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Review article

THE ROLE OF MATRIX METALLOPROTEINASE-1 AND ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE POLYMORPHISMS IN DEVELOPMENT OF CORONARY ARTERY DISEASE

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Abstract

Coronary artery disease (CAD) is one of the major causes of morbidity and mortality worldwide. The main pathophysiological processes involved in the development of CAD include impaired lipid metabolism, coagulation and chronic inflammation of the coronary vessel wall. There are many well-known traditional or conventional risk factors that may contribute to development of CAD like smoking cigarettes, lack of physical activity, diabetes, obesity, arterial hypertension, dyslipidemia (hypercholesterolemia), psychosocial stress etc. Nevertheless, over the last two decades there has been a significant progress in the field of genetic research and enlightening of the genetic basis of development of CAD. Certain genetic polymorphisms have been found to be linked not only to lipid metabolism and coagulation but also to inflammation and response, tissue maintenance, remodeling and degradation of the extracellular matrix. In this review article we discuss some of the most frequently studied gene polymorphisms in the development of CAD – matrix metalloproteinase-1 (MMP-1) and endothelial nitric oxide synthase (eNOS) gene polymorphisms.

Keywords: coronary artery disease, MMP-1, eNOS, gene polymorphism

Introduction

Coronary artery disease (CAD) is one of the leading causes of morbidity and mortality worldwide. It is a form of atherosclerosis that specifically involves coronary arteries although non-atherosclerotic variants of CAD may also exist. Atherosclerosis is a complex disease which occurs early in life and is characterized by a slow progression as a result of interplay between many environmental, lifestyle and genetic factors. The most prevalent risk factors include smoking cigarettes, dyslipidemia (hypercholesterolemia), obesity, arterial hypertension, diabetes, psychosocial stress, lack of physical activity etc., but also age, gender, and family history play a significant role[1,2].

Today, there are many studies which describe atherosclerosis as a chronic inflammatory process. It initially starts with endothelial damage, decreased production of

nitric oxide (a potent vasodilator), increased production of vasoconstricting mediators (endothelin) and endothelial dysfunction. Endothelial dysfunction further leads to increased vessel permeability and subendothelial deposition of lipoproteins (Lp). Lp are involved in the process of oxidation and oxidized Lp are then phagocytized by macrophages (transformed monocytes from the bloodstream) located in the subendothelial tissue. This is a process that gradually turns macrophages into foam cells. Simultaneously, macrophages secrete many adhesive factors as well as pro-inflammatory and anti-inflammatory molecules (cytokines, interleukins) which actually maintain and facilitate the process of inflammation, cellular migration and forming of atherosclerotic plaques. Many cells undergo apoptosis which is a stimulus for intimal migration of the vascular smooth muscle cells. Further inflammation causes hypoxia and increased production of certain matrix metalloproteinases (MMP), a process that may lead to destabilization of atherosclerotic plaques and increase the risk for erosion or rupture-a critical moment for intravascular thrombosis and myocardial infarction[1,2].

Genetic basis of CAD

Observations dating from the middle of the previous century support the claim that the risk for CAD is heritable[1]. The estimated heritability of CAD according to different authors is believed to be around 50%[3-6]. There are many studies like Swedish twin study, Danish Twin Registry and Framingham Heart Study which confirm the role of family history for development of CAD[3,4,6].

In the beginning, some studies have performed research using so-called linkage analysis, which was a preferred method to detect genes responsible for single-gene disorders like familiar hypercholesterolemia. But scientists have hypothesized that CAD is a polygenic disorder and soon it was concluded that more appropriate method for this research was Case-Control Association Study (CCAS) and later Genome-Wide Association Study (GWAS)[3]. GWAS is a technique of genetic research which compares the frequency of every genetic variant in subjects (with disease of interest) with the frequency in healthy individuals. This technique allows a complete genome scanning, which means a precise genotyping with huge number of single nucleotide polymorphisms (SNPs). SNP refers to a variation or change in the DNA sequence (substitution of one base with another) which is frequent in the population[7,8].

