Individualized Folic Acid Supplementation based on Polymorphisms of Methylene tetrahydrofolate Reductase (MTHFR) and Methionine Synthase Reductase (MTRR), Compared with Traditional Folic Acid Supplementation, Reduces Gestational Diabetes Mellitus

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Abstract

Background: Folic Acid (FA) may contribute to the development of gestational diabetes mellitus (GDM), but existing studies are inconsistent. We examined the genotype distributions and allele frequencies of methylenetetrahydrofolate reductase (MTHFR) C677T, A1298C and methionine synthase reductase (MTRR) A66G polymorphisms of pregnant women in China, and compared the effects of individualized folate supplementation and traditional FA supplementation on GDM.

Methods: The genotype distributions and allele frequencies of MTHFR C677T, A1298C and MTRR A66G polymorphisms in 968 pregnant women (case group) were tested. FA metabolism was ranked at four levels, and then pregnant women of different levels are supplemented with different doses of FA at different periods. The case group was followed up for pregnancy complications and compared with 1,940 pregnant women traditionally supplemented with FA in the same hospital (control group).

Results: 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). Compared with control group, the incidence of GDM in the case group were significantly lower, especially in high-risk pregnant women after FA supplementation.

Conclusion: Traditional FA supplementation based on personal habits is controversial, but the use of polymorphisms of genes to clarify the FA metabolism of pregnant women, appropriate, timely and accurate supplementation of FA can effectively reduce gestational diabetes, especially for high-risk pregnant women.

Introduction

Folic acid (FA) is a synthetic form of folate necessary for cell development and biochemical reactions [1]. It is worth noting that a low intake of FA can also increase the risk of adverse pregnancy outcome [2, 3]. Lack of FA in pregnant women will increase the risk of birth defects, especially neural tube malformations [4]. At the same time, the incidence of other birth defects will increase, such as Down’s syndrome, cleft lip and palate, and congenital heart disease. FA supplementation for pregnant women can reduce the prevalence of fetal neural tube defects which often leading to death or disability [5]. Some studies demonstrated that FA supplementation continued throughout pregnancy prevents adverse pregnancy outcomes, whereas some study suggest that high-dose FA may lead to an increased risk of gestational hypertension [1, 6]. In addition, high doses of FA continued throughout pregnancy are not an effective prevention strategy for preeclampsia [7]. Excessive FA supplementation can increase the risk of breast cancer in pregnant women, lead to zinc deficiency in the body and cause abnormal fetal development and cover up vitamin B12 deficiency. Research stated that attention should be given to avoid inappropriate FA supplement use in women who are planning or capable of pregnancy [1, 6]. FA effects may be more relevant in subjects carrying genetic abnormalities of the enzymes of 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 very important to guide pregnant women to accurately supplement FA.

Gestational diabetes mellitus (GDM) is diagnosed when a woman has high blood sugar levels for the first time during pregnancy [8] and the prevalence of GDM is more than 20% in Asian [9]. Higher habitual intakes of supplemental folate before pregnancy were significantly associated with lower GDM risk [10]. But recently research have been conducted that daily intake of FA during early pregnancy was associated with a higher risk of GDM in China [11]. Addition, higher maternal folate coupled with vitamin B12 insufficiency was associated with higher GDM risk in Singapore [12]. However, none of these studies provide accurate FA supplementation for pregnant women based on Polymorphisms of genes.

A number of studies have investigated variations in genes related to folate metabolism [13, 14]. Several key enzymes, including methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR) are involved in the folate metabolic pathway [15]. MTHFR is involved in the one-carbon cycle, and is a crucial enzyme that regulates nucleotide synthesis and DNA methylation [14, 16]. The MTHFR C677T gene polymorphism (rs1801133) and A1298C gene polymorphism (rs1801131) are common gene variants of MTHFR and have been shown to alter the enzyme activity [14, 16]. Methionine as a precursor for S-adenosylmethionine, is produced via the transfer of a methyl group from 5-
methyltetrahydrofolate, which is catalyzed by MTR and MTRR [17, 18]. Similar to MTHFR C677T and A1298C, MTRR A66G is also a common polymorphism, which plays an important role in folate metabolism [14].

