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Genetic variants in phosphatidylethanolamine *N*-methyltransferase (PEMT) and methylenetetrahydrofolate dehydrogenase (MTHFD1) influence biomarkers of choline metabolism when folate intake is restricted

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Abstract

Choline is a required nutrient with roles in liver and brain function, lipid metabolism, and fetal development. Recent data suggest that choline requirements may be altered by polymorphisms in the phosphatidylethanolamine *N*-methyltransferase (PEMT) gene (i.e., 5465G→A; rs7946 and -744G→C; rs12325817) and in the methylenetetrahydrofolate dehydrogenase (MTHFD1) gene (i.e., 1958G→A; rs2236225). This controlled feeding study, conducted in 2000–2001, examined the effects of the PEMT and MTHFD1 genetic variants on biomarkers of choline metabolism in premenopausal Mexican American women (n=43) after a 7-wk period of folate restriction (135μg as dietary folate equivalents, DFE) and after a 7-wk period of folate treatment (400 and 800μg DFE/d combined). Throughout the 14-wk study choline intake remained constant at 349mg/d. The genotype frequencies of the women were 3GG, 19GA, and 21AA for PEMT G5465A; 9GG, 17GC and 17CC for PEMT G-744C; and 9GG, 21GA and 13AA for MTHFD1 G1958A. During folate restriction, homocysteine was adversely influenced by PEMT 5465AA (P=0.001 relative to the G allele) and by MTHFD1 1958AA (P=0.085 relative to 1958GG); whereas the decline in phosphatidylcholine was attenuated by PEMT -744CC (P=0.017 relative to -744GG). During folate treatment, no effects of the genotypes on the response of the measured variables were detected. These data suggest that polymorphisms in genes relevant to choline metabolism modulate parameters of choline status when folate intake is restricted. Additional studies with larger samples sizes are needed to examine the relationship between these genetic variants and varied choline intake in populations with increased demands for choline (i.e., pregnant women).

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Keywords

PEMT; MTHFD1; choline; folate; genetic variants

INTRODUCTION

In 1998, the Institute of Medicine (IOM) established its first choline recommendations, 425 and 550 mg/d for women and men respectively, based on the estimated level of choline intake required to prevent liver damage (1). Choline and its metabolites (i.e. phosphatidylcholine, sphingomyelin, betaine, and acetylcholine) are necessary for methyl group metabolism, lipid transport and metabolism, the structural integrity and signaling functions of cell membranes, and the biosynthesis of the neurotransmitter acetylcholine (2). Disturbances in choline metabolism (i.e., choline deficiency) result in liver dysfunction (3–5) and muscle damage (6) and are associated with altered deoxynucleic acid (DNA) methylation patterns (7) and increased risk of neural tube defects (8,9). Existing data also suggest that choline plays a critical role in fetal development (10,11) and that pregnancy may represent a time of markedly increased maternal choline requirements (12,13).

The human requirement for choline can be met in part through the endogenous biosynthesis of phosphatidylcholine. Specifically, phosphatidylethanolamine *N*-methyltransferase (PEMT) catalyzes the methylation of phosphatidylethanolamine using *S*-adenosylmethionine (SAM) as the methyl donor (14; Figure). As a major consumer of SAM-derived methyl groups, PEMT is also a major producer of homocysteine (15,16); a sulfur containing amino acid linked to many adverse health outcomes. Phosphatidylcholine may also be synthesized by the cytidinediphospho-choline (CDP-choline) pathway utilizing choline as the substrate.

The PEMT gene is highly polymorphic and 98 single nucleotide polymorphisms (SNPs) have been identified in this gene (17). Of those, PEMT 5465G→A and PEMT -744G→C have emerged as functional SNPs, those that affect protein activity and possibly choline requirements and health outcomes. The PEMT 5465G→A polymorphism results in a valine to methionine substitution at position 175, causing a loss of function, and an increased risk of nonalcoholic fatty liver disease (18). The PEMT -744G→C is in the promoter region of the gene, approximately 50 base pairs within the estrogen response element. The -744G→C may affect gene expression and is associated with increased susceptibility to choline deficiency in women possibly through altered estrogen mediated induction of the PEMT gene (19,20).

