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Maternal Gene-Micronutrient Interactions Related to One-Carbon Metabolism and the Risk of Myelomeningocele Among Offspring

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Abstract

Background—Few studies have evaluated interactions between maternal genetic variation in 5,10-methylenetetrahydrofolate reductase (*MTHFR*) and micronutrient intake on the risk of myelomeningocele (MM) in offspring. Therefore, we sought to determine if the role of maternal *MTHFR* C677T and A1298C on MM risk is altered by maternal intake of micronutrients related to one-carbon metabolism.

Methods—The study population consisted of 220 MM case-parent trios recruited from 1996-2006. A dietary questionnaire was used to obtain information on maternal dietary intake on eight micronutrients including folate and cobalamin. TaqMan assays were used to generate *MTHFR* C677T and A1298C genotypes. Log-linear models were used to evaluate the joint effects of maternal genotype and micronutrient intake dichotomized as at or above vs. below the United States Recommended Dietary Allowance (US RDA) on MM.

Results—There was little evidence to suggest maternal *MTHFR* genotypes interacted with micronutrient intake to influence the risk of MM. For instance, the effect of *MTHFR* 677T was similar for mothers with cobalamin intake below US RDA (relative risk [RR]=0.97) vs. at or above US RDA cobalamin intake (RR=0.81, interaction $p=0.87$). However, some differences were noted. For example, the effect of *MTHFR* 1298C appeared to be different between those mothers below US RDA folate intake (RR=0.98) vs. those at or above US RDA folate intake (RR=0.68), but the interaction was not statistically significant (interaction $p=0.27$).

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Conclusions—There did not appear to be strong effects of maternal micronutrient intake on the role of maternal genetic polymorphisms in *MTHFR* C677T and A1298C on MM risk.

Keywords

thiamin; riboflavin; niacin; pyridoxine; folate; cobalamin; myelomeningocele

INTRODUCTION

The discovery of folate (B9) as a factor in reducing the risk of spina bifida (SB) and the resulting mandate in the United States for maternal folic acid supplementation have led to a substantial decline in the prevalence of these serious congenital malformations (Yi et al., 2011). Folate serves as a single carbon donor in one-carbon metabolism to convert homocysteine to methionine with the aid of enzyme cofactors, such as riboflavin (B2), pyridoxine (B6) and cobalamin (B12) (Mason, 2003). Studies have shown that folate deficiency leads to increased maternal plasma homocysteine level, a finding that has been associated with a risk of SB development (Amouzou et al., 2004; Fekete et al., 2010; Czeizel et al., 2011). Recent studies have also suggested that deficiencies in B vitamins involved in one-carbon metabolism may lead to elevated homocysteine levels and, therefore, may increase the risk for SB (Candito et al., 2008; MacFarlane et al., 2011; Zhao et al., 2006). Conversely, enhanced maternal intake of these B vitamins, particularly those involved in folate metabolism may be protective against SB (Carmichael et al., 2010; Chandler et al., 2012).

Related to folate metabolism, many studies have demonstrated that single nucleotide polymorphisms (SNPs) in genes of the folate pathway are associated with the development of neural tube defects (NTDs) (Au et al., 2010; Copp and Greene, 2010; Harris and Juriloff, 2007; Harris and Juriloff, 2010; Juriloff and Harris, 2012). More specifically, studies that have evaluated both maternal and inherited genotypes have reported that *MTHFR* C677T and A1298C are associated with NTDs (Martinez et al., 2009; Yang et al., 2008). *MTHFR* is an enzyme that catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate to convert homocysteine to methionine, in one-carbon metabolism. The *MTHFR* C677T and A1298C variants cause reduced *MTHFR* enzyme activity (van der Put et al., 1998). However, while *MTHFR* C677T has been associated with increased plasma homocysteine levels, the same association is not seen with *MTHFR* A1298C (Crider et al., 2011; van der Linden et al., 2006). Moreover, in mice, *MTHFR* knock out does not lead to NTDs, raising a question about the specificity of the genetic association with NTDs (Pickell et al., 2009). The lack of inclusion of the role of maternal micronutrient intake, especially those micronutrients related to one-carbon metabolism, may explain the equivocal evidence in the literature on the role of *MTHFR* 677T and 1298C genotypes on NTD risk in offspring.

