Correlation between methylenetetrahydrofolate reductase gene C677T polymorphism and preeclampsia in pregnant women
Zu-Qiong Zhang, Shu-Hong HU, Chun-Hua Zhu, Chun-Mei Yang
Department of Obstetrics and Gynecology, Jianshi People’s Hospital in Hubei Province, Enshi, Hubei Province, 445300

ARTICLE INFO
Article history:
Received 2 Nov 2017
Received in revised form 9 Nov 2017
Accepted 12 Nov 2017
Available online 28 Nov 2017

Keywords:
Preeclampsia
Methylenetetrahydrofolate reductase
Homocysteine
Apoptosis
Invasion

ABSTRACT
Objective: To study the correlation between methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism and preeclampsia in pregnant women. Methods: Pregnant women who were diagnosed with preeclampsia in Jianshi People’s Hospital between July 2014 and March 2017 were selected as the PE group of the research, and healthy pregnant women who received antenatal care and gave birth in Jianshi People’s Hospital during the same period were selected as the control group of the research. The MTHFR gene C677T polymorphism in peripheral blood, the contents of homocysteine (Hcy) metabolism indexes and the expression of apoptosis genes and invasion genes were determined. Results: The proportion of MTHFR gene C677T locus TT genotype in peripheral blood of PE group was significantly higher than that of control group while the proportion of CT and CC genotypes were significantly lower than those of control group; Hcy levels in serum and placenta as well as FasL, Caspase-8, Bax, Caspase-9 and Caspase-3 mRNA expression in placenta of PE women with TT genotype were significantly higher than those of PE women with CT genotype and CC genotype while folic acid levels in serum and placenta as well as Notch-1, N-cadherin, Vimentin, CatL and CatB mRNA expression in placenta were significantly lower than those of PE women with CT genotype and CC genotype. Conclusion: MTHFR gene C677T locus mutation can participate in the occurrence of preeclampsia by affecting the Hcy metabolism as well as the expression of apoptosis genes and invasion genes.

1. Introduction
Preeclampsia (PE) is a common complication during pregnancy, abnormal metabolism of homocysteine (Hcy) is an important characteristic of PE patients, and abnormal accumulation of Hcy can affect the sertoli cell proliferation and invasion in the placenta[1,2]. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme that catalyzes the reduction from 5, 10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate, which is involved in metabolism of Hcy and folate in the body. When the expression or activity of MTHFR changes, the metabolism of Hcy and folate can be affected, resulting in the accumulation of Hcy and causing the excessive apoptosis and invasion suppression of cells. In recent years, the study about MTHFR shows that the fourth exon of the gene has significant C677T polymorphism, which is prone to C base mutation to T base and affects the catalytic activity of the products encoded by the gene[3]. The relationship between the MTHFR gene C677T polymorphism and pre-eclampsia has received more and more attention, but it is still unclear about the biological link affected by the gene polymorphism in the process of preeclampsia. In the following studies, we specifically analyzed the correlation of MTHFR gene C677T polymorphism with Hcy metabolism as well as cell apoptosis and invasion in the course of pre-eclampsia.

2. Clinical information and research methods
2.1 General case information
Pregnant women who were diagnosed with preeclampsia in Jianshi People’s Hospital between July 2014 and March 2017 were selected.
as the PE group of the research, all patients conformed to the diagnostic criteria for preeclampsia and the pregnant combined with hypertension before pregnancy, and those combined with gestational diabetes, intrahepatic cholestasis and reproductive system infection during pregnancy were excluded. Healthy pregnant women who received antenatal care and gave birth in Jianshi People’s Hospital during the same period were selected as the control group of the research. PE group included 45 cases, they were 25-36 years old, 28 cases were primiparae, 17 cases were multiparae, and the gravidity was (1.87±0.22); control group included 78 cases, they were 23-35 years old, 46 cases were primiparae, 32 cases were multiparae, and the gravidity was (1.93±0.27). There was no significant difference in general information between the two groups of pregnant women (P>0.05).

2.2 Research methods

2.2.1 Gene polymorphism research methods

The peripheral blood samples were collected from two groups of pregnant women, the genomic DNA extraction kit was used to isolate the whole blood genomic DNA samples, MTHFR gene C677T locus primers were designed, PCR reaction was performed, PCR products received enzyme digestion reaction, enzyme digestion reaction products were collected for agarose gel electrophoresis, and the MTHFR gene polymorphism was judged according to the electrophoresis results, specifically as follows: wild-type CC genotype showed 134 bp band, heterozygote CT genotype showed 59 bp, 75 bp and 134 bp bands, and mutant genotype TT showed 59 bp and 75 bp bands.

