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Maternal and Infant Gene-Folate Interactions and the Risk of Neural Tube Defects

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Abstract

Neural tube defects (NTDs) are common, serious malformations with a complex etiology that suggests involvement of both genetic and environmental factors. The authors evaluated maternal or offspring folate-related gene variants and interactions between the gene variants and maternal intake of folates on the risk of NTDs in their offspring. A case-control study was conducted on mothers and/or their fetuses and infants who were born in California from 1999–2003 with an NTD (cases n = 222, including 24 mother-infant pairs) or without a major malformation (controls n = 454, including 186 mother-infant pairs). Maternal intake of folates was assessed by food frequency questionnaire and genotyping was performed on samples from mothers and infants. For mothers in the lowest folate-intake group, risk of NTDs in offspring was significantly decreased for maternal *MTHFR* SNPs rs1476413, rs1801131 and rs1801133 (odds ratio (OR) = 0.55, 80% confidence interval (CI): 0.20, 1.48; OR = 0.58, 80% CI: 0.24, 1.43; OR = 0.69, 80% CI: 0.41, 1.17, respectively), and *TYMS* SNPs rs502396 and rs699517 (OR= 0.91, 80% CI: 0.53, 1.56; OR = 0.70, 80% CI: 0.38, 1.29). A gene-only effect was observed for maternal *SHMT1* SNP rs669340 (OR = 0.69, 95% CI: 0.49, 0.96). When there was low maternal folate intake, risk of NTDs was significantly increased for infant *MTHFD1* SNPs rs2236224, rs2236225 and rs11627387 (OR = 1.58, 80% CI: 0.99, 2.51; OR = 1.53, 80% CI: 0.95, 2.47; OR = 4.25, 80% CI: 2.33, 7.75, respectively) and *SHMT1* SNP rs12939757 (OR = 2.01, 80% CI: 1.20, 3.37), but decreased for *TYMS* SNP rs2847153 (OR = 0.73, 80% CI: 0.37, 1.45). Although power to detect interaction effects was low for this birth defects association study, the gene-folate interactions observed in this study represent preliminary findings that will be useful for informing future studies on the complex etiology of NTDs.

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Keywords

Congenital Abnormalities; Folic Acid; Genetic Association Studies; Molecular Epidemiology; Neural Tube Defects; Maternal Nutritional Physiological Phenomena; Nervous System Malformations; Nutrigenomics

INTRODUCTION

Several studies have demonstrated that maternal periconceptional folic acid supplementation can reduce the risk of neural tube defects (NTDs) in offspring by up to 70% [Czeizel et al., 1992; MRC, 1991]. In addition, data from countries that have implemented mandatory folic acid fortification of the food supply (at about 0.2 mg/day) show a reduction in the prevalence of NTDs [Botto et al., 2006]. However, food fortification programs currently in place are estimated to prevent only about 9 out of every 100 folic acid-preventable NTDs [Bell et al., 2009]. Thus, elucidation of the mechanisms by which folate reduces the risk of NTDs could be informative for developing improved prevention strategies and reducing the global burden of NTDs.

Given the association between maternal folate status and NTD risk in offspring, recent studies of NTDs have mainly focused on candidate genes that are involved in folate-related pathways [Beaudin et al., 2009; Greene et al., 2009]. However, a specific folate-related genetic variant has not been identified, even as a modest contributor to NTD risk [Detrait et al., 2005]. To more fully investigate the role of folate and folate-related genes as they relate to risk of NTDs, consideration should be given to the possibility that: 1) risk may be mediated via the maternal genotype rather than, or in addition to, the infant genotype, and 2) folate-related genes that contribute to risk of NTDs may do so via interactions with other genes and/or maternal folate status [Mitchell, 1997; Weinberg et al., 1998; Wilcox et al., 1998]. However, few NTD studies have evaluated contributions from both maternal and infant genetic alleles and fewer still have considered allele-folate interactions [Shaw et al., 2002; Volcik et al., 2003a]. In this analysis, we investigated the hypotheses that risk of NTDs is associated with infant and/or maternal genotype for 31 allelic variants in five folate-related genes and those associations are modified by maternal folate intake.

