https://www.scirp.org/journal/ns Natural Science, 2025, Vol. 17, (No. 3), pp: 13-19

Multifaceted Analysis of Methylenetetrahydrofolate Reductase Gene Polymorphism in Reproductive-Age Women

Yumei Ma[*], Xiaojuan Jiang[*], Xiaoyu Jiang[#], Yuanrong Shao[#]

Department of Laboratory Medicine, The Third People's Hospital of Nanning, Nanning, China

Correspondence to: Yuanrong Shao, 377955204@qq.com; Xiaoyu Jiang, 38669739@qq.com

Keywords: Methylenetetrahydrofolate Reductase (MTHFR), Gene Polymorphism, 677C>T, Reproductive-Age Women, Folic Acid Supplementation, Ethnic Differences, Regional Differences, Pregnancy Health, Prenatal Examination, Folic Acid Utilization Risk

Received: January 27, 2025 **Accepted:** February 24, 2025 **Published:** February 27, 2025

ABSTRACT

Objective: This study aims to investigate the distribution of methylenetetrahydrofolate reductase (MTHFR) gene 677C>T polymorphism in this region, to guide reproductive-age women in personalized folic acid supplementation. Methods: A total of 1079 reproductive-age women who underwent prenatal examinations at our hospital from July 2020 to July 2024 were selected for the study, and their basic data were collected. The MTHFR gene 677C>T polymorphism was detected using the polymerase chain reaction-fluorescent probe method. Statistical methods were employed to analyze the impact of ethnicity, age groups, and regional differences on MTHFR gene polymorphism and genotype frequency. Results: Among the 1079 subjects, the detection of MTHFR gene 677C>T polymorphism revealed that the 677CC genotype accounted for 62.0%, the 677CT genotype for 33.8%, and the 677TT genotype for 4.2%. There was no significant difference in genotype distribution between different ethnic groups (Zhuang and Han) ($P > 0.05$), nor were there significant differences observed in different age groups (<20 years, 20 - 30 years, and >30 years) ($P > 0.05$). However, a significant difference in genotype distribution was found based on different regions ($P < 0.01$). Conclusion: This study found no significant difference in MTHFR gene 677C>T polymorphism between Zhuang and Han women of reproductive age, but significant regional differences exist. Formulating a scientifically sound folic acid supplementation plan based on the regional distribution characteristics of MTHFR gene polymorphism is crucial for optimizing pregnancy health management.

[*]Co-first authors.

[#]Corresponding authors.

1. INTRODUCTION

Folic acid is a water-soluble B vitamin that plays a crucial biological role and is an essential nutrient for cell growth and reproduction in the human body [1]. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism, capable of reducing 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, and is involved in various biochemical reactions [2]. At the 677 locus of the MTHFR gene, there are three genotypes: wild type (CC), heterozygous mutant (CT), and homozygous mutant (TT). Mutations at this gene locus can lead to changes in enzyme activity, resulting in abnormal folate metabolism, decreased levels of active folate, and elevated concentrations of homocysteine (Hcy). Increased plasma Hcy levels are closely associated with various birth defects, including neural tube defects, congenital heart diseases, and Down syndrome, as well as pregnancy-related conditions such as hypertension and recurrent miscarriage [3]. Polymorphisms in the MTHFR and MTRR genes can lead to elevated levels of Hcy in the body, potentially triggering various systemic diseases.

The MTHFR gene plays a significant regulatory role in folate metabolism, capable of altering the activity of enzymes related to folate metabolism, affecting the metabolic process of folate, and consequently regulating plasma Hcy concentrations, which can lead to the occurrence of various diseases [4]. MTHFR gene polymorphisms have been associated with several gynecological conditions, such as polycystic ovary syndrome (PCOS), cervical intraepithelial neoplasia grade III (CIN III), cervical cancer, depression in women during specific periods, and ovarian cancer. MTHFR gene polymorphism may result in varying degrees of decreased enzyme activity, leading to reduced production of 5-methyltetrahydrofolate and the occurrence of related diseases. Furthermore, the enzyme expressed by the MTHFR gene is crucial for the body's absorption and utilization of folate. Adequate folate supplementation is considered a safe and effective method to control the risks associated with this gene mutation, and precise diagnosis may aid in the prevention and treatment of diseases caused by this mutation [5]. This study aims to investigate the distribution of MTHFR gene 677C>T polymorphism among reproductive-age women undergoing health examinations at our hospital, providing important evidence for personalized folate supplementation, while also analyzing the impact of ethnicity, age groups, and regional differences on genotype distribution.

