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Research Article
Correlation of Folic Acid Metabolism Gene Polymorphism with Maternal Delivery Outcomes and Neonatal Congenital Diseases During Pregnancy

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Abstract

Background and Objective: Folic acid (FA) plays a vital part in pregnancy. This study analyzes the correlation of FA metabolism gene polymorphism with maternal delivery outcomes to provide reliable guidance for pregnant women. **Materials and Methods:** TaqMan-MGB was used to detect the C677T and A1298C loci of FA metabolism gene MTHFR and A66G locus of MTRR. The correlations of FA risk levels and metabolic gene polymorphism with adverse events (AEs) during pregnancy and final birth outcomes were discussed. **Results:** Significant differences were observed in the risk levels of FA disorders between normal pregnant women and those with oligohydramnios (OH), gestational diabetes mellitus (GDM) or gestational hypertension (GH), same results between pregnant women with the termination of pregnancy, premature birth, dystocia and congenital diseases in the newborn and those with normal delivery, the genotype of MTHFR C677T, MTHFR A1298C and MTRR A66G showed no difference, but the allele of MTHFR C677T differed evidently. Significant differences were also present in the risk levels of FA disorders. **Conclusion:** The risk levels of FA metabolism disorders are strongly related to AEs during pregnancy and adverse pregnancy outcomes.

Key words: Folic acid, pregnancy, MTHFR, hypertension, oligohydramnios

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Folic acid (FA), as a water-soluble vitamin, is essential to the nutritional status of the human body[1]. In the human body, FA mainly has two metabolic modes: Active absorption and diffusion passive absorption and the absorption mainly occurs in the upper small intestine[2]. Nutrients such as glucose and vitamin C can promote the absorption of FA. After absorption, it is stored in the intestinal wall, liver, bone marrow and other tissues and is reduced to physiologically active tetrahydrofolic acid to participate in the synthesis of purine and pyrimidine, playing a vital part in protein synthesis as well as cell mitosis and growth[3,4]. It also has a direct impact on the normal activity of erythrocytes and FA deficiency can trigger a series of erythrocyte disorders and pathological diseases[5]. In addition, as an important nutrient in the body, FA plays a prominent role during pregnancy. Studies have shown that FA deficiency in pregnant women can lead to a variety of defects such as fetal open neural tube malformation, congenital heart disease, simple ventricular dilatation, skeletal system dysplasia and cervix lymphatic hygroma[6,7]. Consequently, it is clinically agreed that FA supplementation during pregnancy is one of the key links to ensure normal delivery.

In recent years, relevant studies on the mechanism of FA metabolism have found that the polymorphism of certain enzymes in FA metabolism plays a crucial role in some people with congenital heart disease, neural tube malformation, premature birth, abortion, down syndrome and male infertility[8]. The metabolic capacity of FA is directly affected by the polymorphism of reductases such as methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) and when the reductase mutates, the utilization rate of FA can be significantly reduced[9]. Moreover, the change of reductase polymorphism can increase the level of homocysteine (Hcy) in the human body, induce vascular endothelial injury and dysfunction, destroy the coagulation and fibrinolysis system and directly cause abortion and stillbirth in severe cases[10]. As the final result of malignant childbirth, adverse pregnancy outcomes affect more than 10% of pregnant women globally, especially in elderly pregnant women[11,12]. Currently, FA metabolism disorders are the focus of research on adverse pregnancy outcomes.

Accordingly, this study analyzes the connection between FA metabolism gene polymorphism and maternal delivery outcomes, aiming at further understanding the significance of FA metabolism disorders in pregnancy and childbirth and providing reliable reference and guidance for future clinical FA supplementation for pregnant women.

MATERIALS AND METHODS

Study population: A retrospective analysis was conducted on 3618 pregnant women admitted to our hospital between January, 2019 and December, 2020. The age of pregnant women ranged from 20-34 years (mean: 26.4±4.2) and the BMI was 22-37 kg m^{-2} (average: 26.4±3.6). Among them, there were 2843 cases of normal pregnancy, 209 cases of oligohydramnios (OH), 319 cases of gestational diabetes mellitus (GDM) and 247 cases of gestational hypertension (GH). The hospital Ethics Committee approved the experimental protocol without reserves and all the participants signed informed consent before enrollment.

