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Associations Between Two Polymorphisms in the Methylenetetrahydrofolate Reductase Gene and Placental Abruption

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Abstract

Objective—Heritable thrombophilias have been implicated as a potential etiology of abruption via vascular disruption at the uteroplacental interface. Polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene have been linked to vascular complications outside of pregnancy, including stroke. Given the underlying thrombotic nature of abruption, we hypothesized that polymorphisms in the MTHFR gene are associated with abruption.

Study design—We examined 2 variants in MTHFR: *677C→T* and *1298A→C* in genomic DNA extracted from maternal blood from the New Jersey-Placental Abruption Study, an ongoing, multicenter case-control study. We identified 195 women with a clinical diagnosis of abruption (cases), and 189 controls matched on race/ethnicity and parity. We assessed allele and genotype frequencies, and their associations with abruption risk after adjusting for confounders through multivariable logistic regression analysis.

Results—The wild-type allele (*C*) frequency of the *677C→T* variant of MTHFR among cases and controls was 69.0% and 64.3%, respectively, and the wild-type allele (*A*) of the *1298A→C* variant was 75.9% and 79.4%, respectively. Distributions of the *677C→T* alleles among controls violated the Hardy-Weinberg equilibrium (P=0.007), while those of the *1298A→C* alleles were in equilibrium (P=0.825). In comparison to the wild-type genotype (*C/C*), the homozygous mutant form (*T/T*) of *677C→T* was not associated with abruption (OR 0.60, 95% confidence interval (CI) 0.33, 1.18). Similarly, the homozygous mutant form (*C/C*) of the *1298A→C* polymorphism was equally distributed between cases and controls (OR 2.28, 95% CI 0.82, 6.35). Plasma homocysteine and vitamin B_{12}, but not folate, concentrations were elevated in cases compared to controls among women

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In this case-control study, 677C→T and 1298A→C polymorphisms in MTHFR were not associated with increased risk of placental abruption.

with the wild-type genotype of MTHFR *677C→T* (P=0.039 for homocysteine; P=0.048 for B_{12} and P=0.224 for folate, respectively).

Conclusions—In this population, neither heterozygosity nor homozygosity for the *677C→T* and *1298A→C* variants in MTHFR was associated with placental abruption.

Keywords

Placental abruption; MTHFR; linkage disequilibrium; case-control; DNA

Placental abruption is a serious obstetrical complication that occurs in approximately 1 in 100 pregnancies.[1-3] Although its occurrence is relatively uncommon, it is a major cause of third trimester bleeding, and accounts for a disproportionately high rate of preterm birth, low birthweight, stillbirth and infant mortality.[4-8] The etiology of abruption is poorly understood, but epidemiologic studies have observed advanced maternal age, multiparity, smoking, crack and cocaine use, intra-amniotic infections, prolonged rupture of membranes, chronic hypertension, preeclampsia, and folate deficiency to be associated with increased risk.[8-18] The strongest risk factor is placental abruption in a prior pregnancy.[1, 2, 19-21]

Recent studies have suggested a genetic predisposition to placental abruption.[22-27] 5,10-Methylenetetrahydrofolate Reductase (MTHFR), an important metabolic enzyme, is required in the conversion of homocysteine to methionine. A mutation in the MTHFR gene is arguably associated with thrombotic events. Homozygosity for the cytosine-to-thymine substitution at nucleotide *677* (*677C→T*), and for the adenine-to-cytosine substitution at nucleotide *1298* (*1298A→C*) in the MTHFR gene have been suggested to be associated with increased risk for abruption. The results from these studies are, however, inconclusive. While some have reported the presence of the mutant genotype of the *677C→T* polymorphism to be associated with increased risk for abruption,[25, 28, 29] others have not.[30-32] Association between the *1298A→C* variant and abruption risk is less well examined. Whether a gene-gene interaction in the *677C→T* and *1298A→C* polymorphisms of the MTHFR gene on the risk of placental abruption exists also remains uncertain. Given the underlying thrombotic nature of abruption, we examined the association between MTHFR polymorphisms (*677C→T* and *1298A→C*) and the risk of placental abruption.

