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Authors

Ilozumba, Mmadili

Cheng, Ting-Yuan

Neuhouser, Marian

et al.

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Associations between Plasma Choline Metabolites and Genetic Polymorphisms in One-Carbon Metabolism in Postmenopausal Women: The Women's Health Initiative Observational Study

Mmadili N Ilozumba,[1] Ting-Yuan D Cheng,[1] Marian L Neuhouser,[2] Joshua W Miller,[3] Shirley AA Beresford,[2,4] David J Duggan,[5] Adetunji T Toriola,[6] Xiaoling Song,[2] Yingye Zheng,[2] Lynn B Bailey,[7] Aladdin H Shadyab,[8] Simin Liu,[9,10] Olga Malysheva,[11] Marie A Caudill,[11] and Cornelia M Ulrich[12,13]

[1]Department of Epidemiology, University of Florida, Gainesville, FL, USA; [2]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [3]Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ, USA; [4]Department of Epidemiology, University of Washington, Seattle, WA, USA; [5]Translational Genomics Research Institute, Phoenix, AZ, USA; [6]Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, St. Louis, MO, USA; [7]Department of Foods and Nutrition, University of Georgia, Athens, GA, USA; [8]Department of Family Medicine and Public Health, University of California, San Diego, La Jolla, CA, USA; [9]Department of Epidemiology, Brown University, Providence, RI, USA; [10]Department of Medicine, Brown University, Providence, RI, USA; [11]Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA; [12]Huntsman Cancer Institute, Salt Lake City, UT, USA; and [13]Department of Population Health Sciences, University of Utah, Salt Lake City, UT, USA

ABSTRACT

Background: Choline plays an integral role in one-carbon metabolism in the body, but it is unclear whether genetic polymorphisms are associated with variations in plasma choline and its metabolites.

Objectives: This study aimed to evaluate the association of genetic variants in choline and one-carbon metabolism with plasma choline and its metabolites.

Methods: We analyzed data from 1423 postmenopausal women in a case-control study nested within the Women's Health Initiative Observational Study. Plasma concentrations of choline, betaine, dimethylglycine (DMG), and trimethylamine N-oxide were determined in 12-h fasting blood samples collected at baseline (1993–1998). Candidate and tagging single-nucleotide polymorphisms (SNPs) were genotyped in betaine-homocysteine S-methyltransferase (*BHMT*), *BHMT2*, 5,10-methylenetetrahydrofolate reductase (*MTHFR*), methylenetetrahydrofolate dehydrogenase (NADP+ dependent 1) (*MTHFD1*), 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*), and 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*). Linear regression was used to derive percentage difference in plasma concentrations per variant allele, adjusting for confounders, including B-vitamin biomarkers. Potential effect modification by plasma vitamin B-12, vitamin B-6, and folate concentrations and folic-acid fortification periods was examined.

Results: The candidate SNP *BHMT* R239Q (rs3733890) was associated with lower concentrations of plasma betaine and DMG concentrations (−4.00% and −6.75% per variant allele, respectively; both nominal $P < 0.05$). Another candidate SNP, *BHMT2* rs626105 A>G, was associated with higher plasma DMG concentration (13.0%; $P < 0.0001$). Several tagSNPs in these 2 genes were associated with plasma concentrations after correction for multiple comparisons. Vitamin B-12 status was a significant effect modifier of the association between the genetic variant *BHMT2* rs626105 A>G and plasma DMG concentration.

Conclusions: Genetic variations in metabolic enzymes were associated with plasma concentrations of choline and its metabolites. Our findings contribute to the knowledge on the variation in blood nutrient concentrations in postmenopausal women. *J Nutr* 2020;150:2874–2881.

Keywords: choline metabolism, one-carbon metabolism, postmenopausal women, genetic variants, betaine, dimethylglycine

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Introduction

Choline is an essential dietary nutrient because of its important role in one-carbon metabolism (1). Through different pathways, choline is metabolized into important end-products, including betaine, dimethylglycine (DMG), and trimethylamine N-oxide (TMAO). Choline is oxidized in the liver to betaine in a 2-step reaction involving choline dehydrogenase (*CHDH*) and betaine aldehyde dehydrogenase (*ALDH7A1*) (1, 2). In turn, betaine plays an important role in one-carbon metabolism as a methyl donor. Betaine is converted to DMG by betaine-homocysteine S-methyltransferase enzymes (*BHMT*, *BHMT2*). The BHMT enzymes also convert homocysteine to methionine simultaneously. Catabolism by the gut microbiome converts choline to trimethylamine, which is absorbed and then converted to TMAO by hepatic flavin monooxygenase isoform (*FMO3*) (1–3). Thus, it is clear that choline metabolism contributes to the folate cycle. Consequently, it can be argued that choline helps maintain DNA stability because studies have shown that low plasma folate concentration is associated with inflammation and DNA instability, which in turn may increase the risk of diseases such as colorectal cancer (4, 5).

