Published in final edited form as:
Am J Med Genet A. 2006 April 1; 140(7): 785–789. doi:10.1002/ajmg.a.31142.

Phosphatidylethanolamine *N*-methyltransferase (*PEMT*) Gene Polymorphisms and Risk of Spina Bifida

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To the Editor

Recently, our group demonstrated a reduction in risk of neural tube defects (NTDs) in infants of mothers who had elevated intakes of dietary choline [Shaw et al., 2004]. We hypothesized that disturbance in choline metabolism that are secondary to either a choline deficiency, or choline pathway genes mutations, may represent risk factors for NTDs. Evidence to further support this hypothesis is derived from in vitro studies. These studies showed that inhibition of choline uptake and metabolism during murine neurulation result in growth retardation and developmental defects, including an increase in the prevalence of NTDs in mouse embryos [Fisher et al., 2001, 2002]. Choline or its derivatives, such as acetylcholine, betaine, phosphocholine, phosphatidylcholine (PtdCho), and sphingomyelin, are nutrients critical to many cellular processes. These include the maintenance of the structural integrity of cell membranes, phospholipid biosynthesis, cholinergic neurotransmission, transmembrane signaling, and lipid-cholesterol transport and metabolism. Choline is also one of the major sources of methyl groups in the diet and has essential roles in methyl-metabolism [Zeisel and Blusztajn, 1994]. The phosphatidylethanolamine *N*-methyltransferase (PEMT, EC 2.1.1.17) pathway contributes approximately one-third of the synthesis of PtdCho, which is the most abundant mammalian phospholipids [Walkey et al., 1999; Reo et al., 2002]. In the absence of dietary supplementation, the PEMT pathway provides the only de novo biosynthesis of choline. This reaction requires *S*-adenosylmethionine (SAM) as the methyl donor [Bremer and Greenberg, 1961], generating *S*-adenosylhomocysteine (SAH), which is subsequently hydrolyzed yielding adenosine and homocysteine (Hcy) [Finkelstein, 1998]. In mice, *PEMT* expression enhances not only plasma Hcy levels, but also Hcy secretion from hepatocytes [Schneider and Vance, 1978]. Hyperhomocysteinemia has been identified as a risk factor for NTDs [Rosenquist and Finnell, 2001]. Knowing the regulatory role of *PEMT* gene in choline metabolism and ultimately in determining Hcy levels, we examined two non-synonymous SNPs, rs7946 (Met212Val) and rs897453 (Val95Ile). We investigated whether *PEMT* gene variants would disturb *PEMT* function, rendering affected individuals more susceptible to spina bifida due to their increased dietary requirements for choline and the dysregulation of Hcy homeostasis. Our analytic strategy investigated potential spina bifida risks associated with *PEMT* gene polymorphisms in a population-based case-control study.

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Data were derived from the California Birth Defects Monitoring Program (CBDMP), a population-based active surveillance system for collecting information on infants and fetuses with congenital malformations [Croen et al., 1991; Schulman and Hahn, 1993]. Included for the study were 360 infants with spina bifida (cases) and 595 non-malformed infants (controls). A subset of samples was derived from a dataset that included detailed maternal interview information on important covariates such as maternal periconceptional vitamin use and choline intake [Shaw et al., 2004]. All samples were obtained with approval from the State of California Health and Welfare Agency Committee for the Protection of Human Subjects. Genomic DNA used for genotyping offspring was collected from newborn screening blood spots and extracted according to the Puregene Genomic DNA Extraction kit (Gentra, Minneapolis, MN) protocol. Two non-synonymous SNPs, Met212-Val (rs7946, A → G) and Val95Ile (rs897453, G → A), were genotyped using a fluorescence-based allele discrimination assay on an ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) following the manufacturer's SNP genotyping protocol. Results were read and interpreted blind to case/control status, and as a measure of confirmation, each assay was done in duplicate. Odds ratios and 95% confidence intervals (CI) were used to estimate risks. These measures were calculated using SAS software (version 9.1). Individuals with wild-type genotypes were considered referents for risk estimation of individuals with heterozygous and homozygous mutant genotypes. Analyses were performed for the overall study group as well as for specific race/ethnic strata, non-Hispanic whites or Hispanic whites. The subset of cases and controls for which maternal interview data were available were used for analyses of possible interactions between *PEMT* genotypes and maternal dietary choline intake. Deviation from Hardy–Weinberg Equilibrium among control infants was evaluated by a chi-square test.

