CORRELATION OF THROMBOEMBOLISM WITH MTHFR C677T MUTANT GENE

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Abstract

Venous thrombosis, including deep venous thrombosis and pulmonary embolism, are important causes of morbidity and mortality, especially to aged people. Thromboembolism diseases are multifactorial illnesses with environment and genetic factors.

The polymorphism of MTHFR gene is associated in many studies with the risk of thromboembolism diseases. We have analysed the MTHFR C677T genotype to a group of individuals with idiopathic thromboembolism. Samples of venous blood were harvested for the extraction of DNA and the genotyping of MTHFR gene was performed through Real-Time PCR method. Then we have analysed the proportion and semnification of the three CC, CT and TT genotypes of MTHFR 677 gene, compared to a group of healthy people without cardiovascular illnesses.

T677T homozygous mutant genotype of MTHFR gene is present in a bigger percentage to patients with thromboembolism compared to the witness lot, suggesting it's predisposing role for thrombophilia.

Key words: venous thrombosis, thromboembolism, methylenetetrahydrofolate reductase gene.

INTRODUCTION

Venous thromboembolism (VTE) makes part of cardiovascular diseases according to ICD-10 (International Classification of Diseases) and comprehends the deep venous thrombosis (DVT) and pulmonary embolism (PE). Deep venous thrombosis complicates in 10% of the cases with pulmonary embolism. The incidence of pulmonary thromboembolism is rated at approximately 100/100.000 cases/year. Pulmonary embolism is a serious complication through its manifestations which may vary from breathing and cardiac disorders to cardiogenic shock or even sudden death. The causes of venous thromboembolism are: age over 60 years, acute myocardial infarction, congestive heart failure, prolonged immobilization, obesity, smoking, major surgeries, orthopaedic surgery, pregnancy and the postpartum period, pills containing estrogens, some neoplazii, prolonged venous catheterization, coagulation disorders and genetic factors. (Gherasim L., 2000)

Family history of thromboembolism diseases is a factor of risk for descendants, but as any other multifactorial illness this is under the influence of genetic and environmental factors.(Zöller B. et al., 2011) In the

studies made on families with venous thromboembolism, the incidence of the siblings was raised, the risk being of 3.08 (2.80-3.39, with a confidence interval of 95%), in comparison with the general population. The risk was bigger at men than women and it was similar to venous thrombosis and pulmonary embolism. (Sørensen H. T. et al, 2011)

Thrombophilia is defined as the tendency to develop arterial or venous thrombosis by raising the coagulability. It exists more genetic polymorphisms as risk factors of venous thrombosis: the polymorphism of prothrombin gene FII G20210 A, the mutation of V Leiden factor, the resistance against activated C protein and S protein, EPCR gene (endothelial cell protein C receptor), the mutation of methylenetetrahydrofolate reductase gene (MTHFR).

The fibrinogen (First factor of coagulability) is a hexameric soluble glycoprotein, the fibrin precursor, composed of three pairs of polypeptide chains Aα, Bβ şi γγ. The fibrinogen is synthesized hepatic, in megakaryocytes it's an important determinant of blood viscosity and platelet aggregation and it has the role of mediation in the final path of thrombus formation. (Arbustini E. et al., 2013)

Three genes, ***FGA*** (OMIM*134820), ***FGB*** (OMIM*134830) and ***FGG*** (OMIM*134850) located on chromosome 4q encode, each of them, one of the three polypeptides Aα, Bβ şi γγ of the fibrinogen. Disfibrinogenemiile are risk factors for thrombosis, but they are found at less than 1% of the patients with venous thrombosis. Several polymorphisms of the gene coding restriction have been highlighted, but these explain just between 1-15% of the plasma fibrinogen value variations in the general population. (Tybjaerg-Hansen A. et al., 1997)

The mutation R16C of FGA gene is the most common cause of "disfibrinogenemie" and was associated with haemorrhage and thrombosis. Approximately 30% of carriers of this mutation presents bleed and 15% presents thrombosis.(Flood V. H. et al, 2006)

F2 (OMIM*176930) is the symbol of the gene which encodes the second factor of coagulability and it's located on chromosome 11p11.2 and has almost 21 kb. Prothrombin or the second factor of coagulability is a dependent glycoprotein of vitamin K, synthesized by liver inactive form. The activation of prothrombin plays an important role in hemostasis and thrombosis by converting fibrinogen (OMIM*134820) into fibrin in order to form the blood clot, stimulation of platelet aggregation and activation of V, VIII and XIII coagulability factors. The mutation of F2 G20210A prothrombin was discovered in 1996 by Poort and his collaborators, it is produced by the substitution of guanine (G) with adenine (A) at the level of base pair 20210, located in the 3' untranslated region of the prothrombin gene. This polymorphism is associated with higher levels of prothrombin,

which will cause a state of hypercoagulability and it may predispose venous thromboembolism and pulmonary thromboembolism.(Poort S. T. et al., 1996)

