One-carbon metabolism enzyme polymorphisms and uteroplacental insufficiency

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OBJECTIVES: This study was undertaken to test novel genetic polymorphisms involved in 1-carbon metabolism for a potential association with increased risk of developing pregnancy complications associated with uteroplacental insufficiency.

STUDY DESIGN: This was a prospective cohort study consisting of 50 women at low risk and 93 women at high risk for having a pregnancy complication develop. Maternal and fetal DNA samples were genotyped for methionine synthase (*MTR*) A2756G, methionine synthase reductase (*MTRR*) A66G and methylenetetrahydrofolate dehydrogenase (*MTHFD1*) G1958A. A chi squared or χ^2 analysis was used to compare genotypes and pregnancy outcome, 1-way analysis of variance and linear regression were used to compare genotype with continuous variables.

RESULTS: The fetal *MTR* 2756 G allele was associated with uteroplacental insufficiency ($P = .022$, likelihood ratio = 10.4) and maternal homocysteine ($P = .017$). The maternal *MTR* A2756G polymorphism was associated with uteroplacental insufficiency ($P = .049$, likelihood ratio = 6.0), but only in mothers not supplementing with high-dose B-vitamins. The maternal MTHFD1 AA genotype was associated with intrauterine growth restriction ($P = .047$, likelihood ratio = 5.8).

CONCLUSION: This study suggests the maternal and fetal *MTR* 2756 G allele is an important risk factor in the development of uteroplacental insufficiency. In addition, the maternal MTHFD1 1958 AA genotype may be associated with intrauterine growth restriction.

Key words: Intrauterine growth restriction;1-carbon metabolism, polymorphisms, preeclampsia, uteroplacental insufficiency

Cite this article as: Furness DLF, Fenech MF, Khong YT, et al. One-carbon metabolism enzyme polymorphisms and uteroplacental insufficiency. Am J Obstet Gynecol 2008;199:276.e1-276.e8.

One-carbon metabolism plays a critical role in determining the availability of folate and methionine that are essential for placental and fetal development. Defects in this pathway reduce the availability of methionine, which is needed for cell growth and the production of S-adenosylmethionine (SAM). SAM is a principal methyl donor inside cells and the methyl groups are key for DNA control of cellular proliferation, cellular migration, differentiation, and cell-to-cell recognition.[1] Single nucleotide polymorphisms (SNPs) in this metabolic pathway can also lead to increased homocysteine (Hcy) causing endothelial cell dysfunction and oxidative stress,[2] which have been associated with uteroplacental insufficiency (UPI), ie, pregnancy complications associated with restricted uteroplacental circulation, including preeclampsia and intrauterine growth restriction (IUGR). Over the past 5 years many studies have demonstrated an association between 2 common methylenetetrahydrofolate reductase (MTHFR) polymorphisms (C677T, and A1298C) and adverse pregnancy outcome, whereas other studies could not confirm this association. This apparent controversy is most likely to be explained because of differences in dietary vitamin intake and vitamin supplementation, specifically folate and riboflavin, between the various populations published so far.[3-5]

Limited data are available on some recently discovered SNPs, which are involved in 1-carbon metabolism. The associations between polymorphisms in methionine synthase (*MTR*) A2756G and methionine synthase reductase (*MTRR*) A66G have been examined in relation to Down syndrome and Hcy metabolism.[6,7] MTR catalyzes the methylation of Hcy to methionine using vitamin B_{12} as a cofactor and 5-methyl-tetrahydrofolate (5-MTHF) as methyl donor (Figure 1).[8] *MTRR* plays a critical role in maintaining vitamin B_{12} (cobalamin) in an active form and consequently may be an important determinant of Hcy concentrations.[9] In addition, the A66G polymorphism in

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Presented at the 28th Annual Meeting of the Society for Maternal–Fetal Medicine, Dallas, TX, Jan. 28-Feb. 2, 2008.

