

GENE POLYMORPHISMS AS MARKERS OF DISEASE SUSCEPTIBILITY

GENSKI POLIMORFIZMI KAO MARKERI PREDISPOZICIJE ZA OBOLJENJA

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Summary: The most widespread diseases of modern man have a polygenic basis, including genetic predisposition and factors in the external environment. Such is the case with cardiovascular disease, malignancy, diabetes and so on. It should be borne in mind that risk factors usually include disorders that are themselves multifactorial, which further indicates the complexity of pathophysiological mechanisms. In the investigation of genetic factors in polygenic diseases studies are underway to determine the association with specific gene polymorphisms. Genetic or DNA polymorphisms are differences in the hereditary basis which are normally found in human populations. The human genome consists of 3×10^9 nucleotide (base) pairs, and it is considered that, on average, every 1000th nucleotide is polymorphic, i.e. varies between two loci or two individuals. The most common type of gene polymorphisms is the single nucleotide polymorphism (SNP). Although gene polymorphisms are an expression of normal variations in the hereditary basis, their effect on the phenotype is interesting, especially the association with proneness to certain diseases. Association studies examine the incidence of certain genetic variants, i.e. genetic polymorphisms in a group of patients, and compare it with the data of a healthy population. The results are often contradictory, so the number of polymorphisms whose role as markers of genetic predisposition has been clearly confirmed is still small. In this paper we review literature data and present experiences from our laboratory in studying genetic polymorphisms as susceptibility factors for the occurrence of thrombophilia and atherosclerosis and its clinical manifestations.

Keywords: gene polymorphisms, thrombophilia, atherosclerosis

Kratak sadržaj: Najrasprostranjenije bolesti savremenog čoveka imaju poligensku tj. multifaktorsku osnovu, koja uključuje genetičke faktore predispozicije i činioce iz spoljne sredine. Takav je slučaj sa kardiovaskularnim bolestima, malignitetom, dijabetesom itd. Treba imati na umu da faktori rizika obično obuhvataju poremećaje koji su sami po sebi takođe multifaktorski, što dodatno ukazuje na kompleksnost patofizioloških mehanizama. U okviru istraživanja genetičkih činioca kod poligenskih bolesti pristupa se studijama asocijacije sa određenim genskim polimorfizmima. Pod genskim, odnosno DNK polimorfizmom podrazumevaju se razlike u naslednoj osnovi koje se normalno sreću u humanim populacijama. Genom čoveka se sastoji od 3×10^9 nukleotidnih (baznih) parova a smatra se da je u proseku svaki 1000. nukleotid polimorfan, tj. da se razlikuje između dva lokusa ili dve osobe. Najčešći tip genskih polimorfizama su polimorfizmi pojedinačnih nukleotida (engl. single nucleotide polymorphism – SNP). Iako genski polimorfizmi predstavljaju izraz normalnih varijacija u naslednoj osnovi, zanimljiv je njihov uticaj na fenotip, a naročito je aktuelno povezivanje sa sklopošću ka određenim bolestima. U studijama asocijacije ispituje se učestalost pojedinih genskih varijanti, tj. genskih polimorfizama u grupi obolelih i upoređuje sa podacima u zdravoj populaciji. Rezultati su često protivrečni, pa je još uvek mali broj polimorfizama sa jasno potvrđenom ulogom genetičkog markera predispozicije. U radu iznosimo iskustva naše laboratorije u ispitivanju genskih polimorfizama kao faktora predispozicije za pojavu trombofilije i ateroskleroze i njenih kliničkih manifestacija.

Ključne reči: genski polimorfizmi, trombofilija, ateroskleroza

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Introduction

Genetic polymorphisms or DNA polymorphisms are an expression of the normal diversity of the hereditary basis of man. Human genome consists of three billion pairs of nucleotides (3×10^9 bp), and it is believed that at least every thousandth nucleotide is

polymorphic, that is, different from person to person, in the general population, i.e. among healthy people (1, 2). The largest number of polymorphisms, about 80%, belong to the type of single nucleotide polymorphisms (SNP), and there are also various tandem repeats (VNTR) and deletion/insertion polymorphisms. DNA polymorphisms are now widely studied and their role as markers of possible genetic predisposition to develop certain diseases is examined. This primarily refers to polygenic disorders, which arise from the interaction between multiple genes and environmental factors, such as a number of disorders related to cardiovascular pathology (3).

