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Dietary consumption of B vitamins, maternal MTHFR polymorphisms and risk for spontaneous abortion

María del Rosario Rodríguez-Guillén[1], **Luisa Torres-Sánchez**[1], **Jia Chen**[2], **Marcia Galván-Portillo**[1], **Irma Silva-Zolezzi**[3], **Julia Blanco-Muñoz**[1], **María A. Hernández-Valero**[4], and **Lizbeth López-Carrillo**[1]

[1]National Institute of Public Health, Morelos, México

[2]Departments of Community and Preventive Medicine, Pediatrics, and Oncological Sciences, Mount Sinai School of Medicine, New York, New York, USA

[3]Mexican Institute of Genomic Medicine, Mexico City, Mexico

[4]Department of Epidemiology and Center for Research on Minority Health, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA.

Abstract

Objective—To asses he association between intake of folate and B vitamins and the incidence of spontaneous abortion (SA) according to the maternal methylenetetrahydrofolate reductase (MTHFR) polymorphisms (677 C>T and 1298 A>C).

Material and Methods—We conducted a nested case-control study within a perinatal cohort of women recruited in the state of Morelos, Mexico. Twenty-three women with SA were compared to 74 women whose pregnancy survived beyond week 20th. Intake of folate and B vitamins respectively, was estimated using a validated food frequency questionnaire. Maternal MTHFR polymorphisms were determined by PCR-RFLP and serum homocysteine levels by HPLC.

Results—Carriers of MTHFR 677TT and 1298AC genotypes respectively showed an increased risk of SA (*OR* 677TT vs. CC/CT=5.0; 95% *CI*: 1.2, 20.9 and *OR* 1298 AC vs. AA=5.5; 95% *CI*: 1.1, 26.6).

Conclusions—Our results support the role of MTHFR polymorphisms as a risk factor for SA, regardless of dietary intake of B vitamins.

Keywords

MTHFR; 677 C>T and 1298 A>C polymorphisms; spontaneous abortion; Mexico

Spontaneous abortion (SA) is defined as the loss of fetal product before 20 weeks of gestation. [1] Lifestyle, diet, and, more recently, maternal genetic characteristics have been proposed as determinants of SA. Specifically, maternal and paternal smoking 2–4 and maternal alcohol and coffee consumption during pregnancy have been associated with a higher risk of SA. 5–7 In addition, low folate maternal serum levels[8] and suboptimal folate metabolism, indicated by the presence of hyperhomocysteinemia, has been also associated with recurrent abortions.[9] The intake of B vitamins that are involved in the folate metabolism pathway in relation to SA has been poorly explored with no conclusive results.[10–12]

*Correspondence: Lizbeth López-Carrillo, Dr.PH, National Institute of Public Health, Av. Universidad No. 655, Col. Sta. María Ahuacatitlán, CP 62508, Cuernavaca, Morelos, México. Tel: +52 (777) 329-3000 ext. 2501; Fax: + 52 (777) 311-2338; lizbeth@insp.mx.

Methylenetetrahydrofolate reductase (*MTHFR*), a key folate-metabolizing gene, is located on chromosome 1. Several non-synonymous single nucleotide polymorphisms (SNPs) are present in the coding region of the gene including positions 677 and 1298.[13] The most studied SNP is the *677 C>T* substitution that results in an amino acid change from alanine to valine at codon 225.[14] The *MTHFR 1298>C* polymorphism results in a substitution of glutamate with alanine.[15,16]

MTHFR enzyme activity is reduced by 35% among the *677CT* carriers and by 50% to 70% among *677TT* carriers.[14] The function of polymorphism 1298A>C has not been consistently demonstrated; however carriers of the heterozygous genotypes (*677CT/1298AC*) show *MTHFR activity* similar to that in NTHFR677TT carriers. [15] The reduction in MTHFR activity increases the levels of homocysteine and in turn, reduces the availability of the DNA methyl groups.[17,18]

