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Original Article

Individualized folic acid supplementation based on MTHFR and MTRR gene polymorphisms reduces the risk of gestational diabetes mellitus in a Chinese population

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Abstract: Objective: Folic acid (FA) may contribute to the development of gestational diabetes mellitus (GDM), but available studies are inconsistent. We studied the genotype distribution and allele frequencies of methylenetetrahydrofolate reductase (MTHFR) C677T, A1298C, and methionine synthase reductase (MTRR) A66G polymorphisms in pregnant Chinese women and compared the effects of individualized and traditional FA supplementation on GDM. Methods: In this retrospective study, genotype distribution and allele frequencies in 968 pregnant women were tested. FA metabolism was tested by dividing patients into four groups, each of which was supplemented with different doses of FA at different times. Pregnancy complications were followed up and compared to 1940 pregnant women traditionally supplemented with FA in the same hospital as a control group. Results: The allele frequencies were 63.3% (C) and 36.7% (T) for MTHFR C677T, 79.3% (A) and 20.7% (C) for MTHFR A1298C and 75.0% (A) and 25.0% (G) for MTRR A66G. The incidence of GDM after FA supplementation was significantly lower in the case group compared to the control group, especially in high-risk pregnancies. Conclusion: Using genetic polymorphisms to elucidate FA metabolism in pregnant women and providing appropriate FA supplementation can be effective in reducing GDM, especially in high-risk groups.

Keywords: Folic acid, gestational diabetes mellitus, polymorphisms, pregnancy

Introduction

Folic acid (FA) is a synthetic form of folate that is essential for cell development and biochemical reactions [1]. Notably, a low intake of FA can also increase the risk of adverse pregnancy outcome [2, 3]. FA deficiency in pregnant women increases the risk of birth defects, especially neural tube malformations [4]. There is also an increased incidence of other birth defects, such as Down's syndrome, cleft lip and palate, and congenital heart disease. FA is also associated with fatty acid metabolism, especially the metabolism of eicosapentaenoic acid and docosahexenoic acid [5]. FA supplementation during pregnancy may influence amino acid and fatty acid metabolism [5]. Furthermore, FA supplementation for pregnant women may reduce the incidence of fetal neural tube defects, which often lead to death or disability [6]. A study (n=6112) comparing women who were provided with a vitamin supply containing 0.8 mg of FA before conception with unsupplemented women at the first prenatal visit, showed a statistically significant effect on the odds of neural tube defects (OR, 0.11 [95% CI, 0.01-0.91]) [6]. Some studies demonstrated that continuous FA supplementation throughout pregnancy may prevent adverse pregnancy outcomes, while other studies suggest that high doses of FA may lead to an increased risk of gestational hypertension [1, 7]. In addition, continuous high doses of FA throughout pregnancy is not an effective prevention strategy for preeclampsia [8]. Excessive FA supplementation can increase the risk of breast cancer in pregnant women, leads to zinc deficiency in the body, causes abnormal fetal development, and covers up vitamin B12 deficiency. Research stated that attention should be taken to avoid

inappropriate FA supplement use in women who are planning or capable of pregnancy [1, 7]. FA effects may be more relevant in subjects carrying genetic abnormalities of the enzymes of the homocysteine metabolic pathway, in particular, the common homozygous thermolabile 5, 10 methylenetetrahydrofolate reductase (MTHFR C677T) [1]. This indicates that, according to polymorphisms of MTHFR and other related genes, it is important to guide accurate FA supplementtion in pregnant women.

Gestational diabetes mellitus (GDM) is diagnosed when a woman first develops hyperglycaemia during pregnancy. The prevalence of GDM is over 20% in Asians [9, 10]. Higher habitual intakes of supplemental folate before pregnancy were significantly associated with a lower risk of GDM [11] but recent research has shown that daily intake of FA during early pregnancy was associated with a higher risk of GDM in China [12]. In addition, higher maternal folate coupled with vitamin B12 insufficiency has been associated with a higher GDM risk in Singapore [13]. However, none of these studies provided accurate FA supplementation for pregnant women based on polymorphisms of genes.