Data from feeding studies showed that the risk of developing choline-deficiency-related disorder has a large variation between individuals, and the finding suggests that genetics play a role in choline metabolism (1). The associations of genetic polymorphisms in choline metabolism enzymes with variation in plasma choline and its metabolites are not fully understood at the population level. There is limited information in the epidemiologic literature, and the sample size in a previous study was small ($n = 75$) (6). Here, we evaluated associations of single-nucleotide polymorphisms (SNPs) in *BHMT* and *BHMT2* with plasma concentrations of betaine and DMG among women enrolled in a case-control study nested within the Women's Health Initiative Observational Study (WHI-OS), a large prospective study of postmenopausal women. Such an investigation is important because postmenopausal women are more likely to develop symptoms of choline deficiency than premenopausal women (7). In addition, our previous analysis in the nested case-control study showed that plasma betaine concentrations were inversely associated with colorectal cancer risk, and plasma TMAO concentrations were positively associated with rectal cancer and with overall colorectal cancer risk among women with lower (compared with higher) plasma vitamin B-12 concentrations (8). These observations highlight the importance of understanding the genetic determinants of choline metabolites. Owing to the overlap of choline metabolism and folate metabolism in achieving and maintaining one-carbon metabolism (9, 10), we also examined SNPs in 4 enzymes [5,10-methylenetetrahydrofolate reductase (*MTHFR*), methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1 (*MTHFD1*), 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*), and 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*)] involved in folate metabolism in relation to the blood biomarkers of choline and its metabolites (1–3). In addition, we explored possible effect modification in these associations due to B-vitamin status and folic acid (FA) fortification periods from 1993 to 1998 that resulted in a change in folate status (8, 11).

Methods

Study population

We analyzed genetic and biomarker data from a nested case-control study of colorectal cancer risk. The study participants were selected from the WHI-OS, a prospective cohort study that recruited 93,676 postmenopausal women from 40 US clinical centers between 1993 and 1998 (12). The study design and baseline characteristics of the WHI-OS have been described in detail previously (3, 5, 12–16). Baseline eligibility requirements included postmenopausal status, age between 50 and 79 y at enrollment, and low likelihood of loss to follow-up within 3 y due to relocation or death resulting from a pre-existing medical condition. For the nested case-control analysis of colorectal cancer risk, incident colorectal cancer cases were selected for a mean ± SD time of 5.2 ± 3.1 y from baseline as of 24 April, 2008 (8, 17). Women were excluded from the case-control study if they had pre-existing intestinal disease, including a history of colorectal cancer, carcinoma in situ, ulcerative colitis, or Crohn disease (1, 3, 13). Risk-set sampling was used to randomly select controls from within the WHI-OS cohort who were alive and free of any type of colorectal cancer, invasive or noninvasive, at the time of case diagnosis. Nine hundred and eighty-eight cases and 988 controls were matched based on age (±3 y), race/ethnicity, enrollment date (±1 y), hysterectomy status, and time of blood draw (±6 mo). The analyses in this report are based on 1423 white participants with complete data on genetics, biomarkers, and covariates (**Supplemental Figure 1**). Written informed consent was obtained from all participants at WHI enrollment and at various follow-up time points in the WHI Extension Study periods. The study was approved by the human subjects review board at the Fred Hutchinson Cancer Research Center, where the WHI Clinical Coordinating Center is located. Additional institutional review board approval was obtained from the University of California, Davis, where some analyte measurements for this report were conducted.

Demographic and health data collection

Demographic characteristics including age, race/ethnicity, education, and household income and health-related characteristics including personal medical history, use of postmenopausal hormones, physical activity, and smoking history were obtained at baseline using standardized questionnaires (12). Data on dietary intake of B vitamins were obtained from a baseline FFQ, and baseline dietary supplement use was assessed using an inventory method (14). Participants' height and weight were measured by trained staff using a standard protocol, and BMI was calculated as weight divided by height squared (kg/m^2).

Blood sample processing and analysis

Blood samples were drawn at study baseline, i.e., at WHI enrollment, after ≥12 h of fasting. Samples were kept at 4°C for ≤1 h before centrifugation (4°C, 10 mins, relative centrifugal force of 1300 x *g*).

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Supplemental Tables 1–3 and Supplemental Figure 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents available at https://academic.oup.com/jn/.
MNI and T-YDC contributed equally to this work.
Address correspondence to MNI (e-mail: milozumba@ufl.edu) or T-YDC (email: tingyuan.cheng@ufl.edu).
Abbreviations used: *BHMT*, betaine-homocysteine S-methyltransferase; CEPH, Centre d'Etude du Polymorphism Humain; DMG, dimethylglycine; FA, folic acid; LD, linkage disequilibrium; MAF, minor-allele frequency; *MTHFD1*, methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1; *MTHFR*, 5,10-methylenetetrahydrofolate reductase; *MTR*, 5-methyltetrahydrofolate-homocysteine methyltransferase; *MTRR*, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase; PLP, pyridoxal-5′-phosphate; SNP, single-nucleotide polymorphism; TMAO, trimethylamine N-oxide; WHI-OS, Women's Health Initiative Observational Study.