First great achievement in this field was sequencing of the human genome in 2000 and additionally in 2005 with the HapMap project. Another great event was the discovery of the first gene locus 9p21 associated with an increased risk for development of CAD in the European population in 2007. Next few years this genetic risk variant was also confirmed in other populations, and now it is estimated to be present in around 75% of the general population[3]. Since 2007, there are more than 200 gene loci in association with CAD that have been detected. Around half of them are thought to be related not to one single risk factor (like dyslipidemia or hypertension), but rather to some pathophysiological mechanism involved in the development of CAD. Such an example is NOS locus responsible for nitric oxide signaling and regulation of the vascular smooth muscle tone, but also associated with genetic CAD risk. There are also many loci whose mechanism they are involved in is not fully understood[2].

The role of MMP-1 gene polymorphisms in CAD

Atherosclerotic plaques have fibrous cap with different thickness which consists of extracellular matrix (ECM) (includes proteins collagen and elastin). Degradation of these proteins may lead to destruction of the fibrous cap and destabilization of the atherosclerotic plaque making it vulnerable and prone to erosion or rupture-a process responsible for occurrence of an acute coronary syndrome (ACS). Major molecules that are involved in the

process of degradation of the ECM and tissue remodeling include matrix metalloproteinases (MMPs)[9].

MMPs are a class (family) of proteolytic enzymes (Zn-dependent endopeptidases) that are involved in the process of degradation of the ECM proteins, remodeling and tissue maintenance[10]. Impaired metabolism of ECM has a significant impact on the vascular remodeling in the process of destabilization of atherosclerotic plaques. Available data suggest that the levels of MMPs are elevated in regions of vulnerable atherosclerotic plaques or during an acute myocardial infarction. It is logical to conclude that genetic abnormalities in terms of overexpression of MMPs may have crucial role in CAD, especially in ACS[9,11,12]. Figure 1 shows the family of MMPs and their involvement in atherosclerotic plaques and myocardial infarction.

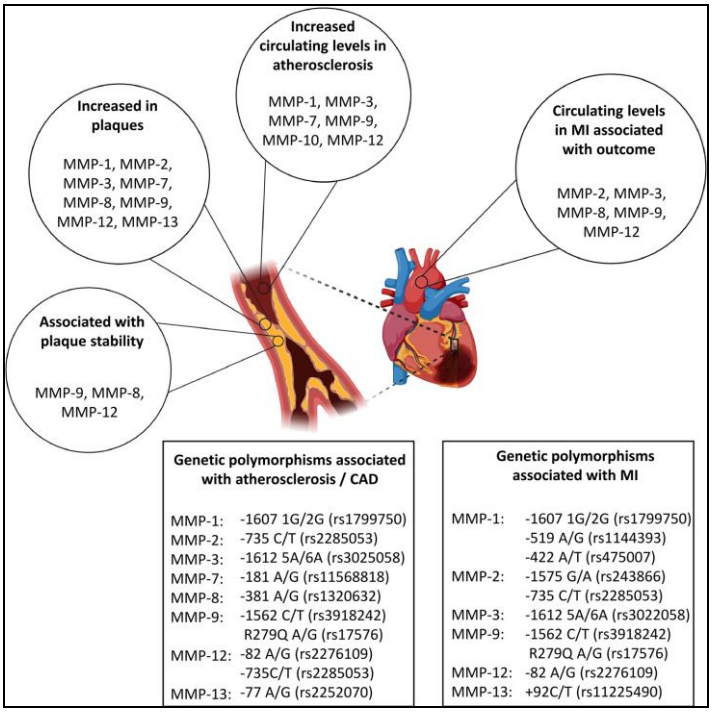

Fig. 1. MMPs in atherosclerosis and myocardial infarction (*Adapted from Bräuninger H. et al.* MMPs in CAD and myocardial infarction. *Basic Research in Cardiology 2023;118:18*)

The activity of MMPs is complex and regulated on different levels: transcription, secretion, activation and inhibition. Inhibition of activity of the MMPs is controlled by another family of tissue inhibitors of MMPs, so-called TIMPs[10]. There are generally several types of MMPs known as collagenases, gelatinases, stromelysins, matrilysins and membrane-type MMPs. They are mostly synthesized in the endothelium and vascular smooth muscle cells as inactive molecules (proenzymes). Biological activity of the MMPs correlates with the MMP gene polymorphisms[13].