METHODS

Study population

This study was based on data from a case-control study conducted in California, which has been described in detail elsewhere [Carmichael et al., 2010]. Cases included live births, stillbirths (fetal deaths at > 20 weeks gestation), and terminations with NTDs (anencephaly and spina bifida) delivered of mothers who resided in Los Angeles, Santa Clara, and San Francisco counties, from July 1999 to June 2003. Spina bifida included cases of lipomyelomeningocele, meningomyelocele, and myelocystocele. For controls, the study included a random sample of live born infants with no major structural malformations, selected from approximately 600,000 live births in the 3 study counties. Potential cases were identified from medical records by the California Birth Defects Monitoring Program (CBDMP), using stringent multiple source and population-based ascertainment approaches as previously described [Schulman et al., 1993]. All cases were reviewed by a medical geneticist to determine whether they met inclusion criteria. Infants diagnosed with single-gene or chromosomal disorders (based on information gathered from medical records) were ineligible. Mothers who had preexisting type I or type II diabetes (5 cases, 1 control) or who reported using seizure medication in the periconceptional interval (1 case, 1 control) were excluded.

Maternal interviews

Mothers were eligible to participate if they were not incarcerated and if their primary language was either English or Spanish. Interviews were conducted using a standardized, computer-based questionnaire, primarily by telephone, in English or Spanish. Women were asked to report on exposures and behaviors occurring two months before through two months after conception (the periconceptional interval), which encompasses the critical period for neural tube development. The interview elicited information on a variety of variables, including sociodemographic factors, a detailed dietary assessment and periconceptional use of folic acid-containing supplements as well as multivitamins containing folic acid. Usual maternal nutrient intake from foods was assessed using a modified version of the National Cancer Institute's Health Habits and History Questionnaire, a semi-quantitative food frequency questionnaire with demonstrated reliability and validity [Block et al., 1986; Block et al., 1990].

DNA collection

Previously collected DNA samples from cases and liveborn controls, as well as their mothers, were available from two sources: buccal brushes and dried newborn screening bloodspots. Samples for mothers of cases that were stillborn or terminated were collected by maternal buccal swab. DNA was not available for stillborn cases or cases that were terminated. Bloodspots were obtained both for infants when a buccal brush had not been acquired and for a subset of infants for whom buccal samples were also available. Bloodspot samples were selected via record matching between CBDMP and the California Genetic Disease Screening Program. Participants that had no DNA sample from either the mother or infant and/or inadequate maternal folate intake information were excluded from analyses (69 cases, 117 controls).

Genetic assays

Genomic DNA was extracted from dried bloodspots and buccal brushes using either protocol A (Puregene DNA Extraction Kit (Gentra, Minneapolis, MN)) or protocol B (NaOH extraction [Richards et al., 1993] along with the QIAquick® Purification kit (Qiagen, Valencia, CA)), depending upon sample type. All samples were quantified using the TaqMan® RNase P method (Applied Biosystems, Foster City, CA). Genotyping of DNA from buccal brush samples was performed on purified, unamplified genomic DNA. Isolated genomic DNA from bloodspots was amplified using GenomiPhi™ multiple displacement amplification according to the manufacturer's instructions (Amersham Biosciences, Sunnyvale, CA). Single nucleotide polymorphisms (SNPs) were assayed using TaqMan (Applied Biosystems, Foster City, CA) and genotypes were read and discriminated on the ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Genotyping was duplicated for all buccal brush samples and for a 5% subset of bloodspot samples for quality control. Three SNPs were excluded in Hispanic infants and one in non-Hispanic White infants due to a genotyping failure rate greater than 20%.

SNP selection

SNPs selected for study included: 1) those previously reported to be associated with NTDs in humans, 2) those resulting in changes in amino acids with functional effects (predicted or known), 3) those with minor allele frequencies (MAFs) ≥ 0.05 in both Hispanic and non-Hispanic White populations, and 4) those with an available genotyping ABI SNP Assay-on-Demand. In total, 31 SNPs from five candidate genes were selected: *DHFR* (rs1650723, rs1677693, rs11951910); *MTHFD1* (rs745686, rs8011839, rs1076991, rs1885031, rs1950902, rs2236224, rs2236225, rs3783728, rs11158542, rs11627387, rs11849530, rs34616731); *MTHFR* (rs1476413, rs1801131, rs1801133); *SHMT* (rs669340, rs1979277,

rs2168781, rs2273026, rs9909104, rs12939757, rs12952556); and *TYMS* (rs502396, rs699517, rs1001761, rs2847153, rs2847149, rs2847326).

