ORIGINAL RESEARCH

Correlation of Folic Acid Metabolic Gene Polymorphisms, Homocysteine, Vitamin B12, and Red Blood Cell Folate with Adverse Pregnancy Outcomes

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ABSTRACT

Objective • This study aims to investigate the relationship between folic acid (FA) metabolic gene polymorphisms, homocysteine (Hcy), vitamin B12 (Vit B12), and red blood cell folate (RBCF) with adverse pregnancy. The findings of this study can help in the prevention and treatment of adverse pregnancy in the future.

Methods • 118 pregnant women admitted to Qingdao Central Hospital between August 2020 and October 2022 were selected for retrospective analysis, including 62 cases of normal delivery (control group, CG) and 56 cases of adverse pregnancy (research group, RG). The single nucleotide polymorphisms of MTHFR C677T, MTHFR A1298C, and MTRR A66G gene loci were tested in both cohorts. Besides, differences in Hcy, Vit B12, and RBCF levels were observed, as well as Hcy, Vit B12, and RBCF alterations in different genotype carriers in the research group.

Results • An elevated proportion of MTHFR 677TT-type gene and MTRR 66GG-type gene carriers and a lower proportion of MTRR 66GG-type gene carriers were found in the research group (χ^2 = 4.458, 4.238, 4.206, P = .035, .040, .040). As indicated by the Logistic regression analysis, carriers of MTHFR 677TT and MTRR 66GG gene had an increased risk of adverse pregnancy outcomes (95%CI=2.881-5.942, 1.427-3.809, P < .001), while MTRR 66AG carriers had a decreased risk (95%CI=0.124-1.849, P < .001). Finally, Hcy levels of MTHFR 677TT and MTRR 66GG gene carriers increased, while Vit B12 and RBCF decreased; the opposite was true for MTRR 66AG gene carriers (P < .001).

Conclusions • FA metabolic gene polymorphisms, Hcy, Vit B12, and RBCF are closely related to adverse pregnancy outcomes, which is of great significance for future clinical evaluation of adverse pregnancy. (*Altern Ther Health Med.* 2024;30(5):284-288)

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INTRODUCTION

During pregnancy, embryonic dysplasias can occur due to various reasons. These include recurrent spontaneous abortion (RSA), repeated fetal arrest, natural death of the fetus in the uterus, pregnancy-induced hypertension, severe umbilical cord torsion, excessive reduction of amniotic fluid, and premature rupture of the amniotic membrane. These conditions are collectively referred to as adverse pregnancy. In severe cases, adverse pregnancy can cause irreversible damage to both maternal and neonatal functions, and can even lead to maternal and infant death, carrying significant potential risks.[1-3] China, has a high occurrence of adverse pregnancy, with an incidence of neonatal diseases (e.g., Down syndrome [DS], neural tube defects [NTDs], and congenital heart disease [CHD]) being approximately about 0.5-2%,[4] which is also the main cause of neonatal death at present.[5] Therefore, preventing the occurrence of adverse pregnancy is of great significance to improve the birth level of newborns and protect the health of mothers.

Folic acid (FA), a water-soluble B vitamin mainly absorbed by the small intestine, is a nutrient that affects the growth and development of the fetus. Due to the need for fetal development during pregnancy, the maternal demand for FA has greatly increased.[1] Studies have shown that FA deficiency in pregnant women can increase the incidence of low birth weight, cleft lip, and palate, CHD, etc. in the fetus, and a lack of FA within three months before and after pregnancy may cause fetal neural tube defects (NTDs).[6] FA has been also indicated to interfere with the levels of homocysteine (Hcy) and vitamin B12 (Vit B12), reduce the occurrence of pregnancy diseases such as RSA and gestational hypertension, and lower the possibility of birth defects such as NTDs and low birth weight infants.[7]

5,10-methylenetetrahydrofolate reductase (MTHFR) and 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (MTRR) are key enzymes in FA metabolism, and their gene polymorphisms can affect enzyme activity, thus influencing FA and Hcy levels in vivo.[8,9]

This study retrospectively analyzed the clinical data of women who underwent pregnancy and preconception examination at Qingdao Central Hospital to explore the association between MTHFR and MTRR gene polymorphisms and the occurrence of adverse pregnancy and to discuss the mechanism of disease occurrence from a genetic perspective, thus laying a foundation for the formulation of personalized clinical intervention programs.