Plasma and serum were collected and stored at −70°C until analysis (5). The laboratory measurements of one-carbon metabolites have been described elsewhere (5, 8, 15). In brief, plasma concentrations of choline and its metabolites (betaine, DMG, and TMAO) were measured in deidentified samples using LC-tandem MS methodology with modifications based on our instrumentation (18, 19). Plasma folate, RBC folate, and plasma vitamin B-12 concentrations were determined by radio assay [SimulTRAC Radioassay Kit Folate (125I), MP Biomedicals, LLC], and plasma pyridoxal-5′-phosphate (PLP) was measured by HPLC with fluorescence detection (13). Total plasma homocysteine was determined by HPLC with postcolumn fluorescence detection (5). The interassay CVs in the blind duplicate control samples for each of the assays were as follows: choline, 7%; betaine, 5%; DMG, 9%; TMAO, 6%; plasma folate, 5%; RBC folate, 10%; vitamin B-12, 6%; PLP, 6%; and homocysteine, 7%.

Genotyping

Genotyping procedures have been reported in an earlier study (3). We genotyped both candidate and tagging SNPs of *BHMT, BHMT2, MTHFR, MTHFD1, MTR*, and *MTRR*. TagSNPs were selected based on linkage disequilibrium (LD) from the CEPH (Centre d'Etude du Polymorphism Humain) population (residents of Utah with Western and Northern European ancestry) from HapMap 2 (data release 24 on the National Center for Biotechnology Information's B36 assembly) (20). The minor-allele frequency (MAF) cutoff was 5% and $r^2 = 0.80$ (21). On each gene, tagSNPs were selected from 10 kb upstream to 5 kb downstream, or tagSNPs were selected through the end of the LD blocks. The Illumina 384-plex BeadXpress GoldenGate genotyping platform was used to genotype SNPs in the study. In terms of quality control, we included 30 CEPH trios from the HapMap project and 5% blinded duplicates (42 case–control pairs). The laboratory personnel were blinded to the status of the cases and controls. The exclusion criteria for the SNPs included <95% concordance with blinded or nonblinded duplicates, 95% call rate, deviations from the expected MAF, or Hardy–Weinberg equilibrium $P < 0.0001$. **Supplemental Table 1** provides the complete list of SNPs examined in this study.

Statistical analysis

We investigated associations between the genetic variants and biomarkers of choline metabolism. Correlations among plasma concentrations of choline metabolites and B vitamins involved in one-carbon metabolism were assessed using Pearson correlation analysis. The choline gene–biomarker associations were tested based on a prior hypothesis. First, we examined associations of genetic variation in *BHMT* and *BHMT2* with plasma betaine and plasma DMG. Second, we examined associations of genetic variation in *MTHFR, MTHFD1, MTR*, and *MTRR* with plasma concentrations of choline, betaine, DMG, and TMAO because these genes are the most relevant to choline metabolism among the genes that were genotyped. In the second analysis, only nonsynonymous SNPs of those genes were examined to reduce the number of comparisons. We conducted multivariable linear regression analyses using the genotype of SNPs as the independent variable and the concentration of biomarkers of choline metabolism as the outcome variable. The models were adjusted for baseline confounding factors selected a priori, including age, BMI, smoking status (never, past, or current), physical activity (as total metabolic equivalents per week), use of postmenopausal hormone therapy (never user, past user, and current user), history of colonoscopy or sigmoidoscopy (yes, no), polyps of colon or rectum removed (yes, no, unknown), family history of colorectal cancer (yes, no, unknown), plasma folate concentration, RBC folate concentration, plasma vitamin B-12 concentration, plasma vitamin B-6 (PLP) concentration, plasma homocysteine concentration, and FA fortification period. These covariates were regarded as known or probable risk factors for colorectal cancer and dietary factors that could affect choline metabolite concentrations. The blood biomarkers and vitamin intake were continuous variables. As dependent variables, the distributions of biomarkers were examined, and logarithmic transformation was performed to improve normality. Genetic variants, i.e., the independent variables, were modeled as continuous variables (0, 1, or 2 alleles). The regression coefficient (β) was converted to a percentage with the formula [exp (β) − 1] × 100% and was interpreted as the estimated percentage difference in the concentration of a biomarker for each additional variant allele. A post hoc analysis was conducted to test for additive compared with recessive inheritance models using the chi-square difference test. The chi-square difference tests were significant for all SNPs; hence, using additive models was adequate for the SNPs in our study.

To explore whether associations between SNPs and plasma choline metabolites were modified by plasma B vitamins involved in one-carbon metabolism, we conducted analyses stratified by high/low plasma concentrations of folate, PLP, and vitamin B-12 based on the median values among the controls. We also examined effect modification due to FA fortification by stratifying by a variable that indicated whether blood draws were obtained prefortification (1993–1995); peri-fortification (1996–1997), when the initial fortification had begun but was not yet mandated; or postfortification (1998) (8). The Wald test was used to evaluate effect modification, including a 2-way interaction term between the SNPs and effect modifiers. Statistical significance was defined as nominal $P < 0.05$ for nonsynonymous or candidate SNPs. For the tagSNPs, to account for multiple comparisons, the Bonferroni correction method was performed at the gene level. The thresholds for significance in these analyses were set at $P < 6.25 \times 10^{-3}$ based on Bonferroni correction for testing 8 tagSNPs in the *BHMT* gene (0.05/8) and at $P < 0.01$ based on Bonferroni correction for testing 5 tagSNPs in the *BHMT2* gene (0.05/5).