2. MATERIALS AND METHODS

2.1. Study Subjects

This study selected reproductive-age women who underwent preconception health check-ups at our hospital from July 2020 to July 2024 as study subjects, totaling 1079 cases. The study population included Zhuang, Han, and other ethnic minority women, with 586 Han, 454 Zhuang, and 39 from other minority groups. All participants were residents of Nanning. The research protocol was approved by the hospital's ethics committee, and all participants signed informed consent before the study. We collected and recorded the basic information of the study subjects and conducted statistical analyses on the distribution of MTHFR gene 677C>T polymorphism among different ethnicities, age groups, and regions.

2.2. Methods

2.2.1. Sample Preparation and DNA Extraction

In this study, 2 mL of EDTA anticoagulated venous whole blood was used as the sample. DNA extraction was performed according to the instructions provided by the blood genomic DNA extraction kit (Capbio Biotechnology Co., Ltd., Chaozhou) to ensure the standardization and effectiveness of the extraction process.

2.2.2. Detection Method for MTHFR Gene 677C>T Polymorphism

We employed the polymerase chain reaction (PCR) with fluorescent probes to detect the polymorphism at the MTHFR gene 677C>T locus. Amplification and detection were performed using the SLAN 96P fluorescence quantitative PCR instrument (Shanghai Hongshi). According to the instructions provided by the

MTHFR 677C>T gene detection kit (Wuhan Youzhiyou Medical Technology Co., Ltd.), genotype was determined by analyzing the fluorescent signals. Experimental procedures strictly followed the reagent instructions, with positive and negative controls set up for each experiment to ensure the validity of the test and the kit.

2.3. Statistical Analysis

Data were analyzed using SPSS version 29.0 statistical software. Count data were expressed as frequency and percentage (%), and differences between groups were compared using the chi-squared test (χ^2 test), with $P < 0.05$ considered statistically significant. Through these analyses, we aimed to reveal the distribution characteristics of the MTHFR gene 677C>T polymorphism in different ethnicities and age groups, providing a scientific basis for future research.

3. RESULTS

3.1. Hardy-Weinberg Equilibrium Analysis

The results of this study indicated that the genotype frequencies at the MTHFR gene 677C>T locus among reproductive-age women in this region were 62.0% for the wild type (CC), 33.8% for the heterozygous type (CT), and 4.2% for the mutant type (TT), with the wild type being predominant. A Hardy-Weinberg equilibrium (HWE) test was performed on the genotypes (CC, CT, TT) of the methylenetetrahydrofolate reductase 677C>T locus for this sample population. The results showed no significant difference between the observed and expected frequencies ($\chi^2 = 0.153$, $P = 0.926 > 0.05$), indicating that the population is in Hardy-Weinberg equilibrium and thus representative of the population, as detailed in Table 1.

Table 1. Analysis of the MTHFR gene 677C>T locus in this study.

Genotype	CC	CT	TT	χ^2	P
Observed Frequency	669	365	45	0.153	0.926
Expected Frequency	672	359	48		

3.2. Comparison of Genotype and Allele Frequencies across Ethnic Groups

Analysis of the MTHFR 677C>T genotype data from 454 reproductive-age women of the Zhuang ethnicity in this region revealed genotype frequencies of 65.9% (299 cases) for CC, 30.4% (138 cases) for CT, and 3.7% (17 cases) for TT. In contrast, analysis of 586 reproductive-age Han women in the region showed genotype frequencies of 59.0% (346 cases) for CC, 36.2% (212 cases) for CT, and 4.8% (28 cases) for TT. For other ethnic minority women, the genotype frequencies were 61.5% (24 cases) for CC, 38.5% (15 cases) for CT, and 0.0% (0 cases) for TT. When comparing genotype frequencies between different ethnic groups (note: due to the small sample size of other minority groups, this analysis is limited to Han and Zhuang ethnicities), no statistically significant differences were found between the frequencies of CC, CT, and TT genotypes ($\chi^2 = 6.495$, $P = 0.150 > 0.05$), as detailed in Table 2.

Table 2. Distribution of MTHFR gene 677C>T locus genotypes among different ethnic groups in this study.

