Does Matrix Metalloproteinase-3 Polymorphism Play a Role in age-Related Macular Degeneration in Patients With Myocardial Infarction?

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Summary

Objective—The aim of our study was to determine if the genotype of the matrix metalloproteinase-3 (MMP-3) gene might carry the risk of age-related macular degeneration (ARMD) in patients with myocardial infarction.

Material and Methods—A total of 499 patients with an acute myocardial infarction or with a history of myocardial infarction were enrolled into the study. They were subdivided into 2 groups: 273 patients with ARMD and 226 patients without ARMD. The control group comprised 560 persons from a random sample of the Lithuanian population. DNA was analyzed using real-time polymerase chain reaction to genotype polymorphism 5A/6A at a position −1171 of the MMP-3 gene promoter.

Results—Of the 499 patients with myocardial infarction, 47% had early-stage ARMD. The patients with ARMD were older than the patients in the group without ARMD (62.1±10.8 vs. 59.6±11.1, P<0.01). The analysis of MMP-3 gene polymorphism did not reveal any differences in the distribution of 5A/5A, 5A/6A, and 6A/6A genotypes between the ARMD group, non-ARMD group, and the control group (24.2%, 52.5%, and 23.3% in the ARMD group; 28.7%, 51.9%, and 19.4% in non-ARMD group; and 25.7%, 49.3% and 25.0%, in the control group, respectively).

Conclusions—MMP-3 gene polymorphism had no predominant effect on the development of ARMD in patients with myocardial infarction.

Keywords

age-related macular degeneration; myocardial infarction; neovascularization; matrix metalloproteinase-3 gene polymorphism

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Introduction

Age-related macular degeneration (ARMD) is a leading cause of irreversible blindness in elderly people. ARMD is the most common cause of visual loss in persons aged more than 60 years in the developed countries (1). Population estimates have placed the prevalence of ARMD approximately at the level of 7% to 10% among adults aged 40-90 years (2, 3). Despite the magnitude of this problem, the etiopathogenesis of ARMD remains poorly understood. ARMD is a disease of multifactorial etiology. Its development is determined by genetic, inherited, and environmental risk factors. Older age, female gender, oxidative stress, long-term exposure to sunlight, low levels of nutritional antioxidants, nutrition habits, and traditional risk factors for ischemic heart disease (IHD) (cigarette smoking, diabetes mellitus, hypertension, obesity, hypercholesterolemia, and alcohol abuse) have been implicated as possible etiological factors for the development of ARMD (4-8).

In general, the most important pathogenetic mechanisms causing the development of ARMD are the formation of drusen, local inflammation, and neovascularization (9). Recent studies have demonstrated that angiogenesis is also associated with an important extracellular remodeling involving different proteolytic systems among which matrix metalloproteinases (MMP) play an essential role (10, 11). MMP-3 (also known as stromelysin-1) is a key member of the MMP family that plays a pivotal role in the physiological and pathological events associated with connective tissue metabolism and remodeling (12, 13). Consequently, *MMP-3* gene polymorphism could have an influence on retinal vascular remodeling and stiffening and plays a role in the development of ARMD. Literature data concerning the impact of MMP-3 on ARMD are scarce and inconsistent. Some results have suggested a possible MMP-3 effect on ARMD pathogenesis (14) and are in conflict with the data from the other study assuming that *MMP-3* expression does not play a role in the development of ARMD (15). To date, there are no studies analyzing the impact of *MMP-3* gene polymorphism on ARMD.

Assuming the fact that MMP-3 could have an influence on retinal vascular remodeling, we hypothesized that the *MMP-3* gene may carry risk in the development of ARMD. To test our hypothesis, the *5A/6A MMP-3* polymorphism in ARMD and non-ARMD patients with myocardial infarction (MI) and a random sample of the Lithuanian population without evidences of IHD and ARMD was evaluated.