F5 (OMIM*612309) gene encodes V factor of coagulability and it is located on chromosome 1q24.2. The V factor(proaccelerin) is a glycoprotein of 330 kDa and it's activated by thrombin(FIIa). The most studied mutation is F5 R506Q, it consists of the replacement of arginine with glutamine, having as effect the resistance against activated vitamin C, a physiological "anticoagulant". Epidemiological data have established clearly the implication of V Leiden factor in venous thromboembolism. This relationship was highlighted for the first time in the LETS study (Leiden Thrombophilia Study), a big dutch study witness-case type, but in several other studies as well. The study revealed a relatively significant risk of 2,7 for venous thromboembolism in carriers of the FV R506Q mutation. The relative risk for thromboembolism related to the other analysed factors was significant for each one; thereby for protein C deficiency was RR=3,1, protein S deficiency was RR=1,6, antithrombin deficiency was RR=5,0, resistance against activated protein C was RR=6,6, von Willebrand and F VIII factor deficiency was RR=4,8, hyperhomocysteinemia RR=2,5 and F II G20210A had RR=2,8.(van der Meer F. J. et al., 1997)

A danish cohort study had observed that heterozygous and homozygous mutants for Leiden factor have a higher risk of 2,7 times, respectively 18 times for the venous thromboembolism than those who don't have the mutation. The absolute risk of venous thromboembolism at 10 years for the non-smoker heterozygous and homozygous with ages below 40 years and who weren't overweight was of 0,7%, respectively 3%. In contrast to smokers, overweight, with ages over 60 years the risk increases significantly to 10%, respectively 51%.(Juul K. et al., 2004)

PROCR or ***EPCR*** (OMIM*600646) gene is the symbol of the gene encoding a protein of 43 kDa (endothelial cell protein C receptor) that is found in vascular endothelial cells and receptor for protein C. The gene is located on chromosome 20q11.22. This protein is generally produced, in big vessels, in the case where there is a high local concentration of protein C that can activate the thrombin-thrombomodulin complexes. Protein C is a protein dependent of vitamin K with a major role in coagulation and it is activated when the thrombin (FII) binds to thrombomodulin, a protein on the surface of endothelial cells. The results of studies confirm that EPCR intervene in the regulation of human coagulation, in particular the A3 haplotype was associated as well with the increase of plasma levels of soluble receptor sEPCR (soluble form of endothelial cell protein C receptor) and with an increased risk of venous thrombosis to men. (Beatrice Saposnik et al., 2004)

Since sEPCR and prothrombin (FII) are inhibitors of activated protein C, the presence of EPCR mutation A3 haplotype has synergistic effect with prothrombin mutation, this context, as well, favors venous thromboembolism. (Navaro S. et al., 2008)

MTHFR (OMIM*607093) is the gene encoding the enzyme reductase methylenetetrahydrofolate and it is located on chromosome 1p36.3 (Goyette P. et al, 1993). There are over 41 polymorphisms of this gene, but the most studied ones are C677T and A1298C. Both of them are produced by the substitution of an amino acid, which will lead to the synthesis of a thermolabile protein, with reduced enzyme activity. According to some studies, MTHFR C677T heterozygous patients do not show hyperhomocysteinemia or increased risk of thrombotic events while MTHFR T677T could develop hyperhomocysteinemia. (LeclercD. et al, 2004) The T677T genotype was associated with a 20% higher risk of venous thrombosis compared with the C677C genotype. (Den Heijer M. et al, 2005)

The risk of thromboembolic disease to patients with hyperhomocysteinemia was highlighted for the first time in 1991. Several studies have shown that patients have a risk 2-4 times higher of venous thrombosis compared with healthy individuals. The risk is 20 times bigger if you associate heterozygous status to hyperhomocysteinemia for V Leiden factor. Oral contraceptives to patients with hyperhomocysteinemia are associated with an increased risk of thrombosis of cerebral veins. (Wald D. S. et al, 2011)

The MTHFR C677T polymorphism is caused by a mutation in the wrong direction, in which the cytosine (C) is replaced by thymine (T) at position 677 of the MTHFR gene. The two alleles are allele C,also knows as non-mutant allele, and allele T, known as mutant allele. Modified codon(A222V) will encode instead of alanine(Ala) the valine(Val), resulting in a thermosensitive protein with reduced enzyme activity(Frosst et al, 1995). MTHFR C677T mutation in numerous studies has been associated with cardiovascular diseases, thromboembolic diseases, recurrent miscarriage, etc.(Shan J. G. et al., 2016, Cao Y. et al, 2013; Xuan C. et al, 2011; Liew S. et al, 2015)

Regarding thromboembolism, results are contradictory, some studies have shown no significant relationship of polymorphism at MTHFR T677T homozygous (OR: 1.38, 95% confidence intervals [CI]: 0.98–1.93), (Simone B. et al, 2013), while other studies estimate a significant risk MTHFR C677T (OR 1.57; 95% CI 1.23-2.00, p = 0.0003) (Gohil R. et al., 2009) The T677T genotype was associated with a 20% higher risk of venous thrombosis compared with the C677C genotype. (Den Heijer M. et al, 2005)

MATERIAL AND METHOD

The purpose of study was to determine the polymorphism of MTHFR C677T gene and establishing the correlation with thromboembolism, to a group of patients, a witness-group of healthy individuals, without cardiovascular medical history.