Eligibility criteria

Inclusion criteria:

- Women or early pregnant women who came to the hospital for pregnancy during the investigation period and agreed to participate after being informed
- No high-risk score
- Accurate gestational age, no pregnancy history, normal glucose tolerance
- No medical and surgical complications, cholestasis, etc.
- Those with the available basic information needed for the study and willing to accept follow-up until discharged after delivery

Exclusion criteria:

- Pregnant women who did not receive routine prenatal examination during pregnancy
- Loss of follow-up during the study
- Those giving birth else where rather than the previously scheduled place
- Those considered unsuitable for the trial by the investigators
- Those suffering from heart, liver, lung, kidney and other important organ diseases and benign tumours (ovarian cysts, uterine fibroids, etc.) during pregnancy

Sample collection and testing: Peripheral venous blood (2 mL) was extracted into EDTA K2 anticoagulation tubes for sample DNA separation using Column DNA Extraction Kits. Genotypes of C677T and A1298C gene loci of MTHFR and A66G gene locus of MTRR in pregnant women were detected by Taqman-MGB. The total volume of each reaction system was 10 μL, including 1 μL of DNA template (20 ng μL^{-1}), 5 μL

of 2×MasterMix, 0.5 μL of 20×primer and 3.5 μL of deionized water. The reaction conditions were 95°C for 15 sec and 60°C for 1 min for 45 cycles. After the reaction, the end-point fluorescence of each well was read on the fluorescence quantitative PCR instrument to determine the genotyping of each sample. And according to the general sequencing reaction kit instructions, FA was divided into four risk levels: Risk-free, as well as low, medium and high-risk.

Endpoints: Correlations of FA risk levels and metabolic gene polymorphism with adverse events (AEs) during pregnancy and final delivery outcomes were analyzed.

Statistical methods: Data analysis was performed using SPSS 22.0. Categorical and continuous variables were recorded as (n/%) and ($\bar{\chi}\pm s$) and compared by chi-square test and T-test, respectively. The correlation was analyzed by Pearson correlation coefficient. p-value <0.05 was considered significant.

RESULTS

Correlation of FA risk levels with AEs during pregnancy: In normal pregnant women, the FA risk level was mainly risk-free (71.79%), while those with low- and high-risk accounted for 17.80 and 1.90%, respectively in Fig. 1a. For pregnant women with OH, 53.59% had a low risk of FA, 18.18% had medium risk and 11.96% had high risk in Fig. 1b. The pregnant women with GDM were mainly at low risk (35.74%) and medium risk (33.86%) of FA, while those at high risk accounted for 19.75% in Fig. 1c. The pregnant women with GH also had a low (33.20%) and medium (39.68%) risk of FA, while those with high risk accounted for 15.79% in Fig. 1d. Therefore, pregnant women with GDM and CH are more susceptible to a high risk of FA.

Association between MTHFR C677T and AEs during pregnancy: There was no significant difference in the distribution of MTHFR C677T gene polymorphism between normal pregnant women and those with OH, GDM or GH, all of whom were mainly CT-type (the proportion of CT-type in normal, OH, GDM and GH pregnant women was 48.40, 49.28, 50.78 and 48.18%, respectively), followed by CC-type (the proportion of CC-type in normal, OH, GDM and GH pregnant women was 34.96, 32.54, 31.03 and 34.82%, respectively), While the TT-type accounted for the least among the 4 groups (the proportion of TT-type in normal, OH, GDM

