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Prevalence of the methylenetetrahydrofolate reductase 677C>T polymorphism in the pregnant women of Yunnan Province, China

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Abstract

Mutations in the methylenetetrahydrofolate reductase (*MTHFR*) gene can result in a reduced ability to utilize folic acid. The *MTHFR 677C>T* polymorphism in particular has been linked to both birth defects and pregnancy-associated diseases. This study aimed to evaluate the prevalence of the *MTHFR 677C>T* mutation among pregnant women in Yunnan Province so as to collect baseline data that may be utilized to guide folic acid supplementation efforts and to support related disease prevention programs. We retrospectively reviewed 3387 pregnant women from Yunnan Province. The *MTHFR 677C>T* polymorphism was identified using polymerase chain reaction (PCR) and DNA sequencing. In total, 1350 (39.9%) subjects were homozygous for the C allele (CC), 1540 (45.4%) subjects were heterozygous (CT), and 497 (14.7%) subjects were homozygous for the T allele (TT). The *MTHFR 677C>T* polymorphism was found to be present within the studied population, with ~60% of these patients being either heterozygous or homozygous for the mutant allele and with an overall T allele frequency of 0.37. The frequency of the T allele was significantly higher among pregnant women with complications relative to women with healthy pregnancies, particularly among women <30 years old. As such, the maternal *MTHFR 677C>T* polymorphism may be a genetic risk factor associated with pregnancy complications and may help identify pregnant women at a high risk of such complications.

Abbreviations: Hcy = homocysteine, MTHFR = methylenetetrahydrofolate reductase, PCR = polymerase chain reaction.

Keywords: birth defects, folate, methylene tetrahydrofolate reductase, polymorphism, pregnancy

1. Introduction

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme responsible for catalyzing 5,10-methylenetetrahydrofolate unidirectional conversion into 5-methyltetrahydrofolate, which in turn functions as a methyl donor during the process of homocysteine to methionine conversion, making this enzyme essential in the context of folate metabolism.[1] The *MTHFR* gene is encoded on chromosome 1p36.3, and has been the focus of extensive study. A study by Frosst et al[2] in 1995 first demonstrated that the 677 C→T mutation in this gene, which leads to an alanine-to-valine substitution, can facilitate the synthesis of a thermolabile *MTHFR* isoform with reduced activity that can result in decreased folate utilization and abnormal folate metabolism. The TT mutation causes the gene to encode for a thermolabile enzyme with a 70% reduction in its activity. Indeed, this *MTHFR 677C>T* polymorphism has been linked with reductions in folate levels in serum, plasma, and red blood cells.[3] Individuals with one or more copies of the T allele may be more predisposed to complex diseases either personally or in their offspring.

This 677C>T mutation has been linked with multiple pathological conditions, including negative gestational events such as spontaneous abortion, fetal death, and neural tube defects.[4–6] Normal serum folate levels have been found to range from 8.07 to 45.3 nmol/L at the Kunming Maternal and Child Health Hospital, with average serum folic acid levels of progestational and pregnant women in Kunming area being 26.86 nmol/L, though 18.01% of these women had serum folic acid levels below the lower limit of the normal reference

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Table 1

Distribution of the MTHFR C677T genotype and allele frequencies among pregnant women in Yunnan.

Single nucleotide polymorphism	Total sample size	Observed genotypic	Count (n%)	Allele frequency		P*
				C	T	
MTHFR C677T	3387	CC	1350 (39.9)	0.63	0.37	.96
		CT	1540 (45.4)			
		TT	497 (14.7)			

* Significance threshold: $P < .05$.

range.[7] It is likely that women of reproductive age may have low dietary folate intake if they are not specifically supplementing their diet with external sources such as fortified cereals.[8] Folate deficiencies can also arise, however, due to specific genetic defects. Few studies to date, however, have specifically examined the relevance of the *MTHFR 677C>T* polymorphism among women in Yunnan Province. Folic acid supplementation is very important for the healthy growth and development of newborns and for the physical health of pregnant women. Therefore, it is important to investigate the population genetic characteristics of folate metabolism-related genes among pregnant women.

The present study was designed with the goal of screening for the *MTHFR 677C>T* mutation among pregnant women in Yunnan Province, China, and to investigate whether this polymorphism is associated with the incidence of pregnancy complications in order to gain clinically relevant insights that can inform effective and scientific counseling strategies for expectant mothers.

2. Materials and methods

2.1. Subjects

The Ethics Committee of Yan'an Hospital of Kunming, Yunnan Province approved the present study. In total, 3387 pregnant women between the ages of 16 and 45 were enrolled in this study from October 2016 to December 2018. All participants were unrelated, non-smokers, non-drinkers, and free of chronic diseases. All women were from Yunnan Province (average elevation: 2000 m).

2.2. Genotyping

Samples of peripheral blood were obtained from all participants, and genomic DNA was isolated from these samples with a TIANGEN Blood DNA Kit (TIANGEN Inc., Beijing, China) based on provided instructions. Sanger sequencing and polymerase chain reaction (PCR) were then used to identify *MTHFR C677T* (rs1801133) variants with the following primers: F-5′-AGTCCCTGTGGTCTCTTCATGC-3′ and R-5′-TAATGAGAATTAGAATCCCTTTTGGAG-3′, with Oligo7.37[9] having been used to design these primers. The following thermocycler settings were used: 95 °C for 3 minutes; 50 cycles of 95 °C for 30 seconds, 60 °C for 45 seconds. After PCR amplification, Sanger sequencing was conducted as a means of determining participant genotype.

2.3. Statistical analysis

MTHFR 677C>T genotype and allele frequencies in women of different health statuses during pregnancy were assessed. Unhealthy pregnancies included those complicated by diabetes, hyperthyreosis, hypothyroidism, hypertension, or thrombocytopenia. SPSS v22.0 was used for all statistical testing in this study. The Hardy–Weinberg equilibrium was assessed via chi-squared test, which was also used to compare *MTHFR 677C>T* polymorphism genotype and allelic frequencies. $P < .05$ was the significance threshold.

3. Results

3.1. MTHFR genotype and allele frequency distributions

The *MTHFR 677C>T* polymorphism was found to be polymorphic in this study population, with C and T allele frequencies of 0.63 and 0.37, respectively (Table 1). The *MTHFR* genotype distributions were consistent with Hardy–Weinberg equilibrium.

3.2. MTHFR genotype and allele frequency distributions in women of differing health status during pregnancy

MTHFR 677C>T genotype and allele frequencies in women of different health status during pregnancy were additionally assessed (Table 2). These analyses revealed that the frequency of the T allele was 46.5% among women that experienced unhealthy pregnancies, whereas it was significantly lower at 36.7% among women that experienced healthy pregnancies ($P = .00$).

Table 2

Distribution of *MTHFR 677C>T* genotype and allele frequencies as a function of health status during pregnancy.

Health status during pregnancy	Genotypes								P*
	CC		CT		TT		Allele frequency		
	n	%	n	%	n	%	C	T	
Unhealthy pregnancy n = 244	68	27.8	125	51.2	51	21.0	53.5	46.5	.000
Healthy pregnancy n = 3143	1282	40.8	1415	45.0	446	14.2	63.3	36.7	

* Significance threshold: $P < .05$.

Table 3

Distribution of *MTHFR 677C>T* genotype and allele frequencies as a function of age and health status during pregnancy among different age groups from Yunnan.

		Genotypes						Allele frequency		
		CC		CT		TT				
Age at pregnancy	Health status during pregnancy	n	%	n	%	n	%	C	T	P^*
<30 yr n=1879	Unhealthy pregnancy	29	27.1	48	44.9	30	28.0	49.5	50.5	.000
	Healthy pregnancy	718	40.5	800	45.2	254	14.3	63.1	36.9	
30–40 yr n=1402	Unhealthy pregnancy	31	26.7	66	56.9	19	16.4	55.2	44.8	.015
	Healthy pregnancy	523	40.7	581	45.2	182	14.2	63.3	36.7	
>40 yr n=106	Unhealthy pregnancy	8	38.1	11	52.3	2	9.5	64.3	35.7	.625
	Healthy pregnancy	41	48.2	34	40.0	10	11.8	68.2	31.8	

* Significance threshold: $P < .05$.

3.3. Age-related differences in MTHFR genotype and allele frequency in women of differing health status during pregnancy

We also assessed *MTHFR 677C>T* genotype and allele frequencies in women of different health status during pregnancy as a function of maternal age (Table 3). This analysis revealed that the frequency of the T allele was higher among women that experienced unhealthy pregnancies relative to women that experienced healthy pregnancies in all age groups (<30 years; 30–40 years; >40 years). The T allele frequency was the highest among women with unhealthy and healthy pregnancies in the <30 years group (50.5%, 36.9%), while among women >40 years old it was 35.7% in those with unhealthy pregnancies and 31.8% in those with healthy pregnancies. We detected significant differences in *MTHFR 677C>T* allele frequencies as a function of health status in women 30 to 40 years and <30 years old ($P < .05$), whereas these differences were not significant in women >40 years old ($P > .05$), possibly due to the limited sample size for this age group.

4. Discussion

Birth defects are a serious public health and social issue in China, and the incidence of such defects is increasing annually according to China's Ministry of Health.[10] Yunnan province has a high rate of overall birth defects, and ranks among the top 5 provinces with respect to the incidence of congenital heart defects. Population monitoring results from 2010 to 2015 in Yunnan province indicated that the incidence of birth defects in this region was higher than the national level in 2010, and that these rates are rising annually.[11] Although the mechanistic basis for these birth defects is unclear, genetic factors, nutritional factors, and environmental factors all play a role. The diets of pregnant women have continued to improve with rising living standards, and as such the etiology of these defects suggests that genetic factors may be one possible cause.

Folate is an essential compound during fetal development, as it functions in key processes including cell division and the transfer of single-carbon units.[12,13] Folate also regulates the overall growth of the fetus and the placenta, as well as the synthesis of neurotransmitters.[14] Research indicates that adequate supplementation of folic acid can prevent birth defects.[15] Moreover, individualized folic acid supplementation has been widely adopted as one of the primary approaches to preventing birth defects. Currently, there are many guidelines that suggest that folic acid should be taken by pregnant individuals.[16,17]

The enzyme MTHFR plays a key role in the folate metabolism pathway and regulates the intracellular folate pool in order to influence DNA synthesis and methylation.[18,19] Mutation of wild-type *MTHFR* at position 677 from C to T impacts the activity of the MTHFR enzyme.[20] Many studies have shown that the *MTHFR 677C>T* mutation is a risk factor associated with many adverse fetal developmental outcomes including spina bifida, congenital heart defects, and malformation of the nervous system.[4,5,21] Moreover, the *MTHFR 677C>T* polymorphism is a commonly known heritable risk factor for elevated blood Hcy levels.[22] However, the T allele frequency of *MTHFR 677C>T* is highly variable throughout the world, with frequencies as high as 64.3% in Europeans and low frequencies of 4.9% to 9.1% among African populations.[23,24] In this study, we conducted the genotyping of 3378 perinatal women as a means of detecting the frequency of the *MTHFR* 677C>T polymorphism in this population (Table 1). This analysis revealed that the T allele had a 37% frequency in our study population.

Recent studies suggest that the *MTHFR* T allele may contribute to the risk of neural-tube defects (NTD), congenital heart defects (CHD), and pregnancy complications.[25–28] Both heterozygous and homozygous *677C>T* genotypes have been found to be associated with elevated plasma Hcy levels.[29,30] Hcy is a biomarker that can be used to identify women at risk of complications and adverse pregnancy outcomes. Elevated Hcy levels in maternal plasma and amniotic fluid may be one cause of NTD.[3,31] Mayor-Olea et al[32] found that the T allele and the TT genotype became more frequent during the last quarter of the 20th century. In the present study, we found that the T allele frequency was lower in the older population cohort (>40 years) compared with younger cohorts (<30 years; 30 to 40 years) regardless of pregnancy health status. In addition, these relatively high mutant allele frequencies may be further complicated by the increasingly sedentary lifestyle of many individuals, unhealthy eating habits, or environmental changes, as such factors can pose a significant health risk.[33] This may also increase the risk of the development of complex diseases that may manifest later in life, at which time the allele has already been passed on to any offspring. In the current study, about 7.2% of women experienced unhealthy pregnancies complicated by conditions such as diabetes, hyperthyreosis, hypothyroidism, hypertension, or thrombocytopenia. We also found that the T allele frequency was higher among women that experienced unhealthy pregnancies relative to women that experienced healthy pregnancies.

These data thus revealed that the *MTHFR 677C>T* polymorphism is associated with health status during pregnancy. Therefore, *MTHFR 677C>T* genotyping before pregnancy and genotype-specific interventions may be an effective means of safeguarding the health of pregnant women.

In summary, folate utilization is important during fetal growth and development, such that folate deficiencies or abnormalities can result in many gestational complications in the fetus and/or the placenta. The *MTHFR 677C>T* polymorphism is closely associated with folate metabolism. Abnormal folate metabolism can lead to an increased risk of birth defects. The data produced in the present study indicate that the *MTHFR 677T* mutation may be one potential cause of health issues among pregnant women and fetuses in Yunnan province. In addition, these *MTHFR 677T* mutations are closely related to the high incidence of birth defects in Yunnan province. It is therefore important that the *MTHFR 677C>T* genotype be characterized in women prior to or during pregnancy as a means of guiding folate supplementation schemes in a scientifically sound manner in order to prevent birth defects or pregnancy-associated diseases. In addition, this information can be used by health planners to better develop programming aimed at further improving current primary prevention measures for birth defects.

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