Received March 12, 2008; revised April 17, 2008; accepted June 5, 2008.

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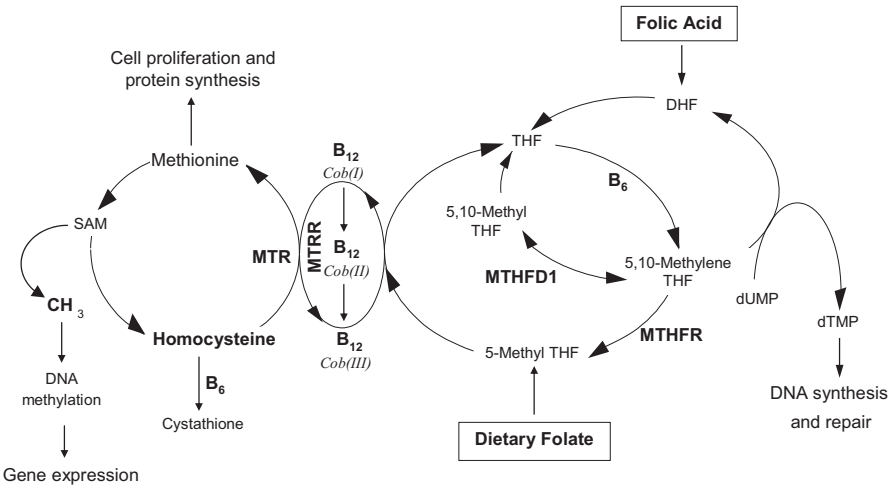
Supported by The Channel 7 Children's Research foundation, the National Institutes of Health, and the Cooperative Research Centre for Diagnostics.

0002-9378/$34.00 • © 2008 Mosby, Inc. All rights reserved. • doi: 10.1016/j.ajog.2008.06.020

FIGURE 1
A simplified scheme of one-carbon metabolism. Key intermediates and enzymes are given



DHF, dihydrofolate; THF, tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MTHFD1, methylenetetrahydrofolate dyhydrogenase; MTR, methionine synthase; MTRR, methionine synthase reductase; SAM, S-adenosylmethionine; B_{12}, vitamin B_{12} (cobalamin, in various oxidative forms); B_6, vitaminB_6; dUMP, uracil; dTMP, thymine; CH_3, methyl group. Vitamin B_{12} acts as an intermediate methyl carrier between methyl-THF and Hcy during the MTR-catalysed remethylation of Hcy to methionine. Vitamin B_{12} cycles between cob(I)alamin and methylcob(III)alamin. However, cobalamin(I) is a strong reductant and can be oxidized to produce an inactive cobalamin(II). MTRR catalyzes the reductive methylation of cobalamin(II) to methylcobalamin(III) using SAM as the methyl donor.

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MTRR appears to represent a risk factor for premature cardiovascular disease[10] and neural tube defects.[11] 5,10-methyl-THF dehydrogenase (MTHFD1) is a nicotinamide adenine dinucleotide phosphate (NADP)-dependent enzyme that catalyzes 3 sequential reactions in the conversion of 1-carbon derivatives of tetrahydrofolate, which are substrates for methionine, thymidylate, and de novo purine synthesis. The G1958A polymorphism has been associated with maternal genetic risk of having a child with neural tube defects[12] and placental abruption[13]; however, to date it has not been studied in relation to preeclampsia or IUGR.

The purpose of this study was to focus on these novel genetic polymorphisms involved in 1-carbon metabolism for a potential association with increased risk of developing UPI, which includes preeclampsia and IUGR.

