STUDYING THE POLYMORPHISM RS17576 MMP-9 GENEIN PATIENTS WITH TYPE 2 DIABETES MELLITUS AFTER MYOCARDIAL INFARCTION

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Annotation. *The aim of our study was to evaluate the role of polymorphism rs17576 MMP-9 gene in patients with type 2 diabetes mellitus after myocardial infarction (MI). In patients with type 2 diabetes mellitus after myocardial infarction 86 Uzbeks have been studied the alleles and genotypes rs17576 MMP-9 gene. According to it, according to the result of the oddsratio (OR - odds ratio), in carriers of the minor allele G the probability of developing the disease decreased by 29% (OR =0.71, 95% CI: 0.45-1.12), this means that the minor allele G has a protective effect against the development of coronary heart disease. On the other hand, the wild-type A allele of the MMP-9 gene polymorphism rs17576 increased the like-lihood ofdeveloping MI by 42% (OR = 1.42; 95% CI: 0.91-2.21) and turned out to be a significant risk factor for developing MI.*

Keywords *Matrix metalloproteinases, Type 2 diabetes mellitus, Myocardial infarction, Gene.*

ИЗУЧЕНИЕ ПОЛИМОРФИЗМА ГЕНА RS17576 MMP-9 У ПАЦИЕНТОВ С САХАРНЫМ ДИАБЕТОМ 2-ГО ТИПА ПЕРЕНЕСЕННОГО ИНФАРКТА МИОКАРДА

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Аннотация. *Целью нашего исследования была оценка роли полиморфизма rs17576 гена MMP-9 у пациентов с сахарным диабетом 2-го типа после инфаркта миокарда (ИМ). У 86 пациентов с сахарным диабетом 2-го типа после инфаркта миокарда были изучены аллели и генотипы rs17576 гена MMP-9. Согласно этому результату, согласно коэффициенту относительного риска (OR — коэффициент относительного риска), у носителей минорного аллеля G вероятность развития заболевания снижается на 29% (OR = 0,71, 95% CI: 0,45–1,12). Это означает, что минорный аллель G оказывает защитное действие против развития ишемической болезни сердца. С другой стороны, аллель A дикого типа полиморфизма гена MMP-9 rs17576 повышал вероятность развития инфаркта миокарда на 42% (OR = 1,42; 95% CI: 0,91–2,21) и оказался значимым фактором риска развития инфаркта миокарда.*

Ключевые слова: *матриксные металлопротеиназы, сахарный диабет 2-го типа, инфаркт миокарда, ген.*

2-TUR QANDLI DIABETLI MIOKARD INFARKTI O‘TKAZGAN BEMORLARDA MMP-9 RS 17576 GENINING POLIMORFIZMINI O‘RGANISH

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Annotatsiya. *Tadqiqotimizning maqsadi miokard infarktidan keyingi 2-turdagi qandli diabet bilan og‘rigan bemorlarda MMP-9 genining rs 17576 polimorfizmining rolini baholash. MMP-9 rs 17576 genining allellari va genotiplari miokard infarkti o‘tqazgan 86 bemorda o‘rganildi. Ushbu natijaga ko‘ra, nisbiy xavf koyeffitsiyentiga ko‘ra (yoki nisbiy xavf koyeffitsiyenti), kichik G allelining tashuvchilari kasallikning rivojlanish yehtimolini 29% kamroq (OR = 0,71, 95% CI: 0,45–1,12). Bu shuni anglatadiki, kichik G alleli yurak tomirlari kasalligining rivojlanishiga qarshi himoya ta’siriga yega. Boshqa tomondan, MMP-9 rs17576 gen polimorfizmining yovvoyi A alleli miokard infarkti yehtimolini 42% ga oshirdi (OR = 1,42; 95% CI: 0,91–2,21) va miokard infarkti uchun muhim xavf omili yekanligini isbotladi.*

Kalit so‘zlar: *matriks metalloproteinazlar, 2-tur qandli diabet, miokard infarkti, gen.*

