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ASSOCIATION OF THE rs1799750 MATRIX METALLOPROTEINASE-1 GENE POLYMORPHISM AND CORONARY ARTERY DISEASE IN YOUNG MACEDONIAN POPULATION

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Abstract

Coronary artery disease (CAD) is very complex disease arising from close interaction of many risk-factors as well as presence of many comorbidities. Pathophysiology mechanisms may be different and encompass endothelial dysfunction, impaired lipid metabolism, chronic inflammation, thrombosis, and mechanisms associated with tissue maintenance and remodeling.

In this research we aim to investigate the association between rs1799750 (-1607 1G/2G) matrix metalloproteinase – 1 (MMP-1) gene polymorphism and CAD in young Macedonian population.

This is an observational, genetic-association study of cases and controls including 57 participants divided into two groups. The first is the group with positive coronary angiography (CA) finding (n=34 participants) and the second is the group with negative CA finding (controls, n=23 participants).

All of them underwent molecular and genetic analyses after performed CA. Complete comparison of the frequencies of genotypes and alleles of the rs1799750 MMP-1 gene polymorphism was used for statistical analysis.

Calculations were performed using Chi-square test (χ^2-test) and Fisher's exact test for analysis of the genotype and allele frequencies of the gene polymorphism using five different models.

The Cochran–Armitage trend test was used to analyze the allelic frequencies with the allelic and additive model. The statistical analyses were performed using XLSTAT 2016, GenAlEx 6.5 and Microsoft Excel 2016 software.

According to the genotypic model, carriers of the heterozygous 1G/2G genotype have 2,8 times higher probability whereas carriers of the 2G/2G genotype have 7,389 times higher probability for development of CAD in comparison to the reference carriers of 1G/1G, respectively ($p<0,05$).

The dominant model has also confirmed that genotype carriers with at least one 2G allele have 4,521 times higher probability for CAD in comparison to homozygous 1G/1G genotype carriers ($p<0,05$).

According to the recessive model, participants with homozygous 2G/2G genotype have statistically significant 3,589 times higher probability for CAD in comparison to participants with at least one 1G allele ($p<0,05$).

Allelic model also proved that carriers of the 2G allele have 3 times higher chances for development of CAD than the carriers of the 1G allele ($p<0,05$).

The last, additive model, confirmed that the risk increases with the number of present 2G allele.

Results from our study clearly show that there is statistically significant genetic association of the rs1799750 MMP-1 gene polymorphism with significant CAD in young Macedonian population. More specifically, presence of genotype 2G/2G as well as allele 2G leads to statistically significant increase of the probability for CAD.

***Keywords*:** CAD, MMP-1, gene polymorphism, rs1799750.

Introduction

Coronary artery disease (CAD) remains the major single cause of morbidity and mortality worldwide. It is very complex disease arising from the close interaction of many risk-factors including lifestyle habits (physical inactivity, dietary habits, smoking, drugs, overweight, increased alcohol intake etc.), environmental factors (air pollution, food pollution…), psychosocial factors (stress), ethnic profile, genetic factors as well as presence of many comorbidities which significantly increase the risk for CAD (arterial hypertension, diabetes, dyslipidemia, chronic kidney disease…).

In regard to these risk-factors, pathophysiology mechanisms leading to development of atherosclerotic CAD may be different and encompass endothelial dysfunction, impaired lipid metabolism, chronic inflammation, intravascular coagulation/thrombosis, and mechanisms associated with tissue maintenance and remodeling. However, genetic factors are probably the most important since they determine and control all previously mentioned mechanisms. They are especially important in young population where exposition to the conventional risk-factors for atherosclerotic CAD is very short or even absent [1, 2].

