

Genotyping of 2 Cholesteryl Ester Transfer Protein Gene Variants Among Coronary Artery Disease Patients

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Abstract

Introduction: Coronary artery diseases (CAD) are still among the top causes of death in most populations. The polymorphisms of the cholesteryl ester transfer protein (CETP) gene can influence the risk of CAD through modulating cholesterol metabolism. In this regard, the current study aimed to determine the role of the 2 important CETP gene polymorphisms in CAD patients.

Methods: To this end, DNA was extracted from the whole blood of 100 CAD patients and 100 healthy controls and then subjected to polymerase chain reaction-restriction fragment length polymorphism for the genotyping of rs5882 and rs708272 polymorphisms.

Results: Based on the results, no meaningful association was found between rs5882 and rs708272 polymorphisms, neither separately nor in combination, and the risk of CAD. However, the risk of CAD significantly increased in male rs5882 polymorphism carriers ($P=0.01$). Finally, no significant association was demonstrated between serum high-density lipoprotein levels and the genotypes or alleles of neither rs5882 nor rs708272 polymorphism.

Conclusion: Despite the finding regarding the lack of an association between CAD and the studied polymorphisms of the CETP gene, the importance of those variants in CETP protein function and CAD pathogenesis warrants further investigation on larger populations.

Keywords: CETP gene, rs5882, rs708272, Polymorphism, Coronary artery disease

Introduction

In spite of recent improvements in population health and preventive lifestyles and pharmaceutical approaches, coronary artery diseases (CAD) remain the most serious causes of human mortality around the world.^{1,2} In addition, CAD includes a group of morbidities such as stable and unstable angina, myocardial infarction, and sudden cardiac death, along with cardiovascular diseases as the most frequent CAD diseases.¹ The most important risk factors are hypertension, smoking, uncontrolled diabetes mellitus, the lack of physical activities, psychological disorders, obesity and high serum cholesterol, and abnormal lipid profile.^{3,4} Further, dyslipidemia plays a major role in the pathogenesis of atherosclerosis. Based on the evidence, most CAD patients have

a related defective hereditary factor in their families. In this regard, identification of variants and polymorphisms within genes involved in lipid metabolism pathways not only helps to clarify the CAD pathogenesis but also may improve CAD screening and early diagnosis and thus prevent the disease.⁵⁻⁷

Cholesteryl ester transfer protein (CETP) exchanges cholesterol and triglyceride between high- and low-density lipoproteins (HDL & LDL), as well as very-low-density lipoproteins (VLDL).⁸ In this way, CETP suppressor drugs and anacetrapib are efficient in enhancing the level and activity of HDL-cholesterol against the atherogenic process.⁹ Various types of polymorphisms and variations have been found within the CETP gene, the importance of which on the CETP glycoprotein function and HDL-C



level is still elusive in different populations.¹⁰⁻¹² rs5882 (I405V) and rs708272 (Taq1B) are the most frequent polymorphisms of the CETP gene which have been studied in various types of heart diseases and CAD.^{11,13}

Owing to the controversy in the results of the association of aforementioned CETP gene polymorphisms with the risk of CAD and the level of lipoproteins in the serum even within the same ethnicities, the present study aimed to primarily assess those associations among a subset of the Iranian population.^{14,15}

Materials and Methods

Population

The case group consisted of 100 patients who referred to the cardiovascular clinics of Baqiyatallah Hospital (Tehran-Iran) during 2017-2019. The inclusion criteria were agreement on filling a consent form, the minimum age of 18 years, and the confirmation of CAD diagnosis through angiography by a cardiologist. On the other hand, patients affected by other cardiac diseases (e.g., valvular diseases, vacuities, and congenital anomalies) and those who were younger than 18 years old or showed unwillingness for participation were excluded from the study. Finally, an equal number of healthy controls with no medical and familial history of sudden death, cardiovascular diseases, or any other chronic diseases were selected to be included in the study.

Blood Sampling

Five milliliters of the whole blood was drawn from all the enrolled samples into ethylenediaminetetraacetic acid canonical tubes. Furthermore, DNA was isolated from collected blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Moreover, the quality and quantity of obtained DNAs were determined using a NanoDrop ND-1000 spectrophotometer.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

The 2 selected polymorphisms of the CETP gene were assessed through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR reaction contained 10 pmol of specific forward and reverse primers (Table 1), Additionally, Taq DNA Polymerase 2x Master Mix RED including 1.5 mM MgCl₂ (Ampliqon,