We calculated polygenic risk scores of the significant SNPs in the *BHMT* and *BHMT2* genes and analyzed their association with plasma concentrations of betaine and DMG, respectively. If a person carried a risk allele for an SNP, a score of 1 was given, otherwise a score of 0 was given. The scores of all the SNPs were summed and the distribution of the total SNP score was divided into quartiles in linear regressions. All statistical tests were 2-sided. Analyses were performed using SAS version 9.3 (SAS Institute Inc.).

Results

Table 1 and **Supplemental Table 2** show the baseline characteristics of the 1423 white postmenopausal women. The mean age of this population was 67.1 y, with a mean BMI of 27.3. One-quarter of participants resided in the Northeast, 21.7% resided in the South, 24.6% resided in the Midwest, and 28.5% resided in the West. Over half (58.3%) of the postmenopausal women had a history of colonoscopy or sigmoidoscopy, whereas 41.7% had no history of colonoscopy or sigmoidoscopy. In terms of smoking status, 48.8% were never smokers, 45.1% were past smokers, and 6.04% were current smokers. Regarding postmenopausal hormone use, 44.4% were never users, 17.6% were past users, and 38.0% were current users. The mean plasma concentrations of the biomarkers were as follows: 9.40 μmol/L for choline, 26.6 μmol/L for betaine, 2.47 μmol/L for DMG, 5.84 μmol/L for TMAO, 536.0 pg/mL for vitamin B-12, 103.3 nmol/L for vitamin B-6, 20.7 ng/mL for plasma folate, and 625.2 ng/mL for RBC folate.

Pearson's *r*s were computed to examine associations among plasma choline metabolites and plasma B vitamins involved in one-carbon metabolism. There were modest correlations between plasma choline and betaine, and betaine and DMG (**Supplemental Table 3**). However, the correlations between plasma betaine concentrations and the concentrations of plasma vitamin B-6, folate, and vitamin B-12 and RBC folate were generally low.

Table 2 shows the associations of nonsynonymous and tagging SNPs in the *BHMT* and *BHMT2* genes with plasma concentrations of betaine and DMG. Individuals with the *BHMT* R239Q (rs3733890; candidate) variant allele had a

TABLE 1 Baseline characteristics of 1423 white postmenopausal women in the Women's Health Initiative Observational Study[1]

Characteristics	*n*	Value
Age, y	1423	67.1 ± 6.77
BMI, kg/m^2	1423	27.3 ± 5.72
Physical activity, total METs per week	1423	13.0 ± 13.0
Residence location (US region)		
Northeast	359	25.2
South	309	21.7
Midwest	350	24.6
West	405	28.5
History of colonoscopy or sigmoidoscopy		
No	593	41.7
Yes	830	58.3
Polyps of colon/rectum ever removed		
No	630	44.3
Yes	177	12.4
Unknown	616	43.3
Family history of colorectal cancer		
No	1053	74.0
Yes	260	18.3
Unknown	110	7.73
Use of postmenopausal hormones		
Never user	632	44.4
Past user	250	17.6
Current user	541	38.0
Smoking status		
Never smoker	695	48.8
Past smoker	642	45.1
Current smoker	86	6.04
Biomarker		
Choline, μmol/L	1423	9.40 (9.28, 9.52)
Betaine, μmol/L	1423	26.6 (26.0, 27.2)
Dimethylglycine, μmol/L	1423	2.47 (2.42, 2.52)
Trimethylamine N-oxide, μmol/L	1423	5.84 (5.39, 6.29)
Vitamin B-12, pg/mL	1423	536.0 (521.8, 550.1)
Vitamin B-6, nmol/L	1423	103.3 (98.1, 108.6)
RBC folate, ng/mL	1423	625.2 (611.6, 638.8)
Plasma folate, ng/mL	1423	20.7 (19.9, 21.5)

[1] Values are means ± SDs for continuous variables, percentages for categorical variables, or means (95% CIs) for plasma choline metabolites and B-vitamin concentrations. Because of rounding, not all percentages add up to 100. MET, metabolic equivalent.

significantly lower concentration of plasma betaine (−4.00%; 95% CI: −6.67%, −1.23% per variant allele). Thus, percentage differences in plasma betaine concentrations per variant allele were 4%. Several variants of tagSNPs in the *BHMT* gene were significantly associated with plasma betaine. The *BHMT* rs1291041 (C>A), rs10944 (A>C), and rs12655567 (C>G) variant alleles were associated with higher concentrations of plasma betaine (9.42%; 95% CI: 6.63%, 12.3%; 8.02%; 95% CI: 5.36%, 10.7%; and 8.15%; 95% CI: 5.48%, 10.9% per variant allele, respectively), whereas the *BHMT* rs9637824 (A>G) and rs558133 (A>C) variant alleles were associated with lower concentrations of plasma betaine (−3.84%; 95% CI: −6.31%, −1.30%; and −5.59%; 95% CI: −8.15%, −2.96% per variant allele, respectively).