Material and Methods

Study Population

A total of 499 patients with an acute MI or with a documented history of MI admitted to the Clinic of Cardiology, Hospital of Lithuanian University of Health Sciences, were randomly enrolled into the study. All the patients had a diagnosis of an acute MI made on the basis of prolonged chest pain, elevated troponin I serum level, ST-segment and T-wave abnormalities on ECG, and typical wall motion abnormalities on the baseline 2-D echocardiogram or previous MI diagnosed based on clinical data of the patient and official medical records. All the patients underwent routine blood testing. The patients completed a standardized questionnaire on IHD risk factors and underwent an ophthalmological examination. Based on the results of the ophthalmological examination, the patients were subdivided into 2 groups: 273 patients with ARMD and 226 without ARMD.

The control group (n=560) consisted of a random sample of Kaunas population without the symptoms of IHD, stroke, and ARMD, taken from the epidemiological Health, Alcohol, and Psychosocial Factors in Eastern Europe study.

The study protocol was approved by the Local Ethics Committee (No. BE-2-14).

Ophthalmological Examination

Visual acuity (VA) was estimated using letter charts and was expressed as decimal notations. All the patients were evaluated by slit lamp biomicroscopy. Biomicroscopy was performed in order to assess the corneal and lenticular transparency.

During each examination, the intraocular pressure was measured. Pupils of the subjects were dilated with 1% tropicamide. After the dilation of the pupils, funduscopy was performed with an ophthalmoscope of the direct monocular type and a slit lamp, using a double aspheric lens of +78 diopters. The results of the examination were recorded on standardized forms, which were developed for this study.

Color fundus photographs were taken with a semiwide angle fundus camera (OPTON SBG, 30 degrees). The photographs were taken focusing to the center of the fovea.

The following exclusion criteria were used: 1) unrelated eye disorders, e.g., high refractive error, cloudy cornea, lens opacity (nuclear, cortical, or posterior subcapsular cataract), keratitis, acute or chronic uveitis, glaucoma, or diseases of the optic nerve; 2) systemic illnesses, e.g., diabetes mellitus, malignant tumors, systemic connective tissue disorders, chronic infectious diseases, or conditions following organ or tissue transplantation; and 3) ungraded color fundus photographs resulting from obscuration of the ocular optic system or because of fundus photograph quality.

The classification of ARMD presented in the Age-Related Eye Disease Study was used (16) according to which early ARMD is defined as a combination of multiple small and few intermediate drusen (63 to 124 μm in diameter) or retinal pigment epithelial abnormalities. Intermediate ARMD is characterized by the presence of extensive intermediate drusen and/or at least one large (giant) druse (125 μm in diameter) or geographic atrophy (GA) not involving the center of the fovea, whereas advanced ARMD by GA involving the fovea and/or or any of the features of neovascular ARMD (16). The diagnosis of early ARMD was made if it was confirmed by 2 experienced ophthalmologists and if no other eye disorders were documented during a detailed ophthalmological examination.

DNA Extraction and Genotyping

DNA extraction and analysis of the gene polymorphism of MMPs were carried out at the Laboratory of Molecular Cardiology, Institute of Cardiology, Lithuanian University of Health Sciences (LUHS). DNA was extracted from 2 mL of peripheral blood (from white blood cells) using a genomic DNA purification kit (AB Ferment) according to the manufacturer's recommendations or the silica gel column method utilizing a genomic DNA extraction kit (SorpoClean™ Genomic DNA Extraction Module, SORPO Diagnostics) according to the manufacturer's recommendations.

The genotyping of *MMP-3* (−1171 *5A/6A*) was carried out using the real-time polymerase chain reaction (PCR) method. The primers and molecular markers for the genotyping of *MMP-3* (−1171 *5A/6A*) (rs3025058) were produced by the German company Metabion according to the primer sequences determined and furnished by the scientists of the Laboratory of Molecular Cardiology at the Institute of Cardiology of LUHS. To ensure internal control, 20 samples were sequenced at the Sequencing Center of the Institute of Biotechnology, and the obtained results confirmed the reiteration and precision of the data. The genotyping was performed using an HT 7900 real-time PCR quantification system (Applied Biosystems, USA). The real-time PCR reagents (2X Maxima™ Probe/ROX qPCR Master mix buffer, fluorescent dye-labeled markers, sterile ddH$_2$O) were taken out from the

environment of −20°C and were thawed at room temperature. The thawed reagents were decentrifuged (10 000 rpm) and stored in an ice tub. An appropriate real-time PCR mix ture of *MMP-3* (−1171 5A/6A) was prepared for determining a single-nucleotide polymorphism.

