected the results. Hospital-based controls may not be representative of the general population, and the diseases they suffered may potentially be related to diet. To minimise the bias, we ensured that controls were recruited from several disease conditions with no apparent relation to dietary causes. In addition, in the present study, allele frequencies were similar to previous studies in Chinese population[39,40], which demonstrated that selection bias may not be a serious problem. The homogeneous ethnic background (99·6% Han Chinese) of the study population also decreased the potential confounding effect from ethnicity. Second, diet information was collected after breast cancer diagnosis, and recall bias may occur in the present study. We tried to interview cases as soon as the diagnosis was made, and 77·6% of cases were interviewed within 3 d of the cases admitted to hospitals. Moreover, pictures about different portion size of foods were provided to assistant participants with quantification of food intake. Third, in the present study, only potentially functional SNP located in exons and SNP with reported MAF >5% in Chinese were selected. Further studies using whole-genome sequencing including all SNP in exons and introns are needed to examine associations between these SNP and breast cancer risk.

In summary, the present study showed that *PEMT* rs7946 and *BHMT* rs3733890 polymorphisms may interact with choline intake on breast cancer risk. Women with low choline intake and wild genotype of *PEMT* rs7946 or *BHMT* rs3733890 had a substantially increased risk of breast cancer. Additional large-scale epidemiological studies are required to prove the present findings.

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None of the authors has any conflicts of interest to declare.

References

1. Davis CD & Uthus EO (2004) DNA methylation, cancer susceptibility, and nutrient interactions. *Exp Biol Med (Maywood)* **229**, 988–995.
2. Ueland PM (2011) Choline and betaine in health and disease. *J Inherit Metab Dis* **34**, 3–15.