Genetic factors for development of atherosclerotic CAD are very heterogenous and some of them are involved in the process of tissue maintenance and remodeling. Atherosclerotic lesions/plaques of the coronary arteries have fibrous caps with certain thickness which stabilize them. They consist of extracellular matrix (ECM) including collagen end elastin. Destruction of these proteins may lead to disruption of the fibrous cap and destabilization of the atherosclerotic plaques making them vulnerable and prone to erosion or rupture – which is basically the pathogenetic mechanism of the acute coronary syndrome (ACS) [3].

Degradation of the ECM proteins is a complex process involving so-called matrix metalloproteinases (MMPs) – a family of zinc-dependent endopeptidases (enzymes). There are five types of MMPs: collagenases, gelatinases, stromelysines, matrilysines and membrane-type MMPs [4].

Literature data suggest that regions of vulnerable atherosclerotic plaques have elevated levels of MMPs [3, 5, 6].

Biological activity of the MMPs is generally determined by and dependent on the MMP gene polymorphisms [7].

MMP-1 is an enzyme that belongs to the class of interstitial collagenases. It is expressed in high levels in the regions of vulnerable atherosclerotic plaques. One of the most important and most researched gene polymorphisms of the MMP-1 is -1607 1G/2G (rs1799750) - insertion-deletion polymorphism at position -1607 in the promoter region [8].

In this study we have decided to investigate the association of this MMP-1 gene polymorphism and CAD in young Macedonian population. The term “young” or “younger” here refers generally to the population starting from 18 up to 45 years old from both sexes.

Materials and methods

Design of the study, subjects and criteria

This is an observational, genetic-association study of cases and controls that includes totally 57 participants with already performed coronary angiography after an appropriate medical indication. Participants are divided in 2 groups: the first group refers to 34 participants with positive coronary

angiography finding (presence of at least one significant coronary lesion) and the second group refers to 23 controls with negative coronary angiography finding (normal finding or finding without significant coronary lesions).

All of them were selected according to the appropriate inclusion and exclusion criteria.

Inclusion criteria are: signed inform consent for participation in the study, participants form both sexes from 18 to 45 years old and previously performed coronary angiography according to an appropriate medical indication. Exclusion criteria are: diabetes, arterial hypertension, dyslipidemia, valvular disease, cardiomyopathies, congenital heart disease, active malignancy, acute or chronic inflammatory and infectious diseases, pregnancy, relative or absolute contraindication for coronary angiography and/or percutaneous coronary intervention. Smoking cigarettes and body mass index (BMI) over 25 kg/m^2 were not exclusion criteria.

All demographic, clinical and laboratory data and samples of 3 ml venous blood have been collected from the participants who were patients and were treated at the University Clinic of Cardiology in Skopje.

Prior to the inclusion in the study, every single participant signed an Inform Consent for participation in the study. The research has been approved by the Ethical Committee at the Faculty of Medicine, University "Ss. Cyril and Methodius" in Skopje, N. Macedonia.

Molecular and genetic analysis

Molecular and genetic analyses have been performed in the Laboratory of Molecular Biology and Genomics at the Faculty of Natural Sciences in Skopje, N. Macedonia. All participants underwent isolation of genomic DNA from 3 ml venous blood samples with Na-EDTA, salting out with NaCl, extraction with chloroform and subsequent precipitation with ethanol (Gemmell and Akiyama, 1996). Genotype determination of the rs1799750 (-1607 1G/2G) MMP-1 gene polymorphism was performed in every single participant by polymerase-chain reaction amplification in real-time (RT-PCR) using TaqMan fluorescent probes with nucleotide sequence specific for the amplificated region of the appropriate gene (5`-end labeled with fluorescent marker FAM or VIC and 3`-end labeled with non-fluorescent quencher (NFQ).

The sequences of oligonucleotide primers for amplification and the sequences of the TaqMan fluorescent probes are shown in the table 1 [9].

