DOI: http://dx.doi.org/10.48305/arya.2023.41105.2846

Role of A Single Nucleotide Polymorphism in Thrombospondine 4 Gene in Premature Myocardial Infarction among Population of Southern Iran

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Original Article

Abstract

BACKGROUND: Coronary Artery Diseases (CAD) are the leading cause of Myocardial Infarction (MI). However, their underlying etiology can be found in the interplay between environmental and genetic factors. On the other hand, it has been shown that Extracellular Matrix (ECM) proteins, such as Thrombospondins (TSP), play a crucial regulatory role in vascular pathologies, including atherogenesis. TSPs are extracellular proteins responsible for intercellular and cell-ECM interactions and are involved in regulating functional responses. Recently, a missense mutation in the TSP-4 gene has been reported to potentially increase the risk of CADs. The present study aimed to investigate the role of rs1866389 Guanosine to Cytosine (G/C) Single Nucleotide Polymorphism (SNP) of the TSP-4 gene on the prevalence of premature MI in southern Iran.

METHOD: The present case-control study included 100 patients with premature MI and 100 healthy individuals. The DNA extracted from the blood samples of the participants underwent Polymerase Chain Reaction (PCR) for the sequence of the TSP-4 gene. Afterward, the frequency of C (mutated) and G (normal) alleles of the TSP-4 gene was evaluated in the case and control groups.

RESULTS: According to our findings, there was no significant intergroup difference in gender, age, and smoking status. However, the case group was significantly higher in the prevalence of Diabetes mellitus (DM), Hyperlipidemia (HLP), and Hypertension (HTN) compared to the control group. Moreover, 22%, 49%, and 29% of the case group had CC, GC, and GG genotypes in the TSP-4 gene, respectively, while the prevalence of CC, GC, and GG genotypes were 10%, 44%, and 46% in the control group. Also, the prevalence of allele C was significantly higher in the case group (47%) compared to the control group (33%, P=0.043), showing its significant association with the increased risk of premature MI (OR = 1.80; 95% CI = 1.01-3.19).

CONCLUSIONS: The rs1866389 G/C SNP of the TSP-4 gene significantly increased the risk of premature MI in the population of southern Iran. Thus, such mutated gene can be used as a target for gene therapy or a marker for early detection of individuals at high risk for CADs.

Keywords: Coronary Artery Disease, Myocardial Infarction, Extracellular Matrix Protein, Thrombospondin Gene

Date of submission: *2022-Oct-29,* ***Date of acceptance:****2023-Feb-07*

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Introduction

As the leading cause of mortality and morbidity throughout the world [1], Coronary Artery Diseases (CAD) increase the chance of Myocardial Infarction (MI), ischemic stroke, and Cardiovascular (CV) deaths in the affected patients. The main pathophysiology of these diseases is the rupture or erosion of an atherosclerotic plaque, leading to partial or complete obstruction of coronary arteries that decreases myocardial perfusion [2]. Moreover, such obstruction can lead to an MI, which can be considered premature in men younger than 55 and women younger than 65 years of age [3]. It has been shown that the interplay between environmental and genetic factors consist the underlying etiology of MI [4, 5]. However, the genetic basis of this disease has attracted the attention of researchers in recent years. It seems that the proteins of the extracellular matrix have a crucial regulatory role in vascular pathologies, making them a recent target for pharmacotherapy [6]. Moreover, it is well established that the interaction between cells and Extracellular Matrix (ECM), as well as the intercellular interactions, play an important role in the early phases of atherogenesis and may lead to other vascular pathologies, such as atherogenesis [7-9].

