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PCSK9 Gene Polymorphisms Associated With the Risk of Myocardial Infarction in Iranian Patients

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Abstract

Introduction: Proprotein convertase subtilisin/kexin type 9 (*PCSK9*) is a key regulatory protein in lipid metabolism and a candidate gene in the etiology of cardiovascular diseases. The present study aimed to evaluate the prevalence and significance of *PCSK9* rs505151 and rs11591147 variants with myocardial infarction (MI) risk in the Iranian population.

Patients and Methods: The frequency of the *PCSK9* rs505151 and rs11591147 variants were compared between 600 cases of MI and 600 healthy age- and sex-matched individuals. Tetra-primer amplification refractory mutation system-polymerase chain reaction (T-ARMS PCR) was used for rs505151, and amplification refractory mutation system-polymerase chain (ARMS-PCR) was utilized to detect the rs11591147 polymorphism. Finally, SPSS and SHEsis software were applied for data analysis.

Results: Carriers of the GG genotype of rs505151 polymorphism (OR: 1.57, 95% CI: 1.05–2.35, $P=0.02$; age-adjusted; OR: 1.54, 95% CI: 1.03–2.32, $P=0.03$) and at least one G-allele including GG+AG vs. AA (OR: 1.54, 95% CI: 1.04–2.28, $P=0.03$; age-adjusted; OR: 1.51, 95% CI: 1.01–2.24, $P=0.04$) have an increased risk of MI. No association between *PCSK9* rs505151 alleles and MI risk was observed. The ratio of individuals with the rs11591147GT variant was higher in healthy individuals vs. patients with MI (48.6% vs. 41.7%), indicating a reduced risk of developing MI (OR: 0.75; 95% CI: 0.59–0.95; $P=0.01$; age-adjusted; OR: 0.74; 95% CI: 0.58–0.95; $P=0.01$). The carriers of at least one T allele (TT+GT vs. GG) (OR: 0.78; 95% CI: 0.62–0.98; $P=0.03$; age-adjusted; OR: 0.78; 95% CI: 0.62–0.98; $P=0.03$) showed a significant reduction in MI risk. The allelic frequencies at this polymorphic site did not differ between MI patients and healthy counterparts. No association was found between the haplotypes constructed from the alleles of these two polymorphisms.

Conclusion: Our study provides the first evidence that *PCSK9* gene polymorphisms may serve as independent prognostic markers for MI patients in Iran.

Keywords: *PCSK9*, Polymorphism, Myocardial infarction, Iran

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Introduction

Myocardial infarction (MI) is a leading cause of global mortality and the most prevalent subgroup of coronary heart diseases (CHDs).[1,2] Among significant risk factors of MI are smoking, hypertension, diabetes mellitus, obesity, and low-density lipoprotein (LDL) and triglyceride (TG) levels.[3,4] Family history, age, and gender are characterized as non-modifiable risk factors.[4] Beside the inevitable role of non-genetic factors in predisposition to MI, higher concordance in monozygotic twins and increase in the incidence of MI in cases with positive family history highlight the footsteps of the genetics in MI etiology.[5] During years, linkage analyses and genome-wide association studies concluded in a huge list of genes and single nucleotide polymorphisms (SNPs) for their potential role in MI pathogenesis.[6-10] Among these, the genes involved in cholesterol homeostasis pathway, including Apo-lipoprotein B-100 (*APOB-100*),[11] LDL receptor (*LDLR*),[12] proprotein convertase subtilisin/kexin type 9 (*PCSK9*),[13,14] and oxidized low-density lipoprotein receptor 1 (*OLR1*),[14] seem the best candidates for their contribution in CHDs.