Table 1. Sequences of the oligonucleotides and probe sequence

Gene polymorphism	Oligonucleotide sequence	Probe sequence or restrictive enzyme
MMP-1	5'-TGCCACTTAGATGAGGAAATTGTAGT-3'	1G 5'-FAM-ATAATTAGAAAGATATGACTTATC-MGB-3'
rs1799750	5'-ACACTTTCCTCCCCTTATGGATTC-3'	2G 5'-VIC-ATAATTAGAAAGGATATGACTTAT-MGB-3'

Amplificability of the samples has been checked by PCR amplification of the region from the gene for β-globin. Oligonucleotide primers and primers of the TaqMan probes belong to the company Thermo-Fischer Scientific. Amplification and reading of the fluorescence in the real time have been performed on the StepOne RT-PCR System (Applied Biosystems) whereas the obtained curves and data have been analyzed with the StepOne software – an integral part of the system. Genotype determination has been performed with the method of allelic discrimination and end-point genotyping of the amplification.

Statistical analyses

Complete comparison of the frequencies of genotypes and alleles of the rs1799750 MMP-1 gene polymorphism with the appropriate demographic, clinical and laboratory data in participants with negative and positive coronary angiography findings was used for statistical analysis. Calculations were performed using Chi-square test (χ^2-test) and Fisher's exact test for analysis of the genotype and allele frequencies of the gene polymorphism using the genotypic, allelic dominant, recessive, heterozygous and superdominant models. The Cochran–Armitage trend test was used to analyze the allelic frequencies with the allelic and additive model. We have also assessed the probability index for chances - odds ratio (OD) - with a confidence interval CI (confidence interval) of 95%. Values of $p<0,05$ here are considered statistically significant. The statistical analyses were performed using XLSTAT 2016, GenAlEx 6.5 and Microsoft Excel 2016 software.

Results

Regarding sex distribution, data showed that out of 57 participants 44 (77,19%) were males and 13 (22,81%) were females. In the group with positive angiography finding 26 (76,47%) were males and 8 (23,53%) were females whereas in the group with negative angiography finding 18 (78,26%) were males and 5 (21,74%) were females (Table 2)

Table 2. Sex distribution

Sex	**Group with positive angiography finding**		**Group with negative angiography finding (controls)**		**Chi square test**
	N	**%**	**n**	**%**	***p***
Males	26	76,47	18	78,26	0,793
Females	8	23,53	5	21,74	
Total	**34**	**100,00**	**23**	**100,00**	

Table 2. Sex distribution

In terms of age distribution, data showed that in the group with positive angiography finding the average age of the participants was 38,3 years and in the group with negative angiography finding the average age of the participants was 39,8 years (Table 3). Since the differences in sex and age distribution between two studied groups were not significant (p=0,793 and p=0,397 respectively), we can conclude that the selection of participants is generally well balanced and allows appropriate genetic-association analysis.

Table 3. Age distribution

Years	**Group with positive angiography finding**	**Group with negative angiography finding (controls)**	**Mann-Whitney test** (two-sided)
n	34	23	0,379
Average	38,30	39,80	
SD	5,57	4,05	
Min age	22	33	
Max age	45	46	

Table 4 shows the distribution of genotypes of the rs1799750 MMP-1 gene polymorphism in participants with positive and negative angiography finding, respectively. It is obvious that the homozygous 2G/2G genotype is dominant in the group with positive angiography finding.

Table 4. Genotypes distribution of the rs1799750 MMP1 gene polymorphism

Genotypes of the rs1799750 MMP-1	Group with positive angiography finding	Group with negative angiography finding (controls)
1G/1G	3	7
1G/2G	12	10
2G/2G	19	6

In addition, Table 5 presents data derived from the coronary angiography in both groups with regard of diagnosis/indication for coronary angiography, number of vessels involved, culprit artery and coronary angiography finding (descriptive finding). As we can clearly see, the most prevalent participants within the group with positive angiography finding were those presenting STEMI, one-vessel disease was the most frequent finding and LAD was the most frequently present culprit artery.

