Common dihydrofolate reductase 19–base pair deletion allele: a novel risk factor for preterm delivery[1–3]

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ABSTRACT

Background: Folate is critical for cell division, a major feature of in utero development. Dihydrofolate reductase (DHFR) is required to convert the folic acid used in supplements and for food fortification and the dihydrofolate produced by thymidylate synthase during DNA synthesis to the reduced folate forms used by the cell.

Objective: We aimed to determine whether a common, recently discovered deletion polymorphism in the *DHFR* gene is a risk factor for preterm delivery or low birth weight.

Design: We studied 324 pregnant women from Camden, NJ. Folate intake was computed from folate supplement intake plus the mean of two 24-h recalls completed during the course of pregnancy. Genomic DNA was extracted from the women's leukocytes and genotyped.

Results: Women with a deletion allele had a significantly greater risk of preterm delivery [adjusted odds ratio (AOR): 3.0; 95% CI: 1.0, 8.8; $P < 0.05$] than did those without a deletion allele. Women with both a *DHFR* deletion allele and low folate intake (<400 μg/d from diet plus supplements) had a significantly greater risk of preterm delivery (AOR: 5.5; 95% CI: 1.5, 20.4; $P = 0.01$) and a significantly greater risk of having an infant with a low birth weight (AOR: 8.3; 95% CI: 1.8, 38.6; $P = 0.01$) than did women without a deletion allele and with a folate intake $\geq$400 μg/d.

Conclusions: The *DHFR* 19–base pair deletion allele may be a risk factor for preterm delivery. In the presence of low dietary folate, the allele may also be a risk factor for low birth weight. This may be a gene-environment interaction. *Am J Clin Nutr* 2005;81:664–8.

KEY WORDS Folate, dihydrofolate reductase, preterm delivery, low birth weight, polymorphism

INTRODUCTION

The need for folate increases during times of rapid cell division and tissue growth, such as fetal development, because of the role of folate in nucleic acid synthesis (1). Dihydrofolate reductase (DHFR) is pivotal because ingested folates must be fully reduced before being metabolized in the body, a reduction carried out by DHFR. Most food folates are fully reduced, but unreduced forms have been found, including folic acid (which is fully unreduced) and dihydrofolates (DHFs; which are partially reduced) (2). In a recent study, blood samples with a total folate concentration $>$50 nmol/L contained an average of 15.7% folic acid, and the upper limit of the range was as high as 31%. Folic acid is used in most vitamin supplements and for food fortification and requires DHFR to participate in cellular reactions. Furthermore, methyltetrahydrofolate, the predominant form of food folate, is readily oxidized to methyl-dihydrofolate and in this form may constitute up to 50% of food folate (3). In the body, DHF is formed chiefly by the action of thymidylate synthase (TYMS) in connection with DNA synthesis. Consequently, the action of DHFR is required not only for the initial reduction of ingested dietary folic acid but more importantly for the continuous reduction of DHF to tetrahydrofolates (THFs). Thus, limitation of maternal DHFR activity could impair the ability of the mother to supply her fetus with a continuous and adequate supply of THF. Dietary and circulating folate have been specifically implicated as risk factors for adverse pregnancy outcome (4, 5). Thus, it is possible that a genetic polymorphism that influences folate metabolism might also affect pregnancy outcome.

We recently discovered a polymorphic 19–base pair (bp) deletion within intron I of *DHFR* (6). Intron 1 is a well known site of regulatory sequences for some genes (7, 8), and a regulatory sequence for intron 1 has been documented for human (9, 10) and mouse (11) *TYMS*, a gene closely related to *DHFR*. Moreover, the mouse *dhfr* gene contains an intron 1 regulatory sequence (12). Because the 19-bp deletion of human *DHFR* removes a potential SP1 transcription factor binding site, it is possible that this new polymorphism acts to decrease *DHFR* transcription and decrease folate availability to the fetus. Moreover, amounts of DHFR protein in CHO cells were significantly higher in the presence of intron 1, and when the intron was deleted, the protein produced was unstable (13). We therefore tested the possibility that this recently discovered *DHFR* 19-bp deletion allele (6) in the mother might contribute to adverse pregnancy outcome.