MATERIALS AND METHODS

Study subjects

In this study, 118 pregnant women admitted to Qingdao Central Hospital between August 2020 and October 2022 were selected, including 62 cases of normal delivery (control group, CG) and 56 cases of adverse pregnancy (research group, RG). Criteria for adverse pregnancy: RSA, preterm birth, fetal malformation, DS, etc. The hospital's ethics committee has reviewed and ratified this research and all subjects signed informed consent.

Criteria for patient inclusion and exclusion

RG: Pregnant women aged 20-30 years with adverse pregnancy outcomes and complete clinical data were included; those with hypertension, heart disease, liver and kidney disease, neoplastic diseases, and mental illness were excluded. CG: 20-30-year-old pregnant women with healthy newborns, complete clinical data, and no adverse pregnancy outcomes were enrolled; the exclusion criteria were the same as in RG.

Genotyping

For both groups, peripheral blood genomic DNA was extracted using silica gel adsorption (K182001, PureLink™ Genomic DNA Miniprep Kit, Thermo Fisher, USA), and single nucleotide polymorphisms of MTHFR C667/A1298C and MTRR A66G gene loci were investigated by Taqman assay using Real-time quantitative PCR (ProFlex PCR instrument, Thermo Fisher, USA). The PCR reaction system consists of genomic DNA (20 ng/μL) 1 μL, Taqman Universal Master Mix (4440049, Thermo Fisher, USA) 5 μL, deionized water 3.5 μL, and Taqman-MGB probe 0.5 μM, respectively. MTHFR C677T probe location: GAAAAGCTGCGTGATGATGAAATCG [G/A] CTCCCGCAGACACCTTCTCCTTCAA. MTHFR A1298C probe location: AAGAACGAAGACTTCAAAGA CACTT [G/T] CTTCACTGGTCAGCTCCTCCCCCCA. MTRR A66G probe location: GGCAAAGGCCATCGCAGA AGAAAT [A/G] TGTGAGCAAGCTGTGGTACATGGAT. The cycling conditions were 20 cycles of 95°C/10 min, 92°C/15s, and 60°C/1 min and 30 cycles of 98°C/15s and 60°C/90s. The terminal fluorescence of the sample well was read using the PCR instrument, and the genotyping results of each sample gene were determined using relevant analysis software.

Determination of Hcy, Vit B12, and red blood cell folate (RBCF)

The peripheral blood samples of patients were sent to the laboratory of Qingdao Central Hospital for Hcy, Vit B12, and RBCF determination using electrochemiluminescence and enzymatic cycling assays. The operation process was strictly by the instructions of the kit.

Outcome measures

Differences in FA metabolism gene polymorphisms between RG and CG, including MTHFR C677T, MTRR 66GG, and MTRR A66G loci, were identified. In addition, differences in Hcy, Vit B12, and RBCF levels between groups were observed, as well as changes in Hcy, Vit B12, and RBCF levels of different genotype carriers in RG.

Statistical analyses

Statistical analyses were conducted using SPSS version 26.0, and a significance level of $P < 0.05$ was used in all analyses. Continuous and categorical variables were described as mean standard deviation ($\bar{x} \pm s$) and frequencies (percentages) [n(%)], respectively. Independent sample t tests were used for inter-group comparisons of continuous variables and ANOVA plus LSD intra-group tests were used for multi-group comparisons; while the Chi-square test was employed to compare categorical variables from two and multiple groups. Related factors were identified using Logistic regression analysis.

RESULTS

Comparison of clinical characteristics and indexes

There was clinical comparability between RG and CG as they showed no evident difference in terms of age, body mass index (BMI), trimester of pregnancy, and gravidity ($P > .05$, Table 1).