A PCR reaction mixture (9 μL) was poured into each well of a 96-well microtiter plate, and then 1 μL of matrix DNA of the samples (~10 ng) and 1 μL of negative control (−K) were added. An optic film was pasted on the 96-well microtiter plate, and it was centrifuged for 15 seconds at 10 000 rpm.

During the genotyping, the following real-time PCR programs were used: Allelic Discrimination and Absolute quantification. Then, the assay was continued following the manual provided by the manufacturer (www.appliedbiosystem.com, Allelic Discrimination Getting Started Guide). After that, the Allelic Discrimination program was completed, and the geno typing results were received. The program determined the individual geno types according to the fluorescence intensity rate of different detectors. A molecular marker labeled with a fluorescent dye, VIC®, or Yakima Yellow® was chosen for the x-axis, and a molecular marker labeled with a fluores cent dye, FAM, was selected for the y-axis.

Statistical Analysis

Continuous data were expressed as means (SD). Categorical data were summarized as frequencies and percentages. The Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of the *MMP* genotypes using the χ^2 test in all groups. The distribution of the *MMP* single nucleotide polymorphism in the ARMD and control group was compared by using the Fisher exact test. In order to predict the impact of age or recurrent event on ARMD development in an individual patient, logistic regression analysis was performed.

The statistical significance level was set at a *P* value of <0.05. The data were analyzed using the standard statistical software SPSS/W 13.0 (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA).

Results

Of the 499 patients with MI, 273 (47%) had early-stage ARMD. Table 1 shows the baseline characteristics of the study population. In general, patients with ARMD were older than non-ARMD patients (*P*<0.001) and had a recurrent MI or recurrent symptoms of unstable angina more frequently (*P*<0.05) (Table 1). Univariate logistic regression analysis revealed that older age and recurrent cardiac events increased a chance to develop ARMD (OR, 1.02; 95% CI, 1.004–1.038; *P*=0.013; and OR, 1.54; 95% CI, 1.043–2.270; *P*=0.030, respectively), but after the adjustment for age, no additional impact of recurrent cardiac events on ARMD development was documented (OR, 0.67; 95% CI, 0.320–1.401; *P*=0.67). Age remained the only independent predictor of ARMD development (OR, 1.02; 95% CI 1.006–1.043; *P*=0.01).

Gender, systolic and diastolic blood pressure, body mass index, prior use of any cardiovascular medication, severity of IHD, and white blood cell and platelet counts were not associated with the development of ARMD.

Cardiovascular risk factors of the study population are presented in Table 2. There were no significant differences in the frequency of any cardiovascular risk factor comparing patients with and without ARMD. Comparing both patients' groups with the control group, a significant difference was observed only in the frequency of arterial hypertension, which was more common in the patients with and without ARMD than in the control group

(*P*<0.001). A history of dyslipidemia and a family history of IHD did not show any differences comparing the groups. Cigarette smoking status, obesity, physical activity, and alcohol consumption showed no associations with ARMD.

Genotype Distribution

The prevalence of *MMP-3 5A/6A* genotypes was 27.7% (*5A/5A*), 52.3% (*5A/6A*), and 20.0% (*6A/6A*) in both patients' groups taken together and 25.7% (*5A/5A*), 49.3% (*5A/6A*), and 25.0% (*6A/6A*) in the control group. The genotype distribution was in the Hardy-Weinberg equilibrium. There were no significant differences in the genotypic distribution of *MMP-3 5A/6A* polymorphism and allele (*5A, 6A*) frequencies comparing the patients with and without ARMD and the control group (Table 3).

Discussion

Our study aimed at determining if the *MMP-3* gene may carry risk in the development of ARMD. Unfortunately, the results showed no differences in the genotypic distribution of *MMP-3* polymorphism comparing the patients with and without ARMD, who were admitted with current or previous myocardial infarction, and the control group. Some previous studies have analyzed the relations between ARMD and some genetic factors; however, most studies analyzed other risk factors for ARMD. For a long time, atherosclerosis has been proposed as a key pathogenic factor for ARMD (17-23). Some investigators have suggested that increased arterial blood pressure, hyperlipidemia, family history of IHD, low physical activity, smoking, and overweight by virtue of their effects on the choroidal circulation and lipid deposition in the Bruch's membrane may be related to the pathogenesis ARMD (3-7, 20, 24) as well as to the development of atherosclerosis and IHD (25-27). ARMD and IHD share the same risk factors, and a few previous studies have reported an association between ARMD and incidence of MI (17, 21, 25). Therefore, we hypothesized that genetic polymorphism of *MMP-3* also may influence the development of ARMD as it has been described in some studies as a risk factor in the development of MI and IHD as well as in the pathogenesis of ARMD.

