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Association Assessment of Paraoxonase 1 Gene Polymorphism with Coronary Artery Disease in Golestan, Iran

Samaneh Abdolahpour [1, a], Azam Bakhshandeh [1, a], Touraj Farazmandfar [1], Mehdi Rayatnavaz [1], Majid Shahbazi [1, *]

[a], These two authors have got equal right as a first author
[1] Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Gorgan, IR Iran

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ABSTRACT

Background: Cardiovascular disease (CVD) is the most common cause of mortality and morbidity worldwide. Coronary artery disease (CAD) is the leading cause of CVD. In CAD, atherosclerotic plaques lead to coronary artery stenosis, hereby impairing myocardial blood and oxygen supply. The oxidative changes in LDL at the vascular wall play a critical role in the development of atherosclerotic lesions. The paraoxonase 1 (PON1) enzyme present in the HDL surface is capable of destroying oxidized LDL and, therefore has an antiatherogenic activity.

Objectives: This study was conducted to investigate the association of two functional polymorphisms in PON1 gene with CAD in northern Iran.

Materials and Methods: This study was a case-control study with the case-base sampling method for the control group. Genomic DNA was extracted from the peripheral blood collected from 305 patients with CAD and 302 healthy controls. Two polymorphisms of PON1(L55M)T > A and PON1(Q192R)A > G, for all samples were genotyped by Single-Specific Primer-PCR. GraphPad6 software was used for statistical analysis.

Results: Logistic regression analysis showed that age, gender, smoking status, and diabetes mellitus were significantly associated with CAD disease ($P < 0.001$). There was a significant association between genotype A/A of PON1(L55M)T > A polymorphism and CAD disease ($P = 0.011$). The results indicated that allele A in this SNP was also significantly associated with the CAD disease ($P = 0.005$). Analysis of dominant genetic model in PON1 (L55M)T > A showed that one copy of A is sufficient for increased risk ($P = 0.016$).

Conclusions: Our findings, at least in the population of Golestan province, indicated that the PON1(L55M) polymorphism might be associated with atherosclerotic disease.

1. Background

Cardiovascular disease (CVD) is the most common cause of mortality and morbidity worldwide. The most common cause of CVD is coronary artery disease (CAD) or atherosclerosis, which accounts for more than half of the deaths from heart diseases. The CAD is currently one of the major health problems in our country, and its incidence rate in Iranian population is respectively 1436 and 1168 per 100,000 people (1). Atherosclerosis is a polygenic and multi-factorial disease, with the estimated contribution of genetic factors over 50% based on epidemiological studies. In CAD, atherosclerotic plaques lead to coronary artery stenosis, thereby impairing the myocardial blood and oxygen supply (2).

The results of some studies on twins showed that there was a higher rate of atherosclerosis-related mortality and morbidity between identical twins (especially male) than non-identical ones; this indicates the importance of genetic factors in the pathogenesis of CAD. Moreover, some recent studies reported an increased risk of 2 to 3 fold of CAD in first-degree relatives. Atherosclerosis before the age of 60 years is considers as an independent risk factor for afflicting

*Corresponding author: Majid Shahbazi, Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Shastkola Road, Falsafi Complex Gorgan, Iran. Zipcode: 4934174611. Tel/Fax: +98-17-32351735, E-mail: shahbazimajid@yahoo.co.uk.

first-degree relatives. As the age of atherosclerosis goes down, the role of inheritance becomes more prominent, so that before the age of 46 years, the inherited factors contribute to 92% to 100% cases of atherosclerosis (3-7).

The process of forming atherosclerotic plaques lasts for decades, and its exact mechanism has not been fully understood. The oxidative changes in LDL on the vascular walls are thought to play a critical role in development of atherosclerotic lesions (8). HDL, with the help of its numerous enzymes, not only prevents LDL oxidation but also hydrolyzes the oxidized LDL. The PON1 enzyme is one of the most important enzymes which play an important role in preventing the formation of atherosclerotic plaques (9). The paroxonase1 (PON1) enzyme present in the HDL surface provides anti-inflammatory and antioxidant properties for HDL which leads to destruction of the oxidized LDL; therefore, it has an antiatherogenic activity. The most important members of this gene family are PON1-PON2-PON3, which is located on chromosome 7 (7q21.3-22.3) (10). The PON1 gene is located on the long arm of the chromosome 7 containing 9 exons and 26 kb in length and more than 200 single-nucleotide polymorphisms (SNPs) have been identified so far. A number of these are located in the coding region, such as L55M and Q192R, that has been demonstrated to affect the catalytic activity of the PON1 enzyme (9, 11).

Despite the prevalence of coronary artery obstruction in Iran, especially in Golestan province, no comprehensive study has been carried out on determining the frequency of PON1 gene polymorphisms in CAD in the Middle East so far.

2. Objectives

We aimed to analyze the association of the frequency of two functional PON1 gene polymorphisms, L55M and Q192R with CAD in Iranian population.

3. Materials and Methods

3.1. Sample Preparation

The study groups in this study consisted of 305 CAD patients and 302 healthy controls aged 32 - 65 years, selected after the angiography, from individuals referred to Angiography Center of Amiralmomenin in Golestan province at northern Iran from 2008 to 2016. The sample size was calculated with a power of 90% as previously described (12). The inclusion criteria for the patient group were having stenosis more than 50% in at least one major coronary artery. Inclusion criteria for the control group were also having normal electrocardiograms at rest, without symptoms of myocardial ischemia during exercise. In agreement with the Helsinki Declaration, all the participants were aware of the study details and signed the relevant written informed consent. This study was approved by the Ethics Committee of Golestan University of Medical Sciences (Ethnicity code: 39459212275). Information on the patient's profile including age, gender, smoking status, blood pressure, diabetes mellitus and ethnic status were extracted from medical records. Blood samples were collected, and genomic DNA was extracted from the whole blood, as described previously (13).

3.2. Genotyping

SNPs of L55M (rs854560) and Q192R (rs662) located in the upstream of the transcription start site were genotyped using a sequence specific primers polymerase chain reaction (SSP-PCR) primers set. The primers were designed by Gene Runner software (version 5.2; Hastings, USA) and PON1 gene sequence information (Table 1). PCR reactions were performed using Taq DNA Polymerase Master Mix (Ampliqon, Copenhagen, Denmark) with 100 ng of DNA and 10 pmol of each primer in a thermal cycler (Techne, Burlington, UK). A primer set was used to amplify human growth hormone (hGH) gene, as the internal control to confirm negative PCR. PCR conditions included initial denaturation (94°C for 4 min), the first 35 cycles (94°C for 30 sec, 55°C for 60 sec and 72°C for 40 sec), and finally 72°C for 5 min. The PCR products were then electrophoresed, as described previously (14).

3.3. Statistical Analysis

Data was analyzed using GraphPad software (version 6; San Diego, CA, United States). Hardy-Weinberg equilibrium and the association between genotypes and disease were analyzed using Chi-square and Fisher's exact tests. Univariate and multivariate logistic regression analysis were performed in order to investigate the risk factors

Table 1. Information of Primers Used in this Study

Description	Primer Name	Sequence (5 ' → 3 ')	Product Size (bp)	GenBank Accession Number
SSP-PCR				
PON1(L55M)T > A	Forward A-allele	GAAAGACAGTCCATTAGGCAGTATCTCCAT	168	NG_008779.1
	Forward T-allele	GAAAGACAGTCCATTAGGCAGTATCTCCAA		
	Reverse generic	TGAACCTATTAAAGAAGAGTGATGTATAGC		
	Forward A-allele	TTTTCTTGACCCCTACTTACA		
PON1(Q192R)	Forward G-allele	TTTTCTTGACCCCTACTTACG	536	
A > G	Reverse generic	ATTGCCTTGATTTACATTTTGGTACA		
hGH	Forward	GCCTTCCCAACCATTCCCTTA	430	NG_011676.1
	Reverse	TCACGGATTTCTGTTGTGTTTC		
Real-Time PCR				
PON1 cDNA	Forward	CCAAGTGAAGTTCGAGTGGTGG	70	NM_00044
	Reverse	TGCCATCGGGTGAAATGTTG	6.5	
PGK1 cDNA	Forward	GCAGATTGTGTGGAATGGTC	101	NM_00029
	Reverse	CCCTAGAAGTGGCTTTCACC	1.3	

of CAD. Variables that showed an association with CAD in univariate analysis were analyzed with a multivariate logistic regression model to examine the independent risk factors of CAD. P-values less than 0.05 were considered significant.