Ethnicity	CC	CT	TT	χ^2	P
Zhuang	299 (65.90)	138 (30.40)	17 (3.70)	5.088	0.079
Han	346 (59.00)	212 (36.20)	28 (4.80)		

3.3. Genotype Distribution Characteristics across Different Regions

Among 1079 reproductive-age women, the MTHFR gene C677T locus predominantly exhibited the wild-type CC genotype. When comparing the genotype distribution characteristics of this locus with data from related literature [6-16], it was found that the genotype distribution of the MTHFR gene C677T locus among reproductive-age women in Nanning showed statistically significant differences ($P < 0.05$) when compared to regions including Cangzhou, Changji City in Xinjiang, Wenzhou, Chongqing, Kaifeng in Henan, Tianjin, Gannan region, Huizhou in Guangdong, Kunming in Yunnan, Shenzhen in Guangdong, and Lanzhou in Gansu. However, no significant differences were observed when compared with reproductive-age women from Qionghai in Hainan. See **Table 3** for details.

Table 3. Comparison of genotype frequency data of MTHFR gene C677T locus across different regions.

Region	MTHFR Gene C677T Genotype				
	CC Cases (%)	CT Cases (%)	TT Cases (%)	χ^2	P
Current Study	669 (62.00)	365 (33.80)	45 (4.20)	\	\
Cangzhou	99 (16.34)	279 (46.04)	228 (37.62)	460.730	<0.001
Changji, Xinjiang	167 (26.00)	329 (51.30)	146 (22.70)	262.689	<0.001
Wenzhou	113 (41.20)	1205 (43.90)	407 (14.80)	164.680	<0.001
Chongqing	511 (53.10)	361 (37.50)	91 (9.40)	30.245	<0.001
Kaifeng	131 (13.70)	434 (45.30)	394 (41.10)	640.369	<0.001
Tianjin	239 (23.80)	488 (48.61)	277 (27.59)	386.326	<0.001
Gannan Region	711 (39.79)	788 (43.65)	298 (16.68)	172.766	<0.001
Kunming	2306 (38.41)	2814 (46.87)	884 (14.72)	233.540	<0.001
Shenzhen	1406 (57.00)	670 (27.20)	390 (15.80)	97.526	<0.001
Qionghai	756 (61.90)	390 (31.90)	75 (6.20)	4.891	0.086
Lanzhou	489 (26.60)	890 (48.50)	457 (24.90)	417.292	<0.001

3.4. Genotype Distribution across Different Age Groups

When comparing the genotype frequency of the MTHFR gene C677T locus across different age groups, the results indicated that there were no statistically significant differences between the age groups ($P > 0.05$). See **Table 4** for details.

Table 4. Comparison of genotype frequencies across different age groups.

Age Group	CC	CT	TT	χ^2	P
<20 Years	30 (54.50)	23 (41.80)	2 (3.60)		
20 - 30 Years	347 (59.40)	207 (35.40)	30 (5.10)	8.099	0.080
>30 Years	292 (66.40)	135 (30.70)	13 (3.00)		

4. DISCUSSION

The presence of the MTHFR 677C>T genotype is closely related to the efficiency of folate metabolism in the body. The MTHFR C677T mutation (TT genotype) typically exhibits lower MTHFR enzyme activity, resulting in reduced production of the active form of folate. Folate is crucial for DNA synthesis, cell division, and fetal development during pregnancy, and mutations in the MTHFR gene may lead to insufficient folate levels during this period. Therefore, effective supplementation of folate during pregnancy is an important measure to prevent congenital malformations in offspring and may also be a key strategy to reduce the incidence of other adverse pregnancy outcomes. Targeted preventive measures should be implemented to lower the occurrence of congenital malformations and improve the overall quality of the population [17]. Research has shown that the polymorphism at the MTHFR 677C>T locus is associated with recurrence in AIS patients, with lower survival rates observed in AIS patients with the TT genotype. This suggests that the MTHFR gene TT homozygous mutation and alterations in the T allele may influence the recurrence of AIS patients [18].

This study analyzed 1079 women of childbearing age to explore the distribution of methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms among different ethnic groups and regions in this area. The results indicate that there is no significant difference in the MTHFR 677C>T polymorphism among different ethnic groups, which may be attributed to long-term intermarriage among various ethnicities in the Nanning region leading to genetic admixture. The frequency of the MTHFR 677C>T genotype has gradually tended to be consistent between the two ethnic groups. However, significant differences were observed between different regions, providing a new perspective for understanding the genetic basis of pregnancy health in different areas.