Materials and Methods

Study design

This study was a prospective cohort study conducted at Commonwealth Scientific and Industrial Research Organisation Human Nutrition Human Nutrition and Women's and Children's Hospital (WCH), Adelaide, South Australia. The study was approved by the Human Experimentation Ethics Committees of both institutions, and participants provided informed consent after the aims and protocols of the study were explained. Pregnant volunteers were enrolled in early pregnancy (before 20 weeks' gestation). When consent was signed, an appointment was made for a fasting blood sample at 20 weeks' gestation. Enrolled patients were given a delivery pack that included the necessary instructions and collection devices for placenta and blood at the time of delivery. Information on diet, socioeconomic factors, supplement intake, obstetric, and medical history was collected. A digital balance was used to record the weights of all mothers to the nearest 100 g. Measurements of height were made by using a stadiometer to the nearest 1 cm. Maternal body mass index (BMI, kg/m^2) was calculated using height and weight.

Recruiting criteria

Inclusion

All patients less than 20 weeks' gestation who attended the antenatal clinics at the WCH were invited to participate in this study.

Exclusion criteria

The exclusion criteria included any maternal or fetal conditions requiring termination of pregnancy, any known major fetal anomaly or fetal demise, multifetal pregnancy, any medical disorder requiring systemic steroids, preexisting renal disease (creatinine >100 mcmol/L), and lack of informed consent.

Patient classification

High-risk classification

Women at high risk of having an adverse pregnancy outcome develop were so classified based on a variety of risk factors identified from their obstetric history. The risk factors included: previous severe preeclampsia/eclampsia, previous severe IUGR (<10 percentile requiring delivery <36 week's gestation, placental abruption, preterm birth (<37 week's gestation), greater than or equal to 3 miscarriages, prior fetal demise, diabetes/insulin resistance and chronic hypertension/and obesity BMI (>30)/polycystic ovaries. Nulliparous women could also be recruited as high risk if they fulfilled 1 of the inclusion criteria (recurrent spontaneous miscarriages, known chronic hypertension, BMI >30, and/or known polycystic ovarian syndrome.)

Low-risk classification

Women who were classified as being at low risk of having an adverse pregnancy outcome develop were healthy women without any known preexisting medical disorder and a prior normal pregnancy (>37 weeks, >10th growth percentile, no hypertension). Healthy nulliparous

women were included as low-risk pregnancy patients.

Clinical diagnosis of pregnancy outcome

UPI was defined as preeclampsia, IUGR, gestational hypertension, and placental abruption. Pregnancy outcomes were defined according to the criteria of the Australasian Society for the Study of Hypertension in Pregnancy.[14] Gestational hypertension is hypertension arising in pregnancy after 20 weeks' gestation without any other feature of the multisystem disorder preeclampsia and resolves within 3 months' postpartum.[14] Gestational hypertension is defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg on 2 occasions ≥6 hours apart.

Preeclampsia is hypertension in pregnancy after 20 weeks' gestation accompanied by the onset of 1 or more of the following: proteinuria ≥300 mg/24 hours or spot urine protein/creatinine ratio ≥30 mg/mmol, renal insufficiency, liver disease, neurologic symptoms (headache, visual disturbances, persistent hyperreflexia), hematologic disturbances (HELLP syndrome), and fetal growth restriction.

Customized percentiles were used to identify growth restriction. Customized percentiles allows calculation of an individual percentile for a particular birth weight, after adjusting for maternal height, weight, parity, and ethnic group and the sex of the baby.[15] IUGR was defined as <10th percentile according to Australian/New Zealand criteria.[16] Normal BMI limits were derived from the 90th and the 10th percentile values for BMI in the population and applied in customized percentile calculation software. The purpose of the limits is to avoid overadjustment to extremes that represent disease, that is, maternal obesity or malnutrition at the upper and lower limits, respectively. The verification and severity of IUGR was based on the reduction of fetal growth velocity on fetal growth trajectory using observed serial ultrasound scans.

Placental abruption was defined as being a clinically evident abruption further confirmed by macroscopic findings on inspection of the placenta. For overlapping diagnosis, preeclampsia had the highest priority, ie, a patient with preeclampsia and IUGR was classified as preeclampsia, therefore all IUGR cases were normotensive IUGR. The diagnoses of pregnancies were based on detailed chart reviews with complete access to all medical records.

