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Reduced folate carrier 80A→G polymorphism, plasma folate, and risk of placental abruption

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Abstract

Folate deficiency and maternal smoking are strong risk factors for placental abruption. We assessed whether the reduced folate carrier [NM_194255.1: c.80A→G (i.e., p.His27Arg)] (*RFC-1*) polymorphism was associated with placental abruption, and evaluated if maternal smoking modified the association between plasma folate and abruption. Data were derived from the New Jersey-Placental Abruption Study—a multicenter, case-control study of placental abruption (2002–2007). Maternal DNA was assayed for the *RFC-1* c.80A→G polymorphism using a PCR-dependent diagnostic test. Maternal folate (nmol/l) was assessed from maternal plasma, collected immediately following delivery. Due to assay limitations, folate levels at ≥ 60 nmol/l were truncated at 60 nmol/l. Therefore, case-control differences in folate were assessed from censored log-normal regression models following adjustment for potential confounders. Distribution of the mutant allele (*G*) of the *RFC-1* c.80A→G polymorphism was similar between cases (52.3%; $n = 196$) and controls (50.5%; $n = 191$), as was the homozygous mutant (*G/G*) genotype (OR 1.1, 95% CI 0.6–2.2). In a sub-sample of 136 cases and 140 controls, maternal plasma folate levels (mean $\pm$ standard error) corrected for assay detection limits were similar between placental abruption cases (63.6 ± 5.1 nmol/l) and controls (58.3 ± 4.7 nmol/l; $P = 0.270$), and maternal smoking did not modify this relationship (interaction $P = 0.169$). We did not detect any association between the *RFC-1* c.80A→G polymorphism and placental abruption, nor was an association between plasma folate and abruption risk evident. These findings may be the consequence of high prevalence of prenatal multivitamin and folate supplementation in this population (over 80%). It is therefore not surprising that folate deficiency may be rare and that the *RFC-1* c.80A→G polymorphism is less biologically significant for placental abruption.

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Introduction

Placental abruption, the premature separation of the placenta prior to delivery of the fetus, is a serious obstetric complication, which affects approximately 1 in 100 pregnancies. Although the etiology of placental abruption remains poorly understood, women of advanced maternal age, multiparity, smokers, cocaine users, chronic hypertension, preeclampsia, prolonged rupture of membranes, previous cesarean delivery, and those with intra-amniotic infections are at greatest risk for placental abruption (Ananth et al. 1999a, b, 2004, 2005, 2006; Cnattingius 1997; Kramer et al. 1997; Kyrklund-Blomberg et al. 2001; Williams et al. 1991a, b). Women with a previous abruption are at 10- to 15-fold increased risk of repeat abruption (Ananth and Cnattingius 2007; Ananth et al. 1996b, 2007b; Rasmussen et al. 1997, 2001), suggesting a possible genetic link.

Although some studies show increased risk of placental abruption among folate-deficient women (Hibbard 1964, 1975; Hibbard and Hibbard 1963; Ray and Laskin 1999; Streiff and Little 1967), others do not (Alperin et al. 1969; Hall 1972; Menon et al. 1966; Whalley et al. 1969). Moreover, the mechanisms that underlie this association also remain unclear. Maternal smoking is one of the most consistently reported risk factors for placental abruption (Ananth and Cnattingius 2007; Ananth et al. 1996a; Williams et al. 1991b), and smoking is known to lower folate and vitamin B₁₂ levels (McDonald et al. 2002; Piyathilake et al. 1994), suggesting an interaction. We recently reported the associations between betaine-homocysteine S-methyltransferase c.742G→A (*BHMT*) and methionine synthase reductase c.66A→G (*MTRR*) polymorphisms and abruption risk (Ananth et al. 2007a) and found that the homozygous mutant form of *BHMT* to be associated with a 2.8-fold increased risk of abruption. In contrast, no association between the *MTRR* and abruption was detected. We also reported the associations between the c.677C→T and c.1298A→C variants of 5,10-methylenetetrahydrofolate reductase (*MTHFR*) and risk of abruption, but found none (Ananth et al. 2007c).