Material and Methods

The New Jersey-Placental Abruption Study (NJ-PAS)

Data for this study were obtained from an ongoing case-control study conducted in Robert Wood Johnson University Hospital, New Brunswick, NJ (since July 2003) and Saint Peter's University Hospital, New Brunswick NJ (since August 2002). Both hospitals serve as large tertiary, level III centers (located within a mile of each other), with a total of approximately 8,000 deliveries annually. The ethics review committee of the Institutional Review Board of both institutions approved this investigation. Further details of the *NJ-PAS* has been described in detail elsewhere.[33]

Placental abruption cases and controls

Placental abruption cases that were eligible for inclusion included women with a clinical diagnosis of abruption before or during delivery, by the attending obstetrician. The definition of placental abruption included the classical signs and symptoms of painful vaginal bleeding or hemorrhage accompanied by documented fetal distress, uterine pain or tenderness, or uterine hypertonicity. In the absence of these clinical hallmarks for abruption, if the delivered placenta showed visual signs of retroplacental bleeding or retroplacental clot/hematoma on the placental surface, then such patients were eligible for inclusion as potential cases. In addition, if an

abruption was visually diagnosed on sonographic examination during routine prenatal care, such cases were considered for inclusion as abruption cases.[34] Women with an abruption were identified by reviewing daily hospital delivery logs at both hospitals, and/or by referral by the physician, nurse, or obstetrics and gynecology residents. Medical and obstetrical labor and delivery charts were carefully reviewed for all abruption cases for confirmation prior to enrollment. The criteria for eligibility included patients with an abruption that delivered at ≥20 weeks, and those that provided consent to participate in the study.

Controls comprised of women that did not have a placental abruption. Controls were identified from viewing daily delivery logs in both hospitals. Controls were matched to cases on parity (nulliparous, primiparous, parity 2, or parity ≥3), and maternal race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, or other race/ethnicity). Following the recruitment of a case, a control patient was sought for recruitment from the same hospital as abruption cases. From the potential pool of eligible controls, we excluded women with a diagnosis of placental previa or stillbirth in the current pregnancy, or with history of placental abruption in any of their previous pregnancies.

Polymorphisms in the 5,10-Methylenetetrahydrofolate reductase gene

The *677C→T* variant of MTHFR occurs in exon 4 and results in an alanine to valine substitution at codon 222.[35, 36] A second MTHFR polymorphism, *1298A→C* in exon 7, results in a glutamate to alanine substitution at codon 429.[37] Both these polymorphisms in the MTHFR gene have been linked with reduced enzyme activity. The *677C→T* mutation is also associated with altered distribution of intracellular folate metabolites.

Genomic DNA was extracted from maternal peripheral blood, precipitated with ethanol, washed, dissolved in a Tris-EDTA buffer, and stored at −20°C. The extracted DNA was then assayed for the 2 mutations in the MTHFR gene using the polymerase chain reaction for DNA amplification and restriction digestion of PCR products with *HinfI* for the *677C→T* and *MwoI 1298A→C*, as previously reported.[35, 38]

Biochemical assays

For the total plasma homocysteine, folate and vitamin B_{12} assays, 1.0 ml blood was drawn into EDTA tubes, and transported on dry ice to the laboratory for assays. The plasma was then separated and stored in Eppendorf tubes and stored at −70°C. These specimens were processed for non-fasting homocysteine metabolism using the Abbott IMX technology. This is based on a fluorescence polarizing immunoassay technique.[39] Plasma folate and vitamin B_{12} were determined using the Abbott Diagnostic IMX based on a microparticle enzyme immunoassays, following the manufacturer's protocols.[39] The coefficients of variation for these assays were <4%.