To date, few studies have examined the interactions between environmental factors (e.g., maternal intake of micronutrients involved in one-carbon metabolism) and maternal genetic factors (e.g., *MTHFR* C677T and A1298C) on the risk of SB in offspring. Further, there are few studies that have examined this interaction in a relatively homogenous case group, for

instance, infants affected solely by MM. The purpose of our multicenter case study, therefore, is to determine if the association between maternal *MTHFR* C677T and A1298C and MM varies by periconceptional maternal micronutrient intake, particularly for the micronutrients involved in one-carbon metabolism. We evaluated the joint effects of maternal gene-micronutrient interactions in a population of 220 well-characterized MM case-parent duos and trios.

MATERIALS AND METHODS

Study Subjects

The study population has previously been described in detail (Au et al., 2008). Briefly, the participants consisted of 220 case-parent trios and duos, with cases affected by MM, recruited from three SB clinics (Houston, TX, Los Angeles, CA and Toronto, ON, Canada) between 1996 and 2006. Participation of both biologic parents was not required. Both males and females and individuals of all racial/ethnic groups were eligible to participate. The inclusion criteria included children diagnosed with MM and whose parents agreed to enroll and provide blood samples and/or saliva for DNA extraction. Exclusions included patients who had syndromic MM. After written informed consent was received from the parents, a blood sample and/or saliva sample was obtained from each participant.

Maternal Surveys

Two surveys were used to collect data from mothers participating in the study. The first survey was used to obtain sociodemographic history, pregnancy history, maternal health history and information on maternal exposures. The second survey, the Diet History Questionnaire (DHQ) from the National Institutes of Health (NIH), was used to obtain information on maternal dietary intake and vitamin supplementation, including prenatal vitamins. The second survey required mothers to recall their diet during the course of the affected pregnancy after the delivery had occurred. The instrument is an updated version of the National Cancer Institute (NCI) Health Habits and History Questionnaire (HHHQ) developed by Dr. Gladys Block and colleagues (Subar et al., 2001). The DHQ has been validated and can be self-administered (Subar et al., 2001; Thompson et al., 2002; Boucher et al., 2006; Johnson et al., 2007). For mothers who had low literacy or who had questions, a nurse coordinator or a trained interviewer under her direction performed the interview in its entirety or helped with specific questions. Information from the DHQ was then analyzed by the program Diet*Calc (National Institutes of Health, National Cancer Institute) to generate estimates of daily dietary intake from food for the following micronutrients: thiamin (mg), riboflavin (mg), niacin (mg), pyridoxine (mg), folate (mcg), cobalamin (mcg), iron (mg) and phosphorus (mg). The daily micronutrient intake was then compared to the United States Recommended Dietary Allowance (US RDA) for pregnant women since the case mothers were queried about their dietary regimen during their affected pregnancies.

Genotyping Methods

Genotyping of *MTHFR* C677T and A1298C have been previously described (Martinez et al., 2009). Briefly, blood samples and/or saliva samples were obtained from patients and both parents when possible. Genomic DNA from blood lymphocytes was extracted utilizing

the Puregene DNA kit (Gentra Systems Inc., Minneapolis, MN). Saliva DNA was extracted using the Oragene DNA collection kit (DNA Genotek Inc., Ottawa, ON, Canada). Samples were genotyped using the TaqMan genotyping assay method (Life Technologies Inc.), following the manufacturer's standard protocol. Sanger sequencing was done on over 10% of samples to verify the genotypes as a quality control measure.