MMP-1 belongs to the interstitial collagenases and it is capable to degrade fibrillar collagen. It is expressed generally by the macrophages in the regions of atherosclerotic plaques prone to erosion or rupture[14]. One of the most studied MMP-1 gene polymorphisms is -1607 1G/2G (rs1799750), which refers to insertion-deletion polymorphism at position -1607 in the promoter region.

The association between MMP-1 gene polymorphisms and risk of CAD remain controversial across the literature, although most of the studies have confirmed their role in the development of CAD and myocardial infarction. Kondapalli *et al.* investigated the association of the -1607 1G/2G MMP-1 gene polymorphism and its serum levels with

susceptibility for CAD and myocardial infarction in asymptomatic first-degree relatives of the Indian population. This study has shown that atherosclerotic plaques of the individuals carrying 2G allele have higher levels of MMP-1 expression and are more prone to occur in vulnerable plaques. Furthermore, the study suggests MMP-1 -1607 1G/2G polymorphism and its serum levels as an independent cardiovascular prognostic marker[14].

Another group of researchers from Poland analyzed the correlation between several genetic polymorphisms including MMP1 1G/2G in Poles under 45 years of age. They found a strong correlation between the presence of this polymorphism and risk of myocardial infarction in young Poles, especially carriers of the 2G allele[15].

One of the largest studies on this issue was performed by Qintao C. *et al*., in which they investigated the role of MMP-1 genetic polymorphism in susceptibility of CAD in the Han Chinese population. Results from this study revealed that there was no significant association between rs1799750 genotype distribution and CAD, although CAD patients had significantly higher frequency of the 2G allele in comparison to healthy persons. This study concluded that there was a potential role of the rs1799750 MMP-1 gene polymorphism in the genetic susceptibility for CAD[16].

There was another study conducted among Iranian Turks investigating the association between MMP-1 -1607 1G/2G (rs1799750) gene polymorphism and CAD. Results of this study showed that 2G allele and 2G/2G genotype frequency were higher in CAD patients older than 50 years in comparison to healthy controls (63.51% *vs*. 45.05%; P=0.006 and 51.35% *vs*. 20.79%; P=0.0004, respectively)[17].

A study conducted among Caucasian Brazilian population investigating MMP gene polymorphisms in patients with CAD detected that they (including-1607 1G/2G polymorphism) had no significant impact on the risk and severity of CAD[5].

The role of eNOS gene polymorphisms in CAD

Nitric oxide (NO) is an important molecule involved in many physiological processes in the human body. It derives from L-arginine with mediation of several isoforms of nitric oxide synthase (NOS) including endothelial NOS (eNOS). Main effects of NO are vasoprotective, and include vasodilation (relaxation of the vascular tonus), inhibition of platelet and leukocyte adhesion to the endothelial cells, and inhibition of the smooth muscle cell proliferation. It also has a protective role against atherosclerosis and superoxide radicals. Production of NO is regulated by several NOS gene polymorphisms. There are several analyzed NOS gene polymorphisms across the literature, among them is also T-786C (rs2070744). It refers to a replacement of thymine with cytosine at nucleotide -786, a genetic variant which reduces promoter activity by 50% and significantly increases the risk for CAD[18-20].

Available literature data suggest that there are contradictory and inconsistent evidence regarding the role of T-786C NOS3 gene polymorphism and susceptibility to CAD, especially with regard to certain ethnicity.

El Saied AM. *et al*. conducted research among Egyptian population analyzing the association between eNOS gene polymorphisms and CAD. They found that both investigated eNOS gene polymorphisms (including T-786C) were not major genetic susceptibility factors for CAD[22]. A group of Algerian scientists also confirmed that T-786C gene polymorphism was not associated with myocardial infarction in Algerian population unlike G894T polymorphism[22].