There was a borderline significant association between the *BHMT2* (rs626105 G>A; candidate) variant allele and a lower concentration of plasma betaine (−2.89%; 95% CI: −5.80%, 0.11% per variant allele). Several variants of tagSNPs in the *BHMT2* gene were significantly associated with plasma betaine. The *BHMT2* rs16876512 (G>A), rs2909856 (A>G), and rs476620 (A>G) variant alleles were associated with lower concentrations of plasma betaine (−9.66%; 95% CI: −13.4%, −5.77%; −4.40%; 95% CI: −6.73%, −1.64%; and −3.84%; 95% CI: −6.31%, −1.31% per variant allele, respectively), whereas the *BHMT2* rs2461248 (A>T) variant allele was associated with a higher concentration of plasma betaine (7.68%; 95% CI: 5.03%, 10.4% per variant allele).

The *BHMT* R239Q (rs3733890; candidate) variant allele was associated with a lower concentration of plasma DMG (−6.75%; 95% CI: −9.47%, −3.95% per variant allele). Several variants of tagSNPs in the *BHMT* gene were significantly associated with plasma DMG. The *BHMT* rs9637824 (A>G), rs642431 (A>C), rs558133 (A>C), and rs492842 (A>G) variant alleles were associated with higher concentrations of plasma DMG (3.99%; 95% CI: 1.20%, 6.86%; 13.3%; 95% CI: 9.80%, 16.9%; 7.36%; 95% CI: 4.33%, 10.5%; and 3.48%; 95% CI: 0.69%, 6.35% per variant allele, respectively), whereas the *BHMT* rs16876500 (G>A) variant allele was associated with a lower concentration of plasma DMG (−7.82%; 95% CI: −11.9%, −3.57% per variant allele).

The *BHMT2* rs626105 G>A (candidate) variant allele was associated with a higher concentration of plasma DMG (13.0%; 95% CI: 9.47%, 16.5% per variant allele). Several variants of tagSNPs in the *BHMT2* gene were significantly associated with plasma DMG. The *BHMT2* rs631305 (G>A) and rs476620 (A>G) variant alleles were associated with higher concentrations of plasma DMG (12.7%; 95% CI: 8.79%, 16.7% and 3.89%; 95% CI: 1.11%, 6.75% per variant allele, respectively), whereas the *BHMT2* rs16876512 (G>A) variant allele was associated with a lower concentration of plasma DMG (−8.53%; 95% CI: −12.5%, −4.40% per variant allele).

Table 3 shows the associations of nonsynonymous SNPs in one-carbon metabolism genes with the concentrations of plasma choline and its metabolites. The *MTRR* S202L (rs1532268; candidate) variant allele was associated with a higher concentration of plasma choline (2.45%; 95% CI: 0.71%, 4.23% per variant allele), whereas the *MTRR* 149M (rs1801394; candidate) variant allele had a borderline significant association with a lower concentration of plasma choline (−1.61%; 95% CI: −3.27%, 0.07% per variant allele). The *MTRR* K377R (rs162036; candidate) variant allele had a borderline significant association with a higher concentration of plasma betaine (4.01%; 95% CI: 0.17%, 8.36% per variant allele). In addition, the *MTRR* S202L (rs1532268; candidate) variant allele had a borderline significant association with a higher concentration of plasma betaine (2.65%; 95% CI: −0.03%, 5.41% per variant allele). The *MTHFD1* rs2236224 G>A (candidate) variant allele was significantly associated with a higher concentration of plasma DMG (3.22%; 95% CI: 0.45%, 6.06% per variant allele), whereas the *MTR* rs116252762 A>G (candidate) variant allele was significantly associated with a lower concentration of plasma DMG (−10.6%, 95% CI = −18.3, −2.25 per variant allele). Genetic variants in the *MTHFR, MTHFD1, MTR,* and *MTRR* genes showed no significant associations with plasma TMAO (data not shown).

Plasma vitamin B-12 status was a significant effect modifier of the association between the genetic variant *BHMT2* rs626105 G>A (candidate) and plasma DMG (*P*-interaction = 0.034; data not shown). The association was stronger in the low plasma vitamin B-12 status group (16.4%; 95% CI: 11.7%, 21.4%) than in the high plasma vitamin B-12

TABLE 2 Associations of candidate and tagging SNPs in *BHMT* and *BHMT2* with plasma concentrations of betaine and DMG[1]