Statistical analysis

We examined the distributions of placental abruption cases and controls in relation to study center, year recruited, maternal age (<19, 19−34, and ≥35 years), maternal education (<12, 12, 13−16, and ≥17 completed years of schooling), prepregnancy body-mass index, smoking and alcohol use before and during pregnancy (yes/no), prenatal care (or any care), as well as the matching factors parity (nulliparous, primiparous, parity 2, and parity ≥3), and maternal race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, or other race/ethnicity). Body-mass index was calculated as the ratio of weight (in kilograms) over squared-height (in meters). We tested differences in these factors between cases and controls based on either the t-test (for continuous variables), or the Fisher's exact or Chi-square test (for categorical variables).

We derived allele and genotype frequencies of the *677C→T* and *1298A→C* variants in MTHFR with bootstrap generated 95% confidence intervals. The bootstrap estimates were based on 10,000 replications. In addition, we evaluated if the observed allele frequencies for the 2 MTHFR polymorphisms were in Hardy-Weinberg equilibrium.[40] Linkage disequilibrium was assessed for the co-segregation of the *677C→T* and *1298A→C* variants.

The association between the 2 MTHFR polymorphisms and abruption was based on the unadjusted odds ratio with 95% confidence interval. This was a matched case-control study by design, all preliminary analyses were based on a matched analysis. However, since the result of these matched analyses (not shown) did not differ from those based on an unmatched analysis we only report the results of the unmatched analysis.

We adjusted the associations between the MTHFR polymorphisms and abruption for several confounders through a multivariable logistic regression analysis. We adjusted the analyses for study site, year recruited, parity and maternal race/ ethnicity, maternal age, smoking and alcohol use during pregnancy, and prepregnancy body-mass index. In addition, we also examined gene-gene interactions in the *677C→T* and *1298A→C* mutations in MTHFR and risk of abruption. Finally, the entire analysis was replicated after stratifying the data on maternal race/ethnicity.

Differences in total plasma homocysteine, folate and vitamin B_{12} distributions were examined between cases and controls. For this analysis, we applied the Box-Cox transformation to the analytes to ensure that the variance stabilization and normality assumptions of the analysis of variance methods were met.[41] General linear models were fitted to the post-transformed analyte data to examine differences between abruption cases and controls as well as within genotypes of the 2 MTHFR polymorphisms.

This case-control study was primarily designed to detect a 2-fold increased frequency of the homozygous mutant genotype (*T/T*) of the *677C→T* polymorphism. We based this on an assumed genotype frequency of 6% among controls and 12% among abruption cases, and a type II error rate of 10% ($1-\beta = 90\%$) . The sample size required to detect an association between the MTHFR *677C→T* polymorphism and abruption with an odds ratio of 2.0 were 180 cases and 180 controls.

Results

A total of 195 abruption cases and 189 controls had complete MTHFR analysis for both the *677C→T* and *1298A→C* variants. As previously reported, distributions of maternal race/ ethnicity and parity were similar between cases and controls, and abruption cases were more likely to be less educated and to be smokers.[33]

Approximately two-thirds of cases and controls carried the wild-type allele of the *677C→T* polymorphism (Table 1). In comparison to the wild-type genotype, the frequencies of the heterozygous (*C/T*) and homozygous (*T/T*) mutant genotypes of *677C→T* were relatively equally distributed between abruption cases and controls. Distributions of the *677C→T* alleles violated the Hardy-Weinberg equilibrium both in cases (P=0.019) and controls (P=0.007). As with the *677C→T* mutation, the *1298A→C* variant of the MTHFR was also not associated with an overall increased abruption risk, and adjustments for confounders had little effect on these associations. Distribution of the *1298A→C* alleles were in equilibrium in both cases (P=0.078) and controls (P=0.825).

We examined the joint effects of the *677C→T* and *1298A→C* variants on the risk of placental abruption (Table 2). Compound heterozygosity for the *677C→T* and *1298A→C* variants of MTHFR (*C/T-A/C*) was not associated with increased risk of abruption.