Comparison of MTHFR C677T genotype and allele frequency distribution

First of all, MTHFR C677T genotyping results showed that the proportion of the TT genotype in RG was 28.57%, higher than that in CG ($P < .05$); while the difference in the proportion of CCFR and CT genotypes was not statistically significant between groups ($P > .05$). Allele frequency detection results revealed a higher proportion of the T gene in RG compared with CG ($P < .05$, Table 2).

Table 1. Clinical characteristics and indexes

	Age	Trimester of pregnancy (weeks)	BMI	Gravidity First pregnancy/non-first
Control group (n=62)	27.37±3.94	26.23±4.01	22.38±3.56	54(87.10)/8(12.90)
Research group (n=56)	27.05±3.39	26.14±4.30	21.89±3.00	50(89.29)/6(10.71)
χ^2	0.641	0.108	0.789	0.135
P value	.467	.914	.432	.714

Table 2. MTHFR C677T genotype and allele frequency distribution

	MTHFR C677T genotype			Allele frequency	
	CC	CT	TT	C	T
Control group (n = 62)	23(37.10)	31(50.00)	8(12.90)	41(66.13)	21(33.87)
Research group (n = 56)	17(30.36)	23(41.07)	16(28.57)	24(42.86)	32(57.14)
χ^2	0.597	0.945	4.458	6.441	
P value	.440	.331	.035	.011	

Comparison of MTHFR A1298C genotype and allele frequency distribution

A1298C genotyping results showed statistical intergroup differences in neither the proportion of AA, AC, and CC genotypes nor the frequency of A and C alleles (P >0.05, Table 3).

Comparison of MTRR A66G genotype and allele frequency distribution

Finally, the A66G genotyping test identified the presence of statistical significance between RG and CG (P < .05), with a markedly reduced proportion of MTRR 66AG gene carriers and an elevated proportion of 66GG gene carriers in RG versus CG (P < .05). Allele frequency detection also revealed a lower proportion of the A gene and a higher proportion of the G gene in RG (P < .05, Table 4).

Multivariate analysis of adverse pregnancy outcomes

Logistic regression analysis was performed on the above gene polymorphisms with differences, with adverse pregnancy (with/without) of pregnant women as the endpoint. Carriers of MTHFR 677TT and MTRR 66GG had an approximately 3.5 fold and 2.6 fold increased risk of adverse pregnancy, respectively (P < .05), while MTRR 66AG genotype carriers had a reduced risk (P < .05. Table 5).

Comparison of Hcy, Vit B12, and RBCF

By further detecting Hcy, Vit B12, and RBCF levels, it was found that Hcy, Vit B12, and RBCF in RG were (8.02±1.38) μmol/L, (517.65±30.27) pg/mL and (1715.73±258.70) nmol/L respectively, showing higher Hcy and lower Vit B12 and RBCF levels compared with CG (P < .05, Figure 1).

Correlation of MTHFR C677T with Hcy, Vit B12, and RBCF

Comparing the levels of Hcy, Vit B12 and RBCF in pregnant women with different MTHFR C677T genotypes in RG, it can be seen that there was no difference in Hcy, Vit B12 and RBCF between 677CC and 677CT gene carriers (P > .05); while the Hcy level of 677T gene carriers was higher and the Vit B12 and RBCF levels were lower than those of the other two genotypes (P > .05, Figure 2)

Correlation of MTRR A66G and Hcy, Vit B12 with RBCF

Among different MTRR A66G carriers, Hcy of 66GG gene carriers was the highest among the three groups, while that of 66AG gene carriers was the lowest (P < .05). The opposite was observed in Vit B12 and RBCF, that is, 66GG gene carriers had the lowest Vit B12 and RBCF levels and 66AG gene carriers had the highest (P < .05, Figure 3).