The mature 63 kDa PCSK9 serine protease is a translational product of the *PCSK9* gene (MIM: 607786) on the short

arm of the human chromosome 1.[15] PCSK9, in a heavy trafficking scene between production and clearance of LDL, plays its critical role as a negative regulator of LDLR through either a post-transcriptional mechanism or preventing the endosome-recycling of the receptor toward cell membrane.[13,16-18] The importance of the gain of function variant rs505151 (exon 12; p.E670G; A23968G) and the loss of function variant rs11591147 (exon 1; p.R46L; G137T) on the PCSK9-related functions in association with CHDs and plasma lipid profile widely investigated. Previous studies demonstrated the significant changes in the level of TG and LDL in the carrier of rs11591147T and rs505151G alleles.[19,20]

The present study was conducted to investigate the association between the PCSK9 rs505151 and rs11591147 polymorphisms with the risk of MI in the Iranian population.

Patients and Methods

Subjects

We performed a hospital-based case-control study enrolling 600 healthy individuals and 600 MI cases from the Iranian population. The study lasted from September 2017 to August 2018. MI cases were selected among the patients admitted in the heart clinic of the Al-Zahra heart hospital, Shiraz, and Vali-e-Asr hospital, Fasa, Iran. The diagnosis was performed according to the result of the cardiac marker troponin I (cTn I) measurement. All patients diagnosed with the concentration of cTn I above than normal amount (0.35 ng/mL). The control group composed of volunteers admitted for routine tests in the laboratories of both hospitals. All individuals signed informed consent before their inclusion in the study.

Demographic and anthropometric characteristics of the individuals were collected filling the standard questionnaire prepared by reviewing the literature.

DNA Extraction and Genotyping

The genomic DNA was extracted using the manual salting-out method.[21] Electrophoresis on 1% agarose gel was the technique of choice to examine the quality of the extracted DNA. Tetra-primer amplification refractory mutation system-polymerase chain reaction (T-ARMS PCR) and amplification refractory mutation system-PCR (ARMS-PCR) used to genotype *PCSK9* rs505151 and rs11591147 polymorphisms respectively. Primers are listed in Table 1. Each PCR reaction was done using 6.25 µL ready to use master mix (YTA, Iran), 0.5 µL of each primer, and 1 µL of DNA. The final volume was reached 12.5 µL adding ddH_2O. The annealing temperatures were 56°C (rs505151) and 60°C (rs11591147). Following electrophoresis of the PCR products on 2.5% agarose gel mixed with DNA safe stain dye for 40 minutes, gels were examined under UV light to determine the bands.

Statistical Analyses

Extracted data from both molecular and demographic analyses were analyzed using SPSS 19.0. Quantitative characters presented as mean ± SD and the qualitative variables presented as frequency (percent). Hardy-Weinberg equilibrium was tested using the chi-square test. Logistic regression analysis was used to explore the association between PCSK9 gene polymorphisms and the risk of MI. The effect of some probable risk factors for MI was checked using logistic regression analysis. Haplotype analysis was performed using The SHEsis software platform (http://analysis.bio-x.cn). In all analyses, the *P* value below 0.05 was considered statistically significant.

Results

Baseline Characteristics of the Study Subjects

Characteristics of the study subjects present in Table 2. The mean age of the MI cases was 60.59 ± 12.60 years distributed in a range of 29–94 years. The mean age of the controls was 57.22 ± 14.27 years (24–93 years). The difference between the age means was statistically significant between cases and controls ($P \geq 0.001$). The difference in body mass index (BMI), weight, and lipid profile were significant between groups (Table 2). Diabetes mellitus, hypertension, smoking, and family history for MI all increased the risk of MI (Table 3).