Several studies have investigated variations in genes related to folate metabolism [14, 15]. Several key enzymes, including methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR) are involved in the folate metabolic pathway [16]. MTHFR is involved in the one-carbon cycle and is a crucial enzyme that regulates nucleotide synthesis and DNA methylation [15, 17]. The MTHFR C677T gene polymorphism (rs1801133) and the A1298C gene polymorphism (rs1801131) are common gene variants of MTHFR and have been shown to alter enzyme activity [15, 17]. Methionine, as a precursor for S-adenosylmethionine, is produced by the transfer of a methyl group from 5-methyltetrahydrofolate, which is catalyzed by MTR and MTRR [18, 19]. Similar to MTHFR C677T and A1298C, MTRR A66G is a common polymorphism, and it plays an important role in folate metabolism [15].

Recent studies have found that FA supplementation resulted in significantly lower complication rates, particularly in GDM, compared to pregnant women who did not supplement with FA [15]. However, there are limited studies of the relationship between FA supplementation and the risk of gestational diabetes in the Chinese population. There is a consensus that FA is necessary and beneficial for pregnant women. However, few studies have used genetic polymorphisms to accurately guide pregnant women to supplement FA during pregnancy. Most studies have been based on experience or common sense in life. In particular, more accurate FA supplementation and better care are key to identifying at-risk pregnant women through genetic testing methods. Therefore, we compared the differences between pregnant women with empirical FA supplementation and those with genetic guidance and precise FA supplementation during pregnancy to identify high-risk pregnant women and to highlight the necessity and importance of genetic testing for accurate guidance of FA supplementation in pregnant women.

Materials and methods

Subjects

The study was approved by the Ethics Committee of the Shaoxing Second Hospital. In this retrospective study, a total of 2908 pregnant women were enrolled between 2014 and 2019. All those enrolled were not taking prescribed medication during their pregnancy and did not drink or smoke. The pregnant women had not been exposed to toxic substances in the environment. The cohort was also free of other major illnesses and had complete clinical information recorded, and the newborn was followed up to 6-8 weeks or more after birth. Clinical information including age, body mass index (BMI), history of abortion, first pregnancy, physical activities, and diseases of the reproductive system during pregnancy was provided by all subjects. Informed consent was obtained from all participants. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Medical Ethics Committee of the Shaoxing Second Hospital.

Genomic DNA extraction and SNP genotyping

Genomic DNA from the blood of pregnant women was isolated using the QIAGEN DNA Blood Maxi Kit (QIAGEN Inc., Valencia, Calif., USA). SNP of MTHFR C677T, MTHFR A1298C, and MTRR A66G was determined by PCR and

Sanger sequencing by ABI 3730XL DNA Analyzer (ABI, USA). Genotyping was performed using direct sequencing of PCR amplicons containing the MTHFR C677T, MTHFR A1298C, and MTRR A66G locus, which was amplified using: forward primer 5'-GGAAGGTGCAAGATCAGAGC-3' and reverse primer 5'-CTGGGAAGAACTCAGCGAAC-3', forward primer 5'-GGCATGTGGTGGCACTG-3' and reverse primer 5'-GGTCTCCCAACTTACCCTTCT-3', forward primer 5'-AGAGTTTCATTCGTACACTCTC-3' and 5'-GGCTCTAACCTTATCGGA-3', respectively.

Assessment of risk and individualized intervention with FA

968 cases were patients who individualized intervention of FA through genetic testing polymorphisms, and 1,940 controls who got empirical supplementation of FA were included in this study.

The ability of pregnant women to metabolise FA was assessed according to the genotype of the three polymorphisms. The risk of abnormal pregnancy outcome was further evaluated and categorized as four levels: unidentified, low, medium, and high, and then supplemented with different doses of FA according to the risk level of abnormal pregnancy outcome and gestational age.