On the other hand, a study performed by Balci S. *et al.* analyzed the possible role of eNOS polymorphisms T-786C and G894T in CAD among Turkish population. Their study came to a conclusion that unlike G894T polymorphism, T-786C variant could be suggested as a CAD risk factor. Furthermore, carriers of TC and CC genotype had a significantly

greater risk for development of CAD (2.1 and 2.842 times greater, p=0.026 and p=0.04, respectively) in comparison to TT genotype[23]. There was another Turkish study conducted by Tangurek B. *et al*. that was investigating the relationship between T-786C eNOS gene polymorphism and CAD in Turkish population. It also confirmed this polymorphism to be a genetic risk factor for CAD and in addition, C-dominant (CC+TC) individuals had almost 3-fold more likelihood to suffer from CAD (OR: 2.902; CI: 1.272–6.622; $P < 0.05$)[24].

An Indian group of scientists also examined the role of this polymorphism in the development of CAD. They found that CC genotype was absent from both study and control group and suggested a possible protective role of TT genotype in the development of insulin resistance and CAD. The study also concluded that reduced plasma NO levels were associated with risk of CAD[25].

Alkharfy KM. *et al.* analyzed the association of eNOS gene polymorphisms and risk of CAD in a Saudi population. They confirmed that the frequencies of TC and CC genotypes of the T-786C gene polymorphism were significantly higher among cases in comparison to controls (50% and 32% *vs*. 34% and 22.5% respectively; p<0.001). This study concluded that eNOS gene polymorphisms (including T-786C) have independent association with CAD in the Saudi population[26].

In addition, the GENICA study, although conducted 20 years ago, confirmed that eNOS T-786C polymorphism (especially C-allele) was associated with a higher risk of CAD in Caucasians[27].

Finally, we would like to present results from two systematic reviews and meta-analyses referring also to the association of eNOS gene polymorphisms and CAD. The first one included more than 130 studies with almost 70,000 participants encompassing five ancestral groups: European, Middle Eastern, Asian, Asian-Indian and African. It included patients with detected CAD and myocardial infarction or ACS as well as CAD-free controls. This systematic review analyzed the most common eNOS gene polymorphisms for CAD: G894T, T-786C and 4b/a. However, more than 7,000 CAD patients and more than 10,000 controls were included in the pool analysis and involved in investigating the association between T-786C gene polymorphism and CAD. Results showed that all three studied eNOS polymorphisms were associated with CAD and more specifically, T-786C gene polymorphism carried the highest CAD risk among participants of Asian ancestry[18]. The second systematic review and meta-analysis was conducted by Liu D. *et al*. and it showed a significant association between eNOS T-786C gene polymorphism and CAD (dominant model) whereas significant differences existed in all genetic models for Caucasians. Additionally, authors of this systematic review have proposed this polymorphism as a possible early marker for detection of CAD.

Conclusion

It is obvious that over the last two decades a tremendous progress has been made in the field of enlightening genetic basis and genetic pathways of developing CAD. There are many discovered gene polymorphisms associated not only with lipid metabolism and thrombosis, but also with inflammation, tissue maintenance and remodeling. There are certainly many of them yet to be discovered. This review article confirms that although still some uncertainties and conflicting evidences are present, in the literature the conclusion prevails that there is an association between MMP-1 -1607 1G/2G and eNOS T-786C gene polymorphisms and risk for CAD. Studies have also confirmed that there are significant disparities among different ethnicity groups concerning presence of these gene polymorphisms and their impact on the risk of CAD. This is especially important in terms of discovering a strong predictive marker for CAD in a young population.

Conflict of interest statement. None declared.