We compared the distributions of plasma total homocysteine, folate and vitamin B_{12} between abruption cases and controls within the different genotypes of the MTHFR mutations (Table 3). Among the wild-type *677C→T* polymorphism (*C/C*), mean homocysteine and vitamin B_{12} concentrations were higher among abruption cases than controls (P=0.039 and P=0.048, respectively). Among women carrying the homozygous mutant genotype of the *1289A→C* polymorphism (*C/C*), mean folate levels were lower among abruption cases than controls (P=0.046).

The associations between MTHFR (*677C→T* and *1298A→C*) and placental abruption stratified on maternal race/ethnicity did not reveal any significant associations (data not shown).

Comment

MTHFR, an important metabolic enzyme, is required in the conversion of homocysteine to methionine. Although the association between an increase in homocysteine level and venous thrombotic events remains equivocal, if there were indeed a thrombotic tendency, then it might be expected to affect placental function. We examined the associations between two variants of MTHFR, *677C→T* and *1298A→C* in relation to abruption. We found no association between the 2 variants of MTHFR and abruption, nor was there any evidence of a joint association between the 2 MTHFR polymorphisms and abruption risk.

The association between increased risk of placental abruption and variant forms of MTHFR remains unclear. In a study by Kupferminc et al.,[28] the authors found no association between MTHFR *677C→T* and abruption, with subsequent studies corroborating these findings.[32] A meta-analysis[26] reported an increased risk for abruption among women carrying the *677C→T* polymorphism of MTHFR (pooled OR 2.3, 95% CI 1.1, 4.9). However, a study of (Black) South African Zulu women reported that the individual *677C→T* variant of MTHFR was not associated with increased risk of abruption, but combined heterozygosity for two MTHFR mutations (*677C→T* and *1298A→C*) was present in 22% and 3.5% of abruption cases and controls, respectively (OR 5.2, 95% CI 1.1, 24.5).[32] These authors demonstrated that the increased risk of abruption was largely driven by an association with the MTHFR *1298A→C* variant (OR 3.2, 95% CI 1.0, 10.4), and speculated that this variant may serve as a susceptibility factor which can be triggered in the presence of the homozygous mutant form of the *677C→T* polymorphism.

Our results are at variance with these findings. In fact, a sub-analysis restricted to African-American women did not reveal such an association. Furthermore, none of our cases or controls was homozygous for both the *677C→T* and *1298A→C* MTHFR polymorphisms. These findings corroborate those of a study of Irish women which showed that the two MTHFR variants *677C→T* and *1298A→C* were not associated with increased risk of abruption.[42] The lack of an association between combined heterozygosity for MTHFR mutations *677C→T* and *1298A→C* and abruption in our study supports the growing body of literature.[25, 31, 43]

A classic condition associated with abruption is hyperhomocystenemia (elevated homocysteine), and low folate levels. With the exception of the wild-type genotype, levels of homocysteine in cases and controls were similar, as were those of plasma folate and vitamin B_{12} (Table 3). MTHFR *677C→T* mutant genotype has been shown to have an impact only in the presence of folate deficiency.[44] However, our study may not have been adequately powered to examine this association. Moreover, folate levels in our study were assessed following delivery (after women experienced the abruption). Assessment of folate levels at or before the time of abruption may provide data that are more useful since MTHFR mutant genotype is known to interact with low folate to increase homocysteine levels.

