Thrombosis Research 135 (2015) 788–795

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Association of folate metabolism gene polymorphisms and pulmonary embolism: A case-control study of West-Siberian population

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ARTICLE INFO

Article history:
Received 16 August 2014
Received in revised form 11 November 2014
Accepted 30 November 2014
Available online 11 December 2014

Keywords:
Pulmonary embolism
Genetically determined risk factors
MTHFR-677
MTHFR-1298
MTR-2756
MTRR-66

ABSTRACT

Introduction: Our objective was to investigate the association between gene polymorphisms of folate cycle (MTHFR 677 C > T, MTHFR 1298 A > C, MTR 2756 A > G, and MTRR 66 A > G) and the risk of pulmonary embolism (PE) in a case-control study.
Materials and methods: 177 PE patients (87 women and 90 men) were compared to a healthy control group (461 people, 123 women, 326 men). All of them are residents of Novosibirsk region. SNPs were genotyped by allele-specific PCR.
Results: The age distributions of our male and female patients were found to be significantly different. For men, the distribution has two maxima, whereas for women it has only one maximum, which is between the two. This fact stimulated us to perform a sex-specific analysis. No statistically significant difference has been found between distributions of the three genes in our PE patients and healthy controls. However, it was discovered that the TT genotype of MTHFR: 677 C > T polymorphism in men increases the risk of PE in comparison to controls. In fact, the difference increases in the age group over 45 years. Also, AA genotype of MTRR 66 A > G polymorphism in women below 45 years decreases the risk of PE. The sex-specific multiple linear regression analysis gave us estimates of the relative PE risk associated with MTHFR 677 C > T, F2: 20210 G > A (Prothrombin), and F5: 1691 G > A (Leiden) mutations.

Introduction

Pulmonary embolism (PE) as a manifestation of venous thromboembolism is one of the most severe cardiovascular conditions. In the absence of treatment mortality reaches 30%; whereas timely appropriate therapy is able to reduce it to 2.8 % [1,2].

The incidence of venous thromboembolism (VTE) varies in different ethnic groups; it is generally high in Africans, has an intermediate position among Europeans and is the lowest in Asia. Thus, 70-120 cases of VTE per 100 thousand population are detected each year in the United States, while only 16-17 cases per 100 thousand are identified in Asia. According to the results of autopsies, PE is recorded in 15% of deaths in North America and less than 1% of deaths in Asia [3].

Many factors contribute to the occurrence of pulmonary thromboembolism, both acquired and inherited. One of the widely discussed risk factors for thrombosis is hyperhomocysteinemia. Homocysteine (Hcy) is an intermediate product of metabolism of methionine in the folate cycle. It is a complex cascade process which requires an adequate intake of folic acid and vitamins B (B6, B12) for its functioning. Homocysteine level in blood depends on the activity of enzyme systems that are involved in its metabolism. They are essentially demethylation reactions with the formation of cysteine and remethylation to methionine. Defects in these pathways lead to hyperhomocysteinemia. Hyperhomocysteinemia is observed in approximately 5 % of the overall population and is associated with the increased risk of many diseases, including cardiovascular and neurodegenerative, congenital defects, diabetes mellitus, autoimmune diseases, osteoporosis, cancer, kidney disease and neuropsychiatric disorders [4]. The most important enzymes of folate cycle involved in homocysteine remethylation are methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR).

In this work the polymorphism of genes regulating the folate cycle was considered. It is known that the enzyme methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10- methylenetetrahydrofolate to 5– methyltetrahydrofolate. It is, in turn, a main circulating form of folate, a methyl group donor for vitamin B12-

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http://dx.doi.org/10.1016/j.thromres.2014.11.021
0049-3848/

dependent remethylation of homocysteine to methionine by the enzyme methionine synthase (MTR). Methylcobalamin, a derivative form of vitamin B12, is an intermediate carrier of methyl group in remethylation of homocysteine, catalyzed by methionine synthase. Methylcobalamin is hereby oxidized, causing methionine synthase (MTR) to lose its activity. Restoration of the enzyme function by its methylation occurs with the participation of the enzyme methionine synthase reductase (MTRR).

There are described several polymorphisms of the gene encoding the enzyme MTHFR. Two singlenucleotide polymorphisms are investigated most often with the respect to the influence on the risk of cardiovascular disease: 1) substitution of cytosine for thymidine nucleotide at position 677 in exon 4 (polymorphism MTHFR 677C > T), and 2) substitution of adenine for cytosine nucleotide at position 1298 in exon 7 (MTHFR 1298 A > C). These replacements are associated with a decreased activity of the enzyme that promotes the accumulation of homocysteine. Also, they are associated with a decrease in the folic acid concentration. In the situation of an adequate supply of folic acid carriage of MTHFR 677 TT homozygote becomes protective against colon cancer and acute lymphocytic leukemia. Additionally, it increases the survival of the fetus carrier of described genotype. On the downside, it is associated with an increased risk of having a child with Down's syndrome [5], and, according to some studies, it increases the risk of venous and arterial thrombosis [6]. Polymorphism MTHFR 1298 A > C is discussed as a genetic risk factor for aborting, especially in folate deficiency, as well as vascular dementia, arterial and venous thrombosis and fetal losses [7]. Polymorphisms of the gene that codes the enzyme methionine synthase, MTR (changing adenine to guanine nucleotides in the coding region of the gene) results in an increased risk of coronary heart disease by 4 times [8]. Several other researchers found no relation of polimorphism MTR 2756A > G neither with cerebrovascular and cardiovascular diseases [9], nor with early development of vascular thrombosis [10].