To determine whether the distribution of genotypes deviated from expected Hardy-Weinberg equilibrium (HWE) frequencies, control mothers and infants were tested using a χ^2 goodness-of-fit test for each race/ethnicity group. When there was evidence that the genotypes for a SNP were not in HWE (χ^2 *P* value < 0.05) in a particular subgroup (e.g. Hispanic mothers), that subgroup was excluded from further analyses (Hispanic mothers (n=17 SNPs excluded); Hispanic infants (n=7 SNPs); non-Hispanic White mothers (n=3 SNPs) and non-Hispanic White infants from (n=7 SNPs).

The analytic subset was comprised of the 76% of cases and 80% of controls from the full study population that had both folate intake information as well as at least one DNA sample available. Of the 222 cases and 454 controls, there were 24 complete mother-infant case pairs and 186 mother-infant control pairs. The remaining observations available for our analytic subset were from infants only (97 cases, 233 controls) or mothers only (101 cases, 35 controls).

Statistical analyses

The association between NTD risk and genotype for each SNP, as well as interactions between each SNP genotype and maternal folate status, were evaluated using a linear (additive) risk model; this model assumes that the effect of the heterozygote genotype lies between the effects of the common and variant homozygote genotypes. The reference genotype in these estimations was chosen as the common homozygote. Given the relatively small number of mother-infant pairs with complete genotype data, these analyses were performed separately for the maternal and infant genotype. Race/ethnicity was included as a term in the models when data from both race/ethnicities were eligible for inclusion. Although not the main focus of this investigation, race/ethnicity and folate intake variables were included as main effects in all models based on their association with risk of NTDs.

Variables defining periconceptional use of folic acid-containing supplements (yes vs. no) and daily dietary maternal intake of folates (dietary folate equivalents) were combined to create a composite variable. Specifically, daily dietary intake in controls was used to establish quartiles of folate intake. The composite variable, “overall intake of folates,” was defined as “low” for women in the lowest quartile of maternal folate intakes (Q1 ≤ 309.49 μg/100g/day) or who did not take supplemental folic acid in the periconceptional period. All other combinations of folate intake (i.e. Q2, Q3 or Q4) and folic acid supplementation (i.e. yes/no) were defined as “high” for folate status.

Parameter estimates and their standard errors obtained from regression models were used to compute odds ratios (ORs) and confidence intervals (CIs). In addition, likelihood ratio tests (LRTs) comparing the full model (parameterized by maternal race/ethnicity, folate intake, genotype and the gene-folate cross term) and reduced models (parameter of interest is dropped from the full model) were used to test hypotheses regarding the significance of both interaction and main effects. If the interaction parameter LRT *P* value was ≥ 0.20, the interaction term was removed from the model and LRTs were carried out for main effects of genotype. The association between the genotype and NTD risk was considered to be significant when the LRT *P* value was < 0.05. Estimates from models with a significant interaction parameter have CIs calculated at 80%, corresponding to the 0.20 *P* value used to assess statistical significance; estimates from models with main effects alone have CIs calculated at 95%. For SNPs with significant interaction terms, “interaction effects” were calculated by exponentiating the sum of the gene, folate, and interaction beta coefficients. Since analyses employed the log-additive model, odds ratios represent the comparison

between heterozygotes and common homozygotes; the square of this OR estimates the log risk in variant homozygotes compared to common homozygotes.

RESULTS

The characteristics of case and control infants and mothers are summarized in Table I. Case infants were more likely to be female than were control infants. Compared to non-Hispanic White mothers, Hispanic mothers were younger at delivery (mean age 27.0 vs. 31.8); however, maternal age at delivery was consistent across cases and controls within maternal race/ethnicity. Case mothers had fewer years of formal education than control mothers, as did Hispanic mothers compared to non-Hispanic White mothers. Maternal periconceptional use of folic acid supplements was higher in non-Hispanic Whites than in Hispanic mothers; differences in daily dietary intake of folate and the composite folate intake variable across these subgroups were more subtle.