SUBJECTS AND METHODS

Subjects

The Camden Study prospectively examines the effects of maternal nutrition and growth in generally healthy pregnant women from one of the poorest cities in the United States (4, 14). Participants include young (aged $\leq$ 18 y) and more mature (aged

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19–29 y) women who have enrolled for prenatal care in Camden clinics. Pregnant women with serious nonobstetric problems (eg, lupus, chronic hypertension, type 1 or type 2 diabetes, seizure disorders, malignancies, or drug or alcohol abuse) are not eligible. In this analysis, we focus on data from 324 women who enrolled and delivered before September 1990. That date precedes the Food and Drug Administration's authorization of the addition of folic acid to grains in 1996 with mandatory compliance by January 1998 (15). All participants gave informed consent. This project was approved by the University of Medicine and Dentistry New Jersey's Institutional Review Boards.

Socioeconomic, demographic, lifestyle, and dietary data were obtained by interview at entry to prenatal care and were updated at week 28 of gestation. Ethnicity was self-identified as African American ($n = 104$), Hispanic (Puerto Rican descent; $n = 213$), or white ($n = 7$). Pregravid weight was determined by recall at entry to prenatal care, and weight was measured at each visit with the use of a beam balance scale. In the Camden study, the correlation between measured weight in the second trimester of pregnancy and recalled pregravid weight is fairly strong ($r > 0.9$) (16). Recorded and recalled weights are generally well correlated ($r = 0.75$–0.98), with the caveat that women with higher body weights tend to underreport their weight (17, 18). Height was measured at entry to prenatal care with the use of a stadiometer. Body mass index (BMI) was computed as pregravid weight divided by height squared (in kg/m^2). Total gestational weight gain was calculated as the difference between reported pregravid weight and weight measured within the 2 wk before delivery.

Information on current and past pregnancy outcomes, complications, and infant abnormalities was abstracted from the prenatal record, the delivery record, delivery logbooks, and the infant's chart. Gestational duration was based on the woman's last normal menstrual period and was confirmed or modified by ultrasound. Preterm delivery (PTD) was defined as delivery at <37 completed weeks of gestation. Low birth weight (LBW) was defined as birth weight < 2500 g.

Information on dietary intake (three 24-h recalls completed during pregnancy) and prenatal multivitamin use was obtained by a registered dietitian experienced in working with this population. Food models were used with the 24-h recalls to quantify portion size; dietary probes (eg, if milk was consumed or used in coffee or tea, whether it was whole milk, 2%-fat milk, 1%-fat milk, or skim milk) were used to further refine intake measures. Data were processed at the Campbell Institute of Research and Technology in Camden, NJ. Campbell's nutrition composition database was derived chiefly from the US Department of Agriculture National Nutrient Database (Internet: http://www.nal.usda.gov/fnic/foodcomp/search/) with some additional input from the US Department of Agriculture Food and Nutrient Database for Dietary Studies, 1.0 (Internet: http://www.barc.usda.gov/bhnrc/foodsurvey/fndds_intro.html), industry sources, and Campbell's internal analyses. To avoid any confounding between changes in intake and gestational duration, data on folate intake were based on diet and supplement use recorded in the first 2 recalls: at entry to care and at 28 wk gestation. Average daily folate intake by week 28 was computed from the mean of the first 2 recalls taken during pregnancy (entry and week 28) and from the frequency of prenatal multivitamin-mineral supplement use (pills per day) between the mother's last menstrual period and week 28. Prenatal multivitamins containing folate (usually 800 μg/d in 1985–1990) were prescribed and used after the women entered prenatal care, which occurred at 19.9 ± 7 wk; only 13.3% of the women used supplements of any kind before then. During pregnancy, 30% of the women never used the multivitamin supplements that had been prescribed and an additional 10% used them sporadically. Folate intake was assessed as a percentage of the recommended dietary allowance (RDA) in effect in 1990, when the RDA for pregnancy was 400 μg/d (19). We characterized folate intake from diet and supplements as low (<400 μg/d) or not low (≥400 μg/d) according to this recommendation.

