BHMT polymorphism and susceptibility to PTE in Chinese patients

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Abstract. – OBJECTIVE: Pulmonary thromboembolism (PTE) is as a common form of venous thrombosis and a potentially fatal cardiovascular disorder, which has become a severe clinical problem with high incidence and mortality. The PTE has a strong genetic basis, which contributes up to half of the variance in PTE incidence and susceptibility single-nucleotide polymorphisms (SNPs) is associated with PTE. Betaine homocysteine methyltransferase (BHMT) is an essential enzyme that catalyzes the remethylating reaction from homocysteine to methionine and participates in conserving methionine and detoxifying homocysteine. In this work, we aimed to explore BHMT polymorphism and susceptibility to PTE in Chinese patients.

PATIENTS AND METHODS: Variant loci of the BHMT gene were screened in serum samples of PTE patients, followed by verification using Sanger sequencing. These polymorphic loci were validated in 16 PTE patients and 16 matched normal patients. The frequency differences between the allele and genotypes were compared using the Hardy-Weinberg equilibrium test and Chi-square test.

RESULTS: A SNP was identified in PTE patients and a heterozygous transition of G>A (Arg239Gln) in rs3733890 was found. The variance difference at rs3733890 between normal patients (2/16, 0.125) and PTE patients (9/16, 0.5625) was significant ($p$<0.01).

CONCLUSIONS: Therefore, we concluded that the BHMT polymorphism, rs3733890 may be a susceptibility SNP for PTE.

Key Words: PTE, BHMT, SNP, Patients, Sanger sequencing.

Introduction

Pulmonary thromboembolism (PTE) is a common form of venous thrombosis and regarded as a potentially fatal cardiovascular disorder, which has become a severe clinical problem with a high incidence and mortality$^{1,2}$. PTE usually occurs during coagulation, hemostasis, and anticoagulation disorders$^{3,4}$. Noteworthy, the combination of environmental and genetic risk factors contributes to the pathogenesis of PTE$^{5}$. Diverse environmental risk factors, including the factor provoking cancer and a non-provoking factor such as body mass index, lead to different treatment strategy for PTE patients$^6$. Despite of great advances that have been achieved in the treatment methods, approximately 50% of PTE patients report$^7$ chronic functional limitations. Therefore, searching for early diagnostic biomarkers and efficient therapeutic targets are imperative.

The PTE has a strong genetic basis, which contributes up to half of the variance in PTE incidence$^9$. Previous studies$^{10}$ have revealed some genetic susceptibility variants in candidate genes that correlated with PTE risk. Other susceptibility single-nucleotide polymorphisms (SNPs), such as SC-L44A2 (rs2288904) gene and TSPAN15 (rs78707713) gene have been identified from genome-wide association studies$^{11-14}$. Betaine homocysteine methyltransferase (BHMT) is an essential enzyme that catalyzes the remethylation reaction from homocysteine to methionine and participates in conserving methionine and detoxifying homocysteine$^{15}$. The aberrant expression and function of BHMT have been proved$^{16,17}$ to be involved in various diseases and tissue development. For instance, BHMT modulates the histone H3 trimethylation (H3K4me3) in neurons, which is implicated in multiple sclerosis (MS) disease pathogenesis$^{18}$. Moreover, multiple studies$^{15,19,22}$ have verified that the SNPs of BHMT is associated with risk of Down syndrome, head and neck cancer, cancer, and coronary artery disease and so on.

Nevertheless, whether BHMT SNPs increases the PTE incidence remains largely unknown. In
BHMT polymorphism and susceptibility to PTE in Chinese patients

In this work, we screened the genotype of BHMT gene in collected PTE patients and determined the correlations between BHMT SNPs and PTE risk.

**Patients and Methods**

**Study Population**

Patients with PTE (n=16) and 16 non-PTE volunteers who received treatment in the First Hospital of Jilin University hospital were recruited in this study. The venous blood samples were collected and centrifuged, after which the plasma was stored at -80°C for subsequent analysis.

**Genotyping**

The integrity of DNA samples was examined by using agarose gel electrophoresis. The DNA was then sonicated and sheared into fragments with a length lower than 200 bp. The DNA fragments were amplified by ligation-mediated PCR (LM-PCR), and the initial library was obtained. Subsequently, the DNA samples were analyzed by whole exome sequencing using an Illumina platform (HiSeq2000, Illumina, San Diego, CA USA). The SNPs of BHMT gene were identified by using SOAPsnp program (Illumina, San Diego, CA, USA).

**PCR Amplification**

Genome DNA was extracted from the blood samples using a DNA extraction kit (TIANGEN, Beijing, China). The purity and concentration of extracted DNA were detected by a spectrophotometer (Thermo, Waltham, MA, USA). The primers for sequencing of BHMT exosomes were designed by Oligo 6 software (available at: https://www.oligo.net/downloads.html) according to the DNA sequences available from the GenBank on National Center for Biotechnology Information (NCBI) website, and synthesized by RiboBio (Guangzhou, China). The DNA samples were amplified by following the process as follows: first the denaturation at 95°C for 5 min, second the 30 cycles at 95°C for 30 sec, 63°C for 20 sec, and 72°C for 60 sec, third the final extension of 5 min at 72°C. Exome sequencing was performed following the process: 95°C for 15 sec, then 35 cycles at 95°C for 15 sec, 50°C for 5 sec, and 60°C for 90 sec.