Assessment of GDM

GDM was diagnosed at the same clinic visit, based on plasma glucose concentrations measured in a fasting state and two hours after a 75 g oral glucose tolerance test (OGTT) was administered. Plasma glucose concentrations were analyzed using the colorimetric method (Advia 2400 Chemistry System, Siemens Medical Solutions Diagnostics; and Beckman LX20 Pro analyzer, Beckman Coulter). Participants were classified as having GDM, if they met one of the following: (1) ≥5.1 mmol/L of fasting plasma glucose concentrations, (2) ≥10 mmol/L and 8.5 mmol/L of plasma glucose concentrations 1-hour and 2-hour post-OGTT, respectively.

Recording of complications

The complications of pregnant women in cases and controls, including GDM, thyroid function, gestational hypertension, abortion, premature birth, macrosomia, and underweight were recorded and analyzed.

Statistical analysis

Statistical analyses were performed using SPSS software (version 19.0; IBM Corp.). Differences were analysed using an unpaired t-test if the continuous variables conformed to a normal distribution. Otherwise, the Mann-Whitney U test was used. When comparing datasets containing multiple groups, one-way analysis of variance was used for normally distributed datasets and a Kruskal-Wallis test was used for non-normally distributed datasets. Categorical variables were summarized as counts and percentages and analyzed using the χ^2 test or Fisher's exact test as appropriate. Two-sided values of $P<0.05$ were considered significant. The age of the subjects is represented by median (range), BMI as mean ± SD, and other indicators by percentages.

Results

Participant characteristics

History of abortion, first pregnancy, diseases of the reproductive system, Body Mass Index (BMI), and age according to maternal characteristics are presented in **Table 1**. Case pregnant women were significantly younger than control pregnant women (median 28 vs. 30, P<0.001). The proportion of case pregnant women with reproductive system diseases was higher than that of control pregnant women (percentage, 5.27% vs. 1.49%, P<0.001). There were no significant differences in BMI, history of abortion, or first pregnancy between the two groups.

Distribution of genotypes and allelic frequencies relative to polymorphisms of the MTHFR and MTRR genes

Table 2 shows the distribution of genotypes and allele frequencies of the polymorphisms in the case group. The distribution of genotype of MTHFR C677T was 39.8% (CC), 47.0% (CT), and 13.2% (TT); those of MTHFR A1298C were 63.4% (AA), 31.7% (AC), and 4.9% (CC), and those of MTRR A66G were 55.6% (AA), 38.7% (AG) and 5.7% (GG). The allele frequencies of MTHFR C677T were 63.3% (C) and 36.7% (T); those of MTHFR A1298C were 79.3% (A) and 20.7% (C), and those of MTRR A66G were 75.0% (A) and 25.0% (G).

Table 1. Clinical characteristics of case and control pregnant women

Clinical characteristic	Cases (n=968)	Controls (n=1940)	*P* value
Age/years	28 (16-44)	30 (17-47)	<0.001
Median (range)			
BMI/Mean ± SD	21.75±2.98	21.69±3.11	0.289
History of abortion	458 (47.31%)	867 (44.69%)	0.181
First pregnancy	333 (34.40%)	692 (35.67%)	0.500
Diseases of the reproductive system	51 (5.27%)	29 (1.49%)	<0.001

Table 2. Distribution of genotype and allele frequencies of polymorphisms

	Genotype	Frequency	Allele	Frequency
MTHFR C677T	CC	385 (39.8%)	C	63.3%
	CT	455 (47.0%)	T	36.7%
	TT	128 (13.2%)		
MTHFR A1298C	AA	614 (63.4%)	A	79.3%
	AC	307 (31.7%)	C	20.7%
	CC	47 (4.9%)		
MTRR A66G	AA	538 (55.6%)	A	75.0%
	AG	375 (38.7%)	G	25.0%
	GG	55 (5.7%)		

FA metabolic capacity and supplementation

FA metabolism was further ranked according to the genotypes of pregnant women, including four levels: unidentified, low, medium, and high. The genotypes and gestational weeks in **Table 3**, guided pregnant women to supplement their individualized FA dosages.

Frequency of complications after supplementation with FA during pregnancy in case and control pregnant women

Table 4 shows the frequency of complications following FA supplementation during pregnancy in case and control groups of pregnant women. We found a significant reduction in GDM in the case group compared to the control group (P<0.001). Macrosomia was also reduced in the case group, compared to the control group (P<0.031). The complications of thyroid function, gestational hypertension, abortion, premature birth, and underweight were not significantly different between these two groups.