Another polymorphism with links to choline metabolism is the 1958G→A SNP in the methylenetetrahydrofolate dehydrogenase (MTHFD1) gene. MTHFD1 is a tri-functional cytoplasmic folate metabolizing enzyme that catalyzes the interconversions of tetrahydrofolate (THF), 10-formyl-THF, 5,10-methenyl-THF, and 5-10 methylene-THF (Figure). The MTHFD1 1958A polymorphism resides in the 10-formylTHF synthetase domain and results in the substitution of an arginine for a glutamate at residue 653 in the protein product (21,22). In pre-menopausal consuming a choline deficient diet, carriers of the variant allele were 15 times more likely than non-carriers to develop choline deficiency (23). The MTHFD1 1958AA is also linked to increased risk of neural tube defects (21,22). It is hypothesized that the MTHFD1 1958G→A genetic variant reduces the production of 5,10-methylenetetrahydrofolate from 10-formyltetrahydrofolate which in turn may lower 5-methyl-THF and impair remethylation of homocysteine to methionine (22–24). Deficiencies in MTHFD1 may also reduce the biosynthesis of phosphatidylcholine and/or increase the demand for choline as a source of one- carbon units.

Folate intake/status may also influence choline metabolism/status. In pre-menopausal Mexican American women, plasma phosphatidylcholine declined in response to folate restriction (135 µg/d as dietary folate equivalents, DFE) and increased after folate treatment (800 µg DFE/d) (25). Notably these changes occurred under conditions of steady choline intake (349 mg/d) and were likely due to changes in the availability of folate derived one-carbon units required for the biosynthesis of phosphatidylcholine through the PEMT pathway. In addition to folate intake, methylenetetrahydrofolate reductase (MTHFR) C677T genotype is a strong genetic modifier of folate and homocysteine status (26) and may influence choline status (25). As an extension of this work, the present study examined the influence of the MTHFD1 and PEMT genetic polymorphisms on biomarkers of choline status in Mexican American women after 7 wk period of folate restriction and after a 7-wk period of folate treatment, alone and together with, the MTHFR C677T genotype.

METHODS

Subjects and Study Design

Healthy pre-menopausal women (n=43; 18–44 y) of self-reported Mexican descent pre-screened for the MTHFR C677T (14CC, 12CT and 17TT) genotype were included in this study. Additional inclusion criteria were non-smoker, non-anemic, non-supplement users, no chronic drug use, no alcohol consumption, no anti-folate medication use, no history of chronic disease such as diabetes or cardiovascular disease, non-pregnant and not planning a pregnancy, non-lactating and a normal blood chemistry profile. Anthropometric data (i.e., height and weight) were also obtained during the screening phase and women with a body mass index (BMI; kg/m²) > 32 were excluded from the study. The women were recruited from Pomona, California and surrounding areas from 2000 through 2001. The screening and experimental procedures were reviewed and approved by the Cal Poly Pomona University Institutional Review Board for human subjects and informed consent was obtained from each study participant.

This was a 14-wk controlled feeding study originally designed to assess the influence of the MTHFR C677T on folate requirements (26) and subsequently on choline status (25). The controlled nature of the original study facilitated the exploration of the effect of additional SNPs on biomarkers of choline status in these women; the primary objective of the present study. For the first 7-wk, all study participants consumed a folate restricted diet providing 135 µg DFE/d followed by randomization to folate treatment with 400 or 800 µg DFE/d for 7-wk (26). Throughout the study, choline intake remained constant at 349 mg/d derived from the diet (149 mg/d) as well as supplement (350 mg/d given every other day).

A five day rotation menu providing 174 mg/d choline, 112 mg/d betaine and 135 µg/d food folate was used throughout the 14-wk study as previously described (25,26). The folate content of the diet was measured via the microbiological assay (27) after trienzyme extraction (28, 29). The choline and betaine content of the diet were measured by liquid chromatography-mass spectrometer after phenol chloroform extraction (30). The diet provided 2110 kilocalories/d derived from carbohydrate (60%), fat (30%) and protein (10%) as estimated by the use of ESHA Food Processor Nutrient Database (version 7.81; 2001, ESHA Research Salem, OR).

Subjects were given supplemental choline (350 mg; Twin Lab[®], Twin Laboratories, Ronkonkoma, NY) every other day to provide total choline intakes (food + supplement) of 349 mg/d. Beginning wk 8, subjects consumed either 156 or 391 µg/d folic acid to achieve total folate intakes of 400 or 800 µg DFE/d. All other nutrients were provided in recommended amounts by the controlled diet or in combination with supplements as previously described (26).