Gene	SNP	Percent conc. difference per variant allele (95% CI)	Nominal P[2]
Betaine			
BHMT	rs3733890 (R239Q, candidate)	−4.00 (−6.67, −1.23)	0.0049
	rs1291041 (C>A, tag)	9.42 (6.63, 12.3)	<0.0001*
	rs9637824 (A>G, tag)	−3.84 (−6.31, −1.30)	0.0032*
	rs16876500 (G>A, tag)	−9.17 (−13.0, −5.19)	<0.0001*
	rs10944 (A>C, tag)	8.02 (5.36, 10.7)	<0.0001*
	rs642431 (A>C, tag)	−3.15 (−6.09, −0.13)	0.0412
	rs558133 (A>C, tag)	−5.59 (−8.15, −2.96)	<0.0001*
	rs12655567 (C>G, tag)	8.15 (5.48, 10.9)	<0.0001*
	rs492842 (A>G, tag)	−3.70 (−6.18, −1.15)	0.0047
BHMT2	rs626105 (G>A, candidate)	−2.89 (−5.80, 0.11)	0.06
	rs16876512 (G>A, tag)	−9.66 (−13.4, −5.77)	<0.0001*
	rs2461248 (A>T, tag)	7.68 (5.03, 10.4)	<0.0001*
	rs2909856 (A>G, tag)	−4.40 (−6.73, −1.64)	0.0015*
	rs476620 (A>G, tag)	−3.84 (−6.31, −1.31)	0.0031*
DMG			
BHMT	rs3733890 (R239Q, candidate)	−6.75 (−9.47, −3.95)	<0.0001
	rs9637824 (A>G, tag)	3.99 (1.20, 6.86)	0.0048*
	rs16876500 (G>A, tag)	−7.82 (−11.9, −3.57)	0.0004*
	rs642431 (A>C, tag)	13.3 (9.80, 16.9)	<0.0001*
	rs558133 (A>C, tag)	7.36 (4.33, 10.5)	<0.0001*
	rs492842 (A>G, tag)	3.48 (0.69, 6.35)	0.0141*
BHMT2	rs626105 (G>A, candidate)	13.0 (9.47, 16.5)	<0.0001
	rs16876512 (G>A, tag)	−8.53 (−12.5, −4.40)	<0.0001*
	rs631305 (G>A, tag)	12.7 (8.79, 16.7)	<0.0001*
	rs2909856 (A>G, tag)	3.01 (0.27, 5.82)	0.0309
	rs476620 (A>G, tag)	3.89 (1.11, 6.75)	0.0059*

[1] Multivariable analyses were adjusted for age, BMI, smoking status (never, past, or current), physical activity (as total metabolic equivalents per week), use of postmenopausal hormone therapy (never user, past user, and current user), history of colonoscopy or sigmoidoscopy (yes, no), polyps of colon or rectum removed (yes, no, unknown), family history of colorectal cancer (yes, no, unknown), plasma folate concentration, RBC folate concentration, plasma vitamin B-12 concentration, plasma vitamin B-6 (pyridoxal-5′-phosphate) concentration, homocysteine concentration, and folic-acid fortification periods. *Statistically significant after Bonferroni correction. The thresholds were 0.00625 (0.05/8) for *BHMT* tagSNPs and 0.01 (0.05/5) for *BHMT2* tagSNPs. *BHMT*, betaine-homocysteine S-methyltransferase; conc., concentration; DMG, dimethylglycine; SNP, single-nucleotide polymorphism.
[2] Statistical significance was defined as nominal $P < 0.05$ for candidate SNPs.

status group (8.93%; 95% CI: 3.89%, 14.2%). Other plasma B vitamins did not modify the associations between genetic variants and plasma choline metabolites (data not shown). FA fortification periods did not modify the associations between genetic variants and plasma choline metabolites (data not shown).

In the polygenic risk score estimation (**Table 4**), women in the highest compared with the lowest quartile of the polygenic risk score of *BHMT* and *BHMT2* genes had a lower concentration of plasma betaine (−15.0%; 95% CI: −20.3%, −9.48%). Women in the highest compared with the lowest quartile of the polygenic risk score of *BHMT* and *BHMT2* genes had a higher concentration of plasma DMG (6.29%; 95% CI: 0.28%, 12.7%), although the pattern of risk estimates did not suggest a dose–response relation.

Discussion

The present study is one of few to assess associations between genetic variations in metabolic enzymes and plasma concentrations of choline metabolites among postmenopausal women in the United States. The candidate SNP *BHMT* R239Q (rs3733890) was associated with lower concentrations of plasma betaine and plasma DMG. Individuals with the candidate SNP *BHMT2* (rs626105 G>A) had a borderline significantly lower concentration of plasma betaine and a significantly higher concentration of plasma DMG. Several variants of tagSNPs in the *BHMT* and *BHMT2* genes were significantly associated with lower plasma betaine and higher plasma DMG concentrations after correction for multiple comparisons. Our study findings imply that the *BHMT* and *BHMT2* genetic variants are gain-of-function variants with respect to their associations with plasma concentration of betaine, because a more active *BHMT* would decrease plasma betaine and increase plasma DMG.