**Statistical Analysis**

We examined Hardy-Weinberg equilibrium with the Chi-square test to determine the differences among the genotype frequencies of the SNPs between the patient group and control group. Statistical analysis was performed by using SPSS software (version 20.0 IBM Corp., Armonk, NY, USA) and a p<0.05 was considered statistically significant.

**Results**

**Baseline Characteristics**

The serum samples from a total of 16 PTE patients and 16 non-PTE volunteers were collected in the First Hospital of Jilin University in China. Table I reveals the baseline characteristics of the 16 PTE patients and 16 non-PTE volunteers. The mean age of non-PTE volunteers was 57 and the mean age of PTE patients was 55. Other clinical features were shown and there was no significant difference between PTE patients and non-PTE volunteers.

**BHMT SNP Identification by Sanger Sequencing**

Next, a SNP of BHMT was identified in the PTE patients by Sanger sequencing (Figure 1). A heterozygous transition of G>A (Arg239Gln) in rs3733890 was found in BHMT.

<table>
<thead>
<tr>
<th>Table I. Baseline characteristics and clinical features of PTE patients and non-PTE controls.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>Cases</td>
</tr>
<tr>
<td>Gender (male/female)</td>
</tr>
<tr>
<td>Mean age</td>
</tr>
<tr>
<td>Malignant tumor</td>
</tr>
<tr>
<td>Surgery/trauma/with&gt;3 days of bed rest (within 1 month)</td>
</tr>
<tr>
<td>With no obvious incentive (spontaneous)</td>
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</tbody>
</table>

N.S., no significance.
Association of the Identified BHMT SNP with PTE

The variance difference of the identified BHMT SNP between 16 non-PTE volunteers and 16 PTE patients was shown in Table II. The variance difference at rs3733890 between normal patients (2/16, 0.125) and PTE patients (9/16, 0.5625) was significant ($p<0.01$).

Discussion

PTE is a common form of venous thrombosis and a potentially fatal cardiovascular disorder, which has become a severe clinical problem with a high incidence and mortality. The PTE has a strong genetic basis, which contributes up to half of the variance in PTE incidence and susceptibility SNPs is correlated with PTE. In this study, we discovered a SNP of BHMT, an essential enzyme that catalyzes the remethylating reaction from homocysteine to methionine, in Chinese PTE patients. Several SNPs were identities to associate with PTE. It has been reported the PLXNA4 susceptibility locus in PTE by using an artificial neural network approach integrating plasma proteomics and genetic data. Protein C susceptibility and polymorphism are associated with PTE. The protein C promoter SNPs are correlated with PTE susceptibility. Polymorphisms A1298C and C677T of MTHFR gene is associated with PTE. Moreover, BHMT plays crucial roles in multiple processes and diseases. It has been reported that the BHMT/betaine methylation axis epigenetically regulates maturation of oligodendrocyte. Epigenetic and genetic regulation of BHMT in hyperhomocysteinaemia is correlated with the efficacy of folate therapy. Polymorphism of BHMT G742A is associated with the systematic risk of Brazilian population. In the current study, a SNP was identified in PTE patients and a heterozygous transition of G>A (Arg239Gln) in rs3733890 was found. The variance difference at rs3733890 between normal patients (2/16, 0.125) and PTE patients (9/16, 0.5625) was significant ($p<0.01$). The effect of BHMT in PTE should be validated and the clinical significance of BHMT should be confirmed in future investigations.

Table II. Frequencies of rs778026543 SNP.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control</th>
<th>Patients</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHMT</td>
<td>Frequency</td>
<td>Frequency</td>
<td>$p$-value</td>
</tr>
<tr>
<td></td>
<td>2/16=0.125</td>
<td>9/16=0.5625</td>
<td><strong>$p&lt;0.01$</strong></td>
</tr>
</tbody>
</table>

**Statistically significant.

Figure 1. Sequence analysis of BHMT genes (exon 6) in PTE patients.

Conclusions

Therefore, we concluded that the BHMT polymorphism, rs3733890 may be a susceptibility SNP for PTE.
Funding
This study was not supported by any funding.

Ethics Approval
The study is authorized by Medical Ethics Committee of First Hospital of Jilin University (Approval No. 20220107).

Informed Consent
All patients participated in this study have signed the informed consents.

Authors’ Contributions
Y.-Q. Jiang and W.-H. Zhang designed the study and experiments; W.-H. Zhang and S.-M. Zhao conducted the experiments; W.-H. Zhang processed the data and wrote the manuscript; Y.-Q. Jiang, S.-M. Zhao, J.-F. Guo, and Y.-P. Liu participated in the revision of manuscript.

Conflict of Interest
We claim no conflict of interest for the current study.

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References