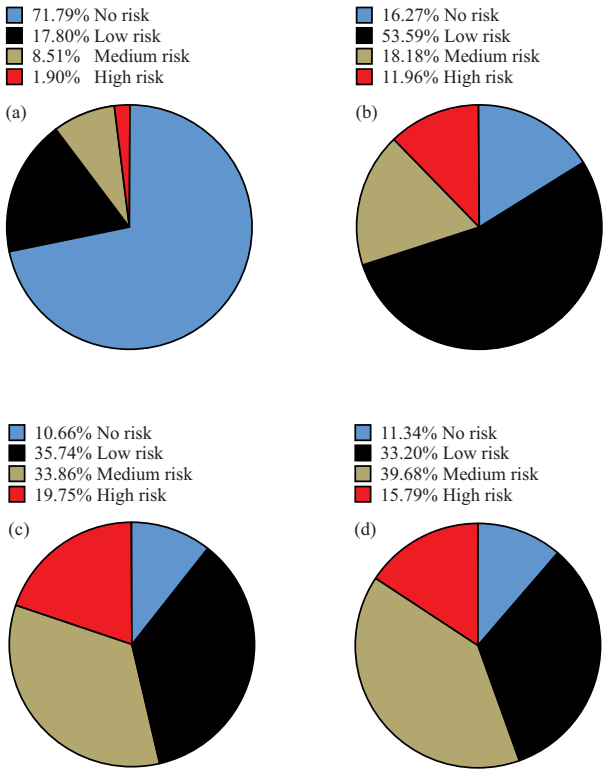

Fig. 1(a-d): Correlation of folic acid risk levels with adverse events during pregnancy, (a) Folic acid risk levels of normal pregnant women, (b) Folic acid risk levels of pregnant women with oligohydramnios, (c) Folic acid risk levels of pregnant women with gestational diabetes mellitus and (d) Folic acid risk levels of pregnant women with gestational hypertension

and GH pregnant women were 16.64, 18.18, 18.18 and 17.00%, respectively) (p>0.05) in Table 1. The C allele frequencies of pregnant women with OH, GDM and GH were 53.59% in Fig. 2b, 45.61% in Fig. 2c and 44.94% in Fig. 2d, respectively. It showed that the C allele frequencies were not significantly different among the three groups (p>0.05), but were all less than that of 64.74% of normal pregnant women in Fig. 2a (p<0.05). The T allele frequencies of pregnant women with OH, GDM and GH were 46.41% in Fig. 2b, 54.39% in Fig. 2c and 55.06% in Fig. 2d, respectively, with no significant difference among the three groups (p>0.05) while all higher than that of 35.26% of normal pregnant women in Fig. 2a (p<0.05). Therefore, in the course of AEs during pregnancy, the C allele of the MTHFR C677T gene will significantly increase, while the T allele will decrease.

Table 1: MTHFR C677T gene polymorphism

	Normal pregnant women	Oligohydramnios	Gestational diabetes mellitus	Gestational hypertension	x^2	p-value
CC	994 (34.96)	68 (32.54)	99 (31.03)	86 (34.82)	2.523	0.866
CT	1376 (48.40)	103 (49.28)	162 (50.78)	119 (48.18)		
TT	473 (16.64)	38 (18.18)	58 (18.18)	42 (17.00)		

Table 2: MTHFR A1298C gene polymorphism

	Normal pregnant women	Oligohydramnios	Gestational diabetes mellitus	Gestational hypertension	x^2	p-value
AA	1942 (68.31)	140 (66.99)	220 (68.97)	172 (69.64)	1.785	0.938
AC	706 (24.83)	50 (23.92)	77 (24.14)	58 (23.48)		
CC	195 (6.86)	19 (9.09)	22 (6.90)	17 (6.88)		