Table 5. Coronary angiography data

. Coronary angiography data	Group with positive angio finding		Group with negative angio finding (controls)	
	n	%	n	%
Diagnosis/Indication for coronary angiography				
Stabile angina	0	0,00	23	100,00
Non-stable angina	2	5,88	0	0,00
NSTEMI	6	17,65	0	0,00
STEMI	26	76,47	0	0,00
Total	**34**	**100,00**	**23**	**100,00**
CAD vessel involved				
No CAD	0	0,00	23	100,00
One-vessel disease	26	76,47	0	0,00
Two-vessel disease	4	11,76	0	0,00
Multi-vessel disease	4	11,76	0	0,00
Total	**34**	**100,00**	**23**	**100,00**
Culprit artery				
No culprit	0	0,00	23	100,00
LM	1	2,94	0	0,00
LAD	16	47,06	0	0,00
Diag	2	5,88	0	0,00
Cx/OM/Ri	5	14,71	0	0,00
RCA	10	29,41	0	0,00
Total	**34**	**100,00**	**23**	**100,00**
Angiography finding				
Normal	0	0,00	9	39,13
No significant lesions	0	0,00	14	60,87
Significant CAD	34	100,00	0	0,00
Total	**34**	**100,00**	**23**	**100,00**

Probability of association of the studied rs1799750 MMP-1 gene polymorphism and CAD was assessed by using statistical analyses. The comparative analysis of genotypic and allelic frequencies was performed by using several standard genetic models. The results of this analysis are shown in Table 6. We can clearly see that there are present statistically significant differences ($p<0,05$) using all genetic models except heterozygous and superdominant.

In the genotypic model the homozygous 1G/1G genotype is used as a reference. Carriers of the 1G/2G genotype have odds ratio (OR) of 2,8 which means they have almost 3 times higher probability to develop significant CAD by the age of 45 in comparison to the carriers of the reference 1G/1G genotype

and this association is statistically significant (p<0,05). Furthermore, if we analyze the carriers of the 2G/2G genotype we can see that according to this model they have even more significant difference in comparison to the reference 1G/1G genotype – 7,389 times higher chance for development of CAD - and this association is also statistically significant (p<0,05).

Table 6. Genetic association of the rs1799750 MMP-1 gene polymorphism and significant CAD in young Macedonian population

Genetic model	**MMP-1 rs1799750 genotype/allele**	**Group with positive angio finding**		**Group with negative angio finding**		χ^2	***p***	***OR* (95% CI)**
Genotypic*	**1G/1G**	3	8,82	7	30,43	5,991	**0,035**	reference
	1G/2G	12	35,29	10	43,48			**2,800 (0,570 - 13,754)**
	2G/2G	19	55,88	6	26,09			**7,389 (1,441 - 37,884)**
	Total	34	100,00	23	100,00			
Dominant	**1G/1G**	3	8,82	7	30,43	4,429	**0,035**	**4,521 (1,028 - 19,880)**
	1G/2G+2G/2G	31	91,18	16	69,57			
	Total	34	100,00	23	100,00			
Recessive	**1G/1G+1G/2G**	15	44,12	17	73,91	4,946	**0,026**	**3,589 (1,135 - 11,344)**
	2G/2G	19	55,88	6	26,09			
	Total	34	100,00	23	100,00			
Heterozygous	**1G/1G**	3	20,00	7	41,18	1,663	0,197	2,800 (0,570 - 13,754)
	1G/2G	12	80,00	10	58,82			
	Total	15	100,00	17	100,00			
Super-dominant	**1G/2G**	12	35,29	10	43,48	0,388	0,533	0,709 (0,240 - 2,096)
	1G/1G+2G/2G	22	64,71	13	56,52			
	Total	34	100,00	23	100,00			
Allelic	**1G**	18	26,47	24	52,17	7,791	**0,005**	**3,030 (1,375 - 6,681)**
	2G	50	73,53	22	47,83			
	Total	68	100,00	46	100,00			
Aditive #	**0 2G**	3	8,82	7	30,43	0,342	**0,010**	/
	1 2G	12	35,29	10	43,48			
	2 2G	19	55,88	6	26,09			
	Total	34	100,00	23	100,00			