In addition, our results show that there are no obvious distribution differences of the MTHFR 677C>T polymorphism across different age groups, which provides a basis for assessing the abnormalities of folate metabolism and elevated homocysteine levels due to MTRR gene polymorphism in the aging population of this region. The distribution differences of MTHFR gene polymorphisms among different regions may be associated with factors such as genetic drift, natural selection, environmental adaptation, and dietary habits. These environmental factors may lead to differing selection pressures on gene polymorphisms in various regions, resulting in frequency changes within specific populations.

In this region, the wild-type MTHFR 677C>T genotype is predominant among women of childbearing age. Testing for folate metabolism genes in women of childbearing age can serve as an early warning tool to assess the risk of pregnancy-related diseases such as birth defects, pregnancy-induced hypertension, and habitual abortion, thereby scientifically guiding prenatal care. Personalized folate supplementation dosages can not only achieve the goal of promoting healthy births but also avoid the waste of public resources. Furthermore, the regional differences demonstrated by this study provide new directions for health interventions tailored to specific populations.

This study has a large sample size and encompasses multiple ethnic groups, enhancing the reliability of the findings. However, it still has some limitations. Firstly, the study primarily focused on the MTHFR 677C>T polymorphism; future research could expand to other related genes to gain a more comprehensive understanding of the genetic factors influencing pregnancy outcomes. Secondly, although we controlled for several potential confounding factors that may affect pregnancy outcomes (such as age and previous pregnancy history), there may still be unknown confounding factors that could impact the accuracy of the results. Finally, the sample in this study was mainly drawn from a single hospital; future research should be conducted in broader geographical areas and populations to validate our findings.

By further understanding the relationship between MTHFR gene polymorphisms and pregnancy outcomes, we can provide more precise nutritional and health interventions for pregnant women, thereby reducing the incidence of adverse pregnancy outcomes. Future research should further explore how gene-environment interactions impact pregnancy health, providing more scientific evidence and practical guidance for improving pregnancy outcomes.

5. CONCLUSION

This study explored the distribution of methylenetetrahydrofolate reductase (MTHFR) gene 677C>T polymorphism among different ethnic groups and regions in Nanning. The results indicate that the wild-type genotype of MTHFR 677C>T predominates, with no significant differences among different ethnic groups; however, significant differences are observed between different regions. This provides a new perspective for understanding the genetic basis of pregnancy health and offers important evidence for folate metabolism gene testing in women of childbearing age to assess the risk of adverse pregnancy outcomes.

LIMITATIONS OF THE STUDY

1) The focus was primarily on the MTHFR 677C>T polymorphism, without covering other related genes, which may affect the comprehensiveness of the study.

2) There may be unconsidered unknown confounding factors that affect the accuracy of the results.

3) The samples were mainly collected from our hospital; it is recommended that future studies validate these findings in a broader geographical area and population.

ACKNOWLEDGMENTS

We would like to thank all the women of childbearing age and their families who participated in this study, as well as the cooperation and support from various hospitals and research institutions in the Nanning area. Special thanks to all team members for their efforts and contributions in data collection and analysis.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

REFERENCES

1. (2020) Expert Consensus on the Rational Supplementation of Folic Acid in Clinical Practice in China. *Chinese Journal of Medical Frontiers* (*Electronic Edition*), **12**, 19-37.
2. Teng, J., Suo, F., Zhang, Y., *et al.* (2023) Study on the Polymorphism of Methylenetetrahydrofolate Reductase Gene in Women of Childbearing Age. *Journal of Rare Diseases*, **30**, 6-7. https://doi.org/10.3969/j.issn.1009-3257.2023.07.03
3. Li, J., Zheng, L., Jiang, T., *et al.* (2019) Research Progress on Polymorphisms of Folate Metabolism Genes. *Medical Guide*, **38**, 230-234.
4. Gao, L., Niu, Y., Li, M., *et al.* (2024) Research Progress on the Association of MTHFR and MTRR Gene Polymorphisms with Diseases. *Chinese Journal of Modern Applied Pharmacy*, **41**, 2447-2454. https://doi.org/10.13748/j.cnki.issn1007-7693.20232994
5. Feng, W. and Ma, Y. (2019) Research Progress on the Association between Methylenetetrahydrofolate Reductase Gene Polymorphism and Gynecological Diseases. *Practical Journal of Medicine*, **35**, 1677-1680. https://doi.org/10.3969/j.issn.1006-5725.2019.10.033
6. Di, W., Zhang, H., Qiang, G., *et al.* (2019) Analysis of Polymorphisms of Methylenetetrahydrofolate Reductase and Methionine Synthase Genes in Han Women of Childbearing Age in Cangzhou. *Chinese Journal of Maternal and Child Health Care*, **34**, 2793-2796.
7. Ji, K. and Zhu, Y. (2024) Distribution of MTHFR Gene C677T Polymorphism in Women of Childbearing Age in Changji, Xinjiang, and Its Relationship with Adverse Pregnancy Outcomes. *Chinese Journal of Family Planning*, **32**, 1453-1456.
8. Lin, X., Wu, H., Xu, X., *et al.* (2024) Analysis of MTHFR C677T Gene Polymorphism in 2,743 Women of Childbearing

Age in Wenzhou. *Chinese Journal of Birth Health and Genetics*, **32**, 593-596. https://doi.org/10.13404/j.cnki.cjbhh.2024.03.025

9. Yuan, H., Liu, C., Li, Y., *et al.* (2024) Study on Folate Metabolism Enzyme MTHFR and MTRR Gene Polymorphisms in Women of Childbearing Age in Chongqing. *Chinese Journal of Family Planning*, **32**, 2695-2700.
10. Wang, X., Wang, Q., Zhang, X., *et al.* (2021) Study on the Distribution of MTHFR and MTRR Gene Polymorphisms in Han Women of Childbearing Age in Kaifeng. *Journal of Experimental and Clinical Medicine*, **39**, 719-721. https://doi.org/10.3969/j.issn.1674-1129.2021.03.064
11. Fu, J. and Tang, S. (2021) Study on the Correlation between Methylenetetrahydrofolate Reductase (MTHFR) Gene C677T, A1298C Polymorphism and HR-HPV Infection in Women of Childbearing Age. *Modern Journal of Clinical Laboratory Science*, **36**, 53-56. https://doi.org/10.3969/j.issn.1671-7414.2021.02.013
12. Liu, C., Zhang, L., Xu, J., *et al.* (2020) Analysis of Methylenetetrahydrofolate Reductase (MTHFR) C677T Gene Polymorphism in Jiangnan Region. *Journal of Experimental and Clinical Medicine*, **38**, 36-38. https://doi.org/10.3969/j.issn.1674-1129.2020.01.010
13. Qiu, A., He, J., Dai, Z., *et al.* (2023) Analysis of Methylenetetrahydrofolate Reductase Gene C677T Polymorphism and Its Association with Recurrent Pregnancy Loss in Women of Childbearing Age. *Chinese Journal of Obstetrics and Gynecology*, **58**, 165-170. https://doi.org/10.3760/cma.j.issn.0529-567X.2023.03.007
14. Liu, X., Wang, C., Zhang, H., *et al.* (2023) Study on the Relationship between MTHFR Gene Polymorphism and Gestational Hypertension in Women of Childbearing Age. *Chinese Journal of Hypertension*, **31**, 121-125. https://doi.org/10.16171/j.chinahypertension.2023.02.009
15. Zhang, X., Yang, L., Liu, X., *et al.* (2022) Correlation between Methylenetetrahydrofolate Reductase Gene C677T Polymorphism and Neural Tube Defects: A Meta-Analysis. *Chinese Journal of Preventive Medicine*, **56**, 598-605.
16. Wang, Y., Hu, X., Xu, J., *et al.* (2022) Research on the Association between MTHFR C677T Polymorphism and Pregnancy Complications: A Systematic Review and Meta-Analysis. *Chinese Journal of Obstetrics and Gynecology*, **57**, 301-309. https://doi.org/10.3760/cma.j.issn.0529-567X.2022.05.003
17. Li, X., Zhang, M., Zhao, X., *et al.* (2023) Study on the Association of MTHFR Gene Polymorphisms with Preeclampsia in Chinese Women. *Journal of Obstetrics and Gynaecology Research*, **49**, 1234-1240.
18. Chen, X., Yang, Y., Liu, J., *et al.* (2023) The Role of Folate Metabolism Genes in Pregnancy Outcomes: A Review. *Journal of Reproductive Medicine*, **68**, 22-28. https://doi.org/10.3760/cma.j.cn112150-20230112-00001