

# Association of *MTHFR* gene C677T polymorphism with pregnancy outcome

L.-L. HUANG, J.-R. TONG, Y. HUANG, Y.-N. WEI, H.-F. CHEN,  
Y. CHEN, J.-Y. SU, L. DENG

Department of Obstetrics, The Second Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, China

*L.-L. Huang and J.-R. Tong have contributed equally to this work, so they are both first authors*

**Abstract. – OBJECTIVE:** The two objectives of the present study were to analyze the correlation between pregnancy outcomes and *methylenetetrahydrofolate reductase (MTHFR)* gene C677T polymorphism, and to provide evidence for clinical improvement of adverse pregnancy outcomes.

**PATIENTS AND METHODS:** 1,995 cases of pregnant women were selected as objects of the study, and underwent *MTHFR* gene C677T polymorphism detection in the Second Affiliated Hospital of Guangxi Medical University from October 2020 to September 2021, in which 919 cases whose pregnancy outcomes could be tracked. According to the result of *MTHFR* gene C677T polymorphism detection, 1,995 cases of pregnant women were classified into a wild-type (CC) group, heterozygous (CT) group, or homozygous (TT) group, and the distributions of *MTHFR* gene C677T polymorphism in pregnant women were analyzed. In addition, according to complications, 919 cases of pregnant women whose pregnancy outcomes could be tracked were divided into the normal pregnancy group (676 cases), GDM group (146 cases), HDP group (47 cases), abnormal fetus group (13 cases), and spontaneous abortion group (37 cases), and the genotype distributions of *MTHFR* gene C677T in each group were analyzed. Besides, according to genotype, 919 cases of pregnant women whose pregnancy outcome could be tracked were divided into CC group (515 cases), CT group (289 cases), and TT group (115 cases), and the correlation between genotype and pregnancy outcomes, such as fetal distress, postpartum hemorrhage, premature birth, and full-term delivery, was then analyzed.

**RESULTS:** For the C677T locus of *MTHFR* gene in the 1,995 cases of pregnant women, there are 1,162 (58.25%) cases of CC genotype, 649 (32.53%) cases of CT genotype, 184 (9.22%) cases of TT genotype. The proportion of TT genotype in GDM, HDP, abnormal fetus, and spontaneous abortion groups were respective-

ly 19.86% (29/148), 25.53% (12/47), 46.15% (6/13), 40.54% (15/37), which were significantly higher than that in normal pregnancy group (7.84%, 53/676), and there were statistically significant differences ( $p < 0.05$ ). The full-term birth rate in TT group (75.65%, 87/115) was lower than those of CC group (91.26%, 470/515) and CT group (89.27%, 258/289), and there were statistically significant differences ( $p < 0.05$ ).

**CONCLUSIONS:** The TT type gene mutation at the C677T site of *MTHFR* gene is closely related to conditions that contribute to a decrease in the number of full-term births and increase the risk of adverse pregnancy outcomes, including GDM, HDP, spontaneous abortion, and fetal abnormalities.

*Key Words:*

Methylenetetrahydrofolate reductase, Gene polymorphism, Pregnancy outcome.

## Introduction

Folic acid is an indispensable substance involved in DNA synthesis, protein metabolism, embryonic development, and additional physiological and biochemical processes during pregnancy. A folic acid metabolism disorder can, therefore, lead to adverse pregnancy outcomes and can endanger the health of mothers and babies. Methylenetetrahydrofolate reductase (*MTHFR*) is a key enzyme in the folate pathway that regulates the conversion of homocysteine to methionine by maintaining methyl pool. C677T transition is a missense mutation with the substitution of cysteine (C) to thymine (T) nucleotide at the site 677 of exon 5 of this gene, which results in changes in protein translation from alanine to valine. C677T polymorphism may not only affect the stability of