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5' CCCACGGTCGGGGTACCTGGGCGGGACGCGCCAGGCCGACTCCCGGCG  3'
   ||||||||||||||                    ||||||||||||||
5' CCCACGGTCGGGGT..................GGCCGACTCCCGGCG  3'
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FIGURE 1. Dihydrofolate reductase 19–base pair polymorphism in intron 1. A portion of the nondeleted allele sequence is shown at the top and the corresponding portion of the deleted allele sequence at the bottom. The deleted portion is indicated by ".....", with flanking sequence to the left and right. The identical base pairs in the 2 strands are indicated by vertical lines.

Sample acquisition and preparation

Maternal white cells were prepared from samples obtained at entry to care, and buffy coat leukocytes were harvested. Genomic DNA was extracted from the subjects' leukocytes by using the QIAamp DNA blood kit (Qiagen, Valencia, CA).

TABLE 1
Background characteristics associated with dihydrofolate reductase genotype during pregnancy[1]

	WT/del, del/del	*WT/WT*	*P*[2]
Parity (%)			0.884
Primipara ($n = 132$)	40.5	41.5	
Multipara ($n = 192$)	59.5	58.5	
Ethnicity (%)			0.088
Puerto Rican ($n = 213$)	64.1	72.3	
African American ($n = 104$)	34.4	23.1	
White ($n = 7$)	1.5	4.6	
Smoking (%)			0.395
Yes ($n = 105$)	31.4	36.9	
No ($n = 218$)	68.6	63.1	
BMI, in kg/m^2 (%)			0.376
<19.8 ($n = 67$)	20.1	23.1	
19.8–26.0 ($n = 188$)	57.5	60.0	
26.1–29.0 ($n = 40$)	13.9	6.2	
>29.0 ($n = 29$)	8.5	10.8	
Previous pregnancy with LBW (%)			0.918
Yes ($n = 31$)	9.6	9.2	
No ($n = 293$)	90.4	90.8	
Previous pregnancy with PTD (%)			0.776
Yes ($n = 33$)	10.4	9.2	
No ($n = 291$)	89.6	90.8	
Daily folate intake, diet + supplements ($n = 323$)(μg/d)	434 ± 17.4[3]	428 ± 34.6	0.863
Daily folate intake, diet only ($n = 323$)(μg/d)	267 ± 13.1	252 ± 25.2	0.620
Energy intake ($n = 323$) (kJ/d)	9706 ± 297	10003 ± 594	0.657

[1] Genotype: *WT*, wild-type allele; *del*, deletion allele. LBW, low birth weight; PTD, preterm delivery.

[2] Categorical variables were compared by chi-square test, continuous variables by ANOVA.

[3] $\bar{x}$ ± SEM of 2 observations (all such values).

TABLE 2
Pregnancy outcome by presence or absence of the dihydrofolate reductase deletion allele[1]

Genotype	Cases	Controls	Total	OR	AOR	95% CI
	n	*n*	*n*			
Preterm delivery						
WT/del, del/del	39	220	259	2.7	3.0	1.0, 8.8[2]
WT/WT	4	61	65	1.0	1.0	—
Total	43	281	324			
Low birth weight						
WT/del, del/del	41	217	258	2.3	2.5	0.9, 6.8
WT/WT	5	60	65	1.0	1.0	—
Total	46	277	323			

[1] Genotype: *del,* deletion allele; *WT,* wild-type allele. Cases, number with preterm delivery or low birth weight; controls, number without preterm delivery or low birth weight; OR, unadjusted odds ratio; AOR, odds ratio adjusted for age, parity, smoking, ethnicity, and BMI.
[2] $P < 0.05$.

DHFR 19-bp deletion polymorphism in intron 1

The deletion and nondeletion alleles form a polymorphic system. Portions of these alleles are shown in **Figure 1**. The A at the 3' end of the deletion segment of the nondeletion allele was reported in one previous study (20) of the *DHFR* gene (GenBank accession #X00855) but was missing in another (21) (GenBank accession #K01612).