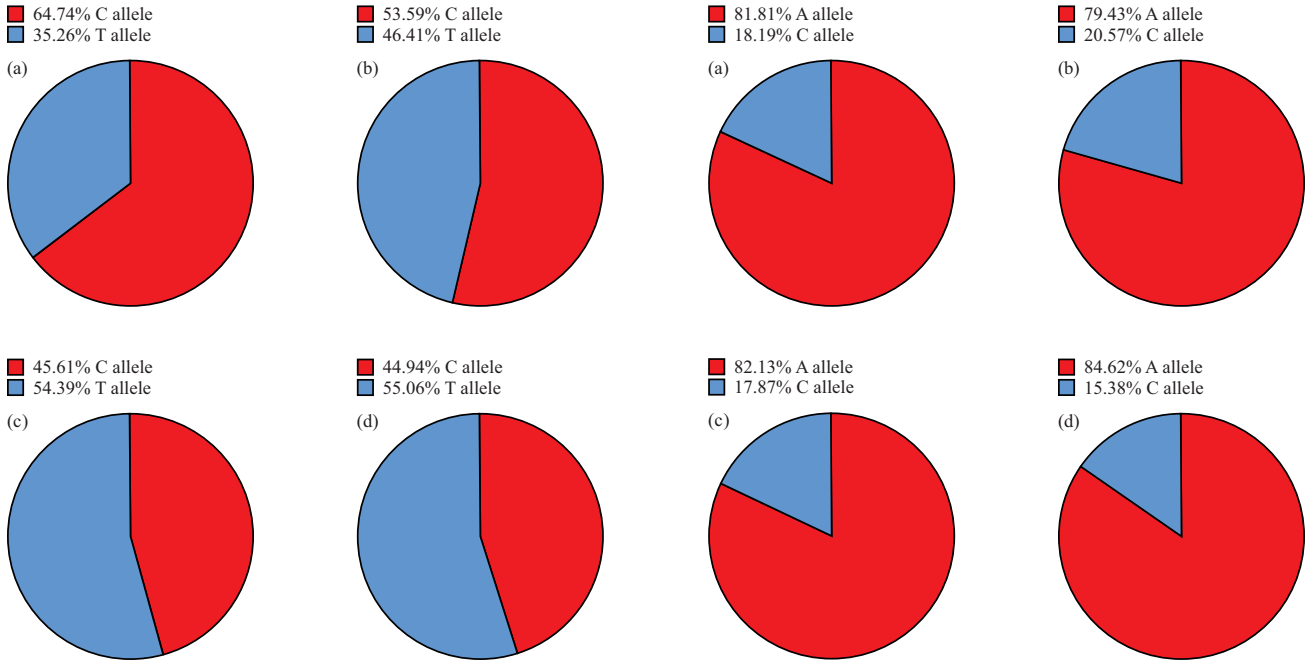

Fig. 2(a-d): MTHFR C677T allele frequency, (a) Allele frequency in normal pregnant women, (b) Allele frequency in pregnant women with oligohydramnios, (c) Allele frequency in pregnant women with gestational diabetes mellitus and (d) Allele frequency in pregnant women with gestational hypertension

Fig. 3(a-d): MTHFR A1298C allele frequency, (a) Allele frequency in normal pregnant women, (b) Allele frequency in pregnant women with oligohydramnios, (c) Allele frequency in pregnant women with gestational diabetes mellitus and (d) Allele frequency in pregnant women with gestational hypertension

Relationship between MTHFR A1298C and AEs during pregnancy: There was no significant difference in the distribution of MTHFR A1298C gene polymorphism among normal pregnant women and those with OH, GDM or GH, all of whom were mainly AA-type (68.31, 66.99, 68.97 and 69.64% for normal, OH, GDM and GH pregnant women, respectively); While the proportion of pregnant women with AC-type (24.83, 23.92, 24.14 and 23.48% for normal, OH, GDM and GH pregnant women, respectively) was higher than that of pregnant women with CC-type (6.86, 9.09, 6.90 and 6.88%, for normal, OH, GDM and GH pregnant women, respectively) ($p>0.05$, in Table 2. The frequencies of A and C alleles in normal pregnant women were 81.81 and 18.19%, respectively in Fig. 3a. While they were 79.43 and 20.57% in pregnant women with OH in Fig. 3b, 82.13 and 17.87% in those with GDM in Fig. 3c and 84.62 and 15.38% in those with GH in Fig. 3d. There was also no significant difference in allele frequencies among the four groups of pregnant women ($p>0.05$), suggesting that the occurrence of AEs during pregnancy is not significantly related to MTHFR A1298C gene polymorphism.

Table 3: MTRR A66G gene polymorphism

	Normal pregnant women	Oligohydramnios	Gestational diabetes mellitus	Gestational hypertension	x^2	p-value
AA	1418 (49.88)	106 (50.72)	168 (52.66)	120 (48.58)	4.354	0.629
AG	1148 (40.38)	88 (42.11)	128 (40.13)	106 (42.91)		
GG	277 (9.74)	15 (7.18)	23 (7.21)	21 (8.50)		

Table 4: Correlation between folic acid metabolism gene polymorphism and pregnancy outcomes

	Termination of pregnancy		Premature delivery		Dystocia		Congenital diseases of the newborn	
	r	p-value	r	p-value	r	p-value	r	p-value
MTHFR C677T	0.124	0.421	0.109	0.511	-0.108	0.394	0.117	0.281
MTHFR A1298C	0.542	0.008	0.634	<0.001	0.693	<0.001	0.624	<0.001
MTRR A66G	0.714	<0.001	0.681	<0.001	0.572	0.004	0.657	<0.001