With respect to the *PEMT* Met212Val genotype, 338 (93.9%) case infants and 554 (93.1%) control infants were successfully genotyped. For *PEMT* Val75Ile, 350 (97.2%) case infants and 566 (95.1%) control infants were successfully genotyped. *PEMT* genotype frequencies among cases and controls are displayed in Tables I and II. A deviation from Hardy–Weinberg Equilibrium was detected in the control group for *PEMT* Met212Val ($\chi^2_{df=1}=10.39$, $P<0.05$). This deviation was eliminated when genotypes of non-Hispanic white and Hispanics were analyzed separately. Among the 356 cases, 37.1% were non-Hispanic whites, 51.2% were Hispanics, and 11.8% were of another race/ethnic background. Among the 593 controls, 49.7% were non-Hispanic whites, 35.9% were Hispanics, and 14.3% were of another race/ethnic background. The minor allele frequency (MAF) for Met212Val was 26.5% in non-Hispanic whites and 40.5% in Hispanics; MAF for Val95Ile was 46.3% in non-Hispanic whites and 29.8% in Hispanics. Neither of the two SNPs, when analyzed separately, was associated with increased risks for spina bifida in the overall population, nor among the two major ethnic groups (Hispanic whites vs. non-Hispanic whites) (Tables I and II). Although the overall study population size permitted adequate statistical power (beta = 0.80, alpha = 0.05, two-sided) to identify even modest increased risks such as 1.6 or more, the statistical power associated with analyses of specific race/ethnic groups was somewhat reduced.

From the study population, a total of 330 cases (91.7%) and 541 controls (90.9%) had compound genotype data available. Odds ratios for the overall study population as well as those for specific maternal race/ethnic groups based on compound genotypes are provided in Table III. Among controls, the two most common compound genotypes were Met212/Met212 + Val95/Ile95 (22.2%), and the double heterozygotes Met212/Val212 + Val95/Ile95 (20.0%). The observed frequencies of these two compound genotypes among non-Hispanic whites were 28.1% and 22.6%, respectively. For the Hispanic control samples, the most common compound genotype was Met212/Val212 + Val95/Val95 (21.9%), followed by the Met212/Met212 + Val95/Ile95 (18.9%) genotype. The frequency of double homozygotes for the more common alleles, Met212/Met212 + Val95/Val95, was higher among cases than in controls in the overall combined population (14.5% vs. 8.9%). This was also observed among the non-Hispanic white

(11.3% vs. 8.5%) and Hispanic (17.3% vs 10.7%) populations. Compared to the "wild-type" genotypes, a few compound genotypes had lower NTD risks (OR = 0.55, 95%CI: 0.33 ~ 0.91 for Met212/Met212 + Val95/Ile95; OR = 0.52, 95%CI: 0.29 ~ 0.92 for Met212/Met212 + Ile95/Ile95; OR = 0.55, 95%CI: 0.33 ~ 0.93 for Met212/Val212 + Val95/Val95; OR = 0.46, 95%CI: 0.26 ~ 0.81 for Val212/Val212 + Val95/Val95) (Table III). Interestingly, we observed a reduced spina bifida risk associated with several compound genotypes when compared to the double wild-type homozygotes, while each SNP showed no significant association with spina bifida when analyzed separately.