The associations reported to date between maternal *MTHFR 677 C>T* and *1298 A>C* polymorphisms and risk of SA has been inconsistent.[19–29] Some authors reported as much as a three-fold increase in SA risk among women carriers of the *677TT* genotype vs. women with the *677CC* or *677CT* genotypes,[23–26] while other authors found no association between *MTHFR 677C>T* and risk of SA.[19,20,22,27] An increase risk for SA was also observed in fetal carriers of the *MTHFR 677T* allele.[21,28,29]

The prevalence of the *MTHFR 677TT* genotype is higher among Mexicans (36–48%) than among European. Many studies on Caucasians show lower frequency than this or African-American populations;[13] in contrast, a much low frequency of the *MTHFR 1298C allele* has been observed in the Mexican population (0 to 2.4% vs. 7.5 to 11.5%) than in Europeans.[30, 31]

The objective of this study was to evaluate the risk of SA according to maternal *MTHFR* polymorphisms (*677 C>T* and *1298 A>C*) and other lifestyle risk factors in a cohort of Mexican women residing in the state of Morelos in Mexico.

Materials and Methods

We conducted a nested case control study within an ongoing perinatal cohort of women that began in January 2001; the cohort had been formed to evaluate the effect of environmental exposure to organochlorine compounds on infant neurodevelopment. Study participants were recruited from four municipalities in the state of Morelos, Mexico, during premarital counseling mandated by Mexican law. To be eligible for the study, the women had to be non-lactating and without a history of chronic disease. A detailed description of the methodology and cohort follow-up is reported elsewhere.[32]

Women were contacted every six weeks by telephone or household visit and asked to report a ten-day delay in their menstrual period to confirm a pregnancy status with a pregnancy test, or clinically document the presence of SA.

Twenty-three women with SA (pregnancy that ended before 20 weeks) with a median gestational age of 10 weeks (min=6 and max=17), were identified among a group of 456 women who became pregnant before July 2004, and 74 women whose pregnancies lasted beyond 20 gestational weeks, with a median gestational ae of 37.2 (min=21 and max=42), were randomly selected as controls.

Home interviews were conducted on both women and their husband/partners to elicit sociodemographic information (e.g., partners'/husband's occupation), reproductive history, and dietary habits before pregnancy, alcohol consumption prior to conception and tobacco use.

During the baseline evaluation, each woman's weight and height were measured to calculate her body mass index (BMI), and a blood sample (7 ml) was obtained to determine her MTHFR genotypes and homocysteine serum levels.

Genotyping of the MTHFR 677 C>T and 1298 A>C Polymorphisms

Genomic DNA was extracted using a QIAamp DNA Blood Mini Kit (QUIAGEN®, Valencia, CA). DNA amplification and genotyping of *MTHFR 677 C>T* and *1298 A>C* were performed by polymerase chain reaction (PCR-RFLP) according to the protocol suggested by Chen et al. [33] and Weisberg et al.16 For quality control purposes, for each batch of PCR-amplified samples, one sample containing a known genotype and one negative control were analyzed. In addition, duplicate analyses of 10% of samples, randomly selected, were performed by a technician who was blinded to the first set of results and reached Kappa correlation coefficients of 1 and 0.954 for the *MTHFR 677 C>T* and *MTHFR A>C 1298* genotype determinations respectively.

Dietary Intake of Folate and B Vitamins

Dietary intake of folate and vitamins B_2, B_6, and B_{12} was estimated by means of a validated, semiquantitative food frequency questionnaire (FFQ) containing 95 different items.[34] For each food item, there was a defined portion, and women were asked to choose from 10 consumption frequency options, which range from never to six times per day. The consumption of 28 individual nutrients (including total caloric intake) was calculated using the Food Intake Analysis 3.0 (FIAS, Texas University, USA). A detailed description of the methods for the calculation of the methods used to calculate nutritional intake has been published elsewhere. 35

On the FFQ the following foods were considered sources of vitamins B_2, B_6, and folate: liver, beans, lentils, green beans, peas, tomatoes, spinach, lettuces, potatoes, cauliflowers, broccoli, chili peppers, beets, carrots, avocados, oranges, papayas, mangos, strawberries, bananas, tangerines, melons, plums, pears, pineapples, apples, peaches, grapes, lamb, tuna, pork and steak; while the dietary intake of vitamin B_{12} was based in the consumption of liver, sardines, tuna, pork, lamb, steak, fish, chocolate, beer, and red and white wines.