Information for each SNP, (chromosome, rs ID, polymorphism type, general genic location, minor allele, genotyping failure rates, genotype distribution and results of testing for HWE in control mothers and infants) is summarized in Supplementary eTable I (See Supporting Information online).

Results from logistic regression models are summarized in Table II. There was no evidence of a nonrandom association (for main or interaction effects) between NTD-affected pregnancies and either maternal or infant variants in *DHFR.*

MTHFD1

An approximate 1.5-fold increase in risk was observed in infant *MTHFD1* SNPs rs2236224 and rs2236225 for infants with the CT genotype in the lowest folate intake category compared to those with the CC genotype in the higher intake group ($OR_{[int\ CC\ vs.\ CT]}$ = 1.58, 80% CI: 0.99, 2.51 (LRT P = 0.07); $OR_{[int\ CC\ vs.\ CT]}$ = 1.53, 80% CI: 0.95, 2.47 (LRT P = 0.10), respectively; Table II). The infant AG genotype in *MTHFD1* SNP rs11627387 appeared to be associated with a four-fold increase in NTD risk when there was low maternal folate intake compared to infants with the GG genotype and high maternal folate intake ($OR_{[int\ GG\ vs.\ AG]}$ = 4.25, 80% CI: 2.33, 7.75 (LRT P < 0.05)).

MTHFR

Mothers had a decreased risk of an NTD-affected pregnancy with low intake of folates and *MTHFR* SNPs rs1476413, rs1801131 and rs1801133 in heterozygous compared to common homozygous mothers in the high folate intake group ($OR_{[int\ GG\ vs.\ AG]}$ = 0.55, 80% CI: 0.20, 1.48 (LRT P = 0.15); $OR_{[int\ AA\ vs.\ AC]}$ = 0.58, 80% CI: 0.24, 1.43 (LRT P = 0.08); $OR_{[int\ CC\ vs.\ CT]}$ = 0.69, 80% CI: 0.41, 1.17 (LRT P = 0.03), respectively).

SHMT1

A maternal gene-only effect for *SHMT1* SNP rs669340 CG appeared to be associated with a reduced risk of an NTD-affected pregnancy compared to mothers with the CC genotype ($OR_{[CC\ vs.\ CG]}$= 0.69, 95% CI: 0.49, 0.96; (LRT P = 0.03), Table II). An interaction effect was observed for risk of NTDs in infants with the *SHMT1* SNP rs12939757 AG genotype that were in the low folate intake group compared to infants with the AA genotype that were in the high folate intake group ($OR_{[int\ AA\ vs.\ AG]}$ = 2.01; 80% CI: 1.20, 3.37 (LRT P = 0.10)).

TYMS

The gene-folate interaction was associated with a reduced risk of NTDs for mothers in the low folate intake group with *TYMS* SNP rs502396 or rs699517 heterozygous genotype compared to the high folate intake group with the common homozygous genotype ($OR_{[int\ TT\ vs.\ CT]}$ = 0.91, 80% CI: 0.53, 1.56 (LRT P = 0.12); $OR_{[int\ CC\ vs.\ CT]}$ = 0.70, 80% CI: 0.38, 1.29 (LRT P = 0.19), Table II). The interaction effect in infants was also protective for risk for SNP rs2847153 ($OR_{[int\ GG\ vs.\ AG]}$ = 0.73, 80% CI: 0.37, 1.45 (LRT P = 0.14)).

DISCUSSION

In this study, 31 SNPs in five folate-related genes were evaluated to determine whether they were associated with the risk of NTDs. Both maternal and infant genotypes were evaluated for each SNP. These analyses provided modest evidence that ten SNPs in four genes were associated with the risk of NTDs via interactions with maternal folate status (*MTHFD1, MTHFR, SHMT1,* and *TYMS*), or via a gene-only association (n=1 SNP in *SHMT1*).

The *MTHFD1* SNP rs2236225 (G1958A; R653Q) is a functional exonic SNP that has been studied in several populations. The maternal and infant genotypes that include the rs2236225 A allele have been associated with increased risk of NTDs (ORs = 1.5–1.8) in some [Brody et al., 2002; De Marco et al., 2006; Parle-McDermott et al., 2006; Shaw et al., 2009], but not all [Doudney et al., 2009; Franke et al., 2009; van der Linden et al., 2007] studies. Although we did not identify an independent association between NTD risk and this variant, infant genotype for this variant and other *MTHFD1* variants (rs2236224, rs2236225 and rs11627387) may be associated with NTD risk via an interaction with maternal folate status.