*MTHFR*, resulting in its degradation. In addition, it may also reduce the normal physiological activity of *MTHFR*, leading to the occurrence of folic acid metabolism disorders<sup>1-4</sup>. Based in the mutation of the wild-type *MTHFR* at position 677 from C to T, the *MTHFR* is divided into homozygous C allele (CC), heterozygous (CT), and homozygous T allele (TT) genotypes<sup>5</sup>. Clinically, the *MTHFR* C677T genotype is used to detect the presence of a folate metabolism disorder, specifically: CC type indicates high protease activity and normal folic acid conversion capacity, CT type indicates moderate protease activity and folic acid conversion activity, and TT type indicates low protease activity and folic acid conversion activity. The presence of a C677T mutation causes a reduction in the utilization rate of folic acid, and folic acid is closely related to hypertensive disorders of pregnancy (HDP), gestational diabetes mellitus (GDM), miscarriage, and fetal abnormalities<sup>6-8</sup>. Therefore, further investigation of the expression of *MTHFR* gene C677T polymorphism in HDP, GDM, abortion, and the abnormal fetus is warranted. In the present study, the pregnancy outcomes of 919 patient participants who underwent *MTHFR* gene C677T polymorphism detection were tracked, and the correlation between *MTHFR* gene C677T polymorphism and pregnancy outcomes was then analyzed with the goal of providing a basis for clinical improvement of adverse pregnancy outcomes.

## Patients and Methods

### General Information

The present study enrolled a total of 1,995 cases of early pregnant women with a singleton pregnancy who underwent *MTHFR* gene C677T polymorphism detection in the Second Affiliated Hospital of Guangxi Medical University between October 2020 and September 2021. Participant age ranged between 18 and 43 years, with an average age of  $30.33 \pm 4.54$  years. The pregnancy outcomes of these patient participants were tracked and recorded. This study was approved by the medical ethics committee of the Second Affiliated Hospital of Guangxi Medical University, and all subjects signed informed consent prior to participation.

### Sample Collection

From the pregnant participants (who were diagnosed with a single live fetus in early pregnancy), a tube of 2-3 mL of fasting cubital venous

blood was drawn and placed in a 2% ethylenediaminetetraacetic acid (EDTA) anticoagulant tube for allele detection of the C677T locus of the *MTHFR* gene.

### Determination of *MTHFR* Gene C677T Polymorphism

*MTHFR* (677C > T) gene detection kit (Microread, Beijing, China) (PCR-chip hybridization method) was used to determine the *MTHFR* genotype. EDTA anticoagulated whole blood was utilized to extract the DNA (according to the instructions of the extraction kit). DNA concentration and purity were determined by ultra-micro spectrophotometer. PCR reaction mix, including DNA template, was then prepared. The PCR amplification conditions were as follows: pretreatment at 50°C for 5 min, pre-denaturation at 94°C for 5 min, denaturation at 94°C for 25 s, annealing at 56°C for 25 s, extension at 72°C for 25 s, a total of 35 samples cycles, and extension at 72°C for an additional 5 min. Hybridization reaction of the PCR products was then performed according to the instructions of the BaiO immunochromogenic kit (Microread, Beijing, China). To determine polymorphism of the *MTHFR* gene C677T, samples and reagent chambers were placed on the sequencer, and the results were then recorded.

### Grouping and Observation Indicators

According to the results of the *MTHFR* gene C677T polymorphism test, 1,995 patients were then divided into either the CC group, CT group, or TT group, and the distributions of *MTHFR* gene C677T polymorphism in pregnant women were analyzed. 919 cases of pregnant women whose pregnancy outcomes could be tracked were divided into HDP group, GDM group, abnormal fetus group, spontaneous abortion group, and normal pregnancy group, and the genotype distributions of *MTHFR* gene C677T in each group were analyzed. The correlation between genotype and pregnancy outcomes, such as fetal distress, postpartum hemorrhage, premature birth, and full-term delivery, was then analyzed.

### Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) 25.0 software (IBM Corp., Armonk, NY, USA). Homogeneous variance and continuous data that were normally distributed were expressed as the mean  $\pm$  standard deviation. Variance analysis was used to compare data among the three study groups.

The LSD test was used for pairwise comparison, the enumeration data were expressed as frequency (rate), and the Chi-square test ( $\chi^2$ ) was used for enumeration data. When the sample size was less than 40 and the theoretical frequency was less than five, Fisher's exact test was performed.  $p < 0.05$  was considered to be statistically significant.

## Results

### ***Distribution of MTHFR Gene C677T Polymorphism***

The present study enrolled a total of 1,995 cases of early pregnant women with a singleton pregnancy who underwent *MTHFR* gene C677T polymorphism detection in the Second Affiliated Hospital of Guangxi Medical University between October 2020 and September 2021. The distribution of *MTHFR* gene C677T polymorphism was 1,162 (58.25%) cases of CC genotype, 649 (32.53%) cases of CT genotype, 184 (9.22%) cases of TT genotype.