The pathogenesis of ARMD is a complex of processes. Recent studies have demonstrated that hypoxia is not the only factor inducing neoangiogenesis. Angiogenesis is also associated with the imbalance between positive and negative regulatory molecules controlling angiogenesis, vascular endothelial grow factors (VEGF), and pigment epithelium-derived factors playing an important role in the choroidal neovascular membrane formation (28, 29) and important extracellular remodeling involving different proteolytic systems, among which MMPs play an essential role (30, 31). MMPs are involved in the different steps of angiogenesis. They facilitate endothelial cell (EC) migration by releasing them from their basement membranes, degrading perivascular extracellular matrix (ECM), and generating ECM degradation products that are chemotactic for EC. Additionally, MMPs increase the bioavailability of VEGF and other peptide growth factors through the degradation of ECM proteins, which sequester growth factors.

MMP-3 is a key member of the MMPs family and plays a role in the physiological and pathological events associated with connective tissue metabolism and remodeling (32). Current evidence suggests that MMPs play a role in early atherosclerosis, plaque rupture, and MI. Some studies have shown the associations between the low-activity *6A* allele and both coronary and carotid atherosclerosis, and increased intima-media thickness (33-35). This suggests that individuals with the *6A/6A* genotype, which leads to the lower production of MMP-3, have an altered matrix turnover favoring the deposition of matrix and accelerated atherosclerosis. Contrary, a number of studies have shown an association between the high-activity *5A* allele and acute coronary events suggesting a role in plaque

rupture due to increased proteolysis (36). Few studies have been conducted to clarify if the *MMP-3* gene may have an influence on retinal vascular remodeling and stiffening and play a role in the development of ARMD. A German study showed that *MMP-3* expression in the retinal pigment epithelium was inducible by oxidative stress; thus, oxidative stress can be considered as one of the risk factors for the development of ARMD (14). Swedish researchers conducted a study in which surgically removed subfoveal fibrovascular membranes from eyes were analyzed for the expression of several MMPs (including MMP-3). Their results supported a role for MMPs in the development of choroidal neovascularization in ARMD (15).

However, our results did not show any differences in the distribution of genotypes comparing the patients with and without ARMD and the control group. Age was the only risk factor for ARMD in our study. These findings confirm the importance of the aging process in the vessels in the development of ARMD (6, 17, 18).

The main limitation of our study is that there were no patients with ARMD without IHD, who could be enrolled as a control group. The other limitation is that the prevalence of ARMD was not evaluated in a random sample of the population. We believe we will have a chance to investigate the impact of genetic *MMP-3* polymorphism on the development of ARMD in patients with ARMD with IHD and without IHD.

Conclusions

In our studied patients' population with current or previous myocardial infarction, *MMP-3* gene polymorphism had no predominant effect on the development of ARMD in patients with myocardial infarction. Larger-scale and repeated studies should be carried out to check this hypothesis.