Determination of Serum Homocysteine Levels

Total serum homocysteine levels were determined in duplicated analysis using a high performance liquid chromatography (HPLC) method previously described by Gilfix et al.[36] Fluorescent intensities were measured with excitation and emission wavelengths set at 385 and 515 nm, respectively. Quality Cotrol (Liquicheck Homocysteine Cotrol-Bilvel, Biorad Inc) showed 98.4% ± 2.7% and 99.1 ± 1.2%, recovery for the low and the high level, respectively. CV between duplicate sample analysis was 5.2 ± 2.8%.

Statistical Analyses

Selected maternal and paternal characteristics were compared between cases and controls. Based on the median values observed in the control group the following categories were created for: maternal age (≤21vs. >21 years), education (≤ 9 vs. >9 years), body mass index (≤ 22.4 vs. >22.4), and paternal age (≤ 24 vs. >24 years). Paternal occupation was categorized as "risky" or "non-risky" according to the occupation types that have been assessed with relation to SA: 37 poultry farmer, bricklayer, painter, maintenance worker, farmer, potter, flower-grower, pesticide applicator, and blacksmith. Due to the skewed dietary distributions, nonparametric tests were used to compare, between cases and controls, the folate and B vitamins intakes respectively. We estimated the Hardy-Weinberg equilibrium test among controls to evaluate the observed and expected frequencies of the genotypes of interest.38 The risk of spontaneous abortion (SA) in relation to dietary consumptions of folate and B vitamins as well as the

presence of maternal methylenetetrahydrofolate reductase (MTHFR) polymorphisms (677 C>T and 1298 A>C) was estimated using odds ratios (ORs) and their respective 95% confidence intervals (CIs) with a recessive hereditary model, and further stratify by early and late abortion (≤10 and >10 weeks). STATA (College Station, Texas, USA) statistical software version 9.0 was used to conduct all of the analyses.

Results

Compared with homemakers, women with paid employment had an almost three-fold borderline significant increased risk of SA (*OR*=2.7; 95% *CI*: 1.0, 7.1). Smoking (before pregnancy) as well as paternal occupation resulted in increased risks of SA respectively; however this was not significant. In contrast, maternal younger age and higher education showed non-significant protection of SA (table I).

As described in table II, non-significant higher dietary intakes of folate and B vitamins were observed among controls compared to cases, except for vitamin B6 intake, which resulted in the border of significance (2.1 vs. 1.8 mg/d; p=0.09). Cases in contrast to controls, showed a non-significant higher median of homocysteine serum levels, as well as the MTHFR 677TT genotype (43.5% vs. 25.7%). No carriers of the *MTHFR 1298CC* genotype were present in this study population. The median values of homocysteine remained different (with no statistical significance) between cases and controls, after further stratification by the alleles of interest (677C: Cases=9.6 vs. Controls= 10.3 μmol/L; 677T: Cases=11.0 vs. Controls= 10.4 μmol/L; p>0.05. 1298A Cases=10.4 vs. Controls= 10.5 μmol/L; 1298C: Cases=9.8 vs. Controls= 8.8 μmol/L; p>0.05.) (data not included in the table). Among the controls, neither of the genotype distributions showed any deviance from the Hardy-Weinberg equilibrium.

Under a recessive inheritance model, a significantly increased adjusted risk of SA (OR=5.0; 95% CI: 1.2, 20.9) was detected among carriers of the *MTHFR 677TT* compared to carries of *MTHFR CC+CT*, that remained after stratifying by abortion type (early or late). Also, the *MTHFR 1298 AC* genotype increased the risk of SA (OR=5.5; 95% CI:1.1, 26.6) when compared with the wild type. This result remained significant among late abortions and it was not evaluated among early abortions due to the small sample size.

