Original Article

MTHFR genetic polymorphism increases the risk of preterm delivery

Yanrong Nan, Hongmei Li

Department of Obstetrics, Affiliated Hospital of Yan’an University, Yan’an, China

Received February 20, 2015; Accepted April 13, 2015; Epub June 1, 2015; Published June 15, 2015

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
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 normal. 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 χ² 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). 

Table 1. Primers sequences
<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR</td>
<td>C677T</td>
<td>forward 5'-TGAAGGAGAAGGTGCTGCGGGA-3' reverse 5'-AGGACCGTGCGGTAGAGTG-3'</td>
</tr>
<tr>
<td></td>
<td>A1298C</td>
<td>forward 5'-CTTTGGGGAGCTGAAGGACTAC-3' reverse 5'-CAGCTTGAGACCATTCGTTT-3'</td>
</tr>
</tbody>
</table>
MTHFR genetic variation in the risk of preterm 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 susceptive genotype for premature delivery occurrence (OR=3.007, 95% CI=1.469-6.447). T allele had a higher distribution 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 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-

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

<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene type/Allele</th>
<th>Cases n=108 (%)</th>
<th>Controls n=108 (%)</th>
<th>χ²</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C677T</td>
<td>CC</td>
<td>26 (24.1)</td>
<td>40 (37.0)</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>44 (40.7)</td>
<td>49 (45.4)</td>
<td>0.982</td>
<td>0.336</td>
<td>1.381 (0.729-2.620)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>38 (35.2)</td>
<td>19 (17.6)</td>
<td>9.115</td>
<td>0.004</td>
<td>3.077 (1.469-6.447)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>96 (44.4)</td>
<td>129 (59.7)</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>120 (55.6)</td>
<td>87 (40.3)</td>
<td>10.101</td>
<td>0.002</td>
<td>1.853 (1.265-2.716)</td>
</tr>
<tr>
<td>A1298C</td>
<td>AA</td>
<td>71 (65.7)</td>
<td>61 (56.5)</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>36 (33.4)</td>
<td>38 (35.2)</td>
<td>0.502</td>
<td>0.561</td>
<td>0.814 (0.460-1.439)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1 (0.9)</td>
<td>9 (8.3)</td>
<td>7.131</td>
<td>0.008</td>
<td>0.095 (0.012-0.775)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>178 (82.4)</td>
<td>160 (74.1)</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>38 (17.6)</td>
<td>56 (25.9)</td>
<td>4.405</td>
<td>0.047</td>
<td>0.610 (0.384-0.970)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Cases locus1-locus2</th>
<th>Controls locus1-locus2</th>
<th>χ²</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-A</td>
<td>81/110</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>C-C</td>
<td>15/19</td>
<td>0.034/0.853</td>
<td>1.072 (0.514-2.237)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-A</td>
<td>97/50</td>
<td>18.524/0.000</td>
<td>2.635 (1.687-4.115)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-C</td>
<td>23/37</td>
<td>0.312/0.653</td>
<td>0.844 (0.466-1.529)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
MTHFR genetic variation in the risk of preterm delivery

Versus 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 677T 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 premature 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 make 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.

Acknowledgements

This study was supported by a grant for the Key project of high-level university construction, Yan’an University.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hongmei Li, First ward, Department of Obstetrics, Affiliated Hospital of Yan’an University, Yan’an, China. E-mail: liheng-moi@sina.com

References

[1] Engelstad HJ, Roghair RD, Calarge CA, Colaizy TT, Stuart S and Haskell SE. Perinatal outcomes of pregnancies complicated by mater-


MTHFR genetic variation in the risk of preterm delivery