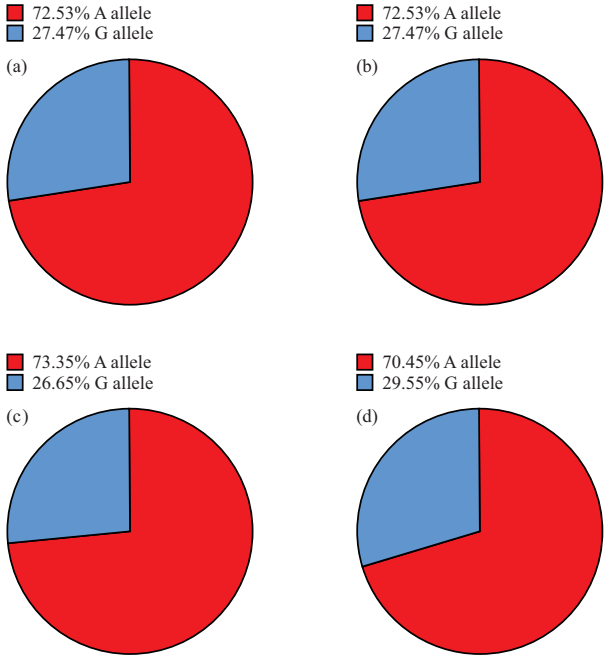

Fig. 4(a-d): MTRR A66G allele frequency, (a) Allele frequency in normal pregnant women, (b) Allele frequency in pregnant women with oligohydramnios, (c) Allele frequency in pregnant women with gestational diabetes mellitus and (d) Allele frequency in pregnant women with gestational hypertension

Relationship between MTRR A66G and AEs during pregnancy: No significant difference was observed in the distribution of MTRR A66G gene polymorphism among normal pregnant women and those with OH, GDM or GH, all of whom were mainly AA-type (49.88, 50.72, 52.66 and 48.58% for normal, OH, GDM and GH pregnant women, respectively) and AG-type (40.38, 42.11, 40.13 and 42.91% for normal, OH, GDM and GH pregnant women, respectively). While pregnant women with GG-type were rare (the proportion of GG-type in normal, OH, GDM and GH pregnant women was 9.74, 7.18, 7.21 and 8.50%, respectively) (p>0.05, in Table 3). The frequencies of A and C alleles in normal pregnant women were 72.53 and 27.47%, respectively in Fig. 4a. While they were 71.29 and 28.71% in pregnant women with OH Fig. 4b, 73.35 and 26.65% in those with GDM in Fig. 4c and 70.45 and 29.55% in those with GH in Fig. 4d. There was also no significant difference in allele frequencies among the four groups of pregnant women (p>0.05), suggesting no significant relationship between the occurrence of AEs during pregnancy and MTRR A66G gene polymorphism.

Correlation of FA risk levels with pregnancy outcomes: Among the 3618 pregnant women, there were 186 cases of termination of pregnancy, 441 cases of preterm delivery, 257 cases of dystocia and 146 cases of neonatal congenital diseases. Most pregnant women with normal delivery had no or low risk of FA, while those with medium- and high-risk accounted for 1.31 and 0.15%, respectively in Fig. 5a. Among pregnant women who terminated pregnancy, most were at moderate risk (46.24%), while 33.33% were at high risk, 10.75% were at low risk and only 9.68% were at no risk in Fig. 5b. Among those with preterm delivery, 40.59% were at medium risk of FA, 25.29% were at low risk, 18.53% were at no risk and 15.59% were at high risk in Fig. 5c. 42.74% of pregnant women with dystocia were at moderate risk, 33.52% were at no risk, 18.16% were at low risk and 5.59% were at high risk in Fig. 5d. In pregnant women with neonatal congenital diseases, 51.37% were at moderate risk, 28.77% were at high risk, 12.33% were at low risk and 7.53% were at no risk in Fig. 5e. The above results indicate that the risk level of FA increased significantly in pregnant women with adverse pregnancy outcomes and is the highest among those with neonatal congenital diseases.

Relationship between FA metabolism gene polymorphism and pregnancy outcome: According to Pearson correlation coefficient analysis, there was no significant relationship between MTHFR C677T and pregnancy outcomes, while both MTHFR A1298C and MTRR A66G were closely related to the termination of pregnancy, premature delivery, dystocia and neonatal congenital diseases in p<0.05, in Table 4.