Table 1. Sequence of the Primers and the Size of the Amplicons

SNPs	Primers	Primer Sequence (5'-3')	Amplicon Size (bp)
rs505151	FO	GGGATGGGGCAGGCTATG	FO-RO: 722 FO-RI (A-allele): 479 FI-RO (G-allele): 278
	RO	CAGAGTGAGTGAGTTCCAGGC	
	FI(G)	AGGCAGCACCAGCGATG**G**	
	RI(A)	CAACGGCTGTCACGGAC**T**	
rs11511947	FO	GCCCTGCTCCTGAACTTC	FO-RO: 440 Allele specific: 195
	RO	GCACTCCACTTCCTCTCTTAC	
	FI(G)	GCTGGTG**T**TAGCCC**T**GC**G**	
	FI(T)	GCTGGTG**T**TAGCCTTA**C****T**	

Variant alleles are bold and underline, modified bases are underline; FO: Forward outer; RO: Reverse outer; FI: Forward inner; RI: Reverse inner

rs505151 Polymorphism and MI

The distribution of the genotypes in this polymorphism (Figure 1) was not in Hardy-Weinberg equilibrium in the control group ($\chi^2 = 29.74$, df = 1, $P = 0.000$). The genotype frequencies in cases were 7.7% (AA), 56.2% (AG), and 36.2% (GG). The alternative frequencies in controls were 11.3% (AA), 52.8% (AG), and 35.8% (GG). The difference in the frequency of the GG genotype was significant before and after adjusting the data for age (Table 4). In the dominant model for the G allele (GG + AG), the reduced in the risk of MI was seen before and after adjustment for age. Neither G nor A alleles were associated with MI risk (Table 5).

Table 2. Characteristics of the Subjects

Variable	MI Cases	Controls	*P* Value[a]
Total	600	600	-
Age range (y)	29-94	24-93	-
Mean of age (y)	60.59±12.60	57.22±14.27	**≤0.001**
Gender ratio (male: female)	339:261	339:261	-
Height (m)	168.87±6.38	169.39±8.87	0.24
Weight (kg)	73.57±8.52	71.25±9.76	**≤0.001**
BMI (kg/m^2)	25.73±2.93	24.69±5.04	**≤0.001**
LDL (mg/dL)	103.36±25.25	96.02±29.05	**≤0.001**
HDL (mg/dL)	40.77±5.67	38.44±16.38	**0.001**
TG (mg/dL)	197.67±44.86	149.26±40.61	**≤0.001**

Abbreviations: LDL, Low density lipoprotein; HDL, High density lipoprotein; TG, Triglyceride.
[a] Student *t* test.
Significant values in bold.

rs11591147 Polymorphism and MI

The genotype frequencies observed for this polymorphism (Figure 2) were in accordance with Hardy-Weinberg equilibrium in the control group ($\chi^2 = 1.83$, df = 1, $P = 0.175$). Genotype frequencies for this polymorphism shown in Table 4. The frequency of the GT genotype was revealed to be different between groups (48.6% vs. 41.7%) and this difference reached the statistical significance before and after adjustment for age. The frequencies of the alleles did not show the difference between MI cases and controls (Table 5).

Haplotype Analysis

The frequencies of the haplotypes in cases and controls are

Table 3. Impact of Some Risk Factors on the Susceptibility of MI

Variable	Case (n=600)	Control (n=600)	*P* Value	OR[b] (95% CI)	*P* Value	OR[b] (95% CI)
Diabetes	299 (49.8)	158 (26.3)	**≤0.001**	2.78 (2.18-3.54)	**≤0.001**	3.27 (2.50-4.28)
Hypertension	409 (68.2)	186 (31)	**≤0.001**	4.76 (3.73-6.08)	**≤0.001**	5.48 (4.19-7.17)
Smoking	335 (55.8)	128 (21.3)	**≤0.001**	2.92 (2.26-3.76)	**≤0.001**	2.96 (2.26-3.90)
Family history	375 (62.5)	176 (29.3)	**≤0.001**	4.01 (3.15-5.11)	**≤0.001**	4.71 (3.60-6.18)

Abbreviation: OR, odds ratio.
[a] Logistic regression analysis before adjustment for age; [b] after adjustment for age.
Significant values in bold.