### ***The Relationship Between MTHFR gene C677T Polymorphism and Pregnancy Complications***

In the 919 cases of pregnant women whose pregnancy outcomes were successfully tracked, the proportions of TT genotype in GDM, HDP, abnormal fetus, and spontaneous abortion were respectively 19.86%, 25.53%, 46.15%, and 40.54%, which were all significantly higher than that of normal pregnancy group (7.84%), and there were statistically significant differences ( $p < 0.05$ ). There was no significant difference in the proportion of CT genotype among the four groups compared with the normal pregnancy group ( $p > 0.05$ ). And the proportions of CC genotype in the abnormal fetus and spontaneous abortion groups were both significantly lower than that of the nor-

mal pregnancy group, and there were statistically significant differences ( $p < 0.05$ ), while there was no significant difference in the CC genotype in GDM and HDP groups compared with that in normal pregnancy group ( $p > 0.05$ ), which was shown in Table I.

### ***The Relationship Between MTHFR Gene C677T Polymorphism and Pregnancy Outcome***

A total of 869 cases (94.56%) were finally delivered, of which 815 were term born and 54 were premature. The full-term delivery of CC, CT, and TT groups were respectively 470 cases (91.26%), 258 cases (89.27%), and 87 (75.65%), in which the full-term birth rates were lower in the TT group than in the CC and CT groups ( $p < 0.05$ ). There was no significant difference in the incidences of premature birth, fetal distress, and postpartum hemorrhage among the three groups ( $p > 0.05$ ), which was shown in Table II.

## Discussion

### ***Distribution of MTHFR Gene C677T Polymorphism in Pregnant Women***

C677T polymorphism of the *MTHFR* gene may affect its stability. The homozygous mutant TT has poor thermal instability, which causes *MTHFR* to be easily degraded and also impacts the normal physiological function of *MTHFR*<sup>9</sup>. Previous studies<sup>10</sup> suggest that the C677T gene TT mutation is more likely to lead to adverse pregnancy outcomes. It has been previously reported<sup>11</sup> that the frequency of the T allele in Europeans is between 22% and 44%, and the frequency is 7% in Saharan blacks living in Africa. In the Chinese Han population, the incidence of *MTHFR* C677T homozygous mutation (TT) is 15%, and heterozygous mutation (CT) is 36% to 40%<sup>5</sup>. The

**Table I.** Association of *MTHFR* gene C677T polymorphism with pregnancy complications.

Group	No. of patients (n)	CC genotype	CT genotype	TT genotype
GDM	146	76 (52.05%)	41 (28.08%)	29 (19.86%)*
HDP	47	21 (44.68%)	14 (29.79%)	12 (25.53%)*
Abnormal Fetus	13	4 (30.77%)*	3 (23.08%)	6 (46.15%)*
Spontaneous Abortion	37	10 (27.03%)*	12 (32.43%)	15 (40.54%)*
Normal Pregnancy	676	404 (59.76%)	219 (32.39%)	53 (7.84%)
In total	919	515 (56.04%)	289 (31.45%)	115 (12.51%)

CC, homozygous C allele; CT, heterozygous T allele; TT, homozygous T allele; GDM, gestational diabetes mellitus; HDP, hypertensive disorders of pregnancy. \*means that compared with the normal pregnancy group,  $p < 0.05$ .

**Table II.** Association of *MTHFR* gene C677T polymorphism with pregnancy outcome.

Group	No. of patients (n)	Premature birth	Full-term birth	Fetal distress	Postpartum hemorrhage
CC	515	31 (6.02%)	470 (91.26%)	28 (5.44%)	8 (1.55%)
CT	289	16 (5.53%)	258 (89.27%)	16 (5.54%)	6 (2.08%)
TT	115	7 (6.09%)	87 (75.65%)	7 (6.09%)	2 (1.74%)
<i>p</i> -value		0.957	< 0.001	0.963	0.863

CC, homozygous C allele; CT, heterozygous T allele; TT, homozygous T allele.

present study identified that the CC genotype was 58.25%, the CT genotype was 32.53%, and the TT genotype was 9.22%. Therefore, there may be regional and ethnic differences in the distribution of *MTHFR* gene C677T polymorphism. Further studies will be needed to explore whether or not this difference in gene distribution will lead to a difference in disease incidence.