This study focuses on a gene involved in folate transport, the reduced folate carrier-1 c.80A→G polymorphism. Mutations in this gene are known to lower plasma folate. We examined allele and genotype frequencies of the c.80A→G of the *RFC-1* gene in placental abruption cases and controls. We examined differences in plasma folate concentrations between abruption cases and controls, and evaluated if maternal smoking modified these associations.

Materials and methods

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

This institutional review board approved, hospital-based, case-control study was initiated in August 2002, with subjects enrolled from Robert Wood Johnson University Hospital, and Saint Peter's University Hospital, New Brunswick, NJ. After enrollees signed consent, an in-person interview was administered which sought details in patients' socio-demographic, behavioral and medical history. Following the interview, a blood draw was performed on subjects; a vast majority of the interviews and blood draws were completed within 24 h following delivery, and all within 72 h of delivery. The study was restricted to women that delivered at or above 20 weeks in gestation. Further details regarding the study cohort, recruitment characteristics and patient profiles have been previously described (Ananth et al. 2007a, c).

Maternal smoking history was based on self-report. We defined smoking exposure when women smoked regularly 3 months before pregnancy or during pregnancy. Data on prenatal multivitamin and folate use, maternal medical and obstetrical conditions, as well as labor and delivery complications were abstracted from prenatal care and obstetrical charts. Folate and

multivitamin use corresponded to supplementation 3 months prior to the last menstrual period and/or during pregnancy.

Placental abruption cases and controls

Cases were comprised of women with a confirmed clinical diagnosis of placental abruption. A clinical definition of placental abruption included the classical hallmarks and symptoms of painful vaginal bleeding or hemorrhage accompanied by documented fetal distress, uterine pain or tenderness, or uterine hypertonicity. In addition, if the freshly delivered placenta showed signs of retroplacental bleeding or retroplacental hematoma/clots on the placental surface, or if abruption was diagnosed prenatally on ultrasound, we recruited these patients as placental abruption cases.

Controls included women without a clinical diagnosis of placental abruption and delivered in one of the two study hospitals as cases. Controls were matched to abruption cases on (self-reported) maternal race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, or other race/ethnicity) and parity (nulliparous, primiparous, or parity ≥ 2). Women diagnosed with placenta previa in the index pregnancy or placental abruption in any of their previous pregnancies was excluded from the eligible pool of controls.

Mutations in the folate pathway gene

Maternal peripheral blood samples were collected in seven vacutainer tubes (BD Vacutainer, Preanalytical Solutions, Franklin Lakes, NJ), and were immediately transported to the laboratory on ice. Three blood samples were collected in buffered citrate solution 0.105 M, three contained K₂ EDTA 7.5 mg, and the final vacutainer contained no additives.

The *RFC-1* c.80A→G; NM_194255.1 (i.e., p.His27Arg) polymorphism was assessed by a PCR-dependent diagnostic test using primers reported by Shaw et al. (2002). A specific 140 bp amplicon was amplified using the sense primer 5'-AGCGGTGGAGAAGCAGGT-3' and the antisense oligonucleotide 5'-GGAGGTAGGGGGTGATGAAG-3'. Genomic amplifications were performed in a 50 μ l reaction with 0.2–2 μ l of genomic DNA template, 250 ng of each specific primer, in the presence of 1.5 mM MgCl₂, 0.2 mM dNTPs and 1.25 U (0.25 μ l) Platinum Taq DNA Polymerase (Invitrogen), in the 1 $\times$ buffer recommended by the manufacturer. Amplification involved an initial denaturation for 2 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 62°C and 2 min at 72°C. After digestion with *HinfI*, bands of 63, 40 and 37 bp were seen on 9% polyacrylamide gels in the presence of the G allele and segments of 100 and 40 bp were obtained for the A allele.