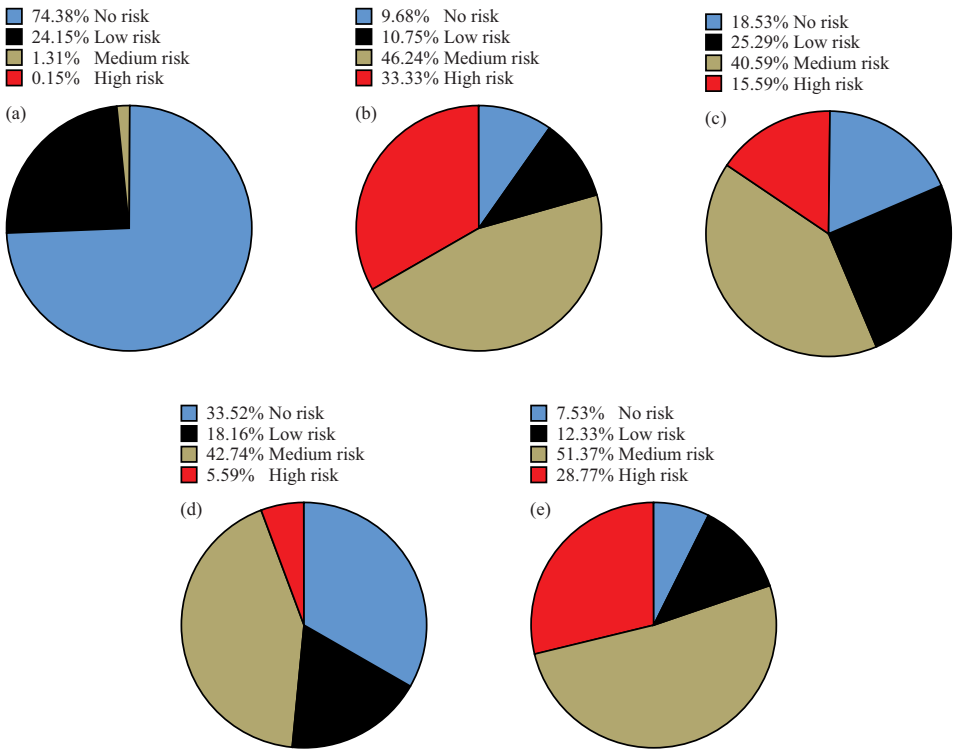

Fig. 5(a-e): Relationship between folic acid risk levels and pregnancy outcomes, (a) Folic acid risk levels in pregnant women with normal delivery, (b) Folic acid risk levels in pregnant women with the termination of pregnancy, (c) Folic acid risk levels in pregnant women with premature birth, (d) Folic acid risk levels in pregnant women with dystocia and (e) Folic acid risk levels in pregnant women with neonatal congenital diseases

DISCUSSION

The FA, a derivative of pteridine, was originally isolated from the liver and is also one of the B vitamins[13]. In the human body, FA is essential in modulating the behaviour and function of erythrocytes, so it also has significant implications for the state of the body during pregnancy and the development of newborns[14]. Studies have shown that adequate FA supplementation before and during pregnancy is the key to reducing the rate of birth defects in newborns[15]. Therefore, the detection of FA metabolism gene polymorphism in pregnant women can not only further understand the role of FA metabolism disorders during pregnancy, but also serve as a reference to provide individualized FA supplementation for them, which is of great significance in preventing AEs during pregnancy and adverse delivery outcomes.

As far as China is concerned, there is a great difference in diet structure between the north and the south and the distribution of FA metabolism gene polymorphism in the human body also varies significantly[16]. The MTHFR and MTRR are the key enzymes in FA metabolism. The polymorphic mutations at C677T and A1298C of the MTHFR gene and A66G of MTRR are the main mechanisms leading to the decrease or deletion of enzyme activity and/or the change of thermal stability[17].

Through the detection of FA-related genes, different FA supplementation schemes can be formulated based on the different requirements and intake of FA and the utilization ability of different pregnant women during pregnancy, which may be more effective in preventing the occurrence of neural tube defects such as neonatal anencephaly and spina bifida[18]. Accordingly, in this study, the polymorphism of the above metabolic genes was detected and their associations with pregnancy were analyzed.