References

1. Malinowski D, Bochniak O, Luterek-Puszyńska K, Puszyński M, Pawlik A. Genetic Risk Factors Related to Coronary Artery Disease and Role of Transforming Growth Factor Beta 1 Polymorphisms. *Genes* 2023; 14(7): 1425. https://doi.org/10.3390/genes14071425.
2. Kessler T, Schunkert H. Coronary Artery Disease Genetics Enlightened by Genome-Wide Association Studies. *JACC: Basic to Translational Science* 2021; 6(7). https://doi.org/10.1016/j.jacbts.2021.04.001.
3. Roberts R, Chavira J, Venner E. Genetic Risk and Its Role in Primary Prevention of CAD. *J Transl Genet Genom* 2022; 6: 388-402. https://dx.doi.org/10.20517/jtgg.2022.07.
4. Khera AV, Kathiresan S. Genetics of Coronary Artery Disease: Discovery, Biology, and Clinical Translation. *Nat Rev Genet* 2017; 18(6): 331-344. doi:10.1038/nrg.2016.160.
5. Dalepiane VLN, Silvello DN, Paludo CA, Roisenberg I, Simon D. Matrix Metalloproteinase Gene Polymorphisms in Patients with Coronary Artery Disease. *Genetics and Molecular Biology* 2007; 30(3): 505-510. https://doi.org/10.1590/S1415-47572007000400001.
6. McPherson R, Tybjaerg-Hansen A. Genetics of Coronary Artery Disease. *Circ Res* 2016; 118: 564-578. doi: 10.1161/CIRCRESAHA.115.306566.
7. Al-Koofee DAF, Mubarak SMH. Genetic Polymorphisms. The Recent Topics in Genetic Polymorphisms. *IntechOpen* 2019. doi: 10.5772/intechopen.88063.
8. Assimes TL, Roberts R. Genetics: Implications for Prevention and Management of Coronary Artery Disease. *JACC* 2016; 68(25): 2797-2818. doi: 10.1016/j.jacc.2016.10.039.
9. Bräuninger H, Krüger S, Bacmeister L, Nyström A, Eyerich K, Westermann D, *et al.* Matrix metalloproteinases in coronary artery disease and myocardial infarction. *Basic Research in Cardiology* 2023; 118: 18. https://doi.org/10.1007/s00395-023-00987-2.
10. Kapoor C, Vaidya S, Wadhwan V, Kaur G, Pathak A. Seesaw of matrix metalloproteinases (MMPs). *J Cancer Res Ther* 2016; 12(1): 28-35. doi: 10.4103/0973-1482.157337.
11. Niu W, Qi Y. Matrix Metalloproteinase Family Gene Polymorphisms and Risk for Coronary Artery Disease: Systemic Review and Meta-Analysis. *Heart* 2012; 98(20): 1483e1491. doi: 10.1136/heartjnl-2012-302085.
12. Ikeda U, Shimada K. Matrix Metalloproteinase and Coronary Artery Diseases. *Clin Cardiol* 2003; 26(2): 55-59. doi: 10.1002/clc.4960260203.
13. Pawlik A, Plucinska M, Kopec M, Głabowski D, Czerewaty M, Safranow K. MMP1 and MMP3 Gene Polymorphisms in Patients with Acute Coronary Syndromes. *IUBMB Life* 2017; 69(11): 850-855. doi:10.1002/iub.1684.
14. Kondapalli MS, Galimudi RK, Gundapaneni KK, Padala C, Cingeetham A, Gantala S, *et al*. MMP 1 circulating levels and promoter polymorphism in risk prediction of coronary artery disease in asymptomatic first degree relatives. *Gene* 2016; 595(1): 115-120. doi: 10.1016/j.gene.2016.09.041.
15. Sakowicz A, Fendler W, Lelonek M, Pietrucha T. Genetic variability and the risk ofmyocardial infarction in Poles under 45 years of age. *Arch Med Sci* 2010; 6(2): 160-167. doi: 10.5114/aoms.2010.13887.
16. Qintao C, Yan L, Changhong D, Xiaoliang G, Xiaochen L. Genetic polymorphism of matrix metalloproteinase-1 and coronary artery disease susceptibility: a case-control study in a Han Chinese population. *Genet Test Mol Biomarkers* 2014; 18(12): 826-831. doi: 10.1089/gtmb.2014.0222.