Index pregnancies

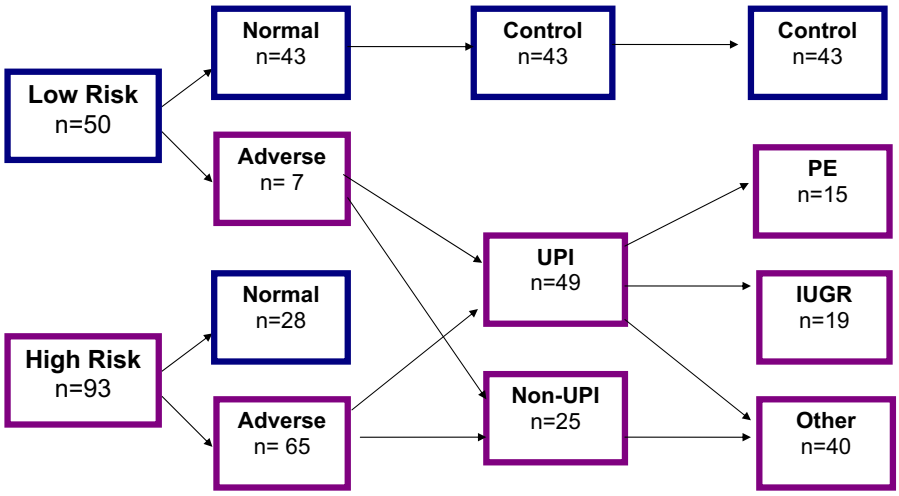
As part of prior patient care, many of the high-risk patients had already been genotyped for MTHFR 677 and 1298 polymorphisms; most of these patients were treated with high-dose folate, vitamin B_{12}, and vitamin B_6 supplements. Because these high-dose vitamin supplementations can mask the phenotypical effects of the MTHFR 677 CT and 1298 AC polymorphism, a separate layer of analysis was introduced based on the so-called index pregnancy, ie, that prior pregnancy that led the clinicians to genotype those particular patients. In the index pregnancies, women were not treated with high dose B-vitamins. This analysis was performed in an attempt to eliminate the influence of high B-vitamin consumption.

Pregnancy groups

One hundred forty-nine women were recruited via the National Institutes of Health (NIH) trial and the WCH antenatal clinics. Of the 149 women recruited, 6 were excluded for the following reasons: 1 moved overseas, 3 dropped out, and 2 miscarried (prior to 20 weeks' gestation). The number of women in each group is shown in Figure 2.

DNA was obtained from 139 mothers and 124 placentas. The missing maternal samples were from 1 low-risk healthy pregnancy with a normal outcome (control) and the remaining from high-risk pregnancies, 1 with a normal outcome and 3 with adverse outcomes, including 1 case of IUGR. Seventeen placental samples could not be collected and 2 samples did not yield sufficient DNA. Two of the missing samples were from low-risk pregnancies with normal outcomes. The remaining were from high-risk pregnancies including 7 normal outcomes and 10 adverse outcomes. Two

FIGURE 2
Classified pregnancy groups



Low risk = healthy women with low risk of having pregnancy complications develop; high risk = women with high risk of having pregnancy complications develop; normal = pregnancies with clinically normal outcomes; adverse = pregnancies with adverse outcome; control = low-risk women with clinically normal outcome; UPI = uteroplacental insufficiency including preeclampsia; IUGR = intrauterine growth restriction, gestational hypertension and placental abruption; Non-UPI = adverse outcomes other than UPI; PE = preeclampsia; Other = adverse outcome other than PE or IUGR, eg, placental abruption and gestational hypertension.