The metabolic basis for the association between NTD risk and rs2236225 is unknown, as the SNP does not appear to modify markers of folate status (e.g., plasma or red blood cell folate or plasma homocysteine levels) [Brody et al., 2002; Carroll et al., 2009; Konrad et al., 2004]. However, recent *in vitro* studies have shown that the *MTHFD1* rs2236225 AA genotype 1) is associated with reduced enzymatic function, 2) can be functionally restored by the introduction of a folate analog, and 3) impairs *de novo* purine biosynthesis based on the metabolic activity of the enzyme as compared to wild type function [Christensen et al., 2009].

The interaction effect for infant tagSNP rs11627387 was the largest detected in our study and suggested that infants with the AG genotype whose mothers had low overall intake of folates had 4 times the NTD risk than infants with the GG genotype whose mothers had high folate intake. Known and predicted effects of the intronic SNP rs11627387 do not suggest an etiologic mechanism underlying this novel finding, indicating that this SNP may be in linkage disequilibrium with the true disease-causing variant(s).

MTHFR encodes a key enzyme in the folate metabolic pathway and the thermolabile valine-containing isoform of this protein (C677T; A222V) has been extensively investigated with respect to NTD risk. However, there is very little evidence that indicates that maternal folate intake interacts with C677T to modify the risk of NTDs [Vollset et al., 2005]. One of the few prior investigations into the relationship between risk of NTDs and C677T-folate interactions did not find evidence of an interactive effect in a pre-fortification California population [Shaw et al., 1998; Volcik et al., 2003a]. In our post-fortification California study population (i.e. independent from the aforementioned California population), we identified three SNPs in the maternal *MTHFR* gene (rs1476413, rs1801131 (A1298C) and rs1801133 (C677T)) that interact with maternal folate intake to decrease the risk of NTDs.

Functional consequences of a common exonic SNP in *SHMT1* (rs1979277 C allele) have been demonstrated in *in vivo* model systems whereby the nuclear import of *SHMT1* is inhibited, potentially affecting *de novo* thymidylate biosynthesis [Woeller et al., 2007]. Association studies on NTDs and *SHMT1* SNP rs1979277 (or SNPs in linkage disequilibrium with rs1979277), have shown conflicting results [Heil et al., 2001; Relton et al., 2004; Wilding et al., 2004]. We did not identify an association between this functional SNP and NTDs, either independently or through a folate interaction. Two other SNPs we evaluated suggested that *SHMT1* may be a modifier of risk of NTDs: 1) a reduced risk was observed for mothers carrying the G allele at SNP rs669340; and 2) an increased risk of NTDs was observed through a folate-gene interaction for infant SNP rs12939757.

Functional studies of *TYMS* provide support for its role in the etiology of NTDs. Two common *TYMS* polymorphisms (a 28-bp tandem repeat in the promoter enhancer region (TSER) and a 6-bp deletion in the 3′-UTR) affect *TYMS* gene expression and enzyme levels [Trinh et al., 2002; Ulrich et al., 2002]. Two association studies on these *TYMS* polymorphisms and NTD risk have, however, provided conflicting results [Volcik et al., 2003b; Wilding et al., 2004]. The most recent association studies conducted on *TYMS* gene variants genotyped a limited number of SNPs [Franke et al., 2009; Martinez et al., 2009; Shaw et al., 2009]. A 2-fold increase in risk of spina bifida in a California population was identified for the 3 infant *TYMS* SNPs rs2847149, rs1001761, and rs502396 in variant homozygotes compared to common homozygotes [Shaw et al., 2009]. In our study, the maternal *TYMS* SNP rs502396 (a tag SNP for rs2847149 and rs1001761) was associated with a decreased risk of NTDs, as was the maternal SNP rs699517 and the infant SNP rs2847153, through an interaction with low maternal folate intake.