Table 4. Frequencies of the Genotypes of the *PCSK9* rs505151 and rs11511947 Gene Polymorphisms in MI Cases and Healthy Controls

		Co-dominant Model		Dominant Model
rs505151	**AA**	**AG**	**GG**	**GG+AG**
Controls = 600, n (%)	68 (11.3)	317 (52.8)	215 (35.8)	532 (88.7)
Cases = 600, n (%)	46 (7.7)	337 (56.2)	217 (36.2)	554 (92.3)
OR[a] (95% CI)	1	1.49 (0.98-2.27)	1.57 (1.05-2.35)	1.54 (1.04-2.28)
P value	-	0.06	**0.02**	**0.03**
OR[b] (95% CI)	1	1.45 (0.95-2.21)	1.54 (1.03-2.32)	1.51 (1.01-2.24)
P value	-	0.08	**0.03**	**0.04**
rs11511947	**GG**	**GT**	**TT**	**TT+GT**
Controls= 600, n (%)	241 (40.2)	291 (48.6)	67 (11.2)	358 (59.8)
Cases = 600, n (%)	278 (46.3)	250 (41.7)	72 (12)	322 (53.7)
OR[a] (95% CI)	1	0.75 (0.59-0.95)	0.93 (0.64-1.35)	0.78 (0.62-0.98)
P value	-	**0.01**	0.71	**0.03**
OR[b] (95% CI)	1	0.74 (0.58-0.95)	0.92 (0.63-1.35)	0.78 (0.62-0.98)
P value	-	**0.01**	0.68	**0.03**

Abbreviation: OR, odds ratio.
[a] Logistic regression analysis before adjustment for age; [b] After adjustment for age.
Significant values in bold.

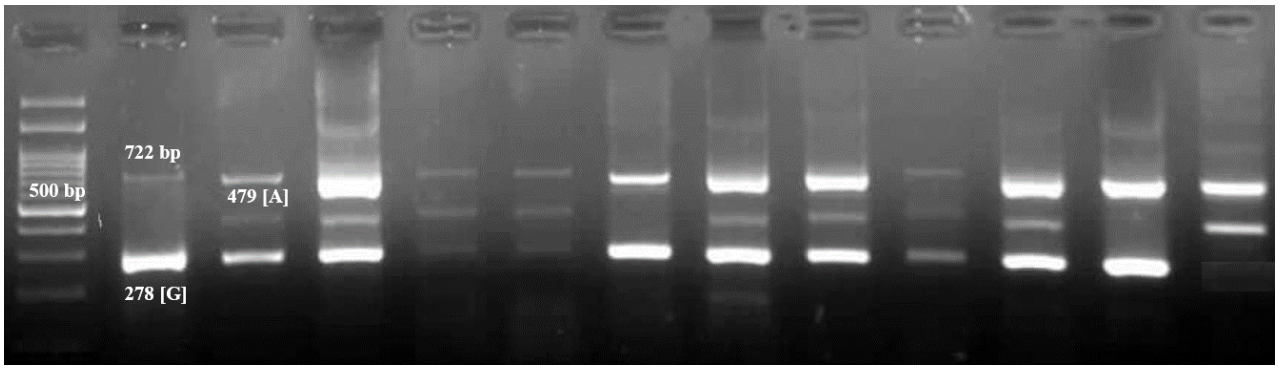

Figure 1. Differentiation of the Three Genotypes of the *PCSK9* rs505151A/G on 2.5% Agarose Gel Using T-ARMS PCR. From left to right, 100bp ladder, homozygote GG genotypes in lanes 2, 7, 12; heterozygote AG genotype in lanes 3-6, 8-11; homozygote AA genotype in lane 13.

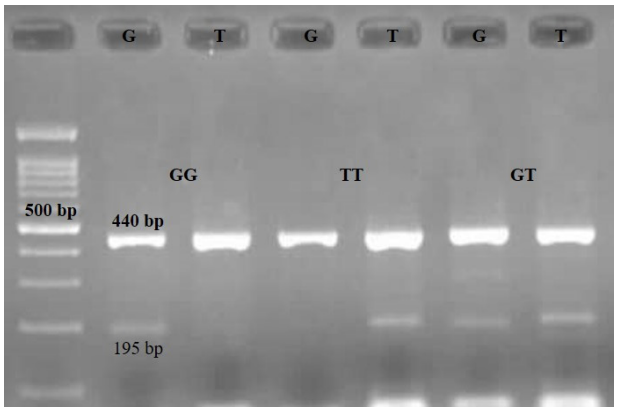

Figure 2. The Size Separation of the ARMS PCR Products of Different Alleles of the *PCSK9* rs11591147G/T Polymorphism. From left to right: 100bp ladder, lane 2 & 3: homozygote GG; lane 4 & 5: homozygote TT genotype; lane 6 & 7: heterozygote GT genotype. ARMS-PCR using to determine different genotypes of the *PCSK9* rs11591147G/T polymorphism.

presented in **Table 6.** No significant difference between the frequencies of the haplotypes found between cases and controls.

Discussion

PCSK9, a newly discovered member of the mammalian serine proprotein convertase family, gets much attention because of its impressive roles in cholesterol metabolism,[22] atherosclerosis[15,23] and so CHD susceptibility.[24] Literature review resulted in many studies investigating the association of the *PCSK9* gene variants and coronary artery disease (CAD) risk in different populations or ethnicities. Regarding these contradictory results, this study for the first time, was conducted to explore the association between *PCSK9* functional variants (rs505151 and rs11591147) and the risk of MI in the Iranian population.

Among several known SNPs in the *PCSK9* gene, the common rs505151A/G (E670G) variant has been confirmed as a gain of function missense alteration. Here, the frequency of the risk rs505151G allele was found to be as high as 0.62 in the healthy population, whereas Huang et al reported the G-allelic distribution of 0.25 among the young adult non-CHD Chinese population.[25] The frequency of this allele in our study was significantly higher than the corresponding frequency reported previously by Scartezini et al for about 0.03 among UK healthy men.[26] Moreover, Hsu et al, Slimani et al, and Cai et al found the frequency of about 0.06 for risk G-allele among Chinese ethnic in Taiwan, Tunisian, and southern Chinese Han population respectively, demonstrating that the majority of these populations carry the wild-type A-allele, in contrast to our study.[27-29] The high variability in the allelic frequency of this polymorphic site may due to differences in ethnic or race. Furthermore, the size of the recruited samples, the sensitivity of the genotyping methods, inclusion and exclusion criteria used in study design, and finally, and most important, the heterogeneous nature of the heart diseases should take into account as the possible answer for the huge amount of variability in the allelic frequencies between populations and inter-ethnicity.

Table 5. Frequencies of the Alleles of the rs505151 and rs11511947 Gene Polymorphisms in MI Cases and Controls

	Alleles	Controls, n (%)	Cases, n (%)	OR (95% CI)	*P* Value[a]
rs505151	A	453 (0.38)	429 (0.36)	1	-
	G	747 (0.62)	771 (0.64)	1.09 (0.92-1.29)	0.310
rs11511947	G	773 (0.65)	806 (0.67)	1	-
	T	427 (0.35)	394 (0.33)	0.88 (0.75-1.05)	0.156

Abbreviation: OR, odds ratio.
[a] Logistic regression analysis.

Table 6. The Haplotype Distributions of *PCSK9* rs505151and rs11511947 Gene Polymorphisms

Haplotype	Controls (Freq)	Vase (Freq)	χ^2	Pearson's *P*	OR (95% CI)
A G	289 (0.24)	276 (0.23)	0.398	0.52	1.01 (0.92-1.12)
A T	166 (0.14)	152 (0.13)	0.700	0.40	0.97 (0.82-1.16)
G G	487 (0.41)	528 (0.44)	2.885	0.08	1.05 (0.97-1.13)
G T	258 (0.21)	244 (0.20)	0.501	0.47	0.99 (0.88-1.13)

All those frequencies <0.03 ignored in analysis.