Discussion

This study detected an increased risk of SA among female carriers of the *MTHFR 677TT* and *MTHFR1298 AC* genotypes, independent of other factors previously associated with increased SA risk (paternal tobacco use and maternal occupation). These results support the findings of recent studies[23–26] that have shown an effect of homozygosity for *MTHFR 677 C>T* on early-late recurrent abortions, but should be interpreted with caution due to the small sample size of this study.

The median levels of all B vitamins examined, i.e. folate, vitamins B2, B6, and B12, were lower in SA cases compared to the controls, although the difference did not reach significance. It is important to point out that multivitamin supplementation is low in the Mexican population, [39] as was the case in this small study population. Thus, these findings need to be replicated in larger studies to fully evaluate the protective role of these vitamins on SA risk.

Similar to the findings in other Mexican populations,[30, 31,40–42] a high percentage of our study population, both cases and controls, were carriers of the *MTHFR 677T* allele (58.7% and 52.0 %, respectively), and a low frequency of the *1298C* allele (11.4% and 9.5%, respectively) was observed. Given our small sample size and he low frequency of the *MTHFR 1298 CC* in the Mexican population (2.3%; 95%CI: 0.9–4.6)30 no carriers of this genotype were present in the

participating women. No information about genotypes of interest in the father or even more in the fetuses was available for this study to evaluate their effects on the risk of SA.

A previous similar study conducted by Borja-Aburto *et al.*43 in Mexico City that included 668 women 16–40 years of age, reported an incidence of SA of 6%, which is equal to our estimation and lower than the 12–15% reported in other, foreign populations.[44] To interpret the estimation of SA occurrence, some methodological considerations should be mentioned: in this study women were mainly primigravidae (~80%), an incidence of SA among primigravidae and women with history of live births is low (5%)44 thus differences in SA throughout studies may partially be attributed to differences in the reproductive history of participants. Also, it is possible that some underestimation of the actual SA incidence exists due to the fact that the majority of pregnancy losses are early as a result of karyotypic abnormalities of conceptus and are difficult to detect without performing a longitudinal assessment of beta human chorionic gonadotrophin (β-hCG),45 that was the case of this study and might have reduced the power to detect significant associations. Balancing these limitations is the strength of our study design, a nested case-control study, where dietary information and sera were obtained at recruitment and allow showing the temporality of the studied variables.

Maternal paid employment was associated with risk of SA in our study population. However, interpretation of this result is limited since we did not collect detailed work-related information such as the number of hours worked, type of exposures, etc. Our findings regarding paternal tobacco use are consistent with the study conducted by Venners *et al*,3 which reported an almost twofold increase of SA among women whose husbands smoked more than 20 cigarettes per day, however the limited sample size precluded the ability to find statistically significant results that should be replicated in future larger cohorts.

The mechanism by which the MTHFR polymorphisms increase the risk of SA is not known. Previously, authors have hypothesized that the increased risk may be due to an increase in homocysteine levels,[18,46] which is consistent with the higher levels of homocysteine that we detected in our cases compared to the controls. Hyperhomocysteinemia reduces the methyl availability;[47] methylation is an important epigenetic characteristic that plays an important role in DNA repair and the stability of the genome. Both *MTHFR677 C>T* and the *MTHFR1298 A>C* polymorphisms have been associated with decreased levels of DNA methylation.[17] Experimental evidence also suggests that aberrant DNA methylation may contribute to the under-development observed in fetal cow clones.[48]

Prevention of SA should consider metabolic dietary differences among the genetically susceptible population. Future larger cohort studies are needed to fully evaluate the interaction between the genetic characteristics and the periconception intakes of folate and other related B vitamins in high risk populations.