A number of 6 young patients with age below 50 years have presented pulmonary thromboembolism. Before the incident they showed no symptoms of cardiovascular disease and have undergone a surgery or an orthopedic surgery.

The control group consists of 6 asymptomatic individuals,apparently healthy, at random pick, of same age and gender with the patients suffering of thromboembolism.

All people have given their informed consent, identical for both groups studied.

Working method:

Both groups of patients were collected 2 ml sample of venous blood from the collection tube, containing EDTA as an anticoagulant. If DNA extraction cannot be done after harvesting, the sample blood can be stored at 2-8° C within 7 days or in a freezer at ≤ - 20° C for maximum two months. After extraction, the DNA can be preserved in a freezer in sterile tubes at -80° C with unlimited validity.

Genomic DNA extraction was performed using the kit Quick gDNA miniprep ™, produced by Zymo Research USA. This technique allows for extraction of genomic DNA of high purity from venous blood collected in EDTA anticoagulant. The aim is to obtain the DNA from the nucleus of white blood cells which will then move in the suspension and then be recovered using a buffer solution. This technique avoids mechanical destruction or enzymatic nucleic acids and RNA contamination.

Analysis method: genotyping was performed by polymerase chain reaction of DNA in real time (real-time PCR). For detection of the polymorphism of the gene methylene-tetrahydrofolate reductase MTHFR C677T, I used the kit from GeneProof® Czech Republic. Real-time PCR (RT-PCR) called quantitative PCR (qPCR), uses oligonucleotides labeled with the fluorescent dye, which will be detected as it occurs amplification and can be monitored in real time, which means that the PCR product does not require further processing. In-vitro amplification of DNA fragments was performed using a thermocycler device Eco Illumina.

RESULTS AND DISSCUSIONS

Part of the patients group with thromboembolism are 3 women and 3 men with ages within the range of 34-50 years.

The control group consist of women and men equally spread, with appropriate ages in comparison to the patients with thromboembolism.

In people with thromboembolism the homozygous and heterozygous mutation of MTHFR gene is present in 5 individuals and only in two of healthy patients, as shown in Table 1 and Fig. 1.

Table 1

MTHFR genotype distributions of all patients

MTHFR genotype distributions of all patients	**Tromboembolism 6 cases**	**Controls 6 cases**	**Total cases 12**
Normal heterozygous genotype MTHFR C677C (wild type)	1	4	5
Mutant heterozygous genotype MTHFR C677T	3	2	5
Mutant homozygous genotype MTHFR T677T	2	-	2

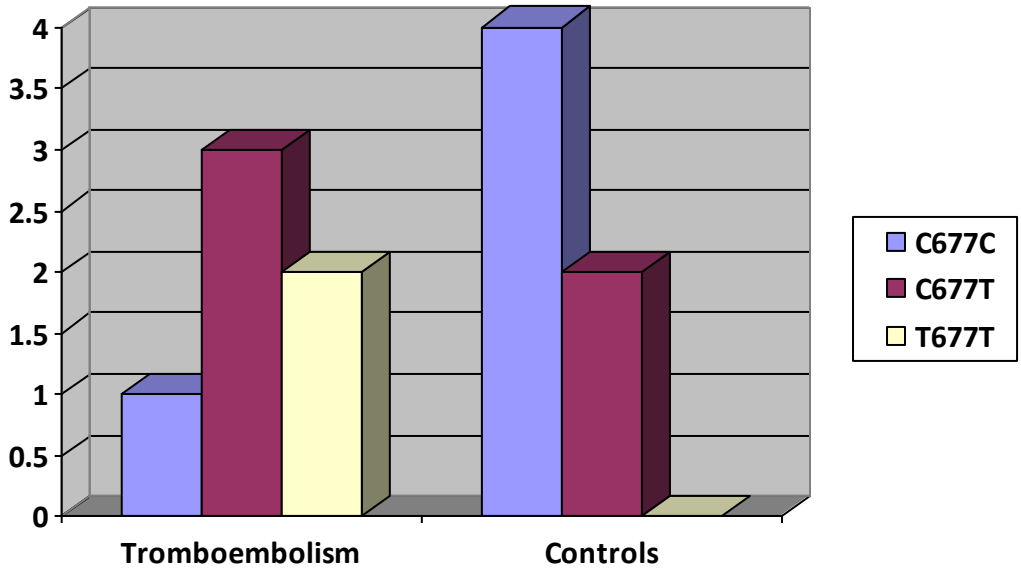

Fig. 1 The genotypic distribution of the MTHFR C677T in the studied groups

CONCLUSIONS

At people with thromboembolism the heterozygous and homozygous mutation of MTHFR gene is present to 5 individuals and at only two of the healthy patients.

The normal genotype MTHFR C677C (wild type) is present to more patients out of the healthy group.

The presence of the mutant allele in heterozygous CT or TT homozygous form, is apparently associated with a higher risk of

thromboembolism. This study can be expanded by similar studies, which may generate conclusions with higher medical significance and relevance.

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