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Role of Gene Polymorphisms of Matrix Metalloproteinases-2, -3 and -13 in the Development of Coronary Artery Atherosclerosis in Patients with Primary Polyosteoarthrosis

Abstract

Rationale. Osteoarthrosis is a chronic non-communicable disease that is observed in more than 10-20% of the world population, and has a leading position in the frequency of patients' disability. In recent years, the development of hypertension, atherosclerosis, as well as cardiovascular complications, has often been reported with underlying progressive osteoarthrosis. The role of matrix metalloproteinases in the course of atherosclerosis in patients with osteoarthrosis is not well understood. **Objective.** To determine the effect of genetic polymorphism of the genes of matrix metalloproteinases (MMPs) -2 (*rs2285053*), -3 (*rs3025058*) and -13 (*rs2252070*) on the development of coronary artery atherosclerosis in patients with primary polyosteoarthrosis. **Methods.** Gene polymorphisms of matrix metalloproteinase-2 (*rs2285053*), -3 (*rs3025058*) and -13 (*rs2252070*) and their relationship with the development of atherosclerosis in patients with osteoarthrosis were defined. **Results.** The study of the polymorphism (*rs2252070 T/C*) of the *MMP-13* gene revealed that the carriage of the homozygous T allele of MMP-13 gene polymorphism was 1.76-fold higher in the group of patients without verified coronary artery atherosclerosis when compared with the group of patients with verified coronary artery atherosclerosis. Therefore, this genotype variant can be positioned as a protective one in relation to the development of atherosclerosis of coronary arteries. The heterozygous variant of *T/C* genotype was more common in the group of patients with verified coronary artery atherosclerosis — 59.1%. Calculation of odds ratio shows that the possibility of coronary artery atherosclerosis in patients with this genotype is 2.7-fold higher than in patients with a homozygous variant. **Conclusion.** Taking into account the results obtained, the heterozygous variant of rs2252070 T/C of matrix metalloproteinase-13 increases the odds of developing coronary artery atherosclerosis in patients with osteoarthrosis.

Keywords: *matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-13, osteoarthrosis, coronary artery atherosclerosis*

Conflict of interests

The authors declare that this paper, its topic, subject and content do not involve competing interests.

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Introduction

Knee and/or hip osteoarthrosis was ranked first in the nosological structure of rheumatic diseases in 2018; its prevalence among all residents of Russia that are 18 years and older was 13% [1]. OA occupies a leading position among diseases that most often lead to the disability of patients. Currently, hypertension [2], atherosclerotic lesions, as well as cardiovascular complications frequently develop in the setting of progressive osteoarthrosis. Genetic predisposition and epigenetic variants with their mutual influence can provoke the development of OA [3] and cardiovascular diseases [4] that often interfere and can be considered as comorbid pathology. This fact is defining for a more severe course of pathological processes [5]. Family history of OA can be the result of the pathological cartilage structure due to a mutation of type II collagen gene CLOL2A1 (localized on chromosome 12); this mutation can lead to the development of cartilage dysplasia and more severe variants of OA [6]. In present-day literature, there are not enough studies of the role of gene polymorphisms of matrix metalloproteinases in the development of OA. In the case of *MMP-31171 5A / 6A* polymorphism, allele *6A*, according to published data, can provoke reduced enzyme synthesis. Therefore, in the presence of variant *5A,* a larger amount of *MMP-3* is formed, which can trigger the rupture of an atherosclerotic plaque [7, 8]. The role of matrix metalloproteinases in the development and progression of atherosclerosis in patients with osteoarthrosis is an urgent problem, but it has not been studied enough.

Objective: to determine the effect of genetic polymorphism of matrix metalloproteinase-2 (*rs2285053*), -3 (*rs3025058*), and -13 (*rs2252070*) genes on the development of coronary artery atherosclerosis in patients with primary polyosteoarthrosis.

Materials and Methods

Study design:

This study enrolled 90 patients diagnosed with primary polyosteoarthrosis (OA). Patients were treated at the Regional Clinical Hospital and at the Clinical Medical Center, Chita, from November 2017 to October 2019. Laboratory tests were conducted at the Laboratory of Experimental and Clinical Biochemistry and Immunology, Research Institute of Molecular Medicine, Chita State Medical Academy.