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TABLE 1
Comparison of maternal age, gestation and birth weight

	Mothers age (y)	Gestational age (d)	Birth weight (g)
Control (n = 43)	31.9 ± 0.8	278.6 ± 1.1	3662.8 ± 60.3
UPI (n = 49)	32.5 ± 1.0	262.6 ± 6.4	3199.6 ± 237.3
t test (2-tailed) *P*	.696	.026	.075
Control (n = 43)	31.9 ± 0.8	278.6 ± 1.1	3662.8 ± 60.3
PE (n = 5)	35.1 ± 1.9	255.1 ± 5.6	2674.0 ± 169.5
t test (2-tailed) *P*	.077	.011	.019
Control (n = 43)	31.9 ± 0.8	278.6 ± 1.1	3662.8 ± 60.3
IUGR (n = 19)	30.9 ± 1.4	251.5 ± 3.2	2301.1 ± 189.6
t test (2-tailed) *P*	.507	.004	>.001

All data represented as mean ± SEM
IUGR, intrauterine growth restriction; *PE*, preeclampsia; *UPI*, uteroplacental insufficiency.

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were classified as UPI, which included 1 case of placental abruption and 1 case of IUGR.

Micronutrient analysis

At 20 weeks' gestation a fasted blood sample was collected from study participants in one 9 mLVacuette-Lithium Heparin tube, one 4 mL Vacuette-K-EDTA, and one 4 mL Vacuette-Serum. Plasma was removed from the Vacuette-K-EDTA and delivered within 2 hours of collection to the Core Laboratory, Division of Laboratory Medicine, Women's and Children's Hospital Adelaide, South Australia, for the quantification of L-homocysteine. Red cell folate, serum folate, serum vitamin B_{12}, and vitamin B_6 quantification was performed by the Institute of Medical and Veterinary Science (IMVS), Adelaide, South Australia.

Genotype analysis

DNA was extracted from maternal blood and fetal cord tissue before genotyping for *MTR* A2756G, *MTRR* A66G, *MTHFD1* G1958A polymorphisms using the Applied Biosystems 7900HT Real Time PCR System and ABI 7300 Sequence Detection System with the SDS ver. 1.9 [software] (Applied Biosystems, Foster City, CA). Each reaction was performed in duplicate with the first column of each plate used as a nontemplate control, confirming lack of nonspecific amplification. Primers for *MTR* A2756G, *MTRR* A66G and *MTHFD1* G1958A were ordered from Applied Biosystems (ABI) as Assays-on-Demand (Applied Biosystems).

Statistics

The X^2 goodness-of-fit test was used to compare allele proportions and genotype with UPI, preeclampsia, and IUGR. The Hardy-Weinberg equilibrium equation was performed for all genotype data. The association between maternal and fetal SNPs within the 1-carbon metabolic pathway with red cell folate (RCF), serum folate (SF), serum vitamin B_{12}, red blood cell vitamin B_6, and plasma Hcy were determined by 1-way analysis of variance (ANOVA). Where a significant association was detected, linear regression was used to test the effects of confounding factors, such as B-vitamin supplementation and smoking. All data analyses were performed by means of the computer-based statistical package of Statistical Product and Service Solution (SPSS) version 14.0 (SPSS, Chicago, IL). Results were reported as mean ± standard error (SE) of the mean and $P < .05$ was considered statistically significant.

Results

Table 1 presents the clinical characteristics of the control patients and those who had UPI, preeclampsia, and IUGR develop. Preeclamptic and IUGR cases were characterized by significantly shorter gestations and lower birth weights.

Maternal allele distribution

There were no significant differences in maternal or fetal allele frequencies among women classified as low-risk healthy pregnancies (controls) and those who had UPI develop, including preeclampsia and IUGR.

Maternal genotype distribution

Genotype frequencies for all the tested polymorphisms were calculated and found to fit the Hardy-Weinberg equilibrium and did not deviate markedly from previously reported frequencies for these polymorphisms.[9,17,18] The maternal *MTHFD1* AA genotype was not associated with preeclampsia, but was significantly associated with the development of IUGR ($P = .047$) as shown in Table 2. The likelihood ratio (LR) was calculated to be 5.8.