2.2.2 Hcy metabolism index detection

The serum samples were collected from two groups of pregnant women, and the contents of Hcy and folic acid were determined by electrochemiluminescence kit; the placenta samples were collected from two groups of pregnant women after delivery, added in RIPA lysate and homogenized to get the tissue protein suspension, and the contents of Hcy and folic acid were determined by electrochemiluminescence kit.

2.2.3 Gene expression detection

The placenta samples were collected from two groups of pregnant women after delivery and added in Trizol lysate to get tissue RNA suspension, the RNA was isolated and synthesized into cDNA by reverse transcription, fluorescence quantitative PCR kit was used to amplify cDNA, and the FasL, Caspase-8, Bax, Caspase-9, Caspase-3, Notch-1, N-cadherin, Vimentin, CatL and CatB mRNA expression were detected.

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>CC genotype</th>
<th>CT genotype</th>
<th>TT genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE group</td>
<td>45</td>
<td>14</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Control group</td>
<td>78</td>
<td>36</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

2.3 Statistical methods

SPSS 17.0 was used to input data. Count data analysis between two groups was by chi-square test, measurement data analysis among three groups was by variance analysis and P<0.05 indicated statistical significance in differences in test and analysis results.

3. Results

3.1 MTHFR genotypes in peripheral blood of two groups of pregnant women

Analysis of the proportion of MTHFR gene C677T locus genotypes in peripheral blood between the two groups of pregnant women was as follows: the proportion of MTHFR gene C677T locus TT genotype in peripheral blood of PE group was greatly higher than that of control group whereas the proportion of CT and CC genotypes were greatly lower than those of control group. Differences in MTHFR gene C677T locus TT genotype, CT genotype and CC genotype in peripheral blood were statistically significant between the two groups of pregnant women (P<0.05).

Table 2.

<table>
<thead>
<tr>
<th>MTHFR genotype</th>
<th>n</th>
<th>Serum</th>
<th>Placenta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hcy</td>
<td>Folic acid</td>
</tr>
<tr>
<td>CC genotype</td>
<td>14</td>
<td>8.68±0.94</td>
<td>9.52±1.05</td>
</tr>
<tr>
<td>CT genotype</td>
<td>10</td>
<td>8.94±0.89</td>
<td>9.84±1.14</td>
</tr>
<tr>
<td>TT genotype</td>
<td>21</td>
<td>11.31±1.58*</td>
<td>6.36±0.79*</td>
</tr>
</tbody>
</table>

*: compared with CC genotype, P<0.05; **: compared with CT genotype, P<0.05.

3.2 Relationship of Hcy metabolism indexes in serum and placenta of PE women with MTHFR gene polymorphism

Analysis of serum Hcy (μmol/L) and folic acid (μg/L) as well as Hcy (nmol/L) and folic acid (ng/L) among PE women with different MTHFR gene C677T locus genotypes was as follows: Hcy levels in serum and placenta of PE women with TT genotype were significantly higher than those of PE women with CT genotype and CC genotype whereas folic acid levels were significantly lower than those of PE women with CT genotype and CC genotype (P<0.05); Hcy and folic acid levels in serum and placenta of PE women with CT genotype were not significantly different from those of PE women with CC genotype (P>0.05).
Abnormal Hcy metabolism is an important characteristic in patients with preeclampsia, and MTHFR is the key enzyme that catalyzes Hcy metabolism in the body. In recent years, studies on the catalytic activity of MTHFR confirm that the No. 4 exon 677 locus of the gene is prone to the mutation from C to T, and the mutation can cause the encoded amino acid to change from alanine to valine, resulting in lower catalytic activity of encoded products. Therefore, the decrease of MTHFR catalytic activity and the disorder of folate, and lead to the increase of Hcy and the decrease of folic acid.

### 3.3 Relationship between apoptosis genes in placenta of PE women and MTHFR gene polymorphism

Analysis of apoptosis genes FasL, Caspase-8, Bax, Caspase-9 and Caspase-3 expression in placenta among PE women with different MTHFR gene C677T locus genotypes was as follows: FasL, Caspase-8, Bax, Caspase-9 and Caspase-3 mRNA expression in placenta of PE women with TT genotype were greatly higher than those of PE women with CT genotype and CC genotype (P<0.05); FasL, Caspase-8, Bax, Caspase-9 and Caspase-3 mRNA expression in placenta of PE women with CT genotype were not significantly different from those of PE women with CC genotype (P>0.05).

### 3.4 Relationship between invasion genes in placenta of PE women and MTHFR gene polymorphism

Analysis of invasion genes Notch-1, N-cadherin, Vimentin, CatL and CatB expression in placenta among PE women with different MTHFR gene C677T locus genotypes was as follows: Notch-1, N-cadherin, Vimentin, CatL and CatB mRNA expression in placenta of PE women with TT genotype were greatly lower than those of PE women with CT genotype and CC genotype (P<0.05); Notch-1, N-cadherin, Vimentin, CatL and CatB mRNA expression in placenta of PE women with CT genotype were not significantly different from those of PE women with CC genotype (P>0.05).