Risk rank of folate metabolism, distribution, and corresponding frequency of gestational complications

Table 5 summarises pregnancy complications including GDM, hypothyroidism, Hyperthyreosis, and gestational hypertension under the folic acid metabolism risk class. In the case group, the percents of pregnant women at the four risk levels including unidentified, low, medium, and high, were 18.4%, 27.0%, 46.9%, and 7.7%, respectively. There was a reduction in GDM among pregnant women at the high risk level compared to those at the unidentified, low and medium levels (percentages of 18.4%, 27.0%, 46.9% and 7.7%). There were no significant differences in hypothyroidism, hyperthyroidism or gestational hypertension among the four risk levels.

Discussion

The folate metabolism pathway plays an important role in cell division, DNA methylation [3, 20], repair, and synthesis [21-23], and it is critically important for maternal health and fetal development [15]. Studies have shown that continuous FA supplementation throughout pregnancy prevented adverse pregnancy outcomes [1]. MTHFR is a key enzyme in folate metabolism [24]. Some genetic polymorphisms encode enzymes that are less efficient and increase serum homocysteine concentrations [24]. This has been associated with inadequate fetal-maternal circulation and an increased risk of adverse pregnancy outcomes [15, 24]. Two polymorphic variants in this gene (C677T and A1298C) have been implicated in a mild form of MTHFR deficiency associated with hyperhomocysteinemia [25]. Recent studies have shown that the C677T and A1298C Single-nucleotide polymorphisms (SNPs) in the MTHFR gene could elevate blood homocysteine [26-28], which may cause fetal nervous system malformation and spina bifida cystica [29]. The MTRR mutation prevents the conversion of homocysteine to methionine and is the main cause of FA and methyl-vitamin deficiency. Among these, A66G is the most important and most studied mutation and has a risk of elevat-

Table 3. Risk rank of folate metabolism and FA supplementation

Risk rank	Genotype (MTHFR C677T/MTHFR A1298C/MTRR A66G)	Folic acid supplementation		
		3 months before conception	Early pregnancy (0-12 weeks)	Late pregnancy (13-40 weeks)
Unidentified	CC AA AA	400 μg/day	400 μg/day	dietary
	CC AC AA			
Low	CT AA AA	400 μg/day	400 μg/day	400 μg/day
	CT AC AA			
Middle	CC CC AA	400 μg/day	800 μg/day	400 μg/day
	CC AA AG			
	CC AC AG			
	CC AA GG			
	CC AC GG			
	CT CC AA			
	CT AA AG			
	CT AC AG			
	CT AA GG			
	CT AC GG			
	TT AA AA			
	TT AC AA			
High	CC CC AG	800 μg/day	800 μg/day	400 μg/day
	CC CC GG			
	CT CC AG			
	CT CC GG			
	TT CC AA			
	TT AA AG			
	TT AC AG			
	TT AA GG			
	TT AC GG			
	TT CC AG			
	TT CC GG			

ed blood homocysteine [30]. The polymorphisms of MTHFR C677T, A1298C, and MTRR A66G allow for the rapid detection of FA uptake and utilisation levels in different individuals, thereby screening out high-risk groups prone to FA deficiency and enabling personalised FA supplementation to reduce the risk of GDM.

The incidence of GDM was significantly lower in the case group than in the control group. In particular, in high-risk pregnant women, there were fewer pregnant women who got GDM. In the case group, there were 75 high-risk pregnant women, but only one had GDM, in the lower of the four risk classes, suggesting that FA supplementation and health care following genetic polymorphism testing may be measures that reduce GDM [15]. A higher intake of habitual FA supplementation before pregnancy was significantly associated with a lower risk of GDM. FA can increase the nitric oxide (NO) levels and restore Type II diabetes-associated-endothelial dysfunction [31]. During pregnancy, marginal folate nutriture alone or in combination with polymorphic alleles of folate genes can impair cellular growth and replication and induce many pregnancy-related complications in the fetus or placenta [15]. The risk of this complication increases with maternal age. Intervention with FA can reduce complications of folic acid metabolism, especially gestational diabetes. To better prevent the development of metabolic complications of folic acid, the dose of FA can be adjusted according to the level of genetic risk and the age of the pregnant woman. We also noted that pregnant women in the control