3. da Costa KA, Niculescu MD, Craciunescu CN, *et al.* (2006) Choline deficiency increases lymphocyte apoptosis and DNA damage in humans. *Am J Clin Nutr* **84**, 88–94.
4. Zeisel SH (2012) Dietary choline deficiency causes DNA strand breaks and alters epigenetic marks on DNA and histones. *Mutat Res* **733**, 34–38.
5. Craig SA (2004) Betaine in human nutrition. *Am J Clin Nutr* **80**, 539–549.
6. Zeisel SH (2006) Choline: critical role during fetal development and dietary requirements in adults. *Annu Rev Nutr* **26**, 229–250.
7. Cho E, Holmes MD, Hankinson SE, *et al.* (2010) Choline and betaine intake and risk of breast cancer among postmenopausal women. *Br J Cancer* **102**, 489–494.
8. Cho EY, Holmes M, Hankinson SE, *et al.* (2007) Nutrients involved in one-carbon metabolism and risk of breast cancer among premenopausal women. *Cancer Epidemiol Biomarkers Prev* **16**, 2787–2790.
9. Xu XR, Gammon MD, Zeisel SH, *et al.* (2008) Choline metabolism and risk of breast cancer in a population-based study. *FASEB J* **22**, 2045–2052.
10. Zhang CX, Pan MX, Li B, *et al.* (2013) Choline and betaine intake is inversely associated with breast cancer risk: a two-stage case-control study in China. *Cancer Sci* **104**, 250–258.
11. Song J, da Costa KA, Fischer LM, *et al.* (2005) Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). *FASEB J* **19**, 1266–1271.
12. da Costa KA, Kozyreva OG, Song J, *et al.* (2006) Common genetic polymorphisms affect the human requirement for the nutrient choline. *FASEB J* **20**, 1336–1344.
13. Li F, Feng Q, Lee C, *et al.* (2008) Human betaine-homocysteine methyltransferase (BHMT) and BHMT2: common gene sequence variation and functional characterization. *Mol Genet Metab* **94**, 326–335.
14. Ainsworth BE, Haskell WL, Whitt MC, *et al.* (2000) Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* **32**, S498–S504.
15. Ainsworth BE, Haskell WL, Herrmann SD, *et al.* (2011) 2011 Compendium of physical activities: a second update of codes and MET values. *Med Sci Sports Exerc* **43**, 1575–1581.
16. Yang YX, Wang GY & Pan XC (2002) *China Food Composition*. Beijing: Peking University Medical Press.
17. Zhang CX & Ho SC (2009) Validity and reproducibility of a food frequency questionnaire among Chinese women in Guangdong province. *Asia Pac J Clin Nutr* **18**, 240–250.
18. Willett WC, Howe GR & Kushi LH (1997) Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* **65**, 1220S–1228S discussion 1229S–1231S.
19. Mostowska A, Myka M, Lianeri M, *et al.* (2011) Folate and choline metabolism gene variants and development of uterine cervical carcinoma. *Clin Biochem* **44**, 596–600.
20. Hazra A, Wu K, Kraft P, *et al.* (2007) Twenty-four non-synonymous polymorphisms in the one-carbon metabolic pathway and risk of colorectal adenoma in the Nurses' Health Study. *Carcinogenesis* **28**, 1510–1519.
21. Koushik A, Kraft P, Fuchs CS, *et al.* (2006) Nonsynonymous polymorphisms in genes in the one-carbon metabolism pathway and associations with colorectal cancer. *Cancer Epidemiol Biomarkers Prev* **15**, 2408–2417.
22. Martinelli M, Scapoli L, Mattei G, *et al.* (2013) A candidate gene study of one-carbon metabolism pathway genes and colorectal cancer risk. *Br J Nutr* **109**, 984–989.
23. Pawlik P, Mostowska A, Lianeri M, *et al.* (2012) Folate and choline metabolism gene variants in relation to ovarian cancer risk in the Polish population. *Mol Biol Rep* **39**, 5553–5560.
24. Finkelstein JD & Martin JJ (1984) Methionine metabolism in mammals. Distribution of homocysteine between competing pathways. *J Biol Chem* **259**, 9508–9513.
25. Morin I, Platt R, Weisberg I, *et al.* (2003) Common variant in betaine-homocysteine methyltransferase (BHMT) and risk for spina bifida. *Am J Med Genet A* **119a**, 172–176.
26. Biselli JM, Zampieri BL, Goloni-Bertollo EM, *et al.* (2012) Genetic polymorphisms modulate the folate metabolism of Brazilian individuals with Down syndrome. *Mol Biol Rep* **39**, 9277–9284.
27. James SJ, Melnyk S, Pogribna M, *et al.* (2002) Elevation in S-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology. *J Nutr* **132**, 2361s–2366s.
28. Xu X, Gammon MD, Zeisel SH, *et al.* (2009) High intakes of choline and betaine reduce breast cancer mortality in a population-based study. *FASEB J* **23**, 4022–4028.
29. Weisberg IS, Park E, Ballman KV, *et al.* (2003) Investigations of a common genetic variant in betaine-homocysteine methyltransferase (BHMT) in coronary artery disease. *Atherosclerosis* **167**, 205–214.
30. Shaw GM, Lu W, Zhu H, *et al.* (2009) 118 SNPs of folate-related genes and risks of spina bifida and conotruncal heart defects. *BMC Med Genet* **10**, 49.
31. Mostowska A, Hozyasz KK, Wojcicki P, *et al.* (2010) Associations of folate and choline metabolism gene polymorphisms with orofacial clefts. *J Med Genet* **47**, 809–815.
32. Zampieri BL, Biselli JM, Goloni-Bertollo EM, *et al.* (2012) BHMT G742A and MTHFD1 G1958A polymorphisms and Down syndrome risk in the Brazilian population. *Genet Test Mol Biomarkers* **16**, 628–631.
33. Zampieri BL, Biselli JM, Goloni-Bertollo EM, *et al.* (2012) Maternal risk for Down syndrome is modulated by genes involved in folate metabolism. *Dis Markers* **32**, 73–81.
34. Amorim MR, Moura CM, Gomes AD, *et al.* (2013) Betaine-homocysteine methyltransferase 742G > A polymorphism and risk of Down syndrome offspring in a Brazilian population. *Mol Biol Rep* **40**, 4685–4689.
35. Park EI & Garrow TA (1999) Interaction between dietary methionine and methyl donor intake on rat liver betaine-homocysteine methyltransferase gene expression and organization of the human gene. *J Biol Chem* **274**, 7816–7824.
36. Henning SM & Swendseid ME (1996) The role of folate, choline, and methionine in carcinogenesis induced by methyl-deficient diets. *Adv Exp Med Biol* **399**, 143–155.
37. Resseguie M, Song J, Niculescu MD, *et al.* (2007) Phosphatidylethanolamine N-methyltransferase (PEMT) gene expression is induced by estrogen in human and mouse primary hepatocytes. *FASEB J* **21**, 2622–2632.
38. Mills JL, Fan R, Brody LC, *et al.* (2014) Maternal choline concentrations during pregnancy and choline-related genetic variants as risk factors for neural tube defects. *Am J Clin Nutr* **100**, 1069–1074.
39. Bi XH, Zhao HL, Zhang ZX, *et al.* (2012) PEMT G523A (V175M) is associated with sporadic Alzheimer's disease in a Chinese population. *J Mol Neurosci* **46**, 505–508.
40. Jin LL, Chen EJ, Hou W, *et al.* (2015) The Association between folate pathway genes and cleft lip with or without cleft palate in a Chinese population. *Biomed Environ Sci* **28**, 136–139.