Limitations and strengths

Despite strong epidemiologic associations between maternal race and abruption risk,[45-47] genetic variations by race in our study were accounted by population stratification by design.[33] Although the study had sufficient power to detect associations between MTHFR and abruption risk, our study may have lacked sufficient power to detect associations stratified by maternal race/ethnicity. Our patient population also comprised of largely high-risk women, as previously reported.[19] Laboratory personnel carrying out the assays for homocysteine, folate and vitamin B_{12} were blinded to case-control status and all assays were performed using automated systems, so the potential for a diagnostic bias is unlikely. All analyses also incorporate adjustments for a variety of confounders, but bias due to residual confounding due to unmeasured factors is likely. Finally, the Hardy-Weinberg equilibrium was violated for the 677C→T genotype of MTHFR in our study. This may have resulted due to our patient population being fairly heterogeneous and comprised of relatively larger proportion of women at high-risk for placental abruption and related obstetrical complications. Thus, some caution in interpretation of our findings for this particular genotype is warranted. Since placental function is determined by both maternal and fetal genes, future studies may benefit from examining associations between fetal MTHFR genotypes, homocysteine pathways and abruption. In addition, whether women carrying the mutant genotypes of the MTHFR polymorphisms are at increased risk for recurrent placental abruption remains unknown, and may be topic worthy of future investigation.

Conclusions

In summary, our study shows no evidence for an association between the MTHFR polymorphisms (*677C→T* and *1298A→C*) and risk of abruption. These data also suggest a lack of distributional changes in the profiles of plasma homocysteine and folate between placental abruption cases and controls.

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APPENDIX

Investigators currently participating or who have participated in the New Jersey-Placental Abruption Study include Cande V. Ananth, PhD, MPH (Principal investigator), Darios Getahun, MD, MPH, Neela Srinivas, MD, MPH, Celeste DeMarco, RN, BSN, Denise Elsasser, MPH, Yu-Ling Lai, RN, and Shelby Pitts, RN (Division of Epidemiology and Biostatistics); John C. Smulian, MD, MPH, Wendy L. Kinzler, MD, Morgan R. Peltier, PhD, Marian Lake, RN, MPH (Division of Maternal-Fetal Medicine), all in the Department of Obstetrics, Gynecology, and Reproductive Sciences, UMDNJ-Robert Wood Johnson Medical School; Claire Philipp, MD (Department of Medicine), UMDNJ-Robert Wood Johnson Medical School; and George G. Rhoads, MD, MPH (Department of Epidemiology) and Dirk F. Moore, PhD (Department of Biostatistics) at UMDNJ-School of Public Health.

Other investigators that were involved with the study included Rima Rozen, PhD and Jacques Genest, MD (McGill University, Montreal, Canada); Susan Shen-Schwarz, MD (Department of Pathology, Saint Peter's University Hospital, New Brunswick, NJ), and Vinay Prasad, MD (Department of Pediatric Pathology, Arkansas Children's Hospital, University of Arkansas Medical Sciences, Little Rock, AR)

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Table 1

Allele and genotype frequencies of MTHFR *677C →T* and *1298A →C* mutations and associations with placental abruption: The New Jersey-Placental Abruption Study

MTFHR allele and genotypes	Abruption cases (n=195) n	%	Controls (n=189) n	%	Odds ratio (95% confidence interval) Unadjusted	Adjusted†
MTHFR *677C →T*						
Allele frequency						
C	269	69.0	243	64.4	1.00 (Reference)	1.00 (Reference)
T	121	31.0	135	35.6	0.77 (0.44, 1.33)	0.61 (0.33, 1.15)
Genotype frequency						
C/C	100	51.3	87	46.0	1.00 (Reference)	1.00 (Reference)
C/T	69	35.4	69	36.5	0.87 (0.56, 1.35)	0.92 (0.55, 1.53)
T/T	26	13.3	33	17.5	0.69 (0.38, 1.23)	0.60 (0.33, 1.18)
MTHFR *1298A →C*						
Allele frequency						
A	296	75.9	300	79.4	1.00 (Reference)	1.00 (Reference)
C	94	24.1	78	20.6	2.32 (0.93, 5.78)	2.30 (0.84, 6.26)
Genotype frequency						
A/A	117	60.0	118	62.4	1.00 (Reference)	1.00 (Reference)
A/C	62	31.8	64	33.9	0.98 (0.63, 1.51)	0.99 (0.61, 1.61)
C/C	16	8.2	7	3.7	2.31 (0.92, 5.81)	2.28 (0.82, 6.35)

P-values for test of Hardy-Weinberg equilibrium among cases and controls were 0.019 and 0.007, respectively, for the *677C →T* and 0.078 and 0.825, respectively, for the *1298A →C* variants of the MTHFR gene.