Inclusion criteria

1. Primary polyosteoarthrosis with damage to three or more groups of joints. Diagnosis was verified based on the clinical classification criteria of the American College of Rheumatology (ACR) taking into account X-ray criteria of Kellgren and Lawrence system [9].
2. The age of subjects ranges from 35 to 55 years.
3. Body mass index (BMI) was under 30 kg/m^2.
4. History of blood pressure (BP) not higher than 179/109 mm Hg with achieved target BP level controlled with antihypertensive drugs (angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers, diuretics, beta-blockers).

The study had the following exclusion criteria:

1. Refusal of the patient to participate in this study
2. Secondary osteoarthrosis
3. Systemic connective tissue diseases
4. Oncological diseases
5. Chronic and acute blood diseases
6. Endocrine diseases
7. Pregnancy and lactation
8. Women in menopause (including surgical)

Description of healthcare intervention:

Molecular genetic analysis was performed using DNA samples extracted from whole blood WBC. Extraction was carried out using a set of reagents for genotyping of polymorphic markers by Real-Time

Table 1. *Polymorphic genetic variants analyzed in the studied patients*

Gene	Chromosomal localization	Single-nucleotide polymorphism
MMP-2	Chr16:55478465 GRCh38 38.1/141	*rs2285053*
MMP-3	Chr11: 1022845217 GRCh38 38.1/141	*rs3025058*
MMP-13	Chr11:10295580 GRCh38 38.1/142	*rs2252070*

PCR (OOO Test-Gene, Russia) according to the manufacturer's recommendations. Expected and observed studies of genotype frequencies were calculated in order to determine compliance with the Hardy-Weinberg equilibrium.

Further analysis included studying association of risk factors, medical history, echocardiography (ECHO-CG), coronary anatomy (according to the results of coronary angiography), lipid profile, and OA characteristics with individual gene polymorphisms. Then, intergenic interactions were analyzed.

Analysis by subgroups:

The patients were divided into 2 groups. Group 1 included 44 patients (mean age 48.79 ± 5.01 years) with primary OA and verified coronary artery atherosclerosis (CA) confirmed by coronary angiography. Group 2 included 46 patients (mean age 44.32 ± 5.4 years) with primary OA and no atherosclerotic changes in vessels according to Doppler ultrasound of brachiocephalic arteries, vessels of lower extremities, no clinical or medical history of coronary heart disease (CHD).

For all study participants, genotypes of *MMP-2, MMP-3, MMP-13* were defined (Table 1).

Ethical review:

All patients signed an informed consent to participate in this study.

This study was approved by the Local Ethics Committee of Chita State Medical Academy of the Ministry of Health of the Russian Federation (No. 86 dated November 1, 2017).

Statistical data analysis:

Microsoft Excel 2016 and Statistica, version 10.0 (StatSoft) were used for statistical data processing. Yates's correction for continuity of Pearson's csquare (c^2) was used to compare discrete values, as well as to calculate the correspondence of the observed frequency distributions of genotypes theoretically expected according to the Hardy Weinberg law. Since the distribution of signs differed from normal distribution, nonparametric statistics methods were used. To compare the two groups, Mann-Whitney U test with Bonferroni correction was used. Differences were considered as statistically significant at $p < 0.05$.

Association assessment was calculated in terms of OR (odds ratio) and RR (relative risk) values indicating 95% confidence interval (CI).

Results

Distribution of genotype frequency in the groups of patients was found to correspond to the Hardy Weinberg continuum.

Genetic test revealed that carriage of a homozygous C allele of polymorphism (*rs2285053 C/T*) of *MMP-2* gene in groups of patients with verified coronary artery atherosclerosis was observed in 77.27% (Table 2).

Analysis of baseline data in study groups showed no difference between the genotypes. Homozygous *T/T* variant of *MMP-2* gene was not found.