Acknowledgments

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Table 1

Baseline Characteristics of the Study Population

Characteristic	ARMD Group n = 273	Non-ARMD Group n = 226	Control Group n = 560
Age, years	62.1 (10.8)	59.6 (11.1)[*]	61.22 (7.82)[†]
Men, n (%)	207 (75.8)	184 (81.4)	450 (80.4)
Recurrent myocardial infarction or angina, n (%)	97 (36.9)	57 (26.1)[*]	…
One-vessel disease	97 (39.1)	101 (47.2)	…
Two-vessel disease	80 (32.3)	61 (28.5)	
Three-vessel disease	71 (28.6)	52 (24.3)	
Educational level, n (%)			
Lower than higher	103 (51.5)	105 (63.3)	
Higher	97 (48.5)	78 (46.7)	…
History of drug treatment, n (%)			
Calcium channel blocker	19 (7.0)	22 (9.7)	
Statin	18 (6.6)	15 (6.6)	…
Beta-blocker	66 (24.2)	49 (21.7)	
ACE	66 (24.2)	47 (20.8)	
Diuretic	10 (3.7)	9 (4.0)	
Antiplatelet (aspirin/clopidogrel)	50 (18.3)	42 (18.6)	
Systolic blood pressure, mm Hg	144.1 (21.9)	143.5 (21.1)	…
Diastolic blood pressure, mm Hg	86.9 (11.4)	87.8 (12.1)	…
Body mass index, kg/m^2	28.8 (4.8)	28.1 (4.4)	28.41 (4.9)
White blood cell count, $\times 10^9$/L	9.24 (3.5)	9.5 (3.0)	…
Neutrophil count, $\times 10^9$/L	7.1 (2.8)	8.0 (2.6)	…
Monocyte count, $\times 10^9$/L	0.7 (0.4)	0.8 (0.8)	…
Lymphocyte count, $\times 10^9$/L	1.8 (0.8)	1.9 (0.9)	…
Platelet count, $\times 10^9$/L	234.1 (52.2)	261.5 (92.3)	…

Values are mean (standard deviation) unless otherwise stated. Ellipses indicate no data available.

ACE, angiotensin-converting enzyme; ARMD, age-related macular degeneration.

[*] $P<0.05$ between non-ARMD and ARMD groups;

[†] $P<0.05$ between patients with ARMD or without ARMD taken together and control group.

Table 2

Cardiovascular Risk Factors in the Study Population With and Without ARMD and the Control Group

Risk Factor	ARMD Group n = 273	Non-ARMD Group n = 226	Control Group n = 560
History of arterial hypertension	228 (83.5)	181 (80.1)	379 (67.9)[*]
Cigarette smoking			
Never	104 (42.8)	80 (39.9)	282 (50.4)
Current or withdrawn	139 (57.2)	137 (60.1)	278 (49.6)
Body mass index			
Normal, <25 kg/m^2	61 (22.3)	56 (24.8)	132 (23.6)
Overweight, 25–30 kg/m^2	114 (41.8)	99 (43.8)	246 (43.9)
Obesity, 30 kg/m^2	98 (35.9)	71 (31.4)	182 (32.5)
History of dyslipidemia	213 (79.5)	160 (75.7)	476 (85.0)
Family history of IHD	59 (35.8)	46 (32.9)	230 (41.7)
Physical activity			
None	98 (61.2)	97 (66.4)	
1–2 times a month	14 (8.8)	9 (6.2)	…
1–2 times a week	16 (10.0)	18 (12.3)	
More than 3 times a week	32 (20.0)	22 (15.1)	
Alcohol consumption			
None	5 (8.3)	5 (6.5)	
Past	7 (11.7)	2 (2.6)	
Less than 1 time a month	35 (58.3)	43 (55.8)	…
1–7 times a week	20 (12.0)	26 (33.8)	
More than 7 times a week	1 (1.7)	1 (1.3)	

Values are number (percentage). Ellipses indicate no data available.

IHD, ischemic heart disease; ARMD, age-related macular degeneration

[*] *P*<0.05 between patients with ARMD or without ARMD taken together and control group.

Table 3

Genotypic Distribution of *MMP-3* Gene Polymorphism and Allele Frequencies in the Study Population With and Without ARMD Versus the Control Group

Genotype	ARMD Group	Non-ARMD Group	Control Group	*P*
5A/5A	54 (24.2)	77 (28.7)	144 (25.7)	NS
5A/6A	117 (52.5)	139 (51.9)	276 (49.3)	NS
6A/6A	52 (23.3)	52 (19.4)	140 (25.0)	NS
Allele frequency				
5A	0.50	0.55	0.50	NS
6A	0.50	0.45	0.50	NS

Values are number (percentage)

5A or *6A* allele frequency was calculated as follows: (2×percentage of *5A/5A* or *6A/6A* genotype frequency+1×percentage of *5A/6A* genotype frequency)/200.

ARMD, age-related macular degeneration; NS, not significant.