In the study of genetic factors in polygenic disease association studies are used that examine the association of certain gene variants, i.e. gene polymorphisms with the occurrence of the disease, and compare data with those of a healthy population (4). Until recently, the studies analyzed the association of polymorphisms in the so-called candidate genes, i.e. genes for which it is assumed that they have a role in the development of disease. Selection of the gene candidates can be done on the basis of their role in disease etiopathogenesis, based on the data obtained from animal models, or based on the results of previously conducted linkage analyses. In recent years, the introduction of microarray technology enabled the study of association in the entire genome (genome wide association studies, GWAS). In fact, only one microarray can simultaneously analyze hundreds of thousands of SNPs, allowing fast and effective scanning of the entire genome for a possible association (3, 4).

The results of studying the importance of genetic polymorphisms in polygenic diseases are often contradictory. There are many reasons for such discrepancies. First of all, the frequency of certain polymorphisms varies in different populations. In addition, the size of the investigated sample and the selection of control groups have a major impact on the result. Therefore, the modern association studies are increasingly collaborative, as a result of association samples from a number of centers that deal with specific issues (4).

Despite the abundance of published results, there is still only a small number of polymorphisms with a clearly confirmed role as vulnerability factors. One example is the polymorphism in the genes for coagulation factor V and prothrombin, which are proven risk factors for thrombophilia. Thrombophilia is defined as an increased tendency to develop thrombosis, which is recurrent, familial, and occurs in an unusual localization and/or at an unusual time. Clinical signs of thrombophilia are thromboembolic events, and among women repeated spontaneous abortion (5).

It has been determined that a variant of coagulation factor V, designated as factor V Leiden, is basically a genetic polymorphism. This is an SNP polymorphism in the 506th codon, where triplet CGA for arginine replaces the CAA triplet for glutamine.

The prevalence of factor V Leiden varies: it is about 1–5% in North America, higher in North and Central Europe, while in the African and Asian population this polymorphism is almost absent. It is believed that this polymorphism originated 21–34000 years ago. Under normal conditions, the APC protein binds to factor V and cuts it into two inactive fragments. It has been determined that the Leiden variant is resistant to the APC protein, which prolongs the action of factor V. The result is a continuation of prothrombin activation and continuous maintenance of the coagulation cascade (4, 5). The gene for prothrombin (coagulation factor II) also has a significant polymorphism 20210G>A. This polymorphism is located in the 3' region of genes that are not translated, and it is supposed to have a regulatory role. Its frequency in European populations is 1–5%, while it is very rare in people of African or Asiatic origin, suggesting that it also comes from a white ancestor. 20210 allele leads to increased activity of prothrombin, and the hyper coagulation state (5, 6). In this way, factor V Leiden and prothrombin 20210 polymorphism significantly increase the risk of thrombophilia and diseases associated with it, such as phlebothrombosis, pulmonary embolism, etc. Meta analyses show that the risk for the first thrombotic event increases 10–20 times in heterozygotes for factor V Leiden and 2–6 times in heterozygotes for allele 20210A. Combined heterozygoteus, possessing both polymorphisms, have as much as 20 times higher risk. These data are particularly important in situations that in themselves predispose to thrombosis and thromboembolism, such as major orthopedic surgery or malignancy (5–7).

The risk is further multiplied in women during pregnancy, as well as, due to the intake of oral contraceptives or hormone replacement therapy, in menopause. Thus, women heterozygous for factor V Leiden who take hormone preparations have 15.6 times higher risk of venous thrombosis, while pregnant women homozygous for this polymorphism show 34 times increased risk of thrombosis. It has been confirmed that the risk of spontaneous abortion is also increased (5–7).

In connection with thrombophilia, the importance of some other polymorphisms is emphasized, such as of a polymorphism in the gene for methylenetetrahydrofolate-reductase, MTHFR 677C>T. This polymorphism leads to a substitution of alanine for valine at position 222 in the polypeptide, which causes a thermolabile form of the enzyme with decreased activity. T allele frequency is the highest in Asian populations, somewhat less in white Europeans and Americans, and the lowest in African populations. In Europe, the incidence of TT homozygosity is 5–15%. People with the 677TT and 677CT genotype have only 30% and 50% MTHFR enzymatic activity, respectively. MTHFR catalyzes the development of methyl-tetrahydrofolate, which is the main methyl group donor in the remethylation of homocysteine to methionine. That is why the MTHFR 677 poly-

morphism is associated with hyperhomocysteinemia, which is considered to be an independent risk factor for thrombophilia and other disorders, such as atherosclerosis, neural tube defects, etc. (6–8).