Denmark) in addition to 50 ng of each genomic DNA sample was adjusted up to the final volume of 25 µL using ddH₂O. After an initial denaturation at 95°C, 30 PCR cycles were performed in a Bio-Rad thermocycler (Bio-Rad Laboratories, Hercules, CA, USA) as the denaturation at 95°C for 30 seconds, annealing temperature specific for each primer pairs for 1 minute, extension at 72°C for 30 seconds, and the final extension at 72 °C for 10 minutes. In addition, annealing temperatures were optimized at 60°C and 62°C for rs5882 and rs708272 polymorphisms, respectively. Further, RFLP reactions were performed on PCR products using the RsaI restriction enzyme for the detection of rs5882 polymorphism producing 268 bps and 40 bps fragments in wild type (G allele). Furthermore, Taq1B was used to digest the PCR products of rs708272 polymorphism producing 535, 361, and 174 bps fragments in wild type (B1 allele). All PCR and RFLP products were resolved on the agarose gel electrophoresis spotted by the SYBR Green I Nucleic Acid Gel Stain except for the RFLP products of RsaI digestion resolved on the polyacrylamide gel electrophoresis 8%.

Biochemical Analysis

To determine the effects of the studied polymorphisms on the lipid profile of enrolled samples, the serum levels of total cholesterol, HDL-C, and LDL-C were measured by colorimetric enzymatic assays using an auto-analyzer (AU 2700 Olympus, First Chemical Ltd, Tokyo, Japan).

Statistical Analysis

The frequency of genotypes and the alleles of the studied polymorphisms were determined between the enrolled patients and healthy controls using Fisher exact and chi-square tests. Additionally, univariate analysis of variance (ANOVA) and multivariate analysis of covariance (ANCOVA) analyses were used to determine the effects of the CETP polymorphisms on the serum HDL-C level. The Hardy-Weinberg equilibrium for genotypic distribution was calculated using Haploview, version 4.0 (Daly Lab at the Broad Institute, Cambridge, MA, the USA). $P < 0.05$ was accepted statistically significant by considering the confidence interval (CI) as 95%.

Results

Table 2 present the frequency and mean of the physical and chemical characteristics of enrolled samples. Similarly, Table 3 provides the frequency of the genotypes and the alleles of the studied polymorphisms (Figures 1 and 2). The results of the chi-square analysis demonstrated no meaningful association between both rs5882 and rs708272 polymorphisms, neither separately nor in combination, and the risk of CAD. Moreover, the risk of CAD failed to increase in either rs5882 or rs708272 polymorphisms considering their smoking status. However, the risk of CAD meaningfully increased in male rs5882 polymorphism carriers ($P = 0.01$, odds

Table 1. Primer Sequences for the Amplification of the 2 Gene Polymorphisms of the CETP Gene

Gene (Polymorphism)	Primer Sequence (5'-3')
CETP (rs5882)	F: GCA GAA CAG TAG TGG CCA AGC AGC G R: GTG ATC ATT GAC TGC AGG AAG CTC TGT A
CETP (rs708272)	F: CAC CCC AGA GAG AGG AGT GCC R: CTG AGC CCA GCC GCA CAC TAA C

Note. CETP: Cholesteryl ester transfer protein.

Table 2. Biochemical and Demographic Characteristics of Patients and Controls

Variable	Case n=100	Control n=100	P value
Age	67.06±7.20	66.86±14.67	NS
Gender (male/female)	63/37	69/31	NS
Height	166.07±7.51	165.55±8.16	NS
Weight	77.64±12.91	78.27±11.14	NS
Smoking (yes/no)	87/13	38/62	<0.001
Total cholesterol	4.9±1.20	4.2±0.93	<0.001
HDL-C (mmol/L)	1.1 ±0.25	1.2±0.21	<0.001
LDL-C (mmol/L)	2.79 ±0.61	2.37± 0.23	<0.001

Note. NS: Not significant; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol.

Table 3. Frequency of the Genotypes of Studied Polymorphisms Among Case and Control Groups

Gene Polymorphisms	Groups		χ^2	P	
	Patient n= 100	Control n= 100			
rs708272	GG	27	36	2.986	0.189
	GA	55	43		
	AA	18	21		
	GA+AA	73	64		
	AA	15	20		
rs5882	GG	45	52	3.337	0.225
	GG	40	28		
	GA+AA	85	80		

ratio =2.68, CI=0.95). In addition, the frequencies of both polymorphisms were in Hardy-Weinberg equilibrium among the analyzed population ($P>0.05$). Eventually, the ANOVA analysis represented no significant association between the serum HDL level and genotypes or alleles of neither rs5882 nor rs708272 polymorphism.