4. Results

The association of some CAD risk factors and ethnic groups with CAD disease is shown in Table 2. Logistic regression analysis showed that age, gender, smoking status, and diabetes mellitus were significantly associated with CAD disease (P < 0.001). No association was seen between blood pressure status and ethnic groups with CAD disease.

Allelic and genotypic frequencies of L55M (rs854560) and Q192R (rs662) at PON1 gene are shown in Table 3. Deviation from the Hardy-Weinberg equation was not observed for both SNPs in either patients group ($\chi2 < 3.65$, df = 1, P = 0.237), and controls group ($\chi2 = 2.77$, df = 1, P = 0.352). As shown in Table 3, there was a significant association between genotype A/A of PON1 (L55M) T > A polymorphism and CAD disease [OR (95% CI): 1.83 (1.15 - 2.90), P = 0.011]. The results indicated that allele A in this SNP is also significantly associated with the CAD disease [OR (95%CI): 1.40 (1.11 - 1.76), P = 0.005]. To investigate the inheritance model of two SNPs in PON1 gene, we considered three recessive, dominant and co-dominant models (Table 3). Analysis of dominant genetic model in PON1(L55M)T > A showed that one copy of A is sufficient for increased risk [OR (95% CI): 1.49 (1.07 - 2.07), P = 0.016], and no significant association was seen in the recessive and co-dominant models of inheritance. In PON1(Q192R)A > G, none of the hereditary models was significant (Table 3).

Table 2. The Association of some CAD Risk Factors and Ethnic Groups with CAD Disease

Features	Logistic Regression			
	Univariate		Multivariate	
	OR (95% CI)	P value	OR (95% CI)	P value
Age (45.8 ± 8.1)	1.79 (1.16 - 2.65)	< 0.001	2.10 (1.21 - 2.87)	< 0.001
Gender	2.47 (1.86 - 3.58)	< 0.001	2.95 (1.94 - 3.46)	< 0.001
Smoking	2.31 (1.36 - 3.65)	< 0.001	2.43 (1.28 - 3.74)	< 0.001
Blood pressure (120 ± 15.6)	1.18 (0.83 - 1.58)	0.316		
Diabetes mellitus	2.21 (1.50 - 3.11)	< 0.001	2.56 (1.64 - 3.38)	< 0.001
Ethnic groups				
Persian	0.61 (0.34 - 1.11)	0.241		
Turkmen	0.71 (0.44 - 1.09)	0.201		
Sistani	0.66 (0.41 - 1.13)	0.172		

Abbreviations: OR, odds ratio; CI, confidence interval.

Table 3. The Genotype and Allele Distributions of Two PON1 Polymorphisms in the CAD and Control Groups

Characteristic	Patients, n (%)	Control, n (%)	OR (95% CI)	P value
PON1(L55M)T > A				
Genotypes				
T/T	108 (35.4)	136 (45.0)	1	-
A/T	133 (43.6)	122 (40.4)	1.37 (0.96 - 1.95)	0.088
A/A	64 (21.0)	44 (14.6)	1.83 (1.15 - 2.90)	0.011
Alleles				
T	349 (57.2)	392 (65.0)	1	-
A	261 (42.8)	212 (35.0)	1.40 (1.11 - 1.76)	0.005
Model of inheritance				
Recessive (A/A vs. A/T + T/T)			1.56 (1.02 - 2.37)	0.053
Dominant (A/A + A/T vs. T/T)			1.49 (1.07 - 2.07)	0.016
Co-dominant (A/T vs. A/A + T/T)			1.14 (0.82 - 1.57)	0.459
PON1(Q192R) A > G				
Genotypes				
A/A	101 (29.6)	117 (29.0)	1	-
A/G	161 (57.8)	144 (60.9)	1.29 (0.91 - 1.83)	0.156
G/G	43 (12.6)	41 (10.1)	1.21 (0.73 - 2.01)	0.520
Alleles				
A	363 (58.5)	378 (59.4)	1	-
G	247 (41.4)	226 (40.5)	1.13 (0.90 - 1.43)	0.289
Model of inheritance				
Recessive (G/G vs. A/G + A/A)			1.04 (0.65 - 1.65)	0.907
Dominant (G/G + A/G vs. A/A)			1.28 (0.91 - 1.78)	0.152
Co-dominant (A/G vs. G/G + A/A)			1.22 (0.89 - 1.68)	0.224