### 4. Discussion

Abnormal Hcy metabolism is an important characteristic in patients with preeclampsia, and MTHFR is the key enzyme that catalyzes Hcy metabolism in the body. In the research, the analysis of MTHFR gene C677T locus polymorphism in peripheral blood of pregnant women with PE showed that the proportion of MTHFR gene C677T locus TT genotype in peripheral blood of PE group was greatly higher than that of control group whereas the proportion of CT and CC genotypes were greatly lower than those of control group. This means that the mutation from MTHFR gene C677T loci allele C to T is associated with the occurrence of preeclampsia, and the occurrence of gene mutation may reduce the catalytic activity of MTHFR and affect the metabolism of Hcy and folic acid. In order to further confirm the effect of MTHFR gene C677T locus polymorphism on Hcy and folic acid metabolism in pregnant women with PE, the Hcy and folic acid contents in serum and placenta were compared among PE patients with different genotypes, and the results showed that Hcy levels in serum and placenta of PE women with TT genotype were significantly higher than those of PE women with CT genotype and CC genotype. This indicates that the mutation from MTHFR gene C677T locus allele C to T in the course of PE will reduce the catalytic activity of MTHFR, thus affect the metabolism of Hcy and folic acid, and lead to the increase of Hcy and the decrease of folic acid. The decrease of MTHFR catalytic activity and the disorder of Hcy metabolism in patients with preeclampsia can cause Hcy to accumulate in the placenta. Hcy promotes inflammation and oxygen free radical generation, and the accumulation of Hcy in the placenta can cause the activation of inflammation and oxidative stress reaction, and will further cause syncytiotrophoblast damage, and affect material transportation function and endocrine function of the placenta. Apoptosis is an important pathological link causing trophoblast injury, and the Bax and FasL are the key molecules mediating the mitochondria apoptosis and death receptor apoptosis pathways in the placenta. Bax can promote the release of cytochrome C and cause Caspase-9 activation, FasL can identify Fas and cause Caspase-8 activation, and the activated Caspase-9 and cytochrome C and cause Caspase-9 activation, FasL can identify Fas and cause Caspase-8 activation, and the activated Caspase-9 and Caspase-3 can cause Caspase-3 activation and ultimately lead to apoptosis through cascade amplification reaction respectively. In order to confirm the effect of MTHFR gene C677T locus polymorphism on trophoblast proliferation and apoptosis in the placenta of PE pregnant women, the apoptosis gene expression in placenta was compared among PE women with different genotypes.
and the results showed that FasL, Caspase-8, Bax, Caspase-9 and Caspase-3 mRNA expression in placenta of PE women with TT genotype were greatly higher than those of PE women with CT genotype and CC genotype. This means that the mutation from MTHFR gene C677T locus allele C to T in the PE course will cause the high expression of apoptosis gene in the placenta, then cause excessive trophocyte apoptosis and lead to the placenta dysfunction. In the process of normal pregnancy, the trophocytes in the placenta have strong invasion ability, and the trophocytes with active proliferation can invade towards uterine decidua, spiral artery and other tissues, which on the one hand, make the placenta anchor in the uterine decidua and exert the corresponding biological functions, and on the other hand, make spiral artery recast and provide adequate blood supply for fetal growth and development. In the course of preeclampsia, the invasion ability of trophocytes is significantly weakened and causes shallow placental implantation and uterine spiral artery recasting. Notch-1 is a kind of transmembrane receptor protein, and it can identify the upstream signaling protein in sertoli cells to start the epithelial mesenchymal transition[13], increase the expression of N-cadherin, Vimentin and other mesenchymal phenotype marker molecules and enhance the cell invasion and movement performance[14,15]; CatL and CatB are the main types of cysteine cathepsins in the placenta, which can degrade extracellular matrix and promote cell invasion[16]. In order to confirm the effect of MTHFR gene C677T locus polymorphism on trophocyte invasion in placenta of pregnant women with PE, the invasion gene expression in placenta was compared among PE patients with different genotypes, and the results showed that Notch-1, N-cadherin, Vimentin, CatL and CatB mRNA expression in placenta of PE women with TT genotype were greatly lower than those of PE women with CT genotype and CC genotype. This indicates that the mutation from MTHFR gene C677T locus allele C to T in PE course can cause the low expression of invasion genes in the placenta, thus inhibit the invasion of trophocytes and cause the shallow placental implantation.

To sum up, it is believed that the mutation from MTHFR gene C677T locus allele C to T is related to the occurrence of preeclampsia; the mutation of this gene can affect the metabolism of Hcy and cause the accumulation of Hcy, thus affecting the expression of apoptosis genes and invasion genes, and resulting in the excessive apoptosis and insufficient invasion of trophocytes.

References