The study participants were provided all of their meals (n=3/d) and snacks (n=1/d) throughout the 14 wk study and were required to consume breakfast and dinner in the metabolic kitchen housed within the Human Nutrition and Food Science Department at Cal Poly Pomona University seven days per week. Lunch and snacks were provided as take-aways and the subjects were instructed to consume all of the requisite foods. Soda and desserts, low in folate/choline, were optional. The supplements were taken with breakfast under the supervision of the investigators. Weight was monitored weekly and any deviation of $\pm 5\%$ from baseline was addressed by modifying energy intake with folate- and choline-free items. Compliance to the dietary regimen was monitored by measuring serum folate concentrations at weekly intervals throughout the study (26). No subject was found to be non-compliant.

Fasting (10h) venous blood samples were collected in EDTA tubes (Vacutainer; Beckton Dickinson, Rutherford, NY) at baseline and weekly thereafter. Plasma was obtained from EDTA blood that was immediately placed on ice and centrifuged within 1-h of the blood draw at $1800 \times g$ for 15 minutes at 4°C . For genotyping purposes, the buffy coat layer representing peripheral leukocytes ($\sim 500 \mu\text{L}$) was removed, dispensed into 1.5 mL cryostat tubes containing $50 \mu\text{L}$ dimethyl sulfoxide (DMSO, Sigma Chemical, St. Louis, MO), mixed by inversion and frozen at -80°C . For choline and homocysteine measurements, plasma was dispensed into 1.5 mL cryostat tubes and stored at -80°C .

Analytical Methods

Choline and its metabolites were determined in plasma via liquid chromatography-mass spectrometry (30) with modifications based on the instrumentation used (25). Plasma total homocysteine (tHcy) was measured with a fluorometric detector after separation from other metabolites by high performance liquid chromatography (31,32). DNA for genotyping was extracted from leukocytes using a QIAmp[®] DNA Blood Mini Kit (QIAGEN, Valencia, CA). Genotyping for PEMT G5465A (rs7946) was performed using a fluorescent TaqMan[®] probe commercially available kit (Applied Biosystems, Foster City, CA). Results were read with a real time polymerase chain reaction (PCR) Chromo 4 (Bio Rad, Hercules, CA) following the manufacturer's protocol. Genotyping for PEMT G-744C (rs12325817) was performed using DNA sequencing on double-stranded DNA templates obtained from genomic DNA PCR amplification. PCR products were purified with QIAquick[®] PCR Purification Kit 250 (QIAGEN). Sequencing reactions were performed by the Beckman Research Institute at the City of Hope (Duarte, CA) using a capillary sequencing machine (Applied Biosystems) and results were interpreted using the Chromatograph Viewer program (Applied Biosystems). Genotyping for MTHFD1 G1958A (rs2236225) was performed using a restriction fragment length polymorphism assay as described by Hol et al. (21). The digested PCR fragments were analyzed by ethidium bromide-stained 2% agarose gel electrophoresis.

Statistical Analysis

The a priori sample size of the original study was calculated based on MTHFR C677T genotype (26); not the genetic variants under investigation in the present study. As a result, the n for some of the genotypes was small (i.e., n=3). This was particularly true after randomization to 400 or 800 μg DFE/d; thus the 400 and 800 μg DFE/d treatment groups were combined. To test for baseline differences (wk 0) in the dependent variables (i.e. age, BMI, choline, homocysteine) between the various genotypes within each gene (i.e., PEMT G5465A), a one-way analysis of variance was performed. To assess the effect of the genotype on the response of the dependent variable, a General Linear Model (GLM) was employed using the differences between the weeks (i.e., wk 7 – wk 0 for folate restriction or wk 14 – wk 7 for folate treatment) as the response. In addition to the genetic variant of interest, MTHFR C677T genotype and the baseline measure (i.e., wk 0 for folate restriction or wk 7 for folate treatment) were included as independent variables. All interactions were tested in the model. The GLM procedure was

followed by post-hoc analyses with adjustments for multiple comparisons. For the PEMT G5465A genotype, carriers of the G allele were combined due to the small number of women with the 5465GG genotype. All analyses were done using SPSS for Windows (version 14, 2006, SPSS Inc, Chicago, IL). Differences were considered to be significant at $P < 0.05$ whereas $P \leq 0.1$ was indicative of trends. Data are presented as mean $\pm$ standard error of the mean for all dependent variables.

RESULTS AND DISCUSSION

The mean age and BMI of the women were 25y (range 18–44y) and 25.2 kg/m² (range = 19.5–32), respectively. All study participants had normal liver function based on measurements of serum concentrations of alanine aminotransferase and aspartate aminotransferase including the two participants whose BMI was 30 or higher. The genotype frequencies were 3GG, 19GA, and 21AA for PEMT G5465A; 9GG, 17GC and 17CC for PEMT G-744C; and 9GG, 21GA and 13AA for MTHFD1 G1958A and were in accordance with the Hardy-Weinberg laws of equilibrium, indicating that no selective mechanisms for a specific genotype existed. At baseline, no differences ($P > 0.05$) in the dependent variables were detected among the various genotypes.