On the other hand, Thrombospondins (TSP) are calcium-binding ECM proteins that are involved in functional cell responses, including apoptosis, adhesion, proliferation, migration, and ECM remodeling, through cell-ECM interactions and extracellular factors, such as cytokines and growth factors [10]. It has been shown that the level of TSPs significantly increases in response to stress. Moreover, cartilage oligomer matrix proteins, or TSP-1, TSP-2, TSP-3, TSP-4, and TSP-5, are the principal members of the TSP family [11-15]. It has been shown that TSP-1, TSP-2, and TSP-4 are responsible for myocyte apoptosis, cardiac remodeling, and CAD in the cardiovascular system by increasing the collagen production and activating the signaling pathway of Tumor Growth Factor β (TGF-β) and matrix metalloproteinase. Moreover, convincing roles have been reported for TSP-1 and TSP-2 in atherogenesis leading to Cardiovascular Diseases (CVD) and MI [16, 17]. The gene encoding TSP-4 is located on chromosome 5q13. A missense mutation converting guanine to cytosine in the TSP-4 gene causes the substitution of proline with alanine at position 387 (A387P) of the final protein, resulting in conformational changes that alter the function of the protein in different aspects [18]. It has been shown that the mutated TSP-4 increases the levels of Interleukin 8 (IL-8), inducing vascular inflammation [19]. Moreover, it increases macrophage accumulation during atherogenesis in mice [20], which is believed to increase the risk of CAD [20, 21]. According to studies on this mutation in different populations, it has a direct effect on the prevalence of CVD in several populations, such as Americans and Egyptians. However, no significant relationship has been found between this mutation and CVD in some studies [22]. Considering the potential association of TSP-4 and atherosclerotic pathologies, the present study aims to investigate the potential association of this single nucleotide polymorphism in TSP-4 (Ala387Pro, rs1866389 G/C) with the prevalence of premature MI in southern Iran. If such a relationship is proved, this mutated protein can be used as a target for pharmacotherapy and a marker for early diagnosis of CAD.

Material and Methods

Study population

The present study included 100 patients (case group) presented to the academic healthcare facilities of the Jahrom University of medical sciences (JUMS) and diagnosed with acute MI, which could be ST Elevation MI (STEMI) or non-ST Elevation MI (NSTEMI), from January 2020 to December 2021. The patients had presented with the symptoms of compressive retrosternal chest pain, cold sweating, nausea, and vomiting and had either increased serum cardiac enzymes or ECG changes suggestive of MI. The diagnosis was made by two cardiologists based on the

latest (2019) guideline by the American Heart Association (AHA) and was confirmed using invasive coronary angiography [23]. Moreover, 100 healthy individuals matched with the case group in gender and age were included in the study as the control group. Also, all participants gave written informed consent, and the study protocol followed the declaration of Helsinki and was approved by the Ethics Committee of the JUMS.

Inclusion criteria

Males younger than 55 years and females younger than 65 years old who were diagnosed with STEMI or NSTEMI.

Exclusion criteria

Death during hospital admission

1. Having conditions that increase the TSP-4 levels, such as known connective tissue disorders and critical illness in the last six months [24]
2. Lack of informed consent and unwillingness to participate

Blood sampling and deoxyribonucleic acid extraction

After the diagnosis of MI, the patients underwent blood sampling from the median cubital vein. The samples (5-10 mL) were collected in tubes containing EDTA to avoid coagulation. Afterward, the Deoxyribonucleic Acid (DNA) content of the samples was extracted from the nucleated blood cells based on the instructions of the kit company (Bioneer Co., Cat.No.: K-3032). Extracted DNA was stored at -20 °C before the Polymerase Chain Reaction (PCR).

Polymerase chain reaction

The present study used the A A T T C C G C A T C T T C A C T T C A C forward primer and the AACCGGTTCTGCTTTGATAAC reverse primer for PCR amplification in order to find the sequence of the mutated TSP-4 gene (rs1866389 G/C (rsA387P) polymorphism, Table 1).

Table 1. Specific primers used in the present study

Sequence	Primer
5′-AATTCCGCATCTTCACTTCAC-3′	Forward
3′-AACCGGTTCTGCTTTGATAAC-5′	Reverse

The PCR was performed using the tubes of the PCR Premix BIONEER and a thermocycler. The reaction volume was 20 µL, including 2.5 µL of the template DNA, 10 pmole of each primer, 1.5 µM of magnesium chloride, and 250 µM of dNTP Taq polymerase enzyme. Moreover, the first phase was performed at 94 °C for 5 minutes. Then, there were 35 cycles, each consisting of a 40-second phase at 94 °C and a 30-second phase at 61 °C. Finally, the last cycle included a 1-minute phase at 72 °C and an 8-minute phase at 72 °C.