Folate assay

Approximately 1.0 ml blood was drawn and aliquoted in EDTA tubes. The plasma was then separated and stored in Eppendorf tubes at -70°C , and shipped in dry ice to the laboratory for folate assays. Plasma folate was determined using the Abbott Diagnostic IMX based on a microparticle enzyme immunoassay, as directed by the manufacturer's protocols (Bissonnette et al. 2001). The coefficient of variation for the folate assay was below 4%.

Sample size and statistical power

This case-control study was designed to detect a 1.75-fold increased frequency of the homozygous mutant genotype (G/G) of the c.80A→G polymorphism in the *RFC-1* gene. We based this on an assumed genotype frequency of 20% among controls and 35% among abruption cases, and a type II error rate of 10%. The sample size required for being able to detect an association between the *RFC-1* c.80A→G polymorphism and abruption with an odds ratio of 2.0 was 198 case-control sets.

Statistical analysis

We tested if the *RFC-1* gene was in Hardy–Weinberg equilibrium among controls using the Chi-square test (Tiret and Cambien 1995). Allele and genotype frequencies, with exact 95% confidence intervals (CI), of the *RFC-1* gene were estimated in cases and controls. Association between the *RFC-1* gene and placental abruption risk was based on the odds ratio with 95% CI. Because this was a matched case–control study by design, all preliminary analyses were based on a matched analysis; since the results of these matched analyses did not differ from those based on an unmatched analysis (not shown) we report the results of the unmatched analysis.

The distribution of maternal plasma folate levels were compared between cases and controls using a “violin” plot (Hintze and Nelson 1998). The violin plot, a generalization of the box-plot, uses a kernel density estimate of the distribution of data. Folate levels were analyzed both as categorical (defined as <25, <10 and <5 percentiles, with cut-offs derived from the control population), and as a continuous variable. Since the folate assay had an upper limit of detection at 60 nmol/l, we treated folate levels at or above 60 nmol/l as being censored. We found a logarithmic transformation for folate to provide a good fit to normality. Therefore, folate levels between cases and controls were compared by fitting a log-normal distribution with right censored data (Cox and Oakes 1984). Further details of this model are shown in Appendix 2.

These preliminary analyses were followed by adjustment for a variety of confounding factors. These included study site, year recruited to study, maternal age (categorized as <20, 20–24, 25–29, 30–34, and ≥35 years), marital status (single or married), lack of prenatal care, smoking during pregnancy, and prepregnancy body-mass index (weight in kilograms over squared-height in inches).

For analysis pertaining to plasma folate, we fitted multivariable general log-normal regression models to assess differences in folate levels between abruption cases and controls. Differences in folate concentrations between cases and controls were also examined within *RFC-1* genotypes by including interaction terms for case–control status and *RFC-1* genotype. Finally, to examine whether these differences were modified by maternal smoking status, interactions among case–control status, *RFC-1* genotype and smoking were introduced in the models. We set the *P* value at <0.05 for main effects and interaction effects as being statistically significant.

All statistical analyses were carried out in SAS version 9.1 (SAS Institute, Cary, NC), and R version 2.5.0 (R Development Core Team 2007).

Results

Of a total recruitment of 218 abruption cases and 210 controls, 196 cases and 191 controls had complete genotyping data on the *RFC-1* c.80A→G polymorphism. Of these, plasma folate assays were completed in 136 abruption cases and 140 controls. The overall incidence of placental abruption in both hospitals (combined) over the study period was 0.96%. The distribution of maternal age, parity, race/ethnicity, marital status, and prepregnancy maternal body-mass index were similar between cases and controls, although placental abruption cases were more likely to smoke than controls (Table 1). These distributions were similar between the full and the reduced sample of cases and controls for which folate data were available.

RFC-1 polymorphism and placental abruption

The *RFC-1* c.80A→G polymorphism was in Hardy–Weinberg equilibrium in both abruption cases (*P* = 0.972) and controls (*P* = 0.875). Frequencies of the *RFC-1* mutant allele (G) in abruption cases and controls were 52.3 and 50.5%, respectively (Table 2). The mutant genotype

(G/G) frequencies of the *RFC-1* polymorphism were similar between cases and controls (OR 1.1; 95% CI 0.6–2.2).