Table 4. Frequency of complications after supplementation with FA during pregnancy in case and control pregnant women

Clinical characteristic	Cases (N=968)	Controls (N=1940)	*P* value
Gestational diabetes mellitus	55 (5.7%)	220 (11.3%)	<0.001
Thyroid function			
Hypothyroidism	15 (1.6%)	19 (1.3%)	0.545
Hyperthyreosis	122 (13.0%)	182 (12.5%)	0.691
Normal	800	1259	
Missing	31	480	
Gestational hypertension	12 (1.2%)	28 (1.4%)	0.657
Premature birth	43	91	0.359
Macrosomia (>4 kg)	40 (4.4%)	131 (6.5%)	0.031
Underweight (<2.5 kg)	8 (0.9%)	16 (0.8%)	0.879
Apgar score (<8)	15 (1.7%)	35 (1.8%)	0.736

Table 5. Risk rank of folate metabolism, distribution, and frequency of gestational complications, according to genotype

Risk rank	MTHFR C677T	MTHFR A1298C	MTRR A66G	N (percent) N=968	Gestational diabetes mellitus	Gestational hypertension	Hypothyroidism	Hyperthyreosis
Unidentified	CC	AA	AA	178 (18.4%)	12 (6.7%)	2 (2.1%)	5/175 (2.9%)	19/175 (10.9%)
	CC	AC	AA					
Low	CT	AA	AA	261 (27.0%)	14 (5.4%)	3 (1.6%)	1/252 (0.4%)	28/252 (11.1%)
	CT	AC	AA					
Middle	TT	AA	AA	454 (46.9%)	28 (6.2%)	6 (1.3%)	7/439 (1.6%)	62/439 (13.7%)
	CC	AA	AG					
	CC	AC	AG					
	CC	AA	GG					
	CC	AC	GG					
	CC	CC	AA					
	CT	CC	AA					
	CT	AA	AG					
	CT	AC	AG					
	CT	AA	GG					
	CT	AC	GG					
High	TT	AA	AG	75 (7.7%)	1 (1.3%)	1 (1.9%)	2/72 (2.8%)	13/72 (17.3%)
	TT	AC	AG					
	CC	CC	AG					
	TT	AA	GG					
	CC	CC	GG					

Note: Thyroid function is missing in 29 cases.

group were older than those in the case group, but that significantly more pregnant women in the case group had reproductive disorders than in the control group. Age is a possible risk factor for pregnancy complications [15]. This suggests that accurate FA supplementation by genetic polymorphism testing may also be of interest for older pregnant women with reproductive disorders.

In this research, precise doses of FA supplementation in high-risk pregnant women, i.e. those with poor FA metabolism, significantly reduced the risk of gestational diabetes. Identifying high-risk pregnant women and individualising FA supplementation based on genetic polymorphisms will generally benefit patients compared to no or blind FA supplementation. Thus, the findings suggest that individual-

ized FA supplementation based on polymorphisms in MTHFR and MTRR may be a powerful measure to reduce GDM compared to conventional FA supplementation. However, this study was derived from a single cohort and needs to be done in other cohorts. Further research is necessary to determine the programmatic details (e.g., supplement dosing durations, and fortification amounts) to improve the effectiveness of prevention efforts.

Conclusions

Based on genetic polymorphisms, pregnant women who were accurately supplemented with FA had a lower risk of developing GDM than those who were not tested, and this event was more pronounced in high-risk pregnant women. It is controversial to supplement too much or too little FA according to individual habits. The use of genetic testing to learn as much as possible about FA metabolism in pregnant women and appropriate, timely, and accurate FA supplementation can be effective in reducing gestational diabetes, which is particularly valuable in high-risk pregnancies.

Disclosure of conflict of interest

None.

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