The PCR products, with a length of 221 bp, were stored at -20 °C before digestion. Afterward, they underwent digestion using the restriction endonuclease Ava II for 24 h at 37 °C to detect the rs1866389 polymorphism, leading to the cleavage of the allele C of the rs1866389 polymorphism into two 143 bp and 78 bp fragments. However, the fragments without allele C stayed intact with a length of 221 bp. Then, electrophoresis was performed in a 2% agarose gel, and the final product was blotted using ethidium bromide and checked under UV light for accuracy (Figure 1).

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 21(Chicago, Illinois, USA.). The present study used the Hardy-Weinberg equilibrium to investigate the frequency of different alleles and genotypes. Moreover, the chi-square test was used to investigate the relationship between TSP-4 polymorphisms and premature MI, while the student t-test was used for intergroup comparisons of

demographics and CVD risk factors. The quantitative variables were reported in mean and Standard Deviation (SD), while the qualitative variables were reported in frequency and percentage (%). The significance level was set at 0.05.

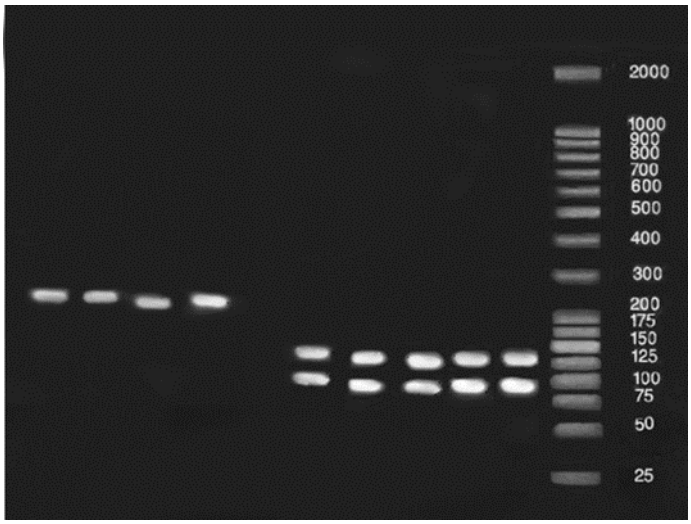

Figure 1. Electrophoresis of the PCR product

Results

The present study included a total of 200 participants, including 100 participants in the case group and 100 participants in the control group. Moreover, the participants' mean age was 41.4±4.94 years and 42.21±7.71 years in the case and control groups, respectively. Also, there were no significant intergroup differences in age (P=0.408), gender (P=0.876), and smoking status (P=0.323).

According to our findings, 30% of the case group had a positive family history of CAD, while only 10% of the control group had a positive family history of CAD, showing a significant difference (P<0.001). Moreover, 25%, 23%, 25%, and 2% of the patients in the case group had Hypertension (HTN), Hyperlipidemia (HLP), Diabetes Mellitus (DM), and renal disease, respectively. However, the prevalence of DM (11%, P=0.010), HTN (8%, P=0.001), HLP (9%, P=0.07), and renal disease (0%, P=0.15) was lower in the control group, which was significant in terms of DM and HTN (Table 2).

The Hardy-Weinberg equilibrium was used to survey the repartition of the genotypes of the TSP-4 gene, demonstrating a success rate of 100%. The distribution of different alleles and genotypes of the rs1866389 gene in the case and control groups is presented in Table 3.

According to our findings, 22%, 49%, and 29% of the participants in the case group had CC, GC, and GG genotypes in the TSP-4 gene, respectively. However, the prevalence of CC, GC, and GG genotypes was 10%, 44%, and 46% in the control group. Thus, the prevalence of the CC genotype was significantly higher in the case group (P=0.021), while the prevalence of the GG genotype was significantly higher in the control group (P=0.013). Moreover, the prevalence of allele C was higher in the case group (47%) compared to the control group (33%), which was significant (P=0.043). Therefore, allele C was associated with an increased risk of premature MI (OR = 1.80; 95% CI = 1.01-3.19).