NTDs have a multifactorial etiology, (i.e. genetic and environmentally mediated). Thus, it is necessary to consider both maternal effects and embryonic effects, as well as potential interactions between the two. The discrimination between maternal and offspring genetic effects relies on genotype information obtained from the mother and the infant so that both genotypes are included in the same statistical model. Given the small number of mother-infant pairs included in this study (24 case pairs) we were not able to model the genotypes simultaneously.

Many of the variants selected for this study had limited prior evidence for their role in the risk of NTDs, either as main effects or through a gene-folate interaction. Thus, some of the results represent a first effort to inform future studies of the etiology of NTDs. Notwithstanding our significant findings, the precision of the interaction estimates are low and must be cautiously interpreted, as some results may be due to spurious rather than true associations. Given our modest sample size, our power to detect interactions was limited. This was an expected limitation and was approached by selecting less stringent *P* values that were uncorrected for multiple testing. The flexibility in our criteria for statistical significance and acceptance of relatively high genotyping error rates, though less stringent, permitted us to utilize our study as a mechanism for informing future studies of NTDs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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TABLE I

Demographic Characteristics and Maternal Periconceptional Intake of Folates Among Neural Tube Defect Cases and Non-malformed Controls

	Hispanic		White, non-Hispanic		Total	
	Cases (n=166) %	**Controls (n=328) %**	**Cases (n=56) %**	**Controls (n=126) %**	**Cases (n=222) %**	**Controls (n=454) %**
Infant sex						
Male	48	55	45	48	47	53
Female	47	45	46	52	47	47
Unknown	5	0	9	0	6	0
Maternal education						
0–11 years	56	43	4	5	42	32
High school diploma/GED	22	30	16	13	21	25
> 12 years	22	27	80	82	36	42
Missing data	1	1	0	1	1	1
Use of folic acid supplements						
Yes	45	54	80	79	54	61
No	55	46	20	21	46	39
Daily dietary intake of folates						
Q1	23	23	27	32	24	25
Q2-Q4	77	77	73	68	76	75
Overall intake of folates						
Low[a]	16	10	4	9	13	10
High[b]	84	90	96	91	87	90
Mean maternal age at delivery, years (SD)	27.15 (5.74)	26.84 (6.13)	31.51 (5.69)	31.90 (5.62)	28.25 (6.02)	28.25 (6.40)

Abbreviations: SD, standard deviation.

[a]Women in the lowest quartile of maternal folate intakes (Q1 ≤ 309.49 μg/100g/day) who did not take supplemental folic acid in the periconceptional period.

[b]All other combinations of folate intake (Q2, Q3 or Q4) and folic acid supplementation (yes/no).

TABLE II

Odds Ratios[a] and Confidence Intervals for Associations Between Maternal and Inherited SNPs in Folate Related Genes and NTD Risk in Offspring, California, 1999–2003

		Maternal Model			Infant Model		
		ORs and CIs			ORs and CIs		
SNP rs ID	Referent Genotype	Genotype[b]	G-E [c]	Interaction Effect[d]	Genotype[b]	G-E [c]	Interaction Effect[d]
DHFR							
rs1650723	GG	0.41[e] 0.12, 1.41	--	--	0.96[f] 0.52, 1.76	--	--
rs1677693	CC	0.72[e] 0.35, 1.46	--	--	0.98 0.66, 1.45	--	--
rs11951910	TT	1.18 0.66, 2.13	--	--	0.85 0.42, 1.72	--	--
MTHFD1							
rs745686	AA	0.81[e] 0.42, 1.56	--	--	1.26[e] 0.65, 2.44	--	--
rs8011839	CC	1.07 0.72, 1.60	--	--	1.14 0.80, 1.62	--	--
rs1076991	AA	0.94 0.68, 1.28	--	--	1.14 0.84, 1.54	--	--
rs1885031	AA	1.50 0.96, 2.35	--	--	N/A[g]		
rs1950902	CC	0.91 0.53, 1.54	--	--	0.85 0.52, 1.40	--	--
rs2236224	CC	1.50[e] 0.83, 2.72	--	--	1.02 0.83, 1.26	0.44 0.24, 0.80	1.58 0.99, 2.51
rs2236225	CC	N/A[g]			0.90 0.73, 1.10	0.48 0.27, 0.86	1.53 0.95, 2.47
rs3783728	TT	0.42[e] 0.11, 1.62	--	--	0.51 0.21, 1.23	--	--
rs11158542	TT	0.91[e] 0.49, 1.69	--	--	0.80[e] 0.16, 3.99	--	--
rs11627387	GG	0.76[e] 0.41, 1.41	--	--	1.11 0.87, 1.41	7.81 3.33, 16.67	4.25 2.33, 7.75