Genotyping of alleles of the *DHFR* deletion polymorphism

Genotyping was by polymerase chain reaction (PCR) amplification with allele-specific primers as previously described (6). For the nondeleted allele, the 3' portion of primer #1 (forward) rests within the 19-bp deletion region, whereas primer #3 (reverse) binds sufficiently far distant in the 3' direction to give a product of 113 bp. For the deleted allele, primer #2 (forward) bridges the gap caused by the 19-bp deletion. Primer #3 (reverse) is the same as for the nondeletion allele, resulting in a product of 92 bp. The PCR reaction was carried out as previously described (6). The products were separated on a 10%-polyacrylamide gel and were visualized with ethidium bromide. Samples were genotyped in duplicate.

Data analysis

Logistic regression was used to examine the effect of the presence or absence of a deletion allele on the outcomes of interest, with control for potential confounding variables (age, parity, ethnicity, smoking, and BMI). Separate models were fit for each outcome of interest by using multiple logistic regression analysis. In logistic regression models, we also examined the interaction between maternal genotype and folate intake for diet and supplements per the prevailing RDA for pregnant women. Adjusted odds ratios (AORs) and their 95% CIs were computed from the logistic regression coefficients and their corresponding covariance matrices (22). Chi-square tests, unadjusted odds ratios (ORs), and analysis of variance also were used to compare unadjusted data by maternal genotype. All computations were done with SAS version 8.0 (SAS Institute Inc, Cary, NC).

RESULTS

We studied 324 pregnant women. Background characteristics associated with the different *DHFR* genotypes are given in **Table 1**. Women with or without a deletion allele did not differ with respect to age (data not shown, $P = 0.8$), parity, smoking, BMI, previous pregnancy with PTD or LBW, total folate intake from diet plus supplements, folate intake from diet, or energy intake (Table 1). The age distribution of the cohort was as follows: ≤15 y, $n = 72$; 16–17 y, $n = 55$; 18–19 y, $n = 111$; 20–23 y, $n = 54$; 24–27 y, $n = 22$; and >27 y, $n = 10$.

The presence of the *DHFR* deletion allele in the mother was associated with a 3-fold increased risk for PTD that was statistically significant ($P = 0.049$) after adjustment for confounding variables (**Table 2**). For LBW, the deletion allele was associated with an AOR of 2.5; the 95% CI included unity and was not significant ($P = 0.07$; Table 2).

Because dietary folate was previously shown to contribute significantly to PTD and LBW in Camden (4), we grouped the 324 women according to whether they carried a *DHFR* 19-bp deletion allele and whether their folate intake was low. For the group with both a deletion allele and low folate intake, the risk of PTD, after control for confounding variables, was significantly elevated (AOR: 5.5; 95% CI: 1.5, 20.4; $P = 0.01$; **Table 3**). Women with both a deletion allele and low folate intake likewise had a significantly elevated risk of LBW (AOR: 8.3; 95% CI: 1.8, 38.6; $P = 0.01$; **Table 4**).

DISCUSSION

The present findings are consistent with our earlier finding that decreased dietary folate is associated with an increased risk of

TABLE 3
Preterm delivery by presence or absence of the dihydrofolate reductase deletion allele and folate intake from diet and supplements[1]

Genotype and folate intake	Cases	Controls	Total	OR	AOR	95% CI
	n	*n*	*n*			
WT/del or *del/del* and low folate	19	56	75	4.9	5.5	1.5, 20.4[2]
WT/del or *del/del* and not low folate	20	164	184	1.8	2.0	0.6, 7.2
WT/WT and low folate	1	17	18	0.9	0.9	0.1, 9.9
WT/WT and not low folate	3	44	6.4	1.0	1.0	—
Total	43	281	324			

[1] Genotype: *del,* deletion allele; *WT,* wild-type allele. Folate intake: low folate, <400 μg/d from diet and supplements; not low folate, ≥400 μg/d. Cases, number with preterm delivery; controls, number without preterm delivery; OR, unadjusted odds ratio; AOR, odds ratio adjusted for age, parity, smoking, ethnicity, and BMI.
[2] $P < 0.01$.