* two-sided χ^2 test # two-sided Cochran-Armitage ordinal test

In the dominant model, genotype carriers with at least one 2G allele have 4,521 times higher probability for CAD in comparison to homozygous 1G/1G genotype carriers and this difference is again statistically significant (p<0,05). According to the recessive model, participants with homozygous 2G/2G genotype have statistically significant 3,589 times higher probability for CAD in comparison to participants with at least one 1G allele (p<0,05).

Heterozygous and superdominant model have not confirmed statistically significant difference in the frequencies of genotypes in both groups (p>0,05).

Regarding the allele comparison, allelic model showed that carriers of the 2G allele have 3 times higher chances for development of CAD than the carriers of the 1G allele (p<0,05). The results of the allelic model are also confirmed with the Cochran-Armitage test in the additive model where it can be seen that

the risk increases with increasing the number of present 2G allele (2 vs 1 vs 0 2G allele). This model does not calculate the odds ratio (OR).

In summary, results from our study clearly show that there is statistically significant genetic association of the rs1799750 MMP-1 gene polymorphism with significant CAD in young Macedonian population. Genotype 2G/2G as well as allele 2G statistically significant increase the probability for development of CAD.

Discussion

Unlike conventional risk-factors, there are many more barely known and unknown risk-factors that may contribute to development of CAD and myocardial infarction, most probably in a combination one to another in very complex pathophysiologic scenario. Genetic factors are certainly (just) a part of them. Last two decades certain advance has been made when many gene polymorphisms associated with different pathogenic mechanisms involved in CAD have been discovered. Some of them are involved in the process of tissue maintenance, degradation and remodeling. In this particular direction different gene polymorphisms of the MMPs have been extensively investigated in different populations so far.

There are conflicting data from the literature regarding the association of the rs1799750 MMP1 gene polymorphism and coronary artery disease. Some of the authors have shown that there is not significant association between this gene polymorphism and CAD or myocardial infarction.

One study in Brazilian population have investigated the link between -1607 1G/2G MMP1 gene polymorphism and CAD and showed that there was not significant impact of this polymorphism on the risk and severity of CAD [10].

The study of Qintao et al. have also investigated the role of MMP1 gene polymorphism in susceptibility for CAD in Chinese Han population. The results from this study have confirmed that there was not significant association between this gene polymorphism and CAD [11].

On the other hand, Kondapalli et al. have proved that atherosclerotic plaques of the Indian carriers of 2G allele express high level of MMP1 and also that they are especially prevalent in vulnerable regions [8].

Another research from Poland have studied correlation between 1G/2G MMP1 gene polymorphism and risk of myocardial infarction in young population. It concluded that young carriers of the 2G allele have strong correlation with myocardial infarction [12].

In this context it is worth mention one study investigating the association between -1607 1G/2G (rs1799750) MMP1 gene polymorphism in Iranian Turks and CAD. The results from this study showed that the frequency of the 2G/2G genotype and 2G allele was significantly higher in participants with CAD [13].

Similarly, our study conducted in young Macedonian population and investigating the association of the rs1799750 gene polymorphism and CAD have shown that carriers of the 2G/2G genotype and 2G allele have significantly higher chances for development of CAD.

Main limitation of the study is small sample size of the both cohorts (cases and controls). But it is intelligible taking into account the strict inclusion and exclusion criteria. Further studies conducted in larger samples are necessary to confirm the results of our study conducted in Macedonian population.

Conclusion

Our study using different genetic models clearly confirms that there is statistically significant association of the rs1799750 MMP1 gene polymorphism and significant CAD in young Macedonian population. Furthermore, presence of genotype 2G/2G as well as allele 2G leads to statistically significant increase of the probability for CAD.

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