A “violin” plot comparing the distribution of plasma folate between cases and controls within the *RFC-1* genotypes is displayed in Fig. 1. While folate levels were fairly similar between cases and controls for the A/G and G/G genotypes, the levels were lower among cases than controls in women with the A/A genotype of the *RFC-1* polymorphism. These data also demonstrate the truncation in folate levels at 60 nmol/l due to assay detection limit. The distributions of plasma folate levels between abruption cases and controls are shown in Table 3. The frequencies of low folate defined as <25, <10 and <5 percentiles were similar between cases and controls, suggesting that low folate was not associated with increased abruption risk.

We examined for an interaction between *RFC-1* genotypes and reduced folate (defined as <25 percentile) on case–control status (Table 4). Among women with the A/A genotype of *RFC-1*, in comparison to women with normal folate, reduced folate was associated with over 2.7-fold (95% CI 1.2–8.4) increased odds of being associated with abruption. However, no association amongst the A/G or G/G genotypes of *RFC-1*, low folate and abruption risk was evident.

We tested for an interaction between maternal smoking and abruption on folate concentrations (Table 5). These data did not reveal any interaction ($P = 0.169$).

Discussion

Studies dating back to the 1960s and 1970s have suggested that folate-deficient women carry an increased susceptibility to develop placental abruption; however, the processes that underlie this phenomenon remain largely unknown. The reduced folate carrier gene is involved in the metabolic pathway and its function lies in the transport of folate. One polymorphism in this folate carrier gene, a replacement of histine by arginine at exon 2 of the *RFC-1* protein c.80A→G, has been associated with lower plasma folate, and arguably higher homocysteine levels (Chango et al. 2000). An association between the mutant genotype of *RFC-1* and neural tube defects was demonstrated in some (De Marco et al. 2001; Morin et al. 2003; Shaw et al. 2002) but not other (Relton et al. 2004) studies.

Our findings suggest that *RFC-1* c.80A→G is not associated with abruption. These data did not show any demonstrable interaction between abruption and maternal smoking on mean folate levels. Although unexpected, we found that women with the normal genotype (A/A) of the *RFC-1* (c.80A→G) and low folate were at 2.7-fold (95% CI 1.2–8.4) increased risk for abruption. This is the first study to investigate the associations between the c.80A→G variant of the *RFC-1* gene, folate deficiency and risk of placental abruption.

Maternal folate and placental abruption

The recognition of maternal folate deficiency is being a risk factor for placental abruption dates back to the early 1960s. Hourihane et al. (1960) were perhaps the first to report the association between folate deficiency and increased risk of abruption. Subsequently, Hibbard and Hibbard (1963) reported, based on a case–control study, a strong association between folate deficiency and risk of abruption (OR 598.2; 95% CI 76.6–4,673.1). A meta-analysis reported a pooled odds ratio of 25.9 (95% CI 0.9–736.3) for abruption in relation to folate deficiency (Ray and Laskin 1999). Our study suggests that low folate levels are not a phenomenon that accompanies women with placental abruption—an observation that provides corroboration with some earlier reports (Alperin et al. 1969; Hall 1972; Menon et al. 1966; Whalley et al. 1969).

Two important factors may have played a critical role to account for the lack of association between low maternal folate and placental abruption in our study. First, the average folate levels of women in our study were, in general, significantly higher than those studies reported in the 1960s and 1970s, largely the consequence of universal folate fortification in common foods in the United States. Nilsen et al. (2008) recently reported that folic acid and other vitamin supplementation during pregnancy was associated with 32% reduction in the risk of developing abruption (OR 0.68; 95% CI 0.56–0.83) in a large population-based study in Norway. However, the prevalence of folic acid and/or vitamin supplementation before or during pregnancy in Norway was considerably lower (35–45%) in comparison to those in our population (over 82%; Table 1). Second, maternal plasma for folate determination were ascertained approximately 1–2 days following delivery, and whether the timing of folate assessment may have impacted our results remains unknown.