Effect of maternal polymorphisms on micronutrient and homocysteine concentrations

The ANOVA results indicate that increased RCF is significantly associated with the *MTHFD1* 1958 G allele ($P = .040$). Serum vitamin B_{12} was significantly associated with the *MTRR* 66 variant G allele ($P = .004$); however, after controlling for confounding factors including folic acid, vitamin B_{12} supplement intake, and smoking, the significant association was no longer detected for either polymorphism.

Fetal genotype distribution

The fetal genotype frequencies for all tested SNPs were calculated and found to fit the Hardy-Weinberg equilibrium equation. When analysing the fetal SNPs, the *MTR* 2756 A > G substitution was significantly associated with UPI ($P = .022$, LR = 10.4) when compared with low-risk healthy clinically normal outcomes (Table 3).

The association of fetal polymorphisms on circulating micronutrients and Hcy concentrations in the mother

The fetal *MTR* A2756G genotype was significantly associated with maternal

plasma Hcy concentration ($P = .022$); after applying linear regression to control for folic acid supplementation and smoking, the significance improves to $P = .017$. The RCF, SF, vitamin B_{12}, and vitamin B_6 were not significantly associated to SNPs in *MTRR* or *MTHFD1* (Table 4).

Index pregnancy analysis

Maternal allele distribution:

There were no significant associations detected between maternal allele frequency in the index pregnancies in relation to the development of UPI.

Maternal genotype distribution:

Table 5 shows the index pregnancy data, which reveals a significant association between *MTR* A2756G polymorphism and the development of UPI ($P = .049$, LR = 6.0).

Comment

This study suggests that the maternal and fetal *MTR* 2756 G allele represents an important risk factor for the development of UPI, which includes preeclampsia and IUGR. The maternal *MTR* 2756 polymorphism investigations in the most recent pregnancies (including women treated with high-dose B-vitamins) did not reveal an association with UPI; however, in the index pregnancy investigation (allowing evaluation of the clinical phenotype without vitamin supplementation) a significant association was detected in relation to UPI. These data suggest that high concentrations of folic acid, vitamin B_{12}, and vitamin B_6 may be able to modify pregnancy outcome, reducing the risk for pregnancy complications associated with UPI. In addition, the fetal *MTR* 2756 GG genotype was significantly associated with increased maternal plasma Hcy concentration and with the development of UPI. The *MTR* A2756G substitution results in an amino acid change of an aspartic acid to a glycine (D919G), at the penultimate position in a long helix that leads out of the cobalamin domain.[19] Having the glycine residue at this position could have an effect on the secondary structure of the protein and therefore have functional consequences.[20] Further studies are needed to elucidate if this allele change impacts on enzyme activity and in turn, 1-carbon metabolism, which may effect placental development and pregnancy outcome.

The other novel finding in this study relates to the maternal *MTHFD1* 1958 AA genotype and IUGR. To date, there have been no studies investigating the effect of MTHFD1 on preeclampsia and/or

TABLE 2
Distribution of maternal polymorphisms in low risk healthy pregnancies (control) and women who had IUGR develop in the current pregnancy

Maternal	Control (n = 42)	IUGR (n = 18)	χ^2 *P*
MTR A2756G			
AA	28 (66.7%)	9 (50.0%)	
AG	10 (23.8%)	8 (44.4%)	.191
GG	4 (9.5%)	1 (5.6%)	
MTRR A66G			
AA	14 (33.3%)	6 (33.3%)	
AG	18 (42.9%)	9 (50.0%)	.669
GG	10 (23.8%)	3 (16.7%)	
MTHFD1 G1958A			
GG	17 (40.5%)	6 (33.3%)	
GA	19 (45.2%)	5 (27.8%)	.047
AA	6 (14.3%)	7 (38.9%)	

Data represent number of women for each genotype and percentage (%) from the total study group.
IUGR, intrauterine growth restriction.