A comparative study of the frequencies of genotypes of *MMP-3* polymorphic loci demonstrated that the carriage of homozygous T allele of (*rs3025058 T/C*) of *MMP-3* gene polymorphism and of heterozygous variant of *T/C* genotype was almost equally distributed in the groups of patients with and without verified coronary artery atherosclerosis (Table 3). Homozygous *C/C* variant of *MMP-3* gene was not found in any of the studied groups. Analysis of baseline data in study groups showed no difference between the genotypes. There were no statistically significant differences in the prevalence of alleles and genotypes in the studied groups.

Table 2. *Distribution of allele frequency and genotypes of MMP 2 rs2285053 C/T gene polymorphism in patients with OA*

Groups of patients	Genotypes, n (%): RR (95% CI)			χ²/p	Alleles		χ²/p
	CC	CT	TT		C	T	
Group I (n=44)	34 (77.27) RR =1.23 (0.58-3.82) OR =1.49	10 (22.72)	0	p_{I-II} 0.56	78 (88.6)	10 (11.4)	p_{I-II} 0.58
Group II (n=46)	32 (69.56)	14 (30.43)	0		78 (84.8)	14 (15.2)	

RR — risk of coronary artery atherosclerosis in the presence of the studied genetic marker in comparison with the group without coronary artery atherosclerosis; 95% CI — confidence interval; OR — odds ratio whether coronary artery atherosclerosis will develop upon detection of this genetic marker or no coronary artery damage will develop

Note: P-value for the difference is < 0.05, OR — odds ratio, RR — relative risk, CI — confident interval

Table 3. *Distribution of allele frequency and genotypes of MMP-3 rs2285053 C/T gene polymorphism in patients with OA*

Groups of patients	Genotypes, n (%), RR (95% CI)			χ² / p	Alleles		χ² / p
	TT	TC	CC		T	C	
Group I (n=44)	26 (59) RR =1 (0.44-2.36) OR =1.02	18 (41)	0	pI-II 0.87	70 (79.54)	18 (20.46)	pI-II 0.87
Group II (n=46)	27 (58.69) RR=0.99 OR=0.98	19 (41.31)	0		73 (79.34)	19 (20.66)	

RR — risk of coronary artery atherosclerosis in the presence of the studied genetic marker in comparison with the group without coronary artery atherosclerosis; 95% CI — confidence interval; OR — odds ratio whether coronary artery atherosclerosis will develop upon detection of this genetic marker or no coronary artery damage will develop

Note: P-value for the difference is < 0.05, OR — odds ratio, RR — relative risk, CI — confident interval

Studying the polymorphism (*rs2252070 T/C*) of *MMP-13* gene revealed that the carriage of homozygous *T* variant of *MMP-13* gene polymorphism was 1.9 times higher in patients without verified coronary artery atherosclerosis compared with the group of patients with severe CA where the prevalence of this genotype amounted to only 31.8% (p = 0.031) (Table 4). Heterozygous variant of *T/C* genotype was more common in patients with verified coronary artery atherosclerosis — 59.1%, which is 1.7 times more often than in the group without coronary artery atherosclerosis (p = 0.036). Calculation of the odds ratio for *MMP-13* genotype showed that carriage of *MMP-13 TC* genotype (CI 95% 1.15-6.36) increases the risk of CA development by 2.7 times.

Discussion

The studies proved the great role of the metalloproteinase component in the degradation of extracellular matrix and, as a result, in the pathogenesis of various CVDs (atherosclerosis, restenosis, cardiomyopathy, myocardial infarction, chronic heart failure, aortic aneurysm) [4, 7]. It was found that the intensity of immune reactivity is a genetic component, and single nucleotide polymorphisms (SNPs) in the sense parts of genes that are responsible for the synthesis of the interleukin component of inflammation and MMP often have an effect on the following: stability of tertiary protein structures, variants of binding of protein components to the substrate. In accordance with the changes occurred,

Table 4. *Distribution of allele frequency and genotypes of MMP-13 rs2285053 C/T gene polymorphism in patients with OA*

Groups of patients Genotypes and alleles, n (%), RR (95% CI)	Group I (n=44)	Group II (n=46)	χ^2/ p_{I-II}
TT	14 (31.81) RR =0.58 (0,15-0.85) OR =0.36	26 (56.52)	**0.031**
TC	26 (59.1) RR =1.65 (1.29-7.42) OR =2.7	16 (34.78)	**0.036**
CC	4 (9.09) RR =1.02 (0.26-4.48) OR =1.05	4 (8.7)	0.76
T	54 (61.36)	68 (73.9)	0.1
C	34 (38.64)	24 (26.1)	