Statistical Analysis

Frequency distributions were determined for demographic variables among the case subjects. Additionally, we tested the distributional characteristics of each micronutrient using the Shapiro-Wilk test for normality. None of the micronutrients demonstrated normality. The medians for each micronutrient were therefore calculated. For each analyzed variant, samples for which a genotype could not be assigned and case-parent trios that had genotype combinations that were inconsistent with Mendelian inheritance were determined. For each subject, the number of genotyping failures (e.g., genotypes that could not be assigned) was determined. These analyses were performed using Intercooled Stata, version 14.1 (StataCorp LP, College Station, TX). Maternal *MTHFR* gene-micronutrient combinations were evaluated using log-linear models among case-parent trios. Specifically, to test the no-interaction null hypothesis, we calculated a 2-degrees-of-freedom likelihood ratio test (LRT) statistic as twice the difference of the log likelihoods for the log-linear model that included two parameters indexing the inherited genotype for single nucleotide polymorphism 1 (SNP1), two parameters indexing the maternal genotype for SNP1, and two interaction terms representing the product of maternal SNP1-exposure and a reduced model that excluded the interaction terms (Lupo et al., 2010; Umbach and Weinberg, 2000). These analyses were run using LEM (Vermunt, 1997) a program for log-linear analysis with missing data that allows information from case-parent trios that have not been completely genotyped (e.g., father not available) to be included in the analysis for any given variant. Despite the fact that 260 mothers were surveyed with the DHQ, 40 children did not have genotypes because of missing samples or difficulty with genotyping DNA, resulting in a total of 220 case-parent trios and duos. Of the total, there were 148 complete genotyped trios (father, mother and child), and 72 genotyped duos (mother and child). We considered a p-value for interaction <0.05 as statistically significant.

RESULTS

The study population (Table 1) consisted of 220 case trios and duos. The case patients were mostly male (55.9%), with Hispanic (43.6%) and non-Hispanic white (43.6%) as the predominant racial/ethnic groups. The majority of mothers were aged 26-30 years (33.2%) or 20-25 years (32.7%). Eighty-five mothers took supplements, and 135 mothers did not take supplements during their pregnancy. A majority of mothers were above the US RDA for the following micronutrients (below/above): thiamin (85/135), riboflavin (71/149), cobalamin (57/163) and phosphorus (31/189). However, in some cases, a majority of mothers were below the US RDA for select micronutrients (below/above): folate (171/49), pyridoxine (128/92) and iron (197/23).

The overall micronutrient intake (Table 2) showed that mothers had a higher median micronutrient intake of thiamin (1.6 mg), riboflavin (1.8 mg), cobalamin (3.7 mcg) and phosphorus (1262.4 mg) compared to the US RDA for pregnant women. In contrast, maternal median intake of niacin (17.9 mg), pyridoxine (1.6 mg), total folate (400.7 mcg) and iron (14.0 mg) were less than the US RDA for pregnant women.

Log-linear models did not reveal significant interactions between maternal *MTHFR* 677T genotypes and micronutrient intake on MM risk in offspring (Table 3). For example, the effect of *MTHFR* 677T was similar for mothers below cobalamin intake (relative risk [RR]=0.97) vs. those at or above intake (RR=0.81, interaction p=0.87). The same was true for folate intake, whereby the effect of *MTHFR* 677T was similar for mothers below intake (RR=0.83) vs. those at or above intake (RR=0.97, interaction p=0.26). However, some differences were observed in our data. For example, the effect of *MTHFR* 677T was positively associated with MM in mothers below niacin intake (RR=1.09), whereas *MTHFR* 677T was negatively associated with MM among those at or above niacin intake (RR=0.70). However, the *MTHFR* 677-niacin interaction was not statistically significant (interaction p=0.44). In fact, no *MTHFR* 677-micronutrient interactions were found to be statistically significant.

As with *MTHFR* 677T genotypes, our results did not reveal significant interactions between maternal *MTHFR* 1298C genotypes and micronutrient intake on MM risk in offspring (Table 4). For instance, the effect of *MTHFR* 1298C was similar for mothers with below thiamin intake (RR=0.80) vs. at or above thiamin intake (RR=0.93, interaction p=0.86). Of note, there did appear to be a modest difference between mothers who were below folate intake (RR=0.98) vs. mothers who were at or above folate intake (RR=0.68), but the interaction was not statistically significant (interaction p=0.27). While other modest differences were observed, none were statistically significant.

DISCUSSION

Overall, our results did not suggest that the role of maternal *MTHFR* genotypes on MM risk in offspring was significantly altered by intake of micronutrients associated with one-carbon metabolism. While there were some modest differences noted in mothers with *MTHFR* 677T genotypes who were at or above the US RDA amounts for select micronutrients, such as niacin, compared to those who were below US RDA amount for the same micronutrients, these differences were not statistically significant. Likewise, in mothers with *MTHFR* 1298C genotypes, there were non-significant differences seen between mothers who were at or above the US RDA intake of select micronutrients, such as folate, vs. those who were below the US RDA intake. However, these results suggest that micronutrients, particularly those involved with one-carbon metabolism, do not appear to have a strong effect on MM risk among mothers with either *MTHFR* 1298C or 677T genotypes.

