THE FREQUENCY OF THR83ALA POLYMORPHISM OF MGP GENE EXON 4 IN PATIENTS WITH ATHEROTHROMBOTIC STROKE

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Abnormal calcium salts depositing in the arterial vessels is considered to be a novel marker of atherosclerosis and related to cerebrovascular disease. Matrix gamma-carboxyglutamic acid protein (MGP) is one of the most potent inhibitors of ectopic mineralization, so it may be associated with calcification of atheromatous plaques, their instability and rupture, and thrombi formation. The human MGP gene is located on chromosome 12p13.1-p12.3 and it consists of 4 exons. Numerous single nucleotide polymorphisms (SNPs) were identified both in the coding and regulatory regions of MGP gene. Polymorphisms of the exons can influences on the structure and function of the mature protein. Considering the central role of MGP in vascular calcification and a similar pathogenesis between coronary artery disease and severe cerebrovascular events, we hypothesized that Thr83Ala polymorphism in the exon 4 of MGP gene might influence the risk of IAS. To validate the hypothesis we performed an analysis of Thr83Ala genotype in patients with IAS and control subjects which represent the north-eastern region of Ukraine. So, the aim of our investigation was to determine whether there is an association between the Thr83Ala polymorphism of MGP gene exon 4 and the risk of IAS development.

Our study group consisted of 170 unrelated Ukrainian patients with a mean ± SD of 64.8 ± 9.5 years who had IAS. Clinical characteristics of patients included generally accepted parameters related to risk factors for atherosclerosis and IAS: body mass index (BMI), blood pressure (BP), fasting blood glucose (FBG), blood plasma lipids and lipoproteins, and some indices of blood coagulation (prothrombin time). The control group consisted of 124 elderly Ukrainian subjects with a mean age of 76.6 ± 10.2 years without a history of IAS and evidences of marked cardio- and cerebrovascular pathologies. DNA for genotyping was extracted from the venous blood using commercially available kits (Isogene Lab Ltd, Russia). To identify MGP gene exon 4 Thr83Ala polymorphism (rs4236) the polymerase chain reaction (PCR) with subsequent restriction fragment length polymorphism (RFLP) analysis was performed. Specific region of the MGP gene was amplified using a pair of specific primers: upstream (sense) – 5` TCAATAGGGAAGCCTGTGATG-3` and downstream (antisense) – 5`-TCAATAGGGAAGCCTGTGATG-3`.

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 5X PCR-buffer, 1.5 mM magnesium sulfate, 200 µM of each dNTP, 20 pM of each primer and 0.5U of Taq DNA polymerase (Fermentas, Lithuania). PCR was carried out in a termocycler GeneAmp PCR System 2700 (Applied Biosystems, USA). Six microlitres (6 µL) of the PCR products (173 bp) were subjected to digestion with 3U Eco477 (Fermentas, Lithuania) and incubated at 37°C for 18 h. The presence of adenine at position 3748 of MGP gene prevented restriction and, in the case of substitution for thymine Eco477, cleaved the amplified fragment of exon 4 into two fragments 127 bp and 46 bp in length. The restriction fragments were separated by electrophoresis and analysed on an ethidium bromide-stained 2.5% agarose gel visualized using ultraviolet transillumination.

All statistical analyses were performed using the Statistical Package for Social Science program (SPSS for Windows, version 17.0, SPSS Inc, Chicago, IL).

The distribution of homozygous carriers of a major allelic variant (Thr/Thr), and heterozygous (Thr/Ala) and homozygous minor allele (Ala/Ala) variants in IAS patients was 39.4%, 48.8% and 11.8%, respectively. The corresponding distributions of variants in the control group were 34.7%, 53.2% and 12.1% ($P>0.05$ by $\chi 2$-test). It was concluded that MGP exon 4 Thr83Ala polymorphism is not associated with the risk of IAS both in men and in women in the Ukrainian population. Only in one subgroup which included non-hypertensive patients, the risk of IAS in heterozygotes was significantly lower as compered with major allele homozygotes. The mechanism of this is not clear and should be a subject of further investigation.

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