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Table I

Association between selected maternal and paternal characteristics and between cases and controls. Morelos, México, 2001–2004

Characteristics	Cases (n=23)	Controls N=74)	OR	95%CI
Maternal				
Age (years)				
> 21	12	33	1.0	
≤ 21	11	41	0.7	0.3–1.9
Education (years)				
≤ 9	11	35	1.0	
> 9	12	39	0.9	0.4–2.5
Previous pregnancies				
None	19	62	1.0	
1–2	4	12	1.1	0.3–3.8
Body mass index				
> 22.4	11	37	1.0	
≤ 22.4	12	37	1.1	04.–2.8
Smoked before pregnancy				
No	11	41	1.0	
Yes	12	33	1.4	0.5–3.5
Occupation				
Household activities	9	47	1.0	
Paid employment	14	27	2.7	1.0–7.1
Paternal				
Age (years)				
≤ 24	11	35	1.0	
> 24	12	39	1.0	0.4–2.5
Occupation*				
Non-risky	14	61	1.0	
Risky‡	5	11	2.0	0.6–6.6
Alcohol intake (cups/week)*				
≤ 1	8	25	1.0	
> 1	9	26	1.1	0.4–3.2
Smoking status*				
No	7	34	1.0	
Yes	12	34	1.7	0.6–4.9

*Due to missing values, numbers for these variables do not equal the total of cases and controls.

†Poultry farmer, bricklayer, painter, maintenance worker, farmer, potter, flower-grower, pesticide applicator or blacksmith.

Table II

Dietary intake of folate and B vitamins, homocysteine serum levels and MTHFR polymorphisms. Morelos, México, 2001–2004

Variables	Cases (n=23)			Controls (n=74)			P values
	P_{10}[a]	Median	P_{90}[a]	P_{10}[a]	Median	P_{90}[a]	
Dietary Intake Folate, µg/d	131.7	341.4	514.2	231.6	392.9	580.8	0.18*
Vitamin B_2, mg/d	1.1	2.1	2.5	1.6	2.2	3.4	0.16*
Vitamin B_6, mg/d	0.9	1.8	2.7	1.2	2.1	3.3	0.09*
Vitamin B_{12}, mg/d	1.9	3.2	6.3	1.8	3.8	8.2	0.52*
Homocysteine serum level (µmol/L)	5.7	10.2	14.8	6.8	9	13.4	0.53*
Polymorphisms		%			%		
MTHFR 677C>T[§#] *CC*		26.1			21.6		0.15‡
CC		30.4			52.7		
TT		43.5			25.7		
MTHFR 1298A>C[§#] *AA*		77.3			81.1		0.69‡
AC		22.7			18.9		

*Based on Mann-Whitney tests

‡Based on χ^2 tests

§Hardy-Weinberg equilibrium test among controls for *MTHFR 677C>T* and *MTHFR 1298 A>C*>0.05.

#1case had missing values for the *MTHFR 1298 A>C*

$P_{10–90}$Percentile 10 and 90

Table III

MTHFR 677C>T and 1298A>C Polymorphisms and risk[1] for spontaneous abortions in a Mexican perinatal cohort. Morelos, México, 2001–2004

Polymorphisms	All		Early Abortions (≤ 10 weeks)				Late Abortions (> 10weeks)			
	OR[1]	95%CI	Cases	Controls	OR[2]	95%CI	Cases	Controls	OR[3]	95%CI
MTHFR 677C>T[*]										
CC+CT	1.0	--	5	55	1.0	--	8	55	1.0	--
TT	5.0	1.2, 20.9	4	19	5.2	0.75,35.6	6	19	5.8	0.82,41.1
MTHFR 1298A>C[‡§]										
AA	1.0	--	8	60	–	–	9	60	1.0	–
AC	5.5	1.1, 26.6	1	14	–	–	4	14	9.7	1.4,68.8

[*] Risk adjusted for maternal occupation, *MTHFR 1298A>C*, folate intake and caloric intake and husbands' smoking status.

[‡] Risk adjusted for maternal occupation *MTHFR* 677C>T, folate intake and caloric intake and husband's smoking status

[§] 1 case had missing values for the *MTHFR 1298A>C*