BHMT is responsible for converting betaine to methionine (and DMG), and its activity would influence methyl group supply. Our findings on *BHMT* R239Q (rs3733890) are consistent with other literature. This candidate SNP is associated with spina bifida, a birth defect causally linked to folate deficiency (22). In a choline feeding trial under conditions of adequate choline and folate intake, the *BHMT* R239Q (rs3733890) genetic variant played a functional role in choline dynamics by partitioning the metabolic endpoint of dietary choline metabolism between betaine and cytidine diphosphate–derived phosphatidylcholine metabolic endpoints (1, 6). However, the gene–biomarker associations may vary

TABLE 3 Associations of candidate SNPs in one-carbon metabolism genes with plasma concentrations of choline and its metabolites[1]

Gene	SNP	Percentage conc. difference per variant allele (95% CI)	Nominal *P*
Choline			
MTRR	rs1801394 (149M, candidate)	−1.61 (−3.27, 0.07)	0.06
	rs1532268 (S202L, candidate)	2.45 (0.71, 4.23)	0.01*
Betaine			
MTRR	rs162036 (K377R, candidate)	4.01 (−0.17, 8.36)	0.06
	rs1532268 (S202L, candidate)	2.65 (−0.03, 5.41)	0.05
Dimethylglycine			
MTHFD1	rs2236225 (R653Q, candidate)	2.45 (−0.23, 5.21)	0.07
	rs2236224 (G>A, candidate)	3.21 (0.45, 6.04)	0.02*
MTR	rs116252762 (A>G, candidate)	−10.6 (−18.3, −2.25)	0.01*

[1] Multivariable analyses were adjusted for age, BMI, smoking status (never, past, or current), physical activity (as total metabolic equivalents per week), use of postmenopausal hormone therapy (never user, past user, and current user), history of colonoscopy or sigmoidoscopy (yes, no), polyps of colon or rectum removed (yes, no, unknown), family history of colorectal cancer (yes, no, unknown), plasma folate concentration, RBC folate concentration, plasma vitamin B-12 concentration, plasma vitamin B-6 (pyridoxal-5′-phosphate) concentration, homocysteine concentration, and folic-acid fortification periods. *Statistical significance was defined as nominal $P < 0.05$ for candidate SNPs. conc., concentration; *MTHFD1*, methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1; *MTR*, 5-methyltetrahydrofolate-homocysteine methyltransferase; *MTRR*, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase; SNP, single-nucleotide polymorphism.

between populations. In individuals with choline deficiency–related organ dysfunction, there was no association between the *BHMT* R239Q (rs3733890) genotype and changes in plasma betaine concentration following dietary choline depletion and repletion protocols (10). For *BHMT2*, we noted that the candidate SNP (rs626105 G>A) was associated with lower betaine but higher DMG concentrations in plasma, which is biologically plausible because the function of *BHMT2* is to convert plasma betaine to plasma DMG. This finding has not been reported in the literature.

As expected, our polygenic risk score was able to predict the plasma concentrations of betaine and DMG in the presence of significant SNPs in the *BHMT* and *BHMT2* genes. Individuals with a high score can have as much as 15% lower plasma betaine concentrations than those with a low score. The difference can be clinically significant. These genes we reported are not rare in the population. For example, 1 in 4 people carry the variant of *BHMT* R239Q (rs3733890) and 1 in 5 people carry the variant of *BHMT2* (rs626105 G>A) (Supplemental Table 1).

TABLE 4 Polygenic risk score of *BHMT* and *BHMT2* genes associated with plasma betaine and DMG concentrations[1]

Quartiles	Percentage concentration difference (95% CI)[2]	*P* value[3]
Betaine		
Quartile 1	Reference	Reference
Quartile 2	−6.99 (−13.0, −0.57)	0.0333
Quartile 3	−15.3 (−20.3, −10.0)	<0.0001
Quartile 4	−15.0 (−20.3, −9.48)	<0.0001
DMG		
Quartile 1	Reference	Reference
Quartile 2	−6.07 (−11.6, −0.15)	0.0445
Quartile 3	3.25 (−2.31, 9.12)	0.2574
Quartile 4	6.29 (0.28, 12.7)	0.0399

[1] The distribution of the total polygenic risk score was divided into quartiles. *BHMT*, betaine-homocysteine S-methyltransferase; DMG, dimethylglycine.
[2] Multivariable analyses were adjusted for age, BMI, smoking status (never, past, or current), physical activity (as total metabolic equivalents per week), use of postmenopausal hormone therapy (never user, past user, and current user), history of colonoscopy or sigmoidoscopy (yes, no), polyps of colon or rectum removed (yes, no, unknown), family history of colorectal cancer (yes, no, unknown), plasma folate concentration, RBC folate concentration, plasma vitamin B-12 concentration, plasma vitamin B-6 (pyridoxal-5′-phosphate) concentration, plasma homocysteine concentration, and folic-acid fortification periods.
[3] Statistical significance was defined as nominal $P < 0.05$ for candidate single-nucleotide polymorphisms.