DISCUSSION

In this study, we conducted a preliminary analysis of MTHFR and MTRR gene polymorphisms in pregnant women with adverse pregnancy outcomes. The proportion of MTHFR 677TT and MTRR 66GG genotypes in pregnant women with adverse pregnancy increased significantly, while

Table 3. MTHFR A1298C genotype and allele frequency distribution

	MTHFR A1298C genotype			Allele frequency	
	AA	AC	CC	A	C
Control group (n = 62)	43(69.35)	15(24.19)	4(6.45)	54(87.10)	8(12.90)
Research group (n = 56)	38(67.86)	12(21.43)	6(10.71)	45(80.36)	11(19.64)
χ^2	0.031	0.128	0.689	0.989	
P value	.861	.721	.406	.320	

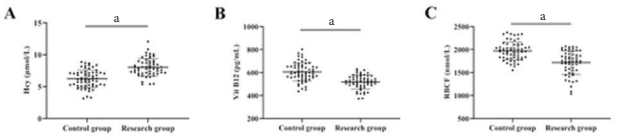
Table 4. MTRR A66G genotype and allele frequency distribution

	MTRR A66G genotype			Allele frequency	
	AA	AG	GG	A	G
Control group (n = 62)	25(40.32)	35(56.45)	2(3.23)	47(75.81)	15(24.19)
Research group (n = 56)	27(48.21)	21(37.50)	8(14.29)	30(53.57)	26(46.43)
χ^2	0.744	4.238	4.206	6.416	
P value	.389	.040	.040	.011	

Table 5. Multivariate analysis of adverse pregnancy outcomes

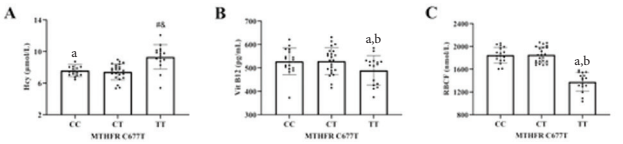
Genotype	β	S.E.	Wald χ^2	P value	OR	95%CI
MTHFR 677TT	0.452	0.164	12.816	<.001	3.541	2.881-5.942
MTRR 66AG	-0.069	0.642	9.762	<.001	0.436	0.124-1.849
MTRR 66GG	0.259	0.227	10.806	<.001	2.601	1.427-3.809

Figure 1. Comparison of Hcy, Vit B12, and RBCF. A: Comparison of Hcy between research and control groups. B: Comparison of Vit B12 between research and control groups. C: Comparison of RBCF between research and control groups.



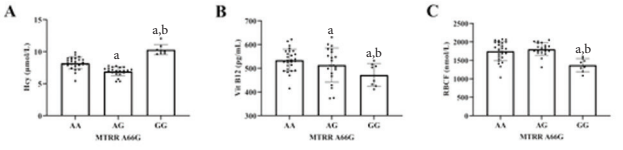
[a]P < .05.

Figure 2. Correlation of MTHFR C677T with Hcy, Vit B12, and RBCF. A: Comparison of Hcy in pregnant women with MTHFR CC, CT, and TT genotypes. B: Comparison of Vit B12 in pregnant women with MTHFR CC, CT, and TT genotypes. C: Comparison of RBCF in pregnant women with MTHFR CC, CT, and TT genotypes.



[a]*vs.* CC-type genes, P < .05
[b]*vs.* CT-type genes, P < .05

Figure 3. Correlation of MTRR A66G and Hcy, Vit B12 with RBCF. A: Comparison of Hcy in pregnant women with MTHFR AA, AG, and GG genotypes. B: Comparison of Vit B12 in pregnant women with MTHFR AA, AG, and GG genotypes. C: Comparison of RBCF in pregnant women with MTHFR AA, AG, and GG genotypes.