Recently research revealed that after supplemented with FA, the complication rates were significantly reduced, especially for GDM, compared with pregnant women without FA supplementation [14]. However, there are limited studies on the relationship between FA supplementation and the risk of gestational diabetes in the Chinese population. There has been consensus on the necessity and benefits of FA supplementation for pregnant women. However, few studies have used polymorphisms of gene to accurately guide pregnant women to supplement FA during pregnancy. Most studies are based on experience or common sense in life. In particular, it is crucial to identify high-risk pregnant women through genetic testing methods, more accurate FA supplementation and more careful care. Therefore, we compared the difference between pregnant women with empirical FA supplementation and pregnant women with genetic guidance and precise FA supplementation during pregnancy to identify high-risk pregnant women and highlight the necessity and importance of genetic testing to accurately guide pregnant women to supplement FA.

**Materials And Methods**

**Study population and SNP genotyping**

The study was approved by the ethics committee of Shaoxing Second Hospital. A total of 2908 pregnant women were enrolled in this study between 2014 and 2019. Clinical information including age, body mass index (BMI), history of abortion, first pregnancy, physical activities, and diseases of reproductive system during pregnancy was completed 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 Shaoxing Second Hospital. Informed consent was obtained from all participants. SNP of MTHFR C677T, MTHFR A1298C and MTRR A66G were determined by PCR and Sanger sequencing by ABI 3730XL DNA Analyzer (ABI, USA).

**Assessment of Potential Risk and Individualized Intervention with FA**

A total of 968 cases of individualized intervention of FA through genetic testing polymorphisms and 1,940 cases of controls with empirical supplementation of FA were included in this study.

According to the genotypes of these three polymorphisms, the FA metabolism ability of pregnant women was evaluated. The risk of abnormal pregnancy outcome was further evaluated and divided into four levels: unidentify, low, middle and high, and then supplemented with different doses of FA according to the risk level of abnormal pregnancy outcome and its gestational age.

**Assessment of GDM**

GDM was diagnosed at the same clinic visit, based on plasma glucose concentrations measured at a fasting state and two hours after a 75g oral glucose tolerance test (OGTT) was administered. Plasma glucose concentrations were analyzed using the colorimetry 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) ≥ 4.5 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.

**Complications Observation and Statistical Analysis**
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 were performed using SPSS 19.0 (IBM, NY, USA). If continuous variables conformed to a normal distribution, unpaired t-tests were used to analyze differences. 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 the Kruskal-Wallis test was used for datasets not normally distributed. Categorical variables were summarized as the counts and percentages, and analyzed using the c2 test or Fisher's exact test, as appropriate. A two-sided values of P<0.05 were considered statistically significant.

**Results**

**Participant characteristics**

History of abortion, first pregnancy, diseases of reproductive system, Body Mass Index (BMI) and age according to maternal characteristics are presented in Table 1. Case pregnant women tended to be younger, which is significant lower than control pregnant women (median 28 vs. 30, P< 0.001). The proportion of case pregnant women with reproductive system diseases is 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, and first pregnancy between the two groups.

| Clinical characteristics         | Cases          | Controls        | 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 reproductive system** | 51 (5.27%)  | 29 (1.49%)      | <0.001  |

**Distribution of Genotypes and Allelic Frequencies Relative to Polymorphisms of the MTHFR and MTRR Genes**

The distribution of genotype and allele frequencies of polymorphisms in the case group are presented in Table 2. The distribution of genotype of MTHFR C677T were 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).
|                        | Genotypes | 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 supplement**

FA metabolism is further ranked according to the genotypes of pregnant women, including four levels: unidentify, low, middle and high. According to the genotype and gestational weeks in Table 3, guide pregnant women to supplement individualized FA dosage.
Table 3
Risk rank of folate metabolism and FA supplementation

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

Frequency complications after supplementation with FA during Pregnancy in the case and control pregnant women

The frequency complications after supplementation with FA during Pregnancy in the case and control pregnant women are presented in Table 4. We found that GDM was significantly reduced in the case group, compared with those in control groups (P< 0.001). The macrosomia was also reduced in the case group, compared with those in control groups (P< 0.031). The
complications of thyroid function, gestational hypertension, abortion, premature birth and underweight were not significantly different between these two groups.

| Clinical characteristics       | 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 (>4kg)             | 40 (4.4%)    | 131 (6.5%)       | 0.031   |
| Underweight (<2.5kg)          | 8 (0.9%)     | 16 (0.8%)        | 0.879   |
| Apgar score (<8)              | 15 (1.7%)    | 35 (1.8%)        | 0.736   |

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

The corresponding gestational complications of GDM, hypothyroidism, hyperthyreosis and gestational hypertension under risk rank of folate metabolism were summarized in Table 5. In case group, the percent of pregant women at four risk including unidentify, low, middle and high, is 18.4%, 27.0%, 46.9% and 7.7%, respectively. Compared with unidentify, low and middle levels in pregnant women, the GDM among the high risk levels in pregnant women were obviously reduce (percent 18.4%, 27.0%, 46.9% vs. 7.7%). There were no significant differences among the four risk levels for hypothyroidism, hyperthyreosis and gestational hypertension in pregnant women.
Table 5
Risk rank of folate metabolism, distribution, and corresponding gestational complications frequency, according to genotypes.