For the subset of subjects whose maternal dietary choline intake data were available, we investigated a possible interaction between maternal dietary choline intake and *PEMT* genotypes. The genotype frequencies in this subset of cases and controls were comparable to those calculated from all study subjects. ORs for infants whose mothers had lower choline intakes (Table IV) did not appear to be substantially different than ORs for infants whose mothers had higher choline intake, therefore we failed to detect an interaction between maternal dietary choline intake and *PEMT* genotypes as modifiers of NTD risk. However, data were sparse for many of these comparisons resulting in imprecise risk estimates.

To our knowledge this is the first epidemiology study to investigate these potential associations. The observation of a reduction in risk associated with a compound genotype, which was not influenced by maternal choline intake, is difficult to interpret. As we recently demonstrated, the mothers' dietary intake of choline was associated with reduced offspring risks for all NTDs, as well as for spina bifida and anencephaly, separately [Shaw et al., 2004]. It has been consistently reported that pregnant women with elevated Hcy levels have an increased risk of having an NTD affected child [Mills et al., 1995]. It is well appreciated that plasma concentrations of Hcy are partially dependent on the function of PEMT, therefore polymorphisms in the *PEMT* gene may serve as genetic modifiers of plasma Hcy levels and risk for NTDs. By searching the NCBI Conserved Domain Database, it was suggested that the Met212-Val polymorphism is situated at a site quite close to the putative AdoMet (SAM) binding motif [Shields et al., 2003]. This site has been highly conserved throughout evolution, speaking to its critical regulatory importance. Haplotype combination studies initially indicated that the combined effects of the *PEMT* Met212Val and Val95Ile polymorphisms might contribute to an abnormal functioning of the *PEMT* gene, which eventually contributes to a failure of neural tube closure in some as yet undetermined fashion. Unlike the genes coding for the enzymes cystathionine beta-synthase (*CBS*), methionine synthase reductase (*MTR*), 5,10-methylenetetrahy-drofolate reductase (*MTHFR*), which are directly involved in folate-Hcy metabolism, the PEMT metabolite profile may be modulated, as its effect is transmitted through the choline pathway. It will be important in future studies to determine the relationship of the polymorphisms to prenatal Hcy levels, and the impact that choline supplementation has on NTD risk. Moreover, studies of additional SNPs within the *PEMT* gene, which may be in linkage disequilibrium with the SNPs we studied, as well as gene variations in other related genes such as other genes in the choline metabolism pathway, choline kinase (*CHK*) and CTP: phosphocholine cytidylyl-transferase (*PCYT1*), should be investigated as potential predictors of increased Hcy levels and risks of birth defects.

Acknowledgments

Grant sponsor: Centers for Disease Control and Prevention, Center of Excellence; Grant number: U50/CCU913241.

The authors are indebted to Dr. George Cunningham, Dr. Fred Lorey, Terry Kennedy, and John Arnopp for making it possible to access newborn blood specimens. We also appreciate the technical support of Ms. Sarah Seth and Ms. Consuelo Valdes.

Abbreviations used

AdoHcy (SAH)	*S*-Adenosylhomocysteine
AdoMet (SAM)	*S*-Adenosylmethionine
CDP-choline	cytidine diphosphocholine
Hcy	homocysteine
NTDs	neural tube defects
PtdCho	phosphatidylcholine
PtdEtn	phosphatidylethanolamine
PEMT	phosphatidylethanolamine *N*-methyltransferase

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TABLE I

PEMT Met212Val Genotype and Risk of Spina Bifida

Genotype	Cases (%)	Controls (%)	OR (95% CI)
Overall			
Met212/Met212	150 (44.4)	240 (43.3)	Referent
Met212/Val212	144 (42.6)	222 (40.1)	1.04[a] (0.78, 1.39)
Val212/Val212	44 (13.0)	92 (16.6)	0.77[a] (0.51, 1.16)
Non-Hispanic white			
Met212/Met212	63 (50.4)	148 (53.8)	Referent
Met212/Val212	55 (44.0)	108 (39.3)	1.20 (0.77–1.86)
Val212/Val212	7 (5.6)	19 (6.9)	0.87 (0.35–2.16)
Hispanic white			
Met212/Met212	69 (39.9)	77 (38.5)	Referent
Met212/Val212	75 (43.3)	84 (42.0)	1.0 (0.64–1.56)
Val212/Val212	29 (16.8)	39 (19.5)	0.83 (0.46–1.48)

[a]Odds ratio adjusted to ethnicity group.