Some studies have reported that micronutrient intake can influence plasma homocysteine levels among individuals with certain *MTHFR* C677T genotypes (Bailey et al., 2002; McNulty et al., 2002; McNulty et al., 2006). For example, one study reported a decrease in plasma homocysteine as cobalamin concentration increased in individuals who were

heterozygous for both *MTHFR* C677T and A1298C (Bailey et al., 2002) even when vitamin B-12 concentrations were within normal range. These data suggest that increasing the intake of micronutrients involved in one-carbon metabolism may mitigate NTD risk in offspring with *MTHFR* C677T polymorphisms. From these studies, it seems plausible that cobalamin and other micronutrients involved in one-carbon metabolism could reduce the risk of NTDs among mothers with *MTHFR* C677T and A1298C genotypes. However, we did not replicate the finding in the current study.

Strengths of our study include the use of the case-parent trio study design. The case-parent trio study design has been shown to provide increased power over the case-control approach when assessing gene-environment interactions (Umbach and Weinberg, 2000). The case-parent trio design is also immune to the effects of population stratification bias in the estimation of inherited genetic effects (RW.ERROR - Unable to find reference:223; Weinberg et al., 1998). Moreover, analyzing the data using the log-linear modeling approach allowed us to include data from incomplete trios or duos (Weinberg et al., 1998). Finally, a particular strength of our study is that the sample population only included individuals who had myelomeningocele. All other NTD phenotypes were excluded as were individuals who had a syndromic diagnosis that included but was not limited to an NTD.

Our study must be considered in light of certain limitations. Because it is a retrospective study, case mothers may not accurately estimate nutritional intake during pregnancy. Since the study only included mothers of affected children and no controls, the type of misclassification would be non-differential and bias findings toward the null. Had there been controls, then it would be a differential misclassification, leading to a bias away from the null. Further, the maternal surveys did not query the mothers on whether the affected pregnancy was planned or unplanned. As planners, mothers may have a better preconception diet than mothers who did not plan their pregnancies. Therefore, the proportion of mothers who did not plan their pregnancies could impact the reliability of the dietary intake estimates. Another limitation of our study is the relatively small sample size, perhaps hampering the detection of relatively modest maternal gene-micronutrient interactions on MM risk in offspring. Because of the small sample size, supplementation was not included in the analysis. Stratification by supplementation would have reduced the sample size even further. Given the lack of significance observed in the current findings, it would be unlikely to detect significance in a smaller sample size. Further, maternal average daily caloric intake was not included in the analysis as the modeling approach used (log-linear model for evaluating gene-environment interactions) does not allow for the inclusion of covariates. However, the lack of inclusion of maternal average daily caloric intake should not lead to confounding as the modeling strategy evaluates differences in allelic distributions between exposed and unexposed mothers.

Despite the study limitations, the results add to the knowledge of NTD causation and encourage initiation of additional projects examining similar objectives. Replication of these findings in potentially larger populations is needed with the possibility of measuring blood levels of maternal micronutrients to obtain accurate serum micronutrient concentrations. The need to evaluate other genetic polymorphisms is also important in elucidating disease risk in light of micronutrient exposure. Additionally, recent studies have shown that paternal diet

could have epigenomic effects on offspring genes that may affect the health status of the offspring (Ost et al., 2014; Wei et al., 2014). These studies may lead to a trend in future study design for gene-micronutrient interactions and birth defects, such as NTDs, to examine the diet of both parents rather than maternal diet alone.

In conclusion, our study did not demonstrate that micronutrient intake altered the maternal genetic effects of *MTHFR* C677T and A1298C on MM risk in offspring. The role of vitamin B micronutrients on MM risk remains unclear. Further studies are needed before targeted intervention strategies based on maternal genotypes and micronutrient intake can be developed.