Discussion

The present study primarily failed to find any association between the risk of CAD and rs708272 polymorphism among a subset of the Iranian population. Neither rs708272 nor rs5882 polymorphisms of the CETP gene demonstrated a meaningful association with the elements of the lipid profile and HDL-C except for the male-specific association of rs5882 polymorphisms with the risk of CAD. In their study, Ghatreh Samani et al found a significant association among rs5882 polymorphism, HDL level, and CAD risk among the Azeri population of Iran.¹⁶ Contrarily, Goodarzynejad et al observed no meaningful correlation between rs5882 polymorphism and the risk of premature CAD (PCAD) among the Iranian population.¹⁷ Interestingly, Heidari-Beni et al reported higher HDL-C levels in the heterozygotes of rs708272 polymorphism in the assessment of 5528 samples aged 10-18 years old.¹⁸ In addition, Mirhafez et al showed a meaningful association between Taq1B polymorphism

and the risk of CAD in the study on 194 Iranian patients without affecting a lipid profile.¹⁹ This discrepancy in the results among the Iranian population indicates that the association of CAD and CETP gene polymorphisms can be influenced by age stratification, type of CAD, and maybe the race of selected samples. Conversely, Shahid et al found no association between rs708272 polymorphism and the risk of CAD among Pakistani CAD patients, which is consistent with our results. Nonetheless, rs708272 polymorphism had a significant effect on increasing and decreasing the levels of triglyceride and HDL-C, respectively.^{14,15} In another study, Arikan et al reported no association between rs5882 and the risk of CAD and lipid profile among the Turkish population, which is in line with our findings without considering participants' gender.²⁰ However, Todur et al represented the gender-dependent effect of rs5882 polymorphism on the LDL level without changing the risk of CAD among the Indian population,²¹ which corroborates with the finding of the current study. In contrast, Iwanicka et al concluded that normal individuals with the T allele of rs708272 polymorphism had higher cholesterol levels, and the C allele was associated with a statistically higher risk of CAD among the Polish population.²² Likewise, Cyrus et al demonstrated the meaningful effect of rs708272 and rs5882 polymorphisms on decreasing and increasing the

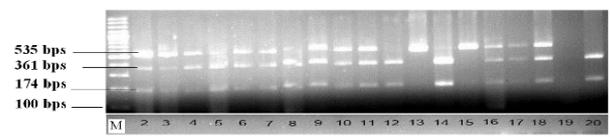


Figure 1. PCR-RFLP of rs708272 Polymorphism Among Case and Controls.

Note. PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; M: Marker (100 bps); Lanes 2, 3, 4, 6-11, and 16-18 represent heterozygotes (535, 361, and 174 bps bands); Lanes 5, 12, 14, and 20 indicate wild homozygotes (361 and 174 bps bands) whereas lanes 13 and 15 display mutant homozygotes with intact uncut PCR products (535 bps band).

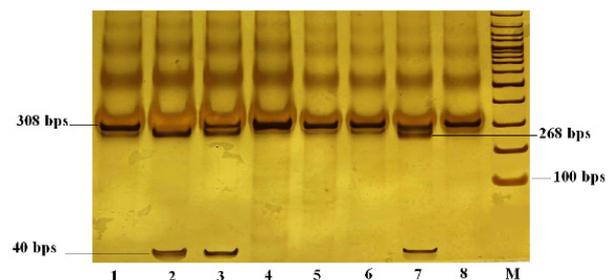


Figure 2. PCR-RFLP of rs5882 Polymorphism Among Case and Controls.

Note. PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; M: Marker (100bps); Lanes 3 and 7 indicate heterozygotes (268, 308, and 40 bps bands); Lanes 1, 4, 5, 6, and 8 represent AA homozygotes (308 bps band) whereas lane 2 denotes GG homozygote (268 and 40 bps bands).

risk of CAD among Saudi Arabian.²³ Based on the results of a randomization meta-analysis study, the carriers of the rs708272-B1B1 genotype had significantly decreased the level of the CETP protein, and therefore, had lower a risk of CAD. Inconsistent results were reported on the effect of rs708272 polymorphism on the level of HDL-C in 2 separate Chinese studies in spite of the same finding regarding the association of that polymorphism with the risk of CAD and coronary atherosclerosis.^{24,25} Abd El-Aziz et al found a significant correlation between rs708272 polymorphism and the risk of PCAD, along with a low serum level of HDL-C among the Egyptian population.²⁶ In their study, Virani et al demonstrated that although CETP gene variants could not considerably change the risk of recurrent myocardial infarction or recurrent revascularization. Based on their results, the mortality rate significantly increased among the AG genotype carriers of rs708272 polymorphism undergoing coronary artery bypass grafting.²⁷

Conclusion

Based on the reported literature on the association between rs708272 polymorphism and less significant rs5882 polymorphism of the CETP gene and the risk of CAD with or without affecting the lipid profile, the results remain inconsistent in different populations, especially those with different ethnicity groups. This probably indicates that other external CