

## Original Article

# **MTHFR genetic polymorphism increases the risk of preterm delivery**

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**Abstract:** Aims: This study aimed to investigate the association between the methylene tetrahydrofolate reductase (*MTHFR*) gene C677T and A1298C polymorphisms and premature delivery susceptibility. Methods: With matched age and gender, 108 premature delivery pregnant women as cases and 108 healthy pregnant women as controls were recruited in this case-control study. The cases and controls had same gestational weeks. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was adopted to analyze C677T and A1298C polymorphisms of the participants. Linkage disequilibrium (LD) and haplotype analysis were conducted by Haploview software. The differences for frequencies of gene type, allele and haplotypes in cases and controls were tested by chi-square test. The relevant risk of premature delivery was represented by odds ratios (ORs) with 95% confidence intervals (95% CIs). Results: TT gene type frequency of C677T polymorphism was higher in cases than the controls ( $P=0.004$ ,  $OR=3.077$ ,  $95\% CI=1.469-6.447$ ), so was allele T ( $P=0.002$ ,  $OR=1.853$ ,  $95\% CI=1.265-2.716$ ). Whereas, CC gene type of A1298C polymorphism had a lower distribution in cases than the controls ( $P=0.008$ ,  $OR=0.095$ ,  $95\% CI=0.012-0.775$ ), so was allele C ( $P=0.047$ ,  $OR=0.610$ ,  $95\% CI=0.384-0.970$ ). Haplotype analysis and linkage disequilibrium test conducted on the alleles of two polymorphisms in *MTHFR* gene, we discovered that haplotype T-A had a higher distribution in cases, which indicated that susceptible haplotype T-A was the candidate factor for premature delivery. Conclusions: Gene type TT of *MTHFR* C677T polymorphism might make premature delivery risk rise while gene type CC of A1298C polymorphism might have protective influence on premature delivery.

**Keywords:** *MTHFR*, polymorphism, premature delivery

## Introduction

Premature delivery, a common clinical disease in gynecology and obstetrics, refers to that the pregnancy is more than 28 weeks but less than 37 weeks [1]. The newborn whose weight ranges from 1000 g to 2499 g is called premature infant [2]. According to the materials, premature infants account for 5%-15% of the newborn while the proportion in developing countries is 25% [3]. The occurrence and death of perinatal infants are often resulted from the immature organ developments and incomplete immune functions, the ratio of which is 75% [4]. In recent years, with the betterment of health-care technology in perinatal period and the improvement of intensive care technology (mechanical ventilation and nutritional therapy) for the premature infants, the survival rate of premature infants has apparently risen [5]. However, the incidence rates of nerve system sequelae, such

as cerebral palsy and obstacles of intelligent, action, seeing and hearing have a rising trend. For a long time, not only does the premature delivery bring about severe physical and spiritual hurt and heavy financial burden for the pregnant and lying-in women, but also it is the main urgent matter for the clinical workers to resolve. Therefore, the prevention and treatment of premature delivery have been the research hotspots.

It was considered that factors, such as abnormalities of chromosome and immune, embryo, matrix and environment were the main causes for premature delivery. Previous researches, which resulted in those studies mainly concentrated on uterine malformation, reproductive tract infection and pregnancy complications. In present studies, the disorder of system of coagulation or fibrinolysis plays an important part in premature delivery [6]. Surveys of plentiful

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**Table 1.** Primers sequences

Gene	Locus	Primer
MTHFR	C677T	forward 5'-TGAAGGAGAAGGTGTCTGCGGGA-3'
		reverse 5'-AGGACGGTGCGGTGAGAGTG-3'
	A1298C	forward 5'-CTTTGGGAGCTGAAGGACTACTAC-3'
		reverse 5'-CACTTTGAGACCATTCCGGTTG-3'

groups have shown that homocysteine (Hcy) level rise or hyperhomocysteinemia damaging system of coagulation or fibrinolysis and influencing uteroplacental circulation selectively, is closely related to the occurrence of premature delivery [7]. Hcy concentration is low if pregnancy is normal while excessive level of Hcy will lead to risk of delivering infant with small gestational age [8]. *MTHFR* is the most common enzyme for influencing Hcy metabolism. At present, we have known that *MTHFR* gene is located in the chromosome 1p36.3 with 11 exons and cDNA is 2220 in length [9]. Studies demonstrated that [10], 5, 10 methylene tetrahydrofolic acid can be restored back to 5-methyltetrahydrofolic acid by *MTHFR*, and then 5-methyltetrahydrofolic acid, as methyl donor, supplies methyl for Hcy metabolism. There are many gene mutations in *MTHFR* and the mutations can lower enzyme activity, which makes Hcy concentration rise [11]. It is beneficial to eugenics to detect the association of *MTHFR* polymorphisms and premature delivery and it will provide new theoretical guidance for premature delivery prevention. In this study, we chose to investigate the association between *MTHFR* C677T and A1298C polymorphisms and premature delivery.

### Materials and methods

#### Research subjects

In this case-control study, 108 premature delivery pregnant women as cases and 108 healthy pregnant women having same pregnancy weeks were enrolled as controls. All subjects were selected in Affiliated Hospital of Yan'an University. The gestational weeks of cases were 18-34 weeks while the controls were 31-34 weeks. The singleton pregnant women in the two groups were primiparas with no hobby of smoking and drinking. Moreover, they did not take drugs like folic acid for more than 4 months. Liver and kidney functions were nor-

mal. Besides, on the cases and controls had no diseases, such as diabetes, hypertension, coronary heart disease, hepatitis, acute and chronic nephritis, placental abruption, placenta previa. This study was granted by the Ethics Committee. Sampling collection process was conducted with the principles of the human genome research ethics. Each participant signed the consent form.

#### DNA extraction

5 ml peripheral venous blood which was processed with EDTA was extracted from every participant, and then the blood was preserved in fridge at -80°C. Phenol- chloroform method was adopted to isolate DNA, and then it was saved at -20°C for spare.

#### Polymerase chain reaction (PCR) amplification

PCR primers were designed by Primer5.0 software and were synthesized by Shanghai Gene Core Bio Tech Co., Ltd. Primers sequences were shown in **Table 1**. Method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was applied to analyze *MTHFR* polymorphisms. The total volume of PCR reaction system was 15 µl, including 1 µl template DNA, 1 µl forward primer, 1 µl reverse primer, 2 µl Taq DNA polymerase, 1 µl 10× buffer solution, 1 µl 4× dNTPs and 8 µl sterile water. PCR reaction was initially performed with 10 min degeneration at 94°C, then followed by 35 circles of 25 s degeneration at 92°C, 1 min annealing at 60°C and 50 s extension at 70°C, finally 5 min extension at 72°C. PCR products were conducted with MspI enzymic digestion, then agarose gel electrophoresis was utilized to determine the gene type of every genetic variation.

#### Statistical analysis

PASW Statistics 18 software was used for carry out  $\chi^2$  test. Differences of gene types and alleles frequencies of every polymorphism in the two groups were compared ( $P < 0.05$  means it has statistical significance). Linkage disequilibrium (LD) and halotype analysis were conducted by Haploview software. Hardy-Weinberg equilibrium (HWE) examination for the controls was tested by PLINK 1.07 software ( $P > 0.05$  means it has statistical significance). Odds

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**Table 2.** Gene types and alleles frequencies of C677T and A1298C polymorphisms

Locus	Gene type/Allele	Cases n=108 (%)	Controls n=108 (%)	$\chi^2$	P value	OR (95% CI)
C677T	CC	26 (24.1)	40 (37.0)	-	-	1
	CT	44 (40.7)	49 (45.4)	0.982	0.336	1.381 (0.729-2.620)
	TT	38 (35.2)	19 (17.6)	9.115	0.004	3.077 (1.469-6.447)
	C	96 (44.4)	129 (59.7)	-	-	1
	T	120 (55.6)	87 (40.3)	10.101	0.002	1.853 (1.265-2.716)
A1298C	AA	71 (65.7)	61 (56.5)	-	-	1
	AC	36 (33.4)	38 (35.2)	0.502	0.561	0.814 (0.460-1.439)
	CC	1 (0.9)	9 (8.3)	7.131	0.008	0.095 (0.012-0.775)
	A	178 (82.4)	160 (74.1)	-	-	1
	C	38 (17.6)	56 (25.9)	4.405	0.047	0.610 (0.384-0.970)

**Table 3.** Linkage disequilibrium and haplotype analysis for alleles of C677T and A1298C polymorphisms

Haplotype	Cases	Controls	$\chi^2$	P	OR (95% CI)
locus1-locus2	2n=216	2n=216		value	
C-A	81	110	-	-	1
C-C	15	19	0.034	0.853	1.072 (0.514-2.237)
T-A	97	50	18.524	0.000	2.635 (1.687-4.115)
T-C	23	37	0.312	0.653	0.844 (0.466-1.529)

ratios (ORs) with 95% confidence intervals (95% CIs) were brought out to represent the relevant risk of premature delivery.

### Results

#### Basic features of subjects

Distributions of *MTHFR* C677T and A1298C polymorphisms in case and control groups were corresponding with HWE. The result of HWE test conducted on the controls showed that the goodness of fit of HWE in every polymorphism was well ( $P>0.05$ ), which indicated that the controls were in balanced state and representative.

#### Association analysis between genotypes and alleles of *MTHFR* gene and premature delivery

Gene types frequencies of *MTHFR* C677T and A1298C polymorphisms were shown in **Table 2**. As for TT gene type frequency of *MTHFR* C677T polymorphism, the cases was higher than the controls and the difference had statistical significance ( $P=0.004$ ). The result illustrated that TT genotype was the susceptible genotype for premature delivery occurrence (OR=3.007, 95% CI=1.469-6.447). T allele had a higher dis-

tribution in case group than in control group and it was closely related with premature delivery ( $P=0.002$ , OR=1.853, 95% CI=1.265-2.716). However, CC gene type of A1298C polymorphism had a lower distribution in case group ( $P=0.008$ , OR=0.095, 95% CI=0.012-0.775), so was rare allele C

( $P=0.047$ , OR=0.610, 95% CI=0.384-0.970) and they took a protective role for premature delivery.

#### Analysis of haplotype in *MTHFR* gene

Haplo View software was used for conducted LD and haplotype analysis on alleles of *MTHFR* C677T and A1298C polymorphisms (**Table 3**). The results demonstrated that 4 haplotypes were consisted in the two SNPs of *MTHFR* gene. The association between the 4 haplotypes and premature delivery was investigated with PASW 18.0 software. We found that there was an apparent difference for T-A haplotype in the two groups ( $P<0.05$ ), which suggested that T-A haplotype made premature delivery risk rise (OR=2.635, 95% CI=1.687-4.115). However, other haplotypes were not closely related to premature delivery ( $P>0.05$ ).

### Discussion

Premature delivery, a common complication in obstetrical department, is divided into 3 types: preterm premature rupture of membranes, spontaneous premature delivery and iatrogenic premature delivery [12]. Factors for premature delivery have not been fully figured out. It is uni-

versally considered that premature delivery is the outcome of multiple factors [13]. Premature delivery is not only associated with situations of lying-in women themselves (cervical incompetence, smoking, reproductive tract infection [14, 15]) but also connected with multiple pregnancy [16, 17], increased weight during pregnancy and etc [18]. At present, the treatment effects for premature delivery are not satisfying. Hence, it is meaningful to detect the controllable factors for influencing premature delivery [19, 20].

Researches in recent years have found that the high Hcy level is closely related to premature delivery [21, 22]. Hcy is a kind of sulfur-containing amino acid and its metabolism is affected by three kinds of key enzymes (MS, CS and *MTHFR*), folic acid and multiple vitamins. *MTHFR* gene is the key enzyme in the circulation of folic acid and Hcy metabolism. *MTHFR* gene mutations can change enzyme activity and folic acid level, which will affect Hcy metabolism [23]. Numerous researches focused on the *MTHFR* C677T polymorphism [24-27]. Stonek et al [28] have discovered that the pregnancy complications of women who carry mutant T of *MTHFR* C677T polymorphism might increase. Frosst et al [29] have found that the base replacement of C→T in *MTHFR* 677 polymorphism can make alanine be replaced by valine, which will increase the thermal instability of enzyme, while lower the thermal stability and activity of enzymes, finally, the Hcy concentration level is risen and the folic acid level is declined. A1298C, another common *MTHFR* mutation, turning the glutamic acid into alanine can influence normal Hcy metabolism, as a result, Hcy level is raised in plasma. Previous researches, shown that A1298C was related to many diseases [30, 31].

In this case-control study, association between two polymorphisms in *MTHFR* gene and premature delivery was detected. HWE results have shown that the controls were representative ( $P>0.05$ ). After calculation, we found that TT gene type frequency of *MTHFR* C677T polymorphism was higher in cases than controls, so was allele T. The results illustrated that they were closely related to premature delivery. CC genotype of A1298 polymorphism had a lower distribution in cases than in controls, the same to allele C. The results suggested that CC genotype and C allele had a protective part for pre-

mature delivery. TT gene type of C677T polymorphism was a candidate factor for premature delivery occurrence ( $P=0.004$ ,  $OR=3.077$ ,  $95\% CI=1.469-6.447$ ). T allele frequency of C677T polymorphism, was obviously different between case and control groups, might made premature delivery risk rise 1.853 times compared with C allele. CC gene type and C allele of A1298C polymorphism had a lower distribution in cases, taking a protective part for premature delivery, decreasing about 0.095 and 0.047 fold risk of premature delivery, respectively.

In this study, haplotype analysis and LD test were conducted on the alleles of *MTHFR* C677T and A1298C polymorphisms. Haplotype T-A was more often discovered in case group ( $P=0.000$ ), which suggested that T-A haplotype was the candidate factor for premature delivery incidence, with the OR was 2.635. Combined with the above results, alleles of T and A were contained in susceptible haplotype T-A and it further indicated that susceptible alleles of T and A in C677T and A1298C polymorphisms might participate in the occurrence of premature delivery.

We can confirm that *MTHFR* polymorphisms are associated with premature delivery. But the molecular mechanism of premature delivery still not definite, that needs an in-depth study. In conclusion, premature delivery is caused by multiple factors. It is necessary to take measures aiming at risk factors to lower the incidence rate of premature delivery.

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### Disclosure of conflict of interest

None.

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