Introduction. Matrix metalloproteinases (MMPs) are a family of protease enzymes consisting of more than 25 individual members. All MMPs have the following functional features: they degrade extracellular matrix (ECM) components; almost all of them are secreted in a latent form and require activation to exhibit proteolytic activity (the exception isMMP-11, which is released into the extracellular matrix as an active enzyme); all MMPs belong to zinc-containing proteins, which is located in the active center of the enzyme, and calcium is required to stabilize their tertiary structure; exhibit high functional activity at neutral pH values. They are regulated enzymes and are inhibited by tissue-specific inhibitors

of metalloproteinases (TIMPs) [1]. MMPs are identified in the myocardium and promote changes in the ECM, resulting in myocardial remodeling. Over the past few decades, increasing evidence from basic and clinical research has demonstrated the important role of MMPs in the progression of left ventricular hypertrophy, remodeling, and mortality after myocardial infarction (MI). In the present study, we examine MMP expression after MI and its role as a possible prognostic marker [2,3]. The nature of collagen in the ECM is determined by the balance between MMPs and TIMPs. Imbalance between MMPs and TIMPs is a major factor responsible for cardiomyocyte and interstitial changes after MI in infarcted and distant regions, since increased levels of some MMPs may contribute to maladaptive remodeling, making it an unfavorable prognostic factor [4,5]. One member of the MMP that may play an important role during acute MI and/or the remodeling process after MI is MMP-9. Active MMP-9 enzymatically degrades numerous ECM substrates, including collagen, fibronectin and laminin, to facilitate ECM turnover and scar formation during cardiac wound healing [6,7]. MMP-9, also known as gelatinase B or collagenase type IV, has a molecular weight of 92 kDa and is one of the important members of MMPs that can contribute to ECM degradation [8]. The aim of our study was to evaluate the role of rs17576polymorphism MMP-9 gene in patients with type 2 diabetes mellitus after myocardial infarction (MI).

Materials and Methods. In patients with type 2 diabetes mellitus after MI 86of Uzbek nationality have been studied the genetic determinants of alleles and genotypes rs17576 of theMMP-9 gene. The control group consisted of 83 healthy individuals— men of Uzbek nationality. The study was performed according to the standards of Good Clinical Practice (Good Clinical Practice) and the Declaration of Helsinki. The study protocol was approved by the ethics committees of all participating clinical centers. Before inclusion in the study all participants provided written informed consent. Study polymorphism rs17576 MMP-9gene gene was conducted using polymerase chain reaction1658 Khusanov R. A. and Nuritdinov N. A.: Studying the Polymorphism rs17576 MMP 9Gene in Patients with Type 2 Diabetes Mellitus after Myocardial Infarction on programmable thermocycler CG-1–96 «Corbett Research» (Australia) and 2720 «Applied Biosystems»(USA), using kits LLC "Medlab" (St. Petersburg), according to the manufacturer's instructions. In our work allele polymorphism G/T894 revealed after digestion of the amplified fragment of 206 bp containing the polymorphic site. Evaluation of deviation of the distribution of genotypes of studied polymorphisms of DNA from the canonical distribution of Hardy-Weinberg equilibrium was performed using the computer program for the analysis of genetic data "GenePop" ("Genetics of Population"). To calculate the "odds ratio" (OR — odds ratio) with 95% confidence intervals (CI— confidence interval), χ2 and p values used statistical package statistical software package «OpenEpi 2009, Version 2.3».

Results. The conducted studies showed that the distribution of alleles in the main and control groups differed significantly. Thus, the percentage of the wild type A allele in the main group was 41.3%, which is 1.25 times higher than in the control group (33.0%). The percentage of the G allele inpatients with prior MI was lower (58.7%) than in the control group (67.0%) (Table 1).

Table 1.

Expected and observed frequencies of distribution of alleles andgenotypes of MMP-9 gene polymorphism (rs17576) in the group of patientswith type 2 diabetes mellitus after MI

Alleles				Allele frequency	
A				0.413	
G				0.587	
Genotype	Genotype frequency				
	ObservedHo	ExpectedHe	χ2	p	df
A/A	0.198	0.179	0.03	0.298	1
A/G	0.43	0.485	0.06		
G/G	0.372	0.344	0.03		
Total	1	1	1.08		

Analysis of the distribution of rs17576 genotypes of theMMP-9 gene in donors of the control group showed that the wild-type homozygous AA genotype was detected in 13.2%of those examined, the heterozygous A/G genotype in 39.8%and the homozygous GG genotype in 47.0% of donors. At the same time, in the main group, 19.8% of patients had a homozygous wild-type AA genotype, 43.0% had a heterozygous A/G genotype, and 37.2% had a homozygous GG genotype. Analysis of the distribution of rs17576 genotypes of theMMP-9 gene in donors of the control group showed that the wild-type homozygous AA genotype was detected in 13.2%of those examined, the heterozygous A/G genotype in 39.8%and the homozygous GG genotype in 47.0% of donors. At the same time, in the main group, 19.8%