Note: RR — risk of coronary artery atherosclerosis in the presence of the studied genetic marker in comparison with the group without coronary artery atherosclerosis; 95% CI — confidence interval; OR — odds ratio whether coronary artery atherosclerosis will develop upon detection of this genetic marker or no coronary artery damage will develop. P-value for the difference is <0.05, OR — odds ratio, RR — relative risk, CI — confident interval

functional activity of synthesized proteins becomes unstable. Genetic polymorphism can have a neutral effect, and can also severely disrupt the functional characteristics of the synthesized protein product. This fact means that genetic polymorphisms under certain conditions predispose or prevent a number of pathologies [7]. At present, the study of genetic polymorphisms predisposing to the occurrence of various diseases is ongoing. Researchers proved that MMPs expressed in myocardium could participate in the degradation of cardiac extracellular matrix that becomes an important factor in the processes of myocardial remodeling [7]. It was found that MMPs in heart tissues are synthesized by fibroblast-like cells and cardiomyocytes mainly in an inactive state; their expression and degree of activity increase during the course of pathological processes in myocardium [10].

If there are no pathological processes, a large number of MMPs (including -2, -3, -13) are synthesized in the joint tissue in small quantities; at the same time the level of MMP component increases rapidly during the course of inflammatory processes. During OA pathogenesis, proinflammatory cytokines (TNF-α and IL-1β) can bind to complementary chondrocyte receptors and cause activation of signaling pathways followed by hyperactivation of the expression of matrix metalloproteinases [3]. The most intense synthesis of MMP-13 takes place in chondrocytes.

MMP-13 plays a major role in the degradation of cartilage tissue, since a large number of catabolic reactions increases its activity. In addition, the study of atherosclerotic plaques allowed the detailed study of two polymorphic variants in the promoter of the gene that encodes this substrate. These polymorphisms include the insertion of additional adenine residue -291 (11A/12A), as well as the transition of -77G/A in the regulatory element of the promoter (*rs17860523*). According to the data [3], the presence of estrogen receptors of a certain type increased the expression of all of the listed polymorphic variants of *MMP-13*, which may be related to joint dysfunction leading to the development of OA in menopausal women.

In our study, analysis of baseline data in the study groups revealed no statistically significant differences in the prevalence of alleles and genotypes of *MMP-2 (rs2285053 C/T) and MMP-3 (rs3025058 T/C*). At the same time, a significant increase in the carriage of the homozygous T allele variant of MMP-13 polymorphism (*rs2252070 T/C*) was found in the group of patients with primary OA without verified atherosclerotic lesions, while there were significantly more carriers of the heterozygous variant of the studied *MMP-13* polymorphism in the group of patients with primary OA with hemodynamically significant coronary artery atherosclerosis.

Conclusions

Thus, in patients with primary osteoarthrosis in combination with hemodynamically significant coronary artery atherosclerosis, the carriage of homozygous T polymorphism of *MMP-13* gene (*rs2252070*) is most likely protective, while the heterozygous variant of *MMP-13* polymorphism (*rs2252070 T/C*) increases the risk of developing hemodynamically significant atherosclerotic lesions of coronary arteries in patients with osteoarthritis by 2.7 times. These data can be used to reach a firmer consensus in terms of additional testing of patients with primary polyosteoarthrosis for the presence of hemodynamically significant coronary artery atherosclerosis, which is very important for successful treatment of this category of patients.

Author Contribution:

Portyannikova, O.O. (ORCID ID: https://orcid.org/0000-0002-2565-3839): The author selected patients for the study taking into account special aspects of clinical and medical histories

Romanova, E.N.: The author developed the study design: ideas, goals and objectives, analyzed local and foreign literature

Govorin, A.V.: The author performed detailed statistical analysis of information

Zwinger S.M. (ORCID ID: https://orcid.org/0000-0001-8030-7659): The author independently carried out blood sampling and a number of laboratory research methods

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