17. Ghaffarzadeh A, Bagheri M, Khadem-Vatani K, Abdi Rad I. Association of MMP-1 (rs1799750)-1607 2G/2G and MMP-3 (rs3025058)-1612 6A/6A Genotypes with Coronary Artery Disease Risk Among Iranian Turks. *J Cardiovasc Pharmacol* 2019; 74(5): 420-425. doi: 10.1097/FJC.0000000000000727.
18. Rai H, Parveen F, Kumar S, Kapoor A, Sinha N. Association of Endothelial Nitric Oxide Synthase Gene Polymorphisms with Coronary Artery Disease: An Updated Meta-Analysis and Systematic Review. *PLoS ONE* 2014; 9(11): e113363. doi: 10.1371/journal.pone.0113363.
19. Colombo MG, Paradossi U, Andreassi MG, Botto N, Manfredi S, Masetti S, *et al*. Endothelial nitric oxide synthase gene polymorphisms and risk of coronary artery disease. *Clin Chem* 2003; 49(3): 389-395. doi: 10.1373/49.3.389.
20. Jaishankar T, Shivasekar M, Mohanakrishnan VV. eNOS Gene Variants and Their Genetic Susceptibility Associated with Coronary Heart Disease. *J Health Sci Med Res* 2023; 41(1): e2022885. doi: 10.31584/jhsmr.2022885.
21. El Saied AM, El Wakf AM, Elmougy R, El Hafez AA, Hussien SM. Lack of Association between Endothelial Nitric Oxide Synthase Gene Polymorphisms and Coronary Artery Disease among Egyptian Population. *International Journal of Biochemistry Research & Review* 2016; 11(2): 1-12, doi: 10.9734/IJBCRR/2016/23818.
22. Meroufel D*,* Médiène-Benchekor S, Dumont J, Benhamamouch S, Amouyel P, Brousseau T. Relationship between endothelial nitric oxide synthase gene polymorphisms and the risk of myocardial infarction in the Algerian population. *Egypt J Med Hum Genet* 2009; 10(1).
23. Balci S, Yildiz PO, Sucu N, Yilmaz DC, Tamer L. T786C and G894T eNOS polymorphisms as a risk assessment of coronary artery disease. *J Cardiol Curr Res* 2017; 8(4): 1-6. doi: 10.15406/jccr.2017.08.00290.
24. Tangurek B, Ozer N, Sayar N, Terzi S, Yilmaz H, Dayi SU, *et al*. The relationship between endothelial nitric oxide synthase gene polymorphism (T-786C) and coronary artery disease in the Turkish population. *Heart Vessels* 2006; 21(5): 285-290. doi: 10.1007/s00380-005-0902-0.
25. Bhutani N, Tangadi D, Bhavsar P. Role of Endothelial Nitric Oxide Synthase (T-786C) Gene Polymorphism in the Development of Coronary Artery Disease. *Galore International Journal of Health Sciences and Research* 2020; 5(1). P-ISSN: 2456-9321.
26. Alkharfy KM, Al-Daghri NM, Al-Attas OS, Alokail MS, Draz HM, Hussain T. Endothelial nitric oxide synthase gene polymorphisms (894G > T and -786T > C) and risk of coronary artery disease in a Saudi population. *Arch Med Res* 2010; 41(2): 134-141. doi: 10.1016/j.arcmed.2010.02.004.
27. Rossi GP, Cesari M, Zanchetta M, Colonna S, Maiolino G, Pedon L, *et al*. The T-786C endothelial nitric oxide synthase genotype is a novel risk factor for coronary artery disease in Caucasian patients of the GENICA study. *J Am Coll Cardiol* 2003; 41(6): 930-937. doi: 10.1016/s0735-1097(02)03012-7.
28. Liu D, Jiang Z, Dai L, Zhang X, Yan C, Han Y. Association between the - 786T>C 1polymorphism in the promoter region of endothelial nitric oxide synthase (eNOS) and risk of coronary artery disease: a systematic review and meta-analysis. *Gene* 2014; 545(1): 175-183. doi: 10.1016/j.gene.2013.09.099.