The PEMT and MTHFD1 genetic variants had subtle effects on some of the measured biomarkers when folate was restricted; effects that were independent ($P > 0.4$) of the MTHFR C677T genotype (Table). Specifically, homozygosity for the PEMT 5465G→A genetic variant (i.e., 5465AA genotype) was associated with a greater increase ($P = 0.001$) in plasma tHcy concentrations relative to G allele carriers; an effect opposite our initial hypothesis which predicted that PEMT deficiency (i.e., the PEMT 5465AA genotype) would decrease the production of homocysteine (15) and thus attenuate the rise in homocysteine. Similarly, the PEMT-744CC genotype attenuated ($P < 0.049$) the decline in plasma phosphatidylcholine although it was predicted to accelerate it. These findings may have occurred by chance. However, an alternative explanation is that the presence of a deleterious SNP changes the metabolic flux of relevant pathways in order to minimize damage. For example, in mice, diminished PEMT activity leads to upregulation of the CDP-choline pathway (33), an alternate pathway for the synthesis of phosphatidylcholine. Thus, when faced with a metabolic insult that impairs PEMT activity (i.e., folate restriction), persons with the PEMT -744CC genotype may be less affected, compared to those with the -744GG genotype, due to their greater dependency on the CDP-choline pathway for phosphatidylcholine biosynthesis. As in mice, the adverse effects of PEMT deficiency may only be observed when choline intake is also restricted (34) or when the demand for phosphatidylcholine is increased by a high fat diet (35).

Homozygosity for the MTHFD1 1958G→A genetic variant (i.e., 1958AA genotype) resulted in a greater increase ($P = 0.086$) in plasma tHcy relative to the 1958GA ($P = 0.033$) and 1958GG ($P = 0.085$) genotypes. These data are consistent with the hypothesis that the MTHFD1 1958G→A SNP reduces the production of 5,10-methylenetetrahydrofolate from 10-formyltetrahydrofolate which in turn may lower 5-methyl-THF and impair remethylation of homocysteine to methionine (22–24).

The response of betaine and choline to folate restriction was not modified ($P > 0.1$) by the PEMT or MTHFD1 genotypes; nor were any of the measured choline status biomarkers modified ($P > 0.1$) by the SNPs under investigation during folate treatment (400 and 800 μg DFE/d; data not shown). The later finding suggests that folate intake may be an important modifier of the influence of these choline-relevant SNPs and is consistent with the intermingling of the folate and choline metabolic pathways.

A limitation of this controlled feeding study was the small sample size. Biomarkers of choline status (i.e., plasma choline, betaine, and phosphatidylcholine) appear to have particularly large between-subject variability even when choline and the intake of other nutrients are tightly controlled. This between subject variability may be related to individual differences in feedback mechanisms that control the endogenous biosynthesis of choline. As such, it is likely that this study was underpowered to detect additional meaningful effects that these genetic variants may have on parameters of choline and one-carbon metabolism. Another limitation was the combining of the 400 and 800 µg DFE/d treatment groups which abolished the ability to investigate the influence of the genetic variants on the background of moderate (400 µg DFE/d) and high (800 µg DFE/d) folate intake levels. Additionally, these data may not be germane to other ethnic/racial groups, other age categories, and the male gender; and it is possible that inclusion of food/beverages were consumed outside of those provided as the women were not housed in a metabolic unit (i.e., they were free-living).

CONCLUSIONS

Knowledge of interactions between genetic variants and select nutrients offers the potential to customize dietary recommendations at the individual level (i.e., personalized nutrition; 36,37) and to ensure that population based dietary recommendations are sufficient for those with the highest needs. Overall, these data support the notion that the PEMT genetic variants, 5465G→A and -744G→C, as well as the MTHFD1 1958G→A genetic variant, are functional SNPs and thus may alter requirements for choline (as well as folate). Although the effects on the measured choline status biomarkers for these genetic variants were modest, greater effects may be observed in populations with lower levels of choline intake and in those with higher demands for choline (i.e., pregnant women; 12,13). Additional studies with larger sample sizes are needed to confirm these findings and to examine the influence of these polymorphisms in populations with habitual low choline/folate intakes and in those with high demands for choline. Until such research has been done, Registered Dietitians should continue to promote achievement of the recommended dietary intake levels for choline and folate as established by the IOM (1).

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Figure 1.

The roles of methylenetetrahydrofolate dehydrogenase (MTHFD1) and phosphatidylethanolamine *N*-methyltransferase (PEMT) in folate, homocysteine and choline metabolism. MTHFD1 plays an indirect role in homocysteine and choline metabolism by providing some of the 5,10-methylene-tetrahydrofolate (THF) that may be channeled toward homocysteine metabolism by 5,10-methyleneTHF reductase (MTHFR). PEMT catalyzes the methylation of phosphatidylethanolamine utilizing S-adenosylmethionine as the methyl donor to produce phosphatidylcholine and S-adenosylhomocysteine (from which homocysteine is derived). Homocysteine may be remethylated by methionine synthase (MS, predominant pathway) or by betaine homocysteine *N*-methyltransferase (BHMT).

Plasma concentrations ($\mu\text{mol/L}$) of total homocysteine and phosphatidylcholine in pre-menopausal women ($n=43$) stratified by phosphatidylethanolamine *N*-methyltransferase (PEMT) G5465A and G-744C genotypes as well as methylenetetrahydrofolate dehydrogenase (MTHFD1) G1958A genotype at baseline (Wk 0) and after 7-wk of folate restriction (Wk 7)^{a-c}

Table

Gene	Metabolite					
	Homocysteine			Phosphatidylcholine		
	Wk 0	Wk 7	Change	Wk 0	Wk 7	Change
PEMT ^{G5465A}						
GG, n=3	5.1 $\pm$ 0.3	6.4 $\pm$ 0.5	1.3 $\pm$ 0.3 ^{yz}	1863 $\pm$ 89	1680 $\pm$ 137	-183 $\pm$ 224
GA, n=19	5.6 $\pm$ 0.2	6.9 $\pm$ 0.2	1.3 $\pm$ 0.1 ^{y**}	1866 $\pm$ 90	1712 $\pm$ 88	-154 $\pm$ 38
AA, n=21	5.5 $\pm$ 0.2	7.6 $\pm$ 0.3	2.1 $\pm$ 0.2 ^z	1821 $\pm$ 80	1703 $\pm$ 66	-117 $\pm$ 50
PEMT ^{G-744C}						
GG, n=9	5.3 $\pm$ 0.3	7.3 $\pm$ 0.4	2.0 $\pm$ 0.3	1863 $\pm$ 133	1660 $\pm$ 103	-203 $\pm$ 73 ^{y*}
GC, n=17	5.8 $\pm$ 0.2	7.6 $\pm$ 0.3	1.8 $\pm$ 0.2	1783 $\pm$ 86	1620 $\pm$ 77	-163 $\pm$ 59 ^{y+}
CC, n=17	5.4 $\pm$ 0.1	6.8 $\pm$ 0.3	1.4 $\pm$ 0.2	1895 $\pm$ 89	1814 $\pm$ 83	-80 $\pm$ 40 ^z
MTHFD1 ^{G1958A}						
GG, n=9	5.5 $\pm$ 0.2	7.0 $\pm$ 0.3	1.5 $\pm$ 0.2 ^{y+}	1679 $\pm$ 104	1597 $\pm$ 102	-82 $\pm$ 72
GA, n=21	5.6 $\pm$ 0.2	7.1 $\pm$ 0.3	1.6 $\pm$ 0.2 ^{y*}	1930 $\pm$ 85	1756 $\pm$ 82	-174 $\pm$ 51
AA, n=13	5.5 $\pm$ 0.3	7.5 $\pm$ 0.4	2.0 $\pm$ 0.3 ^z	1818 $\pm$ 92	1699 $\pm$ 73	-119 $\pm$ 45

^aData are presented as mean $\pm$ standard error of the mean.

^bThe women consumed 350 mg/d of choline throughout the study.

^cThe data were analyzed using the general linear model procedure with the difference (ie, change) between wk 0 and wk 7 for folate restriction and wk 7 and 14 for folate treatment (data not shown) as the response variable. The independent variables included PEMT G5465A genotype, MTHFR C677T genotype and baseline measures (ie., wk 0 for folate restriction). All possible interactions were included in the model and post-hoc analyses with multiple comparisons were employed for the purposes of mean separation. For the PEMT G5465A genotype, an additional analysis was performed after combining the carriers of the G allele. Values in a column with different superscript letters are indicative of differences.

* for $P<0.05$,

** for $P<0.01$; or trends,

⁺ $P<0.1$, relative to homozygous variant genotype. For PEMT G5465A, differences ($P=0.001$) were detected between carriers of the G allele and the AA genotype.