Table 2. Demographic characteristics and the prevalence of CVD risk factors in the case and control groups

Variables		Control group (n=100)	Case group (n=100)	P-value
Gender	Male	72%	71%	0.88 [c]
	Female	28%	29%	
Age (year)		42.2±7.71	41.4±4.94	0.41 [t]
Smoking		27%	25%	0.32 [t]
Positive family history		9%	30%	0.001 [t]*
Diabetes		11%	25%	0.010 [t]*
Hyperlipidemia		9%	23%	0.07 [t]
Hypertension		8%	25%	0.001 [t]*
Renal disease		0%	2%	0.15 [t]

t P-value calculated using the student t-test; c P-value calculated using the Chi-square test. * Indicative of a significant P-value

Table 3. Distribution of different genotypes and alleles of the TSP-4 gene in the study groups

Group	Allele (%)			Hardy-Weinberg Equilibrium	
	CC	CG	GG	C (mutated)	G (normal)
Case (n=100)	22%	49%	29%	47%	53%
Control (n=100)	10%	44%	46%	33%	67%
OR $_{case/control}$ (95% CI)	2.53 (1.13-5.68)	1.39 (0.80-2.41)	0.47 (0.26- 0.86)	1.80 (1.01-3.19)	
P-value	0.021 [c]*	0.23 [c]	0.013 [c]*	0.043 [c]*	

c P-value calculated using the Chi-square test. * Indicative of a significant P-value

Discussion

According to our findings, the prevalence of rs1866389 G/C Single Nucleotide Polymorphism (SNP) in the TSP-4 gene was significantly higher in the participants with premature MI compared to healthy individuals of the same gender and age. The present study was the first to investigate this mutation in the Iranian population. In general, it is believed that smoking, HTN, HLP, and DM are responsible for more than 50% of CAD risk factors, while the remaining stake probably consists of several minor risk factors and genetic factors [25]. Several recent studies have suggested the role of specific genetic variants as CAD risk factors, among them ECM proteins, such as TSP-4, have been widely investigated [8]. It has been shown that TSP-4 has a protective role after MI, reducing the post-MI mortality rate [26]. Moreover, the relationship between the Ala387Prp polymorphism and MI has been studied in different populations. However, such a relationship is still controversial.

A literature review by Han et al. on the role of TSP-4 in CVD in 2020 recommended conducting further studies on this topic. Moreover, a study by Topol et al. in 2001 was the first to report the association between this TSP-4 mutation and increased risk of CAD and MI [27]. Since then, several studies have been reporting such association. However, the available findings need to be more consistent. A study by Wessel et al. in 2004 investigated the prevalence of this polymorphism in the American population, reporting results compatible with those of Topol et al. [28]. Also, Zhoe et al. investigated the relationship between this polymorphism and CAD in the

Chinese Han population [22], while Yamada et al. performed a comprehensive genetic study of the Japanese population. However, they did not find a significant correlation between this polymorphism and MI [29]. Finally, a meta-analysis by Zhang et al. in 2015, which included 13 previous studies, 10,801 cases, and 9,381 controls, on the relationship between SNP mutations in the TSP genes with the prevalence of CADs reported that A387P SNP increased the chance of CADs in Americans [30].

Although only a limited number of genetic variants have proven to play a role in the pathogenesis of CAD, identifying SNPs effective in CAD can be of great importance. Such mutated proteins and genes can be used as a target for pharmacotherapy or a genetic marker for the early detection of high-risk populations.

Conclusion

According to our findings, the rs1866389 G/C SNP of the TSP-4 gene significantly increased the risk of premature MI in the population of southern Iran. Thus, such mutated gene can be used as a target for gene therapy or a marker for early detection of individuals at high risk for CADs. However, there is a need for further studies on larger populations from all regions of Iran to generalize these findings to all Iranian population.

Limitations

The present study had some limitations as well. For example, it was desirable to check the presence of this polymorphism, as well as CADs, in all family members of the case group. However, more financial resources were needed for such measures. Moreover, it was better to investigate the presence of other related SNPs to allow a more reliable prediction of the role of genetics in the development of CADs.

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How to cite this article: Farahbakhsh N, Hooshanginezhad Z, Saleh S, Alaei F, Azizi F, Shojaie M. **Role of a single nucleotide polymorphism in thrombospondine 4 gene in premature myocardial infarction among population of southern Iran.** ARYA Atheroscler 2023; 19(2): 23-9.