TABLE 4
Low birth weight by presence or absence of the dihydrofolate reductase deletion allele and folate intake from diet and supplements[1]

Genotype and folate intake	Cases	Controls	Total	OR	AOR	95% CI
	n	*n*	*n*			
WT/del or *del/del* and low folate	19	56	75	7.5	8.3	1.8, 38.6[2]
WT/del or *del/del* and not low folate	22	161	183	3.1	3.5	0.8, 15.9
WT/WT and low folate	3	15	18	4.4	5.1	0.7, 34.7
WT/WT and not low folate	2	45	47	1.0	1.0	—
Total	46	277	323			

[1] Genotype: *del*, deletion allele; *WT*, wild-type allele. Folate intake: low folate, <400 μg/d from diet and supplements; not low folate, ≥400 μg/d. Cases, number with low birth weight; controls, number without low birth weight; OR, unadjusted odds ratio; AOR, odds ratio adjusted for age, parity, smoking, ethnicity, and BMI.
[2] $P < 0.01$.

PTD and LBW in Camden pregnant women (4, 5) but now raise the question of a gene-environment interaction (Tables 3 and 4). The *DHFR* deletion allele is common in the population; ≈80% of the Camden women in our sample carried the allele (Table 2). These results suggest that the deletion allele in the presence of low folate intake, as defined by the 1989 RDA, is associated with a significantly increased risk of PTD and LBW.

The findings also raise the possibility that even women with folate intakes >400 μg/d may be at higher risk of PTD and LBW if they carry the deletion allele, a situation that is more likely to be seen with wider use of folate supplements and with food folate fortification. For women with the deletion allele and without low folate intake, the adjusted odds ratios for PTD and LBW, though not significant, were increased 2-fold and >3-fold, respectively (Tables 3 and 4). With a larger study, the *DHFR* 19-bp deletion allele may be a risk factor for LBW also; we detected such a trend in the current study ($P = 0.07$; Table 2).

Our data on dietary folate intake and multivitamin use are consistent with data from the second National Health and Nutrition Examination Survey (NHANES II) and the National Natality Survey for a similar time period. In NHANES II, dietary intake was 185 ± 6.2 μg folate/d (median: 154 μg/d) for black women and 210 ± 3.0 μg folate/d (median: 171 μg/d) for white women. About 28% of black women and 18% of white women had folate intakes between 0 and 100 μg/d (23). Use of multivitamins before (14%) and during (65%) pregnancy was likewise uncommon, particularly among those women with characteristics that most resembled the Camden pregnant women: young, black, and unmarried with limited educational attainment (24). In the United States, it is now recommended that women consume 600 μg folic acid/d during pregnancy; this includes 400 μg of synthetic folic acid from supplements or fortified cereals. Unfortunately, even now, less than one-third of women of childbearing age do so (25). Thus, our expectation is that the association of the *DHFR* deletion allele with poor pregnancy outcome may be demonstrable with the folate fortification of the US food supply now in effect.

If the *DHFR* 19-bp deletion allele interferes with the flow of reduced folate from the mother to the fetus, it could work in concert with the elevated ferritin concentrations seen late in pregnancy that are one of the best biomarkers for an increased risk of PTD (26). High maternal concentrations of ferritin are also associated with lower serum and red cell folate (26). Ferritin elevation could contribute to these lower serum and red cell folate concentrations and to PTD by increasing the rate of folate catabolism (27). In the future, controlled clinical trials might be considered for women at high risk of PTD and LBW with a higher dose of a folic acid supplement or with a form of folate that does not require reduction by DHFR.

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WGJ and TOS designed the experiments and wrote the manuscript. TOS and XC collected the participants and samples. WGJ, ESS, and JRS designed the genotyping system. JRS and ESS carried out the genotyping in a blinded fashion. ESS and XC carried out data management. TOS, SB, and XC carried out data analysis. All authors reviewed, criticized, and revised the manuscript and none had any conflict of interest.

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