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Table 1

Demographic Characteristics of Case Patients with Myelomeningocele (MM), Age of Mothers at Time of MM Pregnancy and Proportion of Mothers who are Below or At or Above United States Recommended Dietary Allowance (US RDA) for Micronutrients for Pregnant Women

Characteristic	No. (%)
Cases included	220
Sex	
Male	123 (55.9)
Female	97 (44.1)
Race/ethnicity	
Non-Hispanic white	96 (43.6)
Non-Hispanic black	14 (6.4)
Hispanic	96 (43.6)
Other	14 (6.4)
Age of mothers	
< 20 years	24 (10.9)
20-25 years	72 (32.7)
26-30 years	73 (33.2)
> 30 years	51 (23.2)
Thiamin intake *	
Below	85
Above	135
Riboflavin intake *	
Below	71
Above	149
Niacin intake *	
Below	110
Above	110
Pyridoxine intake *	
Below	128
Above	92
Folate intake *	
Below	171
Above	49
Cobalamin intake *	
Below	57
Above	163
Iron intake *	
Below	197
Above	23
Phosphorus intake *	

Characteristic	No. (%)
Below	31
Above	189

* Number of mothers whose micronutrient intakes are below and at or above United States Recommended Dietary Allowance for Micronutrients for Pregnant Women

Table 2

Mean Daily Dietary Micronutrient Intake of MM Case Mothers included in Assessment

	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Pyridoxine (mg)	Total Folate (mcg)	Cobalamin (mcg)	Iron (mg)	Phosphorus (mg)
Mean	1.7	2.0	20.7	1.8	456.7	4.3	15.7	1369.9
Standard Deviation	0.9	1.0	12.0	1.0	255.7	2.6	9.0	699.7
Median	1.6	1.8	17.9	1.6	400.7	3.7	14.0	1262.4
US RDA	1.4	1.4	18.0	1.9	600.0	2.6	27.0	700.0

Abbreviations: mg = milligrams, mcg = microgram, US RDA= United States Recommended Dietary Allowance

Table 3

Maternal *MTHFR* 677-Micronutrient Interactions and the Risk of Myelomeningocele in Offspring

Micronutrient	Below RDA Intake	At or Above RDA Intake	p for interaction
	RR (95% CI)	RR (95% CI)	
Cobalamin	0.97 (0.54-1.73)	0.81 (0.57-1.16)	0.87
Folate	0.83 (0.58-1.18)	0.97 (0.53-1.77)	0.26
Iron	0.88 (0.63-1.23)	0.60 (0.20-1.80)	0.73
Niacin	1.09 (0.70-1.70)	0.70 (0.45-1.07)	0.44
Phosphorus	1.06 (0.47-2.38)	0.89 (0.63-1.24)	0.61
Pyridoxine	1.04 (0.68-1.58)	0.70 (0.44-1.10)	0.35
Riboflavin	0.78 (0.48-1.29)	0.92 (0.62-1.35)	0.96
Thiamin	0.84 (0.50-1.42)	0.88 (0.60-1.27)	0.65

Abbreviations: RR=relative risk; CI=confidence interval; RDA=recommended dietary allowance. The p-values for interaction are calculated as described in Materials and Methods.

Table 4

Maternal *MTHFR* 1298-Micronutrient Interactions and the Risk of Myelomeningocele in Offspring

Micronutrient	Below RDA Intake	At or Above RDA Intake	p for interaction
	RR (95% CI)	RR (95% CI)	
Cobalamin	0.47 (0.20-1.11)	1.00 (0.68-1.47)	0.41
Folate	0.98 (0.64-1.41)	0.68 (0.34-1.35)	0.27
Iron	0.86 (0.60-1.24)	0.69 (0.19-2.47)	0.39
Niacin	0.60 (0.34-1.06)	1.13 (0.72-1.77)	0.50
Phosphorus	0.27 (0.06-1.19)	0.95 (0.66-1.36)	0.61
Pyridoxine	0.72 (0.43-1.19)	1.03 (0.63-1.67)	0.52
Riboflavin	0.50 (0.21-1.19)	0.97 (0.66-1.43)	0.32
Thiamin	0.80 (0.43-1.46)	0.93 (0.61-1.42)	0.86

Abbreviations: RR=relative risk; CI=confidence interval; RDA=recommended dietary allowance. The p-values for interaction are calculated as described in Materials and Methods.