We hypothesized that genetic polymorphisms in the *MTHFR*, *MTHFD1*, *MTR*, and *MTRR* one-carbon metabolism genes would be associated with plasma concentrations of plasma choline and its metabolites. In the methylation cycle, the methionine synthase reductase (*MTRR*) provides the methylcobalamin (methyl-vitamin B-12) used by methionine synthase (*MTR*) to convert homocysteine to methionine (23). In our study, genetic variation in *MTRR* S202L (rs1532268) was associated with higher plasma concentrations of choline and betaine, whereas *MTRR* K377R (rs162036) was associated with a higher concentration of plasma betaine. Associations of the *MTRR* genetic variants with higher concentrations of plasma choline and betaine could imply that the SNPs or their associated SNPs may lead to a gain of function. In other words, folate and 5-methyl tetrahydrofolate will be used as methyl group donors promoted by *MTRR*, and there are reductions in the use of choline and betaine as methyl group donors. Hence, there might be higher plasma concentrations of choline and betaine in individuals with *MTRR* genetic variants, and the concentrations could be related to disease risk. In our previous analysis using this study population, postmenopausal women with higher compared with lower ratios of betaine to choline had lower colorectal cancer risk overall and lower proximal tumors, lower local/regional tumors, and lower rectal tumors (8).

Our study also noted a significant association between genetic variation in *MTHFD1* (G>A, rs2236224) and a higher concentration of plasma DMG. Our study found a borderline significant association between *MTHFD1* R653Q (rs2236225) and a high concentration of plasma DMG, which could imply greater use of plasma choline as a methyl donor among those with the *MTHFD1* variant as a compensatory mechanism.

Other studies have shown that the *MTHFD1* variant is associated with the risk of neural tube defects (24). In addition, a study that examined the risk of organ dysfunction associated with choline deficiency found that individuals with the *MTHFD1* R653Q genetic variant had increased odds of developing organ dysfunction, manifested by a >5-fold increase of serum creatine kinase activity or an increase in the liver fat content (9). Our finding supports the association between the genetic variant in *MTHFD1* R653Q and the risk of choline-deficiency-related organ dysfunction. Furthermore, our finding that the *MTHFD1* rs2236224 G>A and R653Q genetic alleles were associated with higher concentrations of plasma DMG could imply that the *MTHFD1* genetic variants are gain-of-function variants. In other words, there is a greater use of betaine as a methyl donor, which depletes the plasma concentration of betaine, in individuals with *MTHFD1* genetic variants. In one-carbon metabolism, betaine is an important determinant of plasma homocysteine (25) for which the concentration is also gene-dependent (26).

Plasma vitamin B-12 status was a significant effect modifier of the association between the genetic variant *BHMT2* rs626105 G>A (candidate) and plasma DMG. The association was stronger in the low plasma vitamin B-12 status group than in the high plasma vitamin B-12 status group. *BHMT* is activated in the presence of low availability of plasma vitamin B-12 (27), which could explain why there was a stronger association in the setting of low plasma vitamin B-12 status. In this study population, our previous analysis showed that higher compared with lower plasma TMAO is associated with an increased risk of colorectal cancer in those with lower vitamin B-12 status (8), further strengthening our finding.

Given that feeding studies have shown that having insufficient methyl groups available from choline and betaine for the conversion of homocysteine to methionine causes choline-deficiency-related organ dysfunction, a potential hypothesis is that these genetic variations could cause increased susceptibility to choline deficiency. For example, the phosphatidylethanolamine N-methyltransferase gene (*PEMT*; −744 G>C; rs12325817) was associated with choline-deficiency symptoms in individuals who consumed a low-choline diet (10). We did not genotype the gene or SNPs. However, in the same study, a *BHMT* SNP (G>A; rs3733890) was not associated with susceptibility to choline deficiency. Frank choline deficiency in healthy nonpregnant women is very rare (28) and the WHI study did not assess the symptoms of choline deficiency. Hence, we are unable to determine whether these genotypes are associated with increased susceptibility to choline deficiency in our present study.

A strength of our study was its large sample size. To the best of our knowledge, this is the largest study to date to examine the association of *BHMT* and *BHMT2* genetic variants with choline metabolites in postmenopausal women. We were able to adjust for many potential confounders, including plasma B-vitamin concentration, which serves as another study strength. However, our study was limited by testing for a limited number of genes, especially candidate genes. Future studies should examine a more comprehensive list of genes that could enable a better understanding of the associations of genetic polymorphisms in the *BHMT* and *BHMT2* genes with plasma choline metabolites. Another limitation is the generalizability of our study findings. Our study population was composed of only white women; thus, our results may not be applicable to women of other races. Our study population was also specifically postmenopausal women; therefore, applying our study findings in the context of the associations of these genetic variations with choline metabolites to the general population must be done with caution.

Although the study sample was from a nested case-control study examining colorectal cancer risk, our study focus was not to evaluate this risk. The blood samples from participants were collected at baseline of WHI enrollment, and cases were selected ≥6 mo after baseline. Thus, concentrations of plasma choline metabolites measured in blood collected at baseline were unlikely affected by cases that developed during the follow-up of WHI. However, we cannot exclude the possibility that some indolent cases may have provided blood draw at baseline.

In summary, our study showed associations of genetic variation in metabolic enzymes with plasma concentrations of choline metabolites among postmenopausal white women. Our findings can be clinically meaningful because these variants are common and associated with significantly lower circulating betaine concentrations. Additional studies should examine nutrient–genotype interactions and their potential implications on disease outcomes.

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