Previous studies have reported lower folate levels among smokers during their pregnancy in comparison to non-smokers (McDonald et al. 2002). However, this observation was found to be true only among women that smoked less than an hour prior to folate assessment, whereas folate levels were similar between non-smokers and those who had smoked over an hour prior to folate assessment (Piyathilake et al. 1994). This latter finding suggests that through whatever mechanism smoking reduces plasma folate, the effect is transient. We, however, found no relationship between maternal smoking and folate in both abruption cases and controls.

Limitations of the data

A few limitations of our study call for caution in the interpretation of findings. The *RFC-1* c.80A→G variant was chosen as a potential SNP associated with placental abruption, but other variants in the folate metabolizing pathway may also demonstrate an association with abruption. Second, although this study was adequately powered to detect associations between *RFC-1* and abruption risk, some of the sub-analyses may be restricted by small sample sizes. In particular, the recent recommendation of universal folate fortification in foods and liberal use of prenatal multivitamin supplementation left very few placental abruption cases and controls without this supplementation. Since stratification of analyses by maternal race/ethnicity did not reveal any particular differences (cases and controls were matched on race/ethnicity at recruitment), the number of Hispanic women and those of Asian descent were too few for stable analysis. The possibility of some residual confounding due to unmeasured factors and selection bias owing to referral of high-risk patients recruited to the study are likely.

Plasma folate is highly reflective of recent dietary intake, and we did not elicit dietary data at the time blood was collected for folate assays. Lastly, because placental abruption is apparently a disease of the placenta, and since the placenta permits storage and transport of folate in levels far exceeding those of the maternal circulation (Giugliani et al. 1985), examination of infant *RFC-1* genotypes (and others in the folate metabolism pathway), and maternal–infant interaction in RBC-folate (as an indicator of long-term folate status) on the risk of abruption may provide important clues to understanding the disease etiology.

Conclusions

We found no associations between the *RFC-1* (c.80A→G) polymorphism and placental abruption, nor was there an association between low plasma folate levels and abruption. Folate levels among women enrolled in this study, on average, are generally higher than previous studies. This may be the consequence of dietary folate and nutrient fortification in the United States and prescription of pre-natal vitamins and folic acid supplementation to women before and during their pregnancy. Efforts to segregate the relative maternal and fetal genetic underpinnings may be an important step to understanding this pregnancy complication. Attempts to elucidate the biochemical pathways as to how folate, maternal smoking and

prenatal vitamins interact may help understand their mechanistic pathways to placental abruption and related obstetrical complications.

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Appendix 1

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Other investigators who were involved with the study included 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, Nationwide Children's Hospital, Ohio State University, Columbus, OH).

Appendix 2

We model the distribution of the folate level using a log-normal distribution. Let Y_i^* denote maternal plasma folate (nmol/l) concentration for the i th subject ($1 \leq i \leq n$). The probability density function is given by

$$f(y^*; \mu, \sigma) = \frac{1}{y^* \sigma \sqrt{2\pi}} \exp \left[-\frac{1}{2\sigma^2} (\ln y^* - \mu)^2 \right].$$

Since plasma folate concentrations exceeding 60 nmol/l were censored at that value, the likelihood function (L_i) for estimating the lognormal parameters is a product of terms of the form:

$$L_i(\mu_i; y_i) = f(\mu_i; y_i)^{1-c_i} S(\mu_i; y_i)^{c_i}$$

where

$$S(y; \mu, \sigma) = \int_y^{\infty} f(v; \mu, \sigma) dv$$

is the probability that the concentration y exceeds 60, c_i is a censoring indicator, coded as

$$c_i = \begin{cases} 0 & \text{if } y^* < 60 \\ 1 & \text{if } y^* \geq 60 \end{cases}$$

and $y_i = \min(y_i^*, 60)$. Covariates may be accommodated in a regression model framework by expressing the log-normal mean folate as follows:

$$E(\log Y_i^*) = \beta_0 + \beta_1(\text{CASE}_i) + \beta_2(\text{SMK}) + \beta_3(\text{CASE} \times \text{SMK}) + \sum_{k=4}^p \beta_k(X_{ki})$$

where CASE refers to case–control status (coded as 0 and 1 for controls and cases, respectively), SMK to maternal smoking status, coded as 0 for non-smokers and 1 for smokers, and X_k to the set of confounders. Maximum likelihood estimates of the parameters may be obtained using the “survreg” function in the “survival” package of the R statistical system (R Development Core Team 2007).