[a]*vs.* AA-type genes, P < .05
[b]*vs.* AG-type genes, P < .05

that of MTRR 66AG genotype decreased, indicating a close correlation of MTHFR C677T and MTRR A66G polymorphisms with adverse pregnancy. This is also consistent with previous evidence,[10,11] which can support our view. Meanwhile, through Logistic regression analysis, MTHFR 677TT and MTRR 66GG gene carriers were identified to have an increased risk of adverse pregnancy, while MTRR 66AG gene carriers had a decreased risk, further supporting the importance of MTHFR and MTRR polymorphisms for the occurrence of adverse pregnancy. MTHFR is known to be a key enzyme in FA metabolism, converting 5,10-methylenetetrahydrofolate reductase (MTHFR) into 5-methyltetrahydrofolate (5-MTHF), allowing it to provide methyl for Hcy to form methionine, as well as methyl for the synthesis of DNA, RNA, and proteins in vivo.[12] MTRR cooperates with Vit B12 to maintain the activity of methionine synthetase, enabling MTR to catalyze the synthesis of methionine from Hcy using 5-MTHF as a substrate. At present, there are many locus mutations related to the MTHR gene, among which 677C >T and 1298A>C mutations are the most extensively studied, and the most important mutation in MTRR is 66A>G.[13,14] MTHFR locus mutations lead to a decrease in the activity and stability of the MTHFR enzyme, which reduces the production of 5-MTHF, resulting in abnormal DNA and protein methylation reactions in vivo and hindered conversion of Hcy to methionine.[15] MTRR locus variation reduces MTRR activity, making it impossible to maintain MTR activity and hindering the conversion of Hcy to methionine.[16] Both of them can cause hyperhomocysteinemia, which directly or indirectly leads to the occurrence of adverse pregnancy outcomes.

Similarly, to verify the accuracy of the above viewpoint, we tested the differences in Hcy, Vit B12, and RBCF levels between RG and CG. The results showed a significant elevation of Hcy and reduced Vit B12 and RBCF in RG, which once again validates the importance of the three in maintaining normal pregnancy and delivery. Recent studies have linked adverse pregnancy outcomes such as NTDs, DS, and CHD to elevated plasma Hcy levels. Hyperhomocysteinemia may cause poor chorionic villus vascularization, resulting in embryonic loss and abortion. Elevated Hcy levels have also been associated with stillbirth, preterm birth, and other adverse pregnancy outcomes; high concentration of Hcy in plasma can cause damage and dysfunction of vascular endothelial cells, stimulate the proliferation of vascular smooth muscle cells, destroy the body's coagulation and fibrinolysis system, affect lipid metabolism, etc., so that the body is in a pre-thrombotic state.[17,18] Therefore, an increase in Hcy levels in women with adverse pregnancies is also to be expected. Vit B12 is a coenzyme for various reactions in the body, and its reduced content can induce disorders of the blood, bone marrow, nervous system, and gastrointestinal tract.[19] RBCF is a direct quantitative expression of the FA level, and its influence mechanism on adverse pregnancy may be consistent with the above inference of FA polymorphism.

Finally, Hcy levels were found to increase in MTHFR 677TT and MTRR 66GG gene carriers, while Vit B12 and RBCF decreased, and the opposite was true for MTRR 66AG gene carriers, further demonstrating the above experimental results and the influence of FA polymorphisms on adverse pregnancy. It is suggested that we may be expected to better prevent or treat adverse pregnancy through FA gene polymorphisms, Hcy, Vit B12, and RBCF in the future.

However, we also found other studies inconsistent with the results of this study,[20,21] which may be because FA gene polymorphisms are affected by various factors and may have different expressions in different regions, populations, and age groups, which is still worth further research and analysis for validation. Similarly, due to the limited number of cases, we were unable to further break down the types of adverse pregnancies to more accurately assess the effect of FA polymorphisms on adverse pregnancy outcomes.

CONCLUSION

MTHFR 677TT and MTRR 66GG carriers had increased levels of Hcy, decreased Vit B12 and RBCF, and an increased risk of adverse pregnancy, while MTRR 66AG carriers had a reduced risk. In the future, the risk of adverse pregnancy outcomes can be assessed by detecting maternal FA metabolism gene polymorphisms, Hcy, Vit B12, and RBCF, and effective and accurate targeted treatment programs can be developed to ensure the normal delivery of pregnant women and the health of newborns.

ETHICAL APPROVAL

The study protocol was approved by the Ethics Committee of Qingdao Central Hospital (NO. Lk2022032).

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CONFLICTS OF INTEREST

The authors report no conflict of interest.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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