Firstly, conducted a preliminary analysis of the relationship between FA risk levels and AEs during pregnancy. The results demonstrated that in normal pregnant women, the risk level of FA was generally low, while in those with OH, GDM or GH, the risk level of FA increased markedly, with the notably increased number of cases with high-risk. This also suggests that FA levels are strongly related to AEs during pregnancy and an increase in FA risk levels also predicts a rise in AEs during pregnancy. This is consistent with the previous studies on the risk levels of FA[19,20], which can support the results of this experiment.

Subsequently, found that the polymorphism distribution of FA metabolism genes MTHFR C677T, MTHFR A1298C and MTRR A66G had no evident difference between normal pregnant women and those with AEs during pregnancy, which also confirmed that the three genes were in line with Hardy-Weinberg Equilibrium[21]. Then, we found a notable difference in the allele distribution of MTHFR C677T between pregnant women with AEs during pregnancy and those with normal pregnancy, while no difference in the allele distribution of MTHFR A1298C and MTRR A66G, suggesting that MTHFR gene polymorphism was strongly related to AEs during pregnancy.

As a key enzyme in FA metabolism, MTHFR converts 5, 10-methyl-tetrahydro folic acid into another structure called 5-methyl-tetrahydro folic acid, among which TT genotype is particularly critical for FA metabolism disorders[22]. Studies have shown that MTHFR TT genotype can cause blocked methylation of Hcy, massive accumulation and precipitation of Hcy *in vivo*, as well as a series of vascular malignant diseases such as vascular endothelial injury, vascular smooth muscle hyperplasia and coagulation dysfunction, which is not only the main inducement of hypertension, coronary heart disease and other risk diseases but also the key influencing factor of neonatal congenital vascular diseases[23,24].

The results of this research also thoroughly verified this conclusion, confirming the crucial role of FA metabolic reductase in pregnancy. Further, we made statistics on pregnant women's pregnancy outcomes and found that there were also distinct differences in FA risk levels between pregnant women with premature delivery, dystocia, termination of pregnancy and neonatal congenital diseases and those who delivered normally. This also re-confirms that FA is important not only for pregnant women but also for newborns.

Furthermore, the metabolic genes MTHFR A1298C and MTRR A66G were found to have a close relationship with adverse birth outcomes, indicating that they had a significant potential connection with the adverse delivery outcomes of pregnant women, although they had no significant impact on the AEs during pregnancy. In our opinion, this may be because MTHFR C677T, as the most common polymorphic site mutation of MTHFR, has a direct influence on the mother and the change of its enzyme activity leads to maternally related lesions. MTHFR A1298C and MTRR A66G, on the other hand, indirectly cause changes in DNA methylation ability in pregnant women, which hinders the growth and development of the fetus in the uterus, thus causing adverse pregnancy outcomes[25]. Regarding this point, the research results of Zhang *et al.*[26] are also consistent with our views, which can preliminarily prove ideas. Nevertheless, the effects of the polymorphism of the three metabolic genes need further experimental studies before they can be fully confirmed.

This study made a preliminary analysis of the correlation of FA metabolism gene polymorphism with AEs during pregnancy and pregnancy outcomes, but there are still many deficiencies. For example, the specific impact mechanism of FA metabolism gene polymorphism on pregnant women and newborns, which we proposed above, needs further experimental confirmation. And because the experimental period is too short, we are unable to evaluate whether FA metabolism genes have potential long-term influences on pregnant women who have a normal delivery. Because of the above limitations, we will continue to deepen the related research on folate metabolism genes to obtain more complete experimental results for clinical reference.

CONCLUSION

The risk levels of folate metabolism disorder are strongly related to AEs during pregnancy and adverse pregnancy outcomes and FA metabolism gene polymorphism has important reference significance for the evaluation of risk events of pregnant women and newborns.

SIGNIFICANCE STATEMENT

This study discovered the gene polymorphism that can be beneficial for pregnant women. This study will help the researchers to uncover the critical areas of folate metabolism disorder in pregnancy outcomes that many researchers were not able to explore. Thus a new theory on gene polymorphism related to AEs during pregnancy and adverse pregnancy outcomes may be arrived at.

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