		Maternal Model			Infant Model		
		ORs and CIs			ORs and CIs		
SNP rs ID	Referent Genotype	Genotype[b]	G-E [c]	Interaction Effect[d]	Genotype[b]	G-E [c]	Interaction Effect[d]
rs11849530	AA	0.88[e] 0.40, 1.91	--	--	0.79[e] 0.36, 1.75	--	--
rs34616731	TT	1.02[e] 0.62, 1.68	--	--	1.38[e] 0.87, 2.17	--	--
MTHFR							
rs1476413	GG	1.30 0.99, 1.71	0.31 0.10, 0.95	0.55 0.20, 1.48	1.22 0.74, 2.00	--	--
rs1801131	AA	1.33 1.03, 1.73	0.30 0.11, 0.81	0.58 0.24, 1.43	N/A[g]		
rs1801133	CC	0.74 0.59, 0.91	3.33 1.59, 7.14	0.69 0.41, 1.17	0.66[e] 0.29, 1.47	--	--
SHMT1							
rs669340	CC	0.69 0.49, 0.96	--	--	1.10 0.81, 1.49	--	--
rs1979277	GG	N/A[g]			0.78 0.55, 1.12	--	--
rs2168781	CC	1.15 0.84, 1.59	--	--	0.87 0.65, 1.16	--	--
rs2273026	GG	1.06 0.68, 1.68	--	--	N/A[g]		
rs9909104	TT	1.00[e] 0.62, 1.60	--	--	1.03 0.83, 1.29	--	--
rs12939757	AA	1.44[e] 0.78, 2.66	--	--	1.68 1.34, 2.09	0.46 0.25, 0.86	2.01 1.20, 3.37
rs12952556	TT	N/A[g]			0.88 0.70, 1.10	--	--
TYMS							
rs502396	TT	0.93 0.77, 1.12	0.51 0.28, 0.89	0.91 0.53, 1.56	N/A[g]		
rs699517	CC	0.99 0.79, 1.25	0.46 0.21, 1.01	0.70 0.38, 1.29	0.75 0.55, 1.03	--	--
rs1001761	CC	0.68[e] 0.37, 1.25	--	--	N/A[g]		

		Maternal Model			Infant Model		
		ORs and CIs			ORs and CIs		
SNP rs ID	Referent Genotype	Genotype[b]	G-E [c]	Interaction Effect[d]	Genotype[b]	G-E [c]	Interaction Effect[d]
rs2847153	GG	0.94 0.61, 1.44	--	--	0.95 0.74, 1.23	0.41 0.18, 0.93	0.73 0.37, 1.45
rs2847149	GG	0.76 [e] 0.42, 1.39	--	--	N/A[g]		
rs2847326	TT	0.88 0.61, 1.26	--	--	0.87[f] 0.59, 1.30	--	--

CI, confidence interval; G-E, gene-environment; OR, odds ratio.

[a]Estimated using logistic regression. ORs and CIs (below) are given for evaluated parameters. The referent groups are non-Hispanic Whites and the high folate intake group.

[b]ORs and 95% CIs (below) are given for heterozygotes estimated by a linear (additive) genetic model where the likelihood ratio test for G-E interaction *P* value ≥ 0.20.

[c]G-E ORs and 80% CIs (below) represent the log odds derived from the interaction parameter from the logistic model. ORs are reported for all model parameters where the likelihood ratio test for G-E interaction *P* value < 0.20, otherwise "--" is shown.

[d]For models with a significant G-E estimate, interaction effect ORs were calculated by exponentiating the sum of the gene, folate, and interaction coefficients and represent the risk of NTDs in low folate heterozygotes compared to high folate common homozygotes, otherwise "--" is shown.

[e]Non-Hispanic Whites only included in this model.

[f]Hispanics only included in this model.

[g]N/A specified for models in which controls were shown to deviate from Hardy-Weinberg Equilibrium at the corresponding SNP.