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ASSOCIATION OF POLYMORPHIC VARIANTS OF THE GENE VKORCI WITH ISCHEMIC ATHEROTHROMBOTIC STROKE

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Annually Stroke affects 16 million people and causes the death of nearly 6 million. 88% of all strokes account for ischemic stroke genesis. Disorders in processes of hemostasis and ectopic calcification play the important role in ischemic atherothrombotic stroke development. Today distinguished the group of vitamin K-dependent proteins that includes proteins, which provide hemostasis and proteins that have anticalcifications properties. Residues of glutamic acid, that are the part of structure of vitamin k-dependent proteins, must be carboxylase during the posttranslation modification for the activation of proteins. This reaction is catalyzed by enzyme g-glutamyl carboxylase. Vitamin K is a cofactor in this reaction and it is oxidized from the vitamin K hydroquinone to 2.3 epoxide vitamin K. Enzyme, which restores of vitamin K epoxide to vitamin K hydroquinone is called vitamin K epoxide reductase. Vitamin K epoxide reductase is integral transmembrane protein. It plays the important role in vitamin K-dependent protein activation. VKORC1 gene is localized in 16th chromosome. It consists of 3 exons. Today about 150 polymorphisms of VKORC1 gene have been described. The most important are T2255C and G37300A. That there is no such a data as for Ukrainian populations.

The aim of our research was to perform a case-control study on representatives of the Ukrainian population in order to assess the possible association of the VKORC1 gene polymorphisms with IAS in subjects of both genders.

G3730A polymorphism (rs7294) of VKORC1 gene was determined in 170 patients with AIS and in 124 persons of the control group by using PCR with restriction fragment length analysis. DNA for genotyping was extracted from venous blood using commercially available kits («Isogene Lab Ltd», Russian Federation) according to the manufacturer's protocol. To identify the VKORC1 2nd intron T2255C polymorphism (rs2359612), PCR with subsequent restriction fragment length polymorphism (RFLP) analysis was performed. Specific region of the VKORC1 gene was amplified using a pair of specific primers. Primers were provided by Metabion (Germany). PCR was performed for 33 cycles in a 25 μl volume containing 50 – 100

ng of DNA, 5 µl 5 ´ PCRbuffer, 1.5 mM magnesium sulfate, 200 µM of each dNTP, 20 pM of each primer and 0.5 U of Taq DNA polymerase («Fermentas», Lithuania). PCR was carried out in a termocycler GeneAmp PCR System 2700 («Applied Biosystems», USA). Six microlitres of the PCR products (198 bp) were subjected to digestion with 3 U NcoI («Fermentas») and incubated at 37 oC for 18 h. The presence of cytosine at position 2255 of the gene VKORC1 prevents restriction and in the case of substitution for thymine NcoI cleaves the amplified fragment of the promoter into two fragments of 172 bp and 26 bp in length Statistical analysis was performed by using the software package SPSS-17.

We received the following results. For polymorphism T2255C. Processing of these data using the chi-squared test confirmed a statistically significant difference between the distribution of different variants of the T2255C polymorphism of VKORC1 gene in patients with ischemic atherothrombotic stroke and in healthy people (P = 0.043). Method of logistic regression showed that the risk of stroke in carriers of CC genotype in 2.2 times higher than in patients with genotype TT. Chi-square criteria did not reveal any differences in the distribution of genotypes not in women and not in men. However, the method of logistic regression showed that the risk of stroke in men with the CC genotype in 2.5 times higher than carriers of TT genotype. As for polymorphism G3730A. The frequency of allelic variants was not statistically different in patients with stroke and among healthy people. The frequency of allelic variants was not statistically different in woman and in man.

Based on these results we made following conclusions:

1. Allelic polymorphism T2255C of the VKORC1 gene is an important factor for a genetic predisposition to the development of ischemic atherotrombotic stroke. There is an association of this polymorphism with development of ischemic stroke in the Ukrainian population: the risk of stroke in homozygotes for the minor allele higher than in carriers of the TT genotype.

2. There is no association G3730A polymorphism with ischemic stroke in Ukrainian population.

3. Effect of polymorphism T2255C, but not G3730A polymorphism in the development of ischemic stroke has sexual features: men with a CC genotype in 2.5 times more frequent suffer a stroke than men with genotype TT.

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