Abbreviations: OR, odds ratio; CI, confidence interval

5. Discussion

Cardiovascular disease is recognized as one of the major health problems in Iran and throughout the world. Several theories have been proposed to explain the causative agents of coronary diseases; one of the most important theories is the theory of LDL oxidation. The LDL plaques are deposited in the vascular wall and lead to a decrease in vascular diameter and in myocardial oxygen supply; also, LDL oxidation and lipid peroxide production accelerate this process. PON1 is capable of inhibiting LDL oxidation (15). Functional polymorphisms in PON1 gene are believed to be effective in atherosclerosis and in the spread of certain diseases. Many studies have been conducted to investigate the relationship between this gene and diseases (16). Other diseases include Parkinson's, kidney disease, ocular diseases, systemic lupus erythematosus, abdominal aortic aneurysm, chronic idiopathic pancreatitis, diabetes, aneurysm, Crohn's disease, sarcoidosis, glomerulonephritis, breast cancer, prostate cancer, dementia, Alzheimer's disease, lipid disorders and preeclampsia (17, 18). In studies conducted in India on CAD patients, it has been shown that there was a relationship between PON1 polymorphisms and atrocyclose (9). In another study in Italy, the activity of PON1 enzyme decreased in diseases related to lipid disorders, including familial hypercholesterolemia, Tangier disease and diabetes mellitus (19). However, in another study in the same region, there was no a significant relationship between the genotype distribution of PON1 gene polymorphisms and the development of atherosclerosis (20). With regard to previous studies, it can be understood that studies are still scanty and ambiguous in this field. In contrast to our results, Gupta et al.'s study on India population showed that there was a significant association between PON1(Q192R) polymorphism and CAD, and no significant association was seen between PON1(L55M) polymorphism and CAD (21). In agreement with our results, Taskiran et al.'s study reported that there was a significant association between PON1(L55M) polymorphism and CAD, and no significant association was observed between PON1(Q192R) polymorphism and CAD (22). In contrast to these results, in a study done by Malin et al. in Finland on autopsy samples, the allele T in PON1 (L55M) was demonstrated as an independent risk factor for atherosclerosis, and individuals with T/T genotype had more severe atherosclerotic lesions (23). Mackness et al.'s study in England compared the frequency of PON1 gene polymorphisms in CAD patients and healthy individuals and did not report a significant difference between the genotypes' frequency of PON1 gene (24). Considering the existence of different ethnicities in Golestan province, this population may be considered as a representative population of the whole country. However, the effect of gene polymorphism on protein production may vary according to the type of tissue and cell. Therefore, further studies are needed to investigate the role of PON1 alleles in the production of protein and development of diseases such as CAD.

In conclusion, our findings at least in the population of Golestan province indicate that the PON1(L55M) polymorphism may be associated with atherosclerotic disease.

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Authors' Contribution

Samaneh Abdolahpour and Azam Bakhshandeh genotyped the patients and controls. Mehdi Rayatnavaz was the clinician involved in sample collection and clinical assessment. Touraj Farazmandfar carried out the statistical analysis and wrote the paper. Majid Shahbazi initiated the research program and supervised the project. All authors approved the final manuscript.

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Financial Disclosure

There is no conflict of interest.

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