Fig. 1. Distribution of plasma folate concentration (nmol/l) among abortion cases and controls stratified by reduced folate carrier-1 (c.80A→G) polymorphism (“violin” plot): the New Jersey-Placental Abruption Study, 2002–2007

Table 1

Maternal socio-demographic characteristics in placental abruption cases and controls in the full sample and the reduced sample of women that had completed folate assays: the New Jersey-Placental Abruption Study, 2002–2007

Maternal characteristics	Full sample		Reduced sample	
	Cases (%) (<i>n</i> = 196)	Controls (%) (<i>n</i> = 191)	Cases (%) (<i>n</i> = 136)	Controls (%) (<i>n</i> = 140)
Maternal age (years)				
< 19	2.6	2.1	2.2	2.2
19–24	16.4	15.6	17.8	15.9
25–34	52.3	52.1	51.1	49.3
≥35	28.7	30.2	28.9	32.6
Parity				
Nulliparous	36.4	35.9	37.0	37.0
Primiparous	35.4	34.9	35.6	32.6
Parity ≥2	28.2	29.2	27.4	30.4
Maternal race/ethnicity				
Caucasian	32.8	39.1	34.1	40.6
African-American	18.0	15.6	17.0	15.9
Hispanic	32.8	30.7	32.6	29.0
Other race/ethnicity	16.4	14.6	16.3	14.5
Education < high school	42.1	32.8	43.7	31.9
Single marital status	32.1	27.1	28.2	25.4
Maternal smoking	32.3	28.1	35.6	23.9
Prenatal vitamin/folate	82.1	93.8	88.2	93.4
Prepregnancy BMI [‡]	25.0 ± 5.8	24.7 ± 5.2	25.3 ± 6.1	24.7 ± 5.3
Gestational age (weeks) [‡]	32.7 ± 5.3	37.8 ± 2.7	33.0 ± 5.7	37.9 ± 5.1

[‡] Data reported as mean ± standard deviation. Maternal smoking and prenatal vitamin/folate use includes exposures 3 months prior to pregnancy and/or during pregnancy

BMI body-mass index (kg/m²)

Allele and genotype frequencies of the *RFC-1* (c.80A→G) polymorphism and associations with placental abruption: the New Jersey-Placental Abruption Study, 2002–2007

<i>RFC-1</i> (c.80A →G) alleles and genotypes	All subjects (<i>n</i> = 387) <i>n</i> (%)	Placental abruption: <i>n</i> (%)		Odds ratio (95% confidence interval)	
		Cases (<i>n</i> = 196)		Unadjusted	Adjusted ^a
		Controls (<i>n</i> = 191)			
Allele frequency					
A	374 (48.5)	186 (47.7)	188 (49.5)	1.0 (referent)	1.0 (referent)
G	400 (51.5)	206 (52.3)	194 (50.5)	1.1 (0.8, 1.4)	1.2 (0.9, 1.9)
Genotype frequency					
A/A	91 (24.1)	45 (23.0)	46 (24.1)	1.0 (referent)	1.0 (referent)
A/G	194 (50.1)	97 (49.4)	97 (50.8)	1.0 (0.6, 1.7)	0.9 (0.5, 1.6)
G/G	102 (25.8)	54 (27.6)	48 (25.1)	1.2 (0.7, 2.0)	1.1 (0.6, 2.2)
A/G or G/G	296 (75.9)	151 (77.0)	145 (75.9)	1.1 (0.7, 1.7)	1.0 (0.6, 1.7)

The *RFC-1* alleles were in Hardy Weinberg equilibrium in both cases (*P* = 0.972) and controls (*P* = 0.875)