According to some authors, methionine synthase gene reductase MTRR polymorphism (replacement of adenine to guanine) leads to the increased levels of homocysteine [11,12]. Several researchers have pointed to the relation of the carriage of GG genotype with coronary heart disease [13,14].

The aim of this study was to investigate the association of polymorphisms MTHFR 677 C > T, MTHFR 1298 A > C, MTR 2756 A > G and MTRR 66 A > G with development of pulmonary embolism in patients with venous thrombosis in the West Siberian region of Russia (Novosibirsk region).

Materials and Methods

Selection of Cases and Controls

The study included 177 patients (87 women and 90 men, average age 52.4 ± 14.7 years), admitted to the Novosibirsk Research Institute of Circulation Pathology in the period between April 2012 and January 2014 for treatment (catheter fragmentation with localized fibrinolysis and further systemic fibrinolysis, thrombectomy, and placement of an inferior vena cava filter if necessary). All of the patients had a verified diagnosis of acute massive and submassive pulmonary embolism. Signs of venous thrombosis were confirmed in all patients on admission and they were in different areas. Factors provoking thromboembolism were marked as follows: 45% of patients had obesity, 58 % had varicose disease, 12.5% of patients had a trauma in pre-PE period, 19.8 % of patients smoked, 17% of women took estrogen containing drugs, 40% of patients already had a previous history of venous thrombosis.

For all our patients and controls we determined several rare risk factors, such as a deficiency of protein C and antithrombin deficiency. Nobody from our patients and controls had the protein C deficiency, neither the antithrombin deficiency. Unfortunately, we have not measured a protein S activity, but this risk factor usually is even less frequent then the protein C deficiency or the antithrombin deficiency.

The exclusion criteria were renal insufficiency, gastrointestinal disorders, such as chronic inflammatory disease, autoimmune disease, severe form of psoriasis, malignancy, hematological diseases, pregnancy for women.

The selection of patients may be illustrated by the following numbers. According to statistics from the main regional hospital, in 2013 all hospitals of Novosibirsk received 284 PE patients (44 of them died during the treatment). The population of Novosibirsk region is 2.730 million people, hence the hospitals get 10.4 PE patients per 100000 people every year. It is important to remember that annual incidence of PE events in a typical European country is 10 times bigger, and is about 100 cases per 100000 people every year.

Most likely, it means that Novosibirsk hospitals deal with heavy cases exclusively, while other cases are treated conservatively without hospitalization, or remain unassigned.

The control group consisted of 449 apparently healthy blood donors from Novosibirsk (123 women, 326 men) who had no history of thromboembolic events and had a similar ethnic background. Written informed consent was obtained from all study participants.

Blood Sampling and Genotype Determination

Blood for genetic testing was sampled from the cubital vein in the morning on an empty stomach. DNA was isolated from EDTA-stabilized whole blood according to the manufacturer's instructions to the reagent kit Probe-GS-Genetics (DNA technology, Russia). Genotyping of genes polymorphisms of folate cycle, MTHFR 677 C > T, MTHFR 1298 A > C, MTR 2756 A > G and MTRR 66 A > G was performed (the study was examiner-blind) by PCR method in real time mode according to the instructions of the test system Genetics Folate Metabolism (DNA technology, Russia). Registration and accounting of amplification results were performed in real time mode using the amplificatory DT-96 (DNA technology, Russia).

Serum tHcy Assay

Fasting venous blood samples were collected into the clot activator tubes, centrifuged and analyzed immediately. Serum tHcy concentration was measured by automated chemiluminescent microparticle immunoassay using Architect Homocysteine Reagent Kit on Architect Plus (Abbott, USA) analyzer.

Statistical Analysis

Statistical analysis was performed using a standard software package Statistica 8 and DeFinetti program, available on the website of the Institute of Human Genetics (Munich, Germany, http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl).

Conformity of genotype distribution in the examined groups to Hardy-Weinberg distribution was assessed using the criterion χ^2. To estimate the relative risk a criterion of ratio chances OR (odds ratio) with 95% confidence intervals (CI) was used. Statistical significance of differences was set at a probability value of $p < 0.05$.

Results and Discussion

Age Distributions

The age distributions of PE patients and controls are presented in Figs. 1 and 2, respectively. According to Fig. 1, male and female distributions are almost equal for healthy donors, but they are very different for the patients under 55 years. While the distribution for male patients has two maxima with a minimum age about 40-45 years, the distribution for female patients has maximum at this age. This fact indicates that