#### **The Relationship Between *MTHFR* Gene C677T Polymorphism and Pregnancy Complications**

Mutation of *MTHFR* gene C677T reduces its stability and leads to reduced conversion of homocysteine to methionine. Abnormal metabolism of homocysteine may subsequently result in elevated levels of homocysteine in the body. Clinically, the *MTHFR* C677T genotype is often associated with a risk of high levels of homocysteine, during which the CC genotype indicates a low risk of hyper-homocysteine, the CT genotype indicates a moderate risk of hyper-homocysteine, and the TT genotype is correlated with a high risk of hyper-homocysteine. High levels of homocysteine are closely associated with adverse pregnancy outcomes such as GDM and HDP, and this supports previous observations<sup>12,13</sup> showing that *MTHFR* C677T homozygous TT increases the risk of GDM and HDP in pregnant women. Al-Achkar et al<sup>8</sup> showed that *MTHFR* gene C677T polymorphism increases the risk of recurrent spontaneous abortion (RSA), which suggests that the *MTHFR* C677T gene is a susceptibility gene for RSA<sup>14</sup>. Additionally, pregnant women who carry the TT genotype could be at risk for fetal abnormalities in their neonates such as neural tube defects, congenital heart disease, Down syndrome, and congenital cleft lip and palate<sup>15,16</sup>. In this study, the proportions of TT genotype of GDM, HDP, abnormal fetus, and spontaneous abortion were respectively 19.86%, 25.53%, 46.15%, and 40.54%, which were significantly higher than that of the normal pregnancy group (7.84%), and there were statistically significant differences. The present

data suggest that the *MTHFR* C677T homozygous TT genotype indicates a risk for GDM and HDP. These findings support previous studies<sup>6,7,12</sup> on *MTHFR* gene C677T polymorphism in GDM and HDP, and they suggest that *MTHFR* gene C677T polymorphism can be used to predict and diagnose the risk of GDM and HDP. For pregnant patients with the TT genotype, additional attention should be paid to the encouragement of folic acid supplementation in order to help prevent the occurrence of GDM and HDP.

#### **Association of *MTHFR* Gene C677T Polymorphism with Pregnancy Outcome**

At present, there are few reports about the occurrence of adverse pregnancy outcomes, such as premature birth, fetal distress, and postpartum hemorrhage, directly caused by the C677T polymorphism of *MTHFR* gene. It is generally accepted that the mutation of *MTHFR* gene at C677T increases the risk of HDP and GDM, thus indirectly leading to the occurrence of premature birth, fetal distress, and postpartum hemorrhage. This study, is consistent with previous reports that the C677T polymorphism of *MTHFR* gene is associated with premature birth, fetal distress, and postpartum hemorrhage. However, the full-term delivery rate of 75.65% in TT group was lower than that of CC (91.26%) and CT (89.27%) groups, and there were statistically significant differences. These results indicate that the TT genotype is more likely to lead to spontaneous abortion and abnormal fetal outcomes, which explains the decrease in number of pregnant women with the TT genotype who were able to carry the pregnancy to full term delivery. This finding suggests that the TT genotype is closely associated with adverse pregnancy outcomes.

### **Conclusions**

This study confirmed that the TT type gene mutation at the C677T locus of the *MTHFR* gene is closely related to GDM, HDP, spontaneous

abortion, and fetal abnormalities, and that it leads to a decrease in the number of full-term births and an increase in the risk of adverse pregnancy outcomes. Therefore, *MTHFR* gene C677T polymorphism should be detected before or during pregnancy, in order to guide folic acid supplementation to reduce the incidence of complications during pregnancy and help prevent the occurrence of adverse pregnancy outcomes.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

### Ethics Approval

This study was approved by the Hospital Medical Ethics Committee.

### Informed Consent

Signed informed consent was obtained from all patients.

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### Authors' Contributions

Li Deng and Junyou Su performed the conception and design of study; Yan Huang and Yanni Wei analyzed and interpreted the patient data; Hongfei Chen and Yan Chen performed the examination of the patients, Lingling Huang was a major contributor in writing the manuscript, Junru Tong revised the manuscript. All authors read and approved the final manuscript.

### Availability of Data and Materials

All data generated or analyzed during this study are included in this article and its supplementary material.

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