TABLE II

PEMT Val75Ile Genotype and Risk of Spina Bifida

Genotype	Cases (%)	Controls (%)	OR (95% CI)
Overall			
Val75/Val75	146 (41.7)	234 (41.3)	Referent
Val75/Ile75	158 (45.1)	250 (44.2)	1.01[a] (0.76, 1.35)
Ile75/Ile75	46 (13.1)	82 (14.5)	0.90[a] (0.59, 1.36)
Non-Hispanic white			
Val75/Val75	38 (29.2)	78 (27.6)	Referent
Val75/Ile75	65 (50.0)	148 (52.3)	0.90 (0.56, 1.46)
Ile75/Ile75	27 (20.8)	57 (20.1)	0.97 (0.53, 1.77)
Hispanic white			
Val75/Val75	88 (50.0)	104 (51.2)	Referent
Val75/Ile75	75 (42.6)	77 (37.9)	1.15 (0.75, 1.76)
Ile75/Ile75	13 (7.4)	22 (10.8)	0.69 (0.33, 1.47)

[a]Odds ratio adjusted to ethnicity group.

TABLE III

PEMT Met212Val and Val95Ile Compound Genotype, Maternal Ethnicity, and Risk of Spina Bifida

Combined genotype	Cases (%)	Control (%)	OR (95% CI)
Overall			
Met212/Met212 + Val95/Val 95	48 (14.5)	48 (8.9)	Referent
Met212/Met212 + Val95/Ile95	66 (20.0)	120 (22.2)	**0.55 (0.33–0.91)**[a]
Met212/Met212 + Ile95/Ile95	34 (10.3)	66 (12.2)	**0.52 (0.29–0.92)**[a]
Met212/Val212 + Val95/Val95	54 (16.4)	98 (18.1)	**0.55 (0.33–0.93)**[a]
Met212/Val212 + Val95/Ile 95	75 (22.7)	108 (20.0)	0.69 (0.42–1.14)
Met212/Val212 + Ile95/Ile95	10 (3.0)	11 (2.0)	0.91 (0.35–2.34)
Val212/Val212 + Val95/Val95	35 (10.6)	76 (14.0)	**0.46 (0.26–0.81)**[a]
Val212/Val212 + Val95/Ile95	8 (2.4)	14 (2.6)	0.57 (0.22–1.49)
Val212/Val212 + Ile/95/Ile95	0	0	—
Non-Hispanic white			
Met212/Met212 + Val95/Val95	14 (11.3)	23 (8.5)	Referent
Met212/Met212 + Val95/Ile95	31 (25.0)	76 (28.1)	0.67 (0.31–1.47)
Met212/Met212 + Ile95/Ile95	18 (14.5)	45 (16.7)	0.66 (0.28–1.55)
Met212/Val212 + Val95/Val95	17 (13.7)	39 (14.4)	0.72 (0.30–1.72)
Met212/Val212 + Val95/Ile95	29 (23.4)	61 (22.6)	0.78 (0.35–1.74)
Met212/Val212 + Ile95/Ile95	8 (6.5)	7 (2.6)	1.88 (0.56–6.31)
Val212/Val212 + Val95/Val95	5 (4.0)	13 (4.8)	0.63 (0.19–2.16)
Val212/Val212 + Val95/Ile95	2 (1.6)	6 (2.2)	—
Val212/Val212 + Ile/95/Ile95	0	0	—
Hispanic white			
Met212/Met212 + Val95/Val95	29 (17.3)	21 (10.7)	Referent
Met212/Met212 + Val95/Ile95	28 (16.7)	37 (18.9)	0.55 (0.26–1.16)
Met212/Met212 + Ile95/Ile95	10 (6.0)	19 (9.7)	**0.38 (0.15–0.99)**[a]
Met212/Val212 + Val95/Val95	34 (20.2)	43 (21.9)	0.57 (0.28–1.18)
Met212/Val212 + Val95/Ile95	37 (22.0)	35 (17.9)	0.77 (0.37–1.58)
Met212/Val212 + Ile95/Ile95	2 (1.2)	3 (1.5)	—
Val212/Val212 + Val95/Val95	22 (13.1)	35 (17.9)	**0.45 (0.21–0.99)**[a]
Val212/Val212 + Val95/Ile95	6 (3.6)	3 (1.5)	—
Val212/Val212 + Ile/95/Ile95	0	0	—