^aOdds ratios were adjusted for study site, year recruited to study, maternal age, education, marital status, maternal smoking and prepregnancy body-mass index

Table 3

Distribution of plasma folate concentrations among placental abruption cases and controls: the New Jersey-Placental Abruption Study, 2002–2007

Maternal plasma folate	Cases (<i>n</i> = 136) <i>n</i> (%)	Controls (<i>n</i> = 140) <i>n</i> (%)	Odds ratio (95% confidence interval)	
			Unadjusted	Adjusted ^a
Bottom 25% (< 31 nmol/l)	34 (25.2)	35 (25.4)	1.0 (0.6, 1.7)	0.7 (0.4, 1.3)
Bottom 10% (< 24 nmol/l)	22 (16.3)	15 (10.9)	1.5 (0.7, 3.1)	1.0 (0.4, 2.3)
Bottom 5% (< 18 nmol/l)	15 (11.1)	7 (5.1)	2.2 (0.9, 5.6)	1.3 (0.5, 3.9)

Plasma folate concentration (nmol/l) were based on a censored log-normal distribution (see text and appendix)

^aOdds ratios were adjusted for study site, year recruited to study, maternal age, education, marital status, maternal smoking, and prepregnancy body-mass index

Interaction between *RFC-1* (c.80A→G) genotypes and low maternal plasma folate on placental abruption: the New Jersey-Placental Abruption Study, 2002–2007

<i>RFC-1</i> (c.80A→G) genotype	Plasma folate < 25th percentile	Cases (<i>n</i> = 136) <i>n</i> (%)	Controls (<i>n</i> = 140) <i>n</i> (%)	Odds ratio (95% confidence interval)	
				Unadjusted	Adjusted
A/A	Normal	17 (58.6)	24 (70.6)	1.0 (referent)	1.0 (referent)
	Low	12 (41.4)	10 (29.4)	1.9 (0.7, 5.5)	2.7 (1.2, 8.4)
A/G	Normal	57 (82.6)	52 (73.2)	1.0 (referent)	1.0 (referent)
	Low	12 (17.4)	19 (26.8)	0.6 (0.3, 1.3)	0.5 (0.2, 1.2)
G/G	Normal	29 (75.0)	29 (82.1)	1.0 (referent)	1.0 (referent)
	Low	9 (25.0)	6 (17.1)	1.6 (0.5, 5.5)	0.4 (0.1, 2.1)
A/G or G/G	Normal	86 (81.1)	81 (77.1)	1.0 (referent)	1.0 (referent)
	Low	21 (18.9)	25 (22.9)	0.8 (0.4, 1.5)	0.5 (0.2, 1.1)

The cut-off for low plasma folate (<25 percentile) was 31.0 nmol/l. Odds ratios were adjusted for maternal age, education, and prepregnancy body-mass index. Adjusted *P* value for interaction between *RFC-1* (c.80A→G) polymorphism and low plasma folate: *P* = 0.365

Table 5

Distribution of plasma folate among placental abruption cases controls stratified by maternal smoking: the New Jersey-Placental Abruption Study, 2002–2007

Maternal smoking	Mean $\pm$ standard error plasma folate (nmol/l)		<i>P</i> value
	Cases (<i>n</i> = 136)	Controls (<i>n</i> = 140)	
Total (<i>n</i> = 276)	63.6 $\pm$ 5.1	58.3 $\pm$ 4.7	0.270
Non-smokers (<i>n</i> = 194)	64.3 $\pm$ 5.2	59.1 $\pm$ 5.0	0.136
Smokers (<i>n</i> = 82)	61.3 $\pm$ 6.5	56.4 $\pm$ 5.8	0.575
<i>P</i> value	0.190	0.504	

Plasma folate concentration (nmol/l) were based on a censored log-normal distribution (see text and appendix). Folate comparisons were adjusted for marital status and education. All reported *P* values are adjusted. Adjusted *P* value for interaction between case–control status and maternal smoking on plasma folate: *P* = 0.169