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TABLE 3
Distribution of various fetal polymorphisms in 1-carbon metabolism from low-risk healthy (control) pregnancies and those who had UPI develop in the current pregnancy

Fetal	Control (n = 41)	UPI (n = 47)	χ^2 *P*
MTR A2756G			
AA	23 (56.1%)	26 (55.3%)	
AG	18 (43.9%)	14 (29.8%)	.022
GG	0 (0.0%)	7 (14.9%)	
MTRR A66G			
AA	16 (39.0%)	15 (31.9%)	
AG	20 (48.8%)	25 (53.2%)	.692
GG	5 (12.2%)	7 (14.9%)	
MTHFD1 G1958A			
GG	14 (34.1%)	13 (27.7%)	
GA	18 (43.9%)	24 (51.1%)	.662
AA	9 (22.0%)	10 (21.3%)	

Data represent number of fetal genotypes and percentage (%) from the total study group.
UPI, uteroplacental insufficiency.

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TABLE 4
Association of fetal polymorphisms with circulating micronutrients and homocysteine concentrations in the mother

	Hcy μmol/L	RCF nmol/L	SF nmol/L	B_{12} pmol/L	B_6 Status[a]
MTR A2756G					
AA (n = 66)	4.5 ± 0.2	665.3 ± 28.8	27.0 ± 1.2	231.1 ± 19.2	42.8 ± 1.9
AG (n = 41)	4.6 ± 0.2	624.7 ± 36.7	25.7 ± 1.7	253.6 ± 22.4	42.6 ± 2.9
GG (n = 11)	5.9 ± 0.5	679.9 ± 74.9	26.3 ± 3.4	180.5 ± 19.5	38.2 ± 4.4
ANOVA *P*	.023	.636	.811	.325	.677
Adjusted *P* value[b]	.017				
MTRR A66G					
AA (n = 42)	5.0 ± 0.3	637.0 ± 38.0	25.0 ± 1.7	208.5 ± 22.3	43.4 ± 2.6
AG (n = 61)	4.6 ± 0.2	682.7 ± 28.8	28.2 ± 1.2	251.2 ± 17.4	40.9 ± 2.1
GG (n = 14)	4.7± 0.3	566.6 ± 65.1	23.1 ± 2.8	237.9 ± 51.0	44.2 ± 4.4
ANOVA *P*	.346	.220	.124	.348	.679
MTHFD1 G1958A					
GG (n = 38)	4.5 ± 0.3	708.8 ± 35.0	29.2 ± 1.7	228.1 ± 25.6	39.4 ± 2.4
GA (n = 53)	4.7 ± 0.2	655.2 ± 34.0	25.0 ± 1.4	248.4 ± 21.2	42.8 ± 2.6
AA (n = 26)	5.1 ± 0.3	565.9 ± 41.3	25.3 ± 1.8	213.8 ± 18.3	46.1 ± 2.2
ANOVA *P*	.318	.054	.117	.590	.280

All data are represented as mean ± SEM.
B_{12}, serum vitamin-B_{12}; B_6, red cell vitamin-B_6; Hcy, L-homocysteine in plasma; RCF, red cell folate; SF, serum folate.
[a] Based on PPA activity which is inversely related to vitamin-B_6 concentration in RBCs.
[b] Controlled for folic acid intake and smoking using linear regression analysis.
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TABLE 5
Distribution of various maternal genotypes involved in 1-carbon metabolism genes in low-risk healthy normal (control) pregnancies and those with UPI in their index pregnancy