of patients had a homozygous wild-type AA genotype, 43.0% had a heterozygous A/G genotype, and 37.2% had a homozygous GG genotype. Similarly, the percentage of genotypes AA, AG and GG in patients with FC-2 was 17.8; 37.8 and 44.4%, respectively, while in patients with FC-3 these figures were21.9; 48.8 and 29.3%. Similarly, for the tested polymorphism, it was found that the level of the heterozygous genotype in the main group of patients was slightly lower than the expected result(0.43/0.485; D = -0.113), on the other hand, the expected and observed results for the heterozygous genotype in the control group did not differ significantly (0.398/0.427; D=-0.067).According to these indicators, the difference between the observed - empirical and expected - theoretical results in the main and control groups was not statistically significant ($\chi 2<3.84$; $p>0.05$), which showed that the results determined during the study correspond to Hardy-Weinberg. Thus, using the results obtained during the study, the pathogenetic significance of the rs17576 polymorphism of the MMP-9gene in patients with myocardial infarction associated with type 2 diabetes mellitus was analyzed. According to it, according to the result of the odds ratio (OR - odds ratio), in carriers of the minor allele G the probability of developing the disease decreased by 29% (OR = 0.71, 95% CI:0.45-1.12), this means that the minor allele G has a protective effect against the development of coronary heart disease. On the other hand, the wild-type A allele of theMMP-9 gene polymorphism rs17576 increased the likelihood of developing MI by 42% (OR = 1.42; 95% CI:0.91-2.21) and turned out to be a significant risk factor for developing MI. Although chi-square did not reveal a statistically significant positive association between the distribution of alleles of the rs17576 polymorphism of theMMP-9 gene and the development of the disease MI ($\chi 2$ =2.4, p = 0.12), the chi-square index was higher than the random variation between the factor and the disease, as well as the wild allele of the MMP-9 gene, which indicates polymorphism rs17576 (A) demonstrates a tendency to develop MI. This suggests that the reason for the lack of association between the MMP-9 gene in our study may be due to the relatively small number of patients studied. When analyzing the rs17576 polymorphism of the MMP-9 gene for the pathogenetic significance of various genotypes in the development of the disease, the wild type A/A genotype showed a 61% increase (OR=1.61, 95% CI 0.71-3.69) in the probability of developing MI. Similarly, the heterozygous A/G genotype increased the likelihood of developing the disease by 14% (OR=1.14; 95% CI 0.62-2.11), while the homozygous G/G genotype reduced the likelihood by33% (OR=0,67; 95% CI 0.62-2.11). This means that wild homozygous A/A and heterozygous A/G genotypes increase the risk of developing MI, on the other hand, the homozygous G/G genotype has a protective effect on the development of the disease, but the presented results were not statistically significant ($\chi 2<3.84$, $p>0.05$).

Discussion. MMP-9 is secreted by macrophages of a fibrous capsule and is supposed to be involved in remodeling processes after it, and can also increase the risk of myocardial infarction associated with atherosclerosis and the breakdown of plaques [9]. MMP-9 contributes to the vulnerability of plaques, and high expression of MMP-9 is associated with the destabilization of coronary plaques. Thus, the MMP-9 is able to spread inflammatory signals with it, therefore it is necessary to maintain its activity at an adequate level, otherwise it can cause uncontrolled inflammation, as well as destabilize potentially dangerous coronary plaques [10].In this regard, we investigated one of the common polymorphisms of the MMP -9 gene - RS17576 A> G (orGl279arg), located on the exon 6, is a replacement A with G, which leads to a replacement of unrowned glutamine with appositively charged arginine in a catalytic domain MMP -9[11]. Consequently, this mutation can change enzymatic functional proportions and/or the level of expression, which can play an important role in the pathogenesis of them, as well as in remodeling after transferred [12].

Conclusions. This means that wild homozygous A/A and heterozygous A/G genotypes increase the risk of developing MI. According to it, according to the result of the odds ratio (OR- odds ratio), in carriers of the minor allele G the probability of developing the disease decreased by 29% (OR = 0.71,95% CI: 0.45-1.12), this means that the minor allele G has a protective effect against the development of coronary heart disease. On the other hand, the wild-type A allele of the MMP-9 gene polymorphism rs17576 increased the like-lihood of developing MI by 42% (OR = 1.42; 95% CI:0.91-2.21) and turned out to be a significant risk factor for developing MI.

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