All these studies shared an important characteristic in common, no association with genotypes and/or alleles of the *PCSK9* rs505151 polymorphism with development and/or severity of CAD or CHD was not found.[25-28] In contrast to the above-mentioned studies, a study has been conducted in China by He et al who attempted to reveal whether the rs505151 SNP in the *PCSK9* gene plays a role as a molecular marker for CAD. Although the frequency of the G allele was as low as 5.6% in the non-CHD healthy group, a significant association was shown between GG and AG genotypes and G allele and greater risk of CHD.[30] In the recent study, Reddy et al showed an increase in the risk of CAD among the north Indian population in the carriers of AG and AG+GG (dominant model for G allele) and the G allele.[31] Confirming evidence on the correlation between the rs505151G allele and risk of CAD have provided from two meta-analyses performed by Adi et al[32] and Qiu et al.[19] Our results were consistent with the four latter studies showing an increased in the risk of MI in carriers of rs505151GG and GG+GA genotypes before and after adjusting the data for age.

rs11591147G/T is the second well-characterized rare variant in the *PCSK9* gene. Multiple studies substantiate the role of this polymorphism on CAD [33] and MI.[34,35] The frequency of the rs11591147T allele varies between populations, e.g. 2-4.5% in Caucasians.[22,32,33] Unlike other Caucasians, the frequency of the rs11591147T allele was estimated lower in Italy by approximately 1.42%.[36] Cai et al found no R46L variant in the population of southern Han Chinese population.[27] The frequency of the rs11591147T allele in our healthy population was considerably higher than previous reports (33%). The association analysis in our study population resulted in the protective effect of the GT heterozygote genotype and the dominant model of inheritance for T allele (TT+GT vs. GG) on the risk of MI. similar results were extracted from the study by Kathiresan et al which noticed the heterozygosity for rs11591147 *PCSK9* variation associated with lower risk of MI.[34] Benn et al showed a 58% reduction in the risk of MI in carriers of the rs11591147T allele (46L).[35] Provide more confidence on the protective influence of rs11591147T allele against MI, a comprehensive case-control study with 1880 Patients with premature MI (age <45 years) and 1880 healthy controls were performed by Guella et al in Italy. The results of the study did not support the protective effect of the rs11591147T allele against MI. Follow the recruitment of 1056 elderly controls, the difference was significant toward the protection against MI.[36] A meta-analysis by Qiu et al confirmed previous reports on the role of rs11591147T (46L) allele in protecting carriers from cardiovascular disease susceptibility.[19] Unlike the above studies supporting the reduces in the risk of cardiovascular disease in carriers of the rs11591147T allele, Polisecki et al in the study of an elderly population with a high prevalence of cardiovascular disease did not find any statistically significant difference for T-allele frequency between groups.[37]

Conclusion

In conclusion, this is the first study to assess the allelic and genotype frequencies of the PCSK9 rs505151 and rs11591147 variants among the Iranian population. A higher risk of MI was reported for the homozygote rs505151GG genotype and also in the dominant model for GG+AG before and after adjusting the data for age. While the protective effect of the heterozygote HT and the TT+GT genotype was seen against MI for the rs11591147 variant. Diabetes mellitus, hypertension, smoking, and family history for MI were found the age-independent risk factors for MI.

Ethical Approval

This article has been extracted from MSc thesis in the field of Molecular Genetics approved by the Islamic Azad University, Arsanjan Branch, Iran. The study was approved by the Local Ethics Committee of our Department.

Conflict of Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

FNZ contributed to design, contributed to acquisition, analyses, drafted the manuscript. MN contributed to conception and design, contributed to analysis and interpretation and critically revised manuscript. The content of the paper was approved by all authors.

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Branch.

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