Maternal	Control (n = 41)	UPI (n = 24)	$\chi^2 P$
MTR A2756G			
AA	27 (65.9%)	9 (37.5%)	
AG	10 (24.4%)	13 (54.2%)	.049
GG	4 (9.8%)	2 (8.3%)	
MTRR A66G			
AA	14 (34.1%)	5 (20.8%)	
AG	17 (41.5%)	16 (66.7%)	.144
GG	10 (24.4%)	3 (12.5%)	
MTHFD1 G1958A			
GG	17 (41.5%)	5 (20.8%)	
GA	19 (46.3%)	13 (54.2%)	.169
AA	5 (12.2%)	6 (25.0%)	

Data represents number of maternal genotypes and percentage (%) from the total study group.
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IUGR. Parle-McDermott et al[13] investigated *MTHFD1* G1958A in an Irish population and found a significant association with placental abruption and the occurrence of neural tube defects.[12] Studies in relation to MTHFD1 are in the early stages and further investigations, including larger subject numbers are needed. In addition, various polymorphisms within the gene and functional tests are required to understand the impact on enzyme activity and the possible risk associated with pregnancy complications.

There have been a number of previous studies that have investigated polymorphisms within *MTHFR*, *MTR*, and *MTRR* genes and related these with pregnancy diseases in various populations.[21-27] Previous studies have shown an association with *MTR* (A2756G) and *MTRR* (A66G) genetic polymorphisms and the levels of circulating Hcy concentration[28-36]; however, with the exception of the fetal *MTR* A 2756G, an association

was not detected in this study. The neutral influence of these polymorphisms on plasma Hcy may be explained by adequate status in folate, vitamin B_{12}, and vitamin B_6 from supplement intake or dietary sources. Supplementing with folic acid, vitamin B_{12}, vitamin B_6, and vitamin B_2 enhance specific enzyme functions, reducing plasma Hcy concentrations, and therefore may have masked the effect of the tested genetic variants. A number of studies[9,28,29,37] have suggested that maternal environmental factors have a much greater influence than genetic factors on the levels of circulating Hcy during pregnancy. In addition, in vitro studies[3] have shown that folate status is more important in explaining variations in Hcy concentration compared with genotype, which supports the notion of modifying possible risks of pregnancy complications with substrate and cofactor nutrients in 1-carbon metabolism.

A limitation of this study is that in the analysis of the index pregnancy data are not available to ascertain if these women were supplementing with B-vitamins. Although it is highly probably that these women were not supplementing with high-dose B-vitamins because (a) the uptake of periconception folate has been very low in South Australia over the past decade,[38] and (b) at that stage, these women had not yet had a genetic polymorphism associated with B-vitamin metabolism diagnosed.

The data appeared to indicate that the maternal *MTHFD1* AA genotype was associated with increased RCF; however, after controlling for confounding factors such as folic acid, vitamin B_{12} supplement intake and smoking, the relationship was no longer significant. Moreover, the *MTRR* 66 GG genotype was associated with increased serum vitamin B_{12}, but again the significance was lost after logistic regression was used to control for vitamin supplement intake and smoking. These results support the plausibility of modifying risk nutritionally; however, controlled interventions are needed to determine precisely which genotypes benefit, which vitamin combinations are best and at what dosage. In this study, both RCF and SF were measured providing comprehensive information on folate status. SF becomes low in the early stages of folate deficiency before the reduction of tissue folate stores. RCF is a direct measure of tissue folate stores.

This is the first study to include methionine synthase (*MTR*) A2756G, methionine synthase reductase (*MTRR*) A66G, and methylenetetrahydrofolate dehydrogenase (*MTHFD1*) G1958A MTR in association with Hcy, folate and B-vitamin cofactors involved in 1-carbon metabolism in both mother and fetus in relation to preeclampsia and IUGR risk. Prospective randomized control trials are urgently needed to elucidate the impact of B-vitamin supplementation in pregnancies in couples with polymorphisms in important enzymes involved in 1-carbon metabolism. If confirmed, research of this type may pave the way for personalized nutritional advisories in the obstetrics of the future. ■

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