Results of extensive cost-benefit studies showed no support for universal polymorphism screening prior to introduction of hormone therapy, during pregnancy or after major orthopedic surgery. Instead, they recommend selective testing of patients with a history of previous thromboembolism (6, 7). To women with thrombophilia, for the prevention of spontaneous abortions low-dose aspirin and heparin are recommended. There is no universal agreement on the need for preventive therapy in elective orthopedic surgery (6, 7).

The Laboratory of Molecular Genetics at the Institute of Human Genetics, School of Medicine, Belgrade, in cooperation with the Institute of Hematology, Clinical Center of Serbia, analyzes the polymorphisms factor V Leiden, prothrombin 20210G>A and MTHFR 677C>T in the cases of thrombophilia. DNA for analysis is extracted from peripheral blood. Detection of the polymorphism is performed by the PCR method followed by digestion of PCR products by specific restriction enzymes. In factor V Leiden we use the MnlI enzyme that recognizes the wild type allele, in prothrombin 20210 polymorphism the HindIII enzyme recognizing the mutant allele, while the MTHFR 677T allele is detected by cutting DNA with the restriction enzyme HinfI.

The most experience we have in the analysis of polymorphism MTHFR 677C>T. In our research we found no significant association of 677T allele with the occurrence of pulmonary embolism. In the group of patients with pulmonary embolism the MTHFR 677TT genotype frequency was 10.5%, versus 11% in the control group of healthy people. Although the carriers of TT genotype had no significantly higher levels of serum homocysteine, the prevalence of hyperhomocysteinemia was higher in this than in the two other (CC and CT) genotypes.

In another study we analyzed the MTHFR 677 polymorphism in patients with stroke but without other risk factors for this disease. We found a significantly higher frequency of the TT genotype (19% in patients versus 11% in control).

Atherosclerosis is the second polygenic disorder that attracts attention in the field of genetic polymorphisms. Numerous genes and gene products have been identified that have a probable or a clear stake in the development of the disorder. According to the function of proteins encoded by these genes, the division can be made into three main groups: 1) factors that control lipid transport and metabolism (apoE, C-III, LDL receptor, lipoprotein (a)), and total

cholesterol level, 2) vasoactive factors, such as angiotensin converting enzyme (ACE), 3) factors of coagulation, platelet adhesion and fibrinolysis, such as plasminogen activator inhibitor-1 and platelet glycoproteins Ib and IIIa (2). The correlation of genetic variation caused by hepatic lipase and HDL levels, variations of apoE and LDL levels, apoCIII/apoAV and triglyceride levels, and paraoxonase 1 and myocardial infarction has been proved (7). One of the most studied is the insertion/deletion (I / D) polymorphism in the ACE gene. There is a presence (I) or absence (D) of 287 bp segment in intron 16 of the ACE gene. Numerous studies show that people who in both loci have the D variant, homozygotes DD, have a significantly higher risk for developing atherosclerosis, hypertension and myocardial infarction, but there are results which deny the link (3, 4, 9). In young people without other risk factors the importance of homocysteine as an atherogenic factor is indicated, as well as in connection with this polymorphism in the genes that control its metabolism (MTHFR, CBS, etc.). In addition, a large family has recently been analyzed in which an autosomal dominant form of coronary artery disease was demonstrated. In all cases, deletion of seven amino acids in the polypeptide chain of transcription factor MEF2A (myocyte-specific enhancer factor 2) was found (3). Future tests should prove whether these or similar changes exist in other cases of coronary artery disease, or turn to a few specific families.

In our laboratory we have examined the relationship between the I/D polymorphism in the ACE gene and abdominal aortic aneurysm (AAA), a common vascular disease with high fatality rate. The study included a total of 91 unrelated individuals: 61 patients with AAA, and 30 healthy controls comparable for sex and age. They were genotyped for the ACE I/D polymorphism by standard PCR analysis (9). Allele and genotype frequencies were compared between patients and controls using Chi-square test, and Kruskal-Wallis test was used to examine differences in the aneurysm diameter between genotypes.

The genotype distribution of ACE I/D was significantly different between patients and controls. D allele was significantly more frequent in AAA patients compared to healthy controls (0.71 vs. 0.60, $p < 0.05$). The association between the DD genotype and the presence of AAA was found to be marginally significant in a DD vs. ID+II model (OR=2.358, $p = 0.049$). Average aneurysm diameter was not significant association between the ACE DD genotype and susceptibility to AAA, reported by other authors (9–12).

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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