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

Note: Thyroid function is missing in 29 cases.

Discussion
The folate metabolism pathway plays an important role in cell division [3, 19] and DNA methylation, repair and synthesis [20–22], and it is critically important for the health of pregnant women and the development of fetuses [14]. Research showed that FA supplementation continued throughout pregnancy prevents adverse pregnancy outcomes [1]. MTHFR is a key enzyme in folate metabolism [23]. Some genetic polymorphisms code for a less efficient enzyme, increasing serum concentrations of homocysteine [23]. This has been associated with inadequate feto-maternal circulation and increased risk of adverse pregnancy outcome [14, 23]. Two polymorphic variants in this gene (C677T and A1298C) have been implicated in a mild form of MTHFR deficiency associated with hyperhomocysteinemia [24]. Recently studies have showed that the C677T and A1298C Single-nucleotide polymorphisms (SNPs) of the MTHFR gene could elevate blood homocysteine [25–27], which may cause
fetal nervous system malformation and spina bifida cystica [28]. The MTRR mutation prevents the conversion of homocysteine to methionine and is the main cause of FA and methyl vitamin deficiency. Among them, A66G is the most important and most studied mutation and has the risk of elevating blood homocysteine [29]. Through the polymorphisms of MTHFR C677T, A1298C and MTRR A66G, it is possible to detect the level of FA absorption and utilization by different individuals as soon as possible, thereby screening high-risk groups prone to FA deficiency, and realizing personalized FA supplements to reduce the risk of pregnancy syndrome and birth defects in newborns.

The rate of GDM in the case group is significantly lower than the control group. Especially in high-risk pregnant women, there are fewer pregnant women with GDM. In the case group, there are 75 of pregnant women at high risk, but only a pregnant women have GDM, which is the lower among pregnant women with other three risk levels. FA has a significant effect on GDM [14]. A higher intake of habitual FA supplementation before pregnancy is significantly associated with a lower risk of GDM [10]. FA can increase the nitric oxide (NO) level and restore Type II diabetes associated-endothelial dysfunction [30]. In addition, we also noticed that pregnant women in the control group were older than those in the case group, but there were significantly more pregnant women in the case group with reproductive system diseases than the control group. Age may be a possible risk factor for pregnancy complications [14]. This indicates that for older pregnant women with reproductive system diseases, accurate FA supplementation through gene polymorphism testing may be more important.

In this research, the pregnant women at high risk, that is pregnant women with poor FA metabolism, the precise FA supplement dose significantly reduces the risk of gestational diabetes. Therefore, compared with pregnant women who have not supplemented with FA [14] or supplemented with FA according to common sense, it is clinically beneficial to identify high-risk pregnant women and individualized supplementation of FA according to polymorphisms of genes. As a consequence, the results show that, compared with traditional FA supplementation, individualized FA supplementation based on the polymorphisms of MTHFR and MTRR may be a powerful measure to reduce GDM.

**Conclusions**

According to Polymorphisms of genes, pregnant women who accurately supplemented FA had a lower risk of diabetes during pregnancy than non-tested pregnant women, and this event was more pronounced in high-risk pregnant women. Too much or too little FA supplement according to personal habits is controversial, and the use of genetic testing to clarify the FA metabolism of pregnant women, appropriate and timely and accurate supplementation of FA can effectively reduce gestational diabetes, especially for high-risk pregnant women.

**Declarations**

**AUTHOR CONTRIBUTIONS:**

JMZ, XYY and LD conceived and designed the study. XYY, BYD, YW, XQX and AQY acquired the data. JMZ and XYY analyzed and interpreted the data. LD and XYY drafted the article.

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**CONFLICTS OF INTEREST:**

All authors report no conflict of interest related to the submitted work.

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**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of Shaoxing Second Hospital, Shaoxing, China. All participants signed an informed consent. All methods were carried out in accordance with relevant guidelines and regulations.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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