† Odds ratios were adjusted for study site, year recruited, maternal race/ethnicity, parity, maternal age, education, prenatal care, pregnancy body-mass index and smoking during pregnancy

Table 2

Interaction between MTHFR *677C→T* and *1298A→C* genotypes and the risk of placental abruption: The New Jersey-Placental Abruption Study

MTHFR *677C→T*	MTHFR *1298A→C*	Placental abruption: n (%) Cases (n=195)	Controls (n=189)	Odds ratio (95% confidence interval) Unadjusted	Adjusted
C/C	*A/A*	45 (23.1)	39 (20.6)	1.00 (Reference)	1.00 (Reference)
C/C	*A/C*	39 (20.0)	41 (21.7)	0.85 (0.46, 1.56)	0.73 (0.35, 1.53)
C/C	*C/C*	16 (8.2)	7 (3.7)	1.98 (0.74, 5.31)	1.81 (0.57, 5.70)
C/T	*A/A*	46 (23.6)	46 (24.3)	0.87 (0.48, 1.57)	0.84 (0.41, 1.73)
C/T	*A/C*	23 (11.8)	23 (12.2)	0.87 (0.42, 1.78)	0.83 (0.35, 1.95)
C/T	*C/C*	0 (0.0)	0 (0.0)	—	—
T/T	*A/A*	26 (13.3)	33 (17.5)	0.68 (0.35, 1.33)	0.52 (0.23, 1.17)
T/T	*A/C*	0 (0.0)	0 (0.0)	—	—
T/T	*C/C*	0 (0.0)	0 (0.0)	—	—

Odds ratios were adjusted for study site, year recruited to study, maternal race/ethnicity, parity, maternal age, education, prenatal care, prepregnancy body-mass index, and smoking during pregnancy

Table 3

Distribution (mean ± standard deviation) of total plasma homocysteine, folate, and vitamin B_{12} among abruption cases and controls by MTHFR *677C →T* and *1298A →C* genotypes

	Plasma homocysteine			Plasma folate			Plasma vitamin B_{12}		
	Cases (n=136)	Controls (n=136)	P-value†	Cases (n=136)	Controls (n=136)	P-value†	Cases (n=136)	Controls (n=136)	P-value†
MTHFR *677C →T*									
C/C	5.9 ± 2.0	5.3 ± 2.0	0.039	45.6 ± 16.2	46.2 ± 14.9	0.923	293 ± 143	267 ± 98	0.048
C/T	5.5 ± 2.2	5.6 ± 2.4	0.954	39.5 ± 14.7	41.4 ± 16.0	0.620	265 ± 128	250 ± 109	0.042
T/T	6.3 ± 2.7	6.0 ± 1.7	0.670	41.3 ± 18.9	42.6 ± 14.9	0.665	243 ± 105	213 ± 71	0.267
MTHFR *1298A →C*									
A/A	5.8 ± 2.2	5.5 ± 1.8	0.492	39.9 ± 16.4	44.4 ± 15.7	0.677	268 ± 125	257 ± 97	0.398
A/C	5.9 ± 1.8	5.8 ± 2.7	0.863	46.2 ± 15.8	41.8 ± 14.7	0.225	273 ± 145	235 ± 102	0.175
C/C	6.6 ± 3.2	5.2 ± 1.7	0.485	48.3 ± 13.9	53.3 ± 11.8	0.046	329 ± 140	288 ± 105	0.224

Values were adjusted for study site, year recruited to study, maternal race/ethnicity, parity, maternal age, education, prenatal care, pre-pregnancy body-mass index and smoking during pregnancy

† Tests of significance were performed after Box-Cox transformations were applied to homocysteine, folate, and vitamin B_{12} concentrations