[a]Confidence interval of OR does not contain 1.0.

TABLE IV

PEMT Met212Val and Val95Ile Compound Genotype, Maternal Choline Intake, and Risk of Spina Bifida

Combined genotype	Cases (%)	Control (%)	OR (95% CI)
Overall			
Met212/Met212 + Val95/Val95	10 (10.3)	30 (9.1)	Referent
Met212/Met212 + Val95/Ile95	18 (18.6)	73 (22.2)	0.74 (0.31–1.79)
Met212/Met212 + Ile95/Ile95	10 (10.3)	37 (11.3)	0.81 (0.30–2.20)
Met212/Val212 + Val95/Val95	18 (18.6)	64 (19.5)	0.84 (0.35–2.05)
Met212/Val212 + Val95/Ile95	25 (25.8)	71 (21.6)	1.06 (0.45–2.47)
Met212/Val212 + Ile95/Ile95	6 (6.2)	5 (1.5)	3.60 (0.90–14.39)
Val212/Val212 + Val95/Val95	9 (9.3)	40 (12.2)	0.68 (0.24–1.87)
Val212/Val212 + Val95/Ile95	1 (1.0)	9 (2.7)	0.33 (0.04–2.97)
Val212/Val212 + Ile/95/Ile95	0	0	—
Lower 25% percentile			
Met212/Met212 + Val95/Val95	4 (12.9)	9 (11.0)	Referent
Met212/Met212 + Val95/Ile95	6 (19.4)	18 (22.0)	0.75 (0.17–3.35)
Met212/Met212 + Ile95/Ile95	2 (6.5)	9 (11.0)	0.50 (0.07–3.45)
Met212/Val212 + Val95/Val95	6 (19.4)	17 (20.7)	0.79 (0.18–3.56)
Met212/Val212 + Val95/Ile95	6 (19.4)	17 (20.7)	0.79 (0.18–3.56)
Met212/Val212 + Ile95/Ile95	3 (9.7)	1 (1.2)	6.75 (0.53–86.56)
Val212/Val212 + Val95/Val95	4 (12.9)	9 (11.0)	1.00 (0.19–5.29)
Val212/Val212 + Val95/Ile95	0	2 (2.4)	—
Val212/Val212 + Ile/95/Ile95	0	0	—
Higher 25% percentile			
Met212/Met212 + Val95/Val95	6 (9.1)	21 (8.5)	Referent
Met212/Met212 + Val95/Ile95	12 (18.2)	55 (22.3)	0.76 (0.25–2.30)
Met212/Met212 + Ile95/Ile95	8 (12.1)	28 (11.3)	1.00 (0.30–3.32)
Met212/Val212 + Val95/Val95	12 (18.2)	47 (19.0)	0.89 (0.30–2.70)
Met212/Val212 + Val95/Ile95	19 (28.8)	54 (21.9)	1.23 (0.43–3.51)
Met212/Val212 + Ile95/Ile95	3 (4.6)	4 (1.6)	2.63 (0.46–15.11)
Val212/Val212 + Val95/Val95	5 (7.6)	31 (12.6)	0.57 (0.15–2.09)
Val212/Val212 + Val95/Ile95	1 (1.5)	7 (2.8)	0.50 (0.05–4.90)
Val212/Val212 + Ile/95/Ile95	0	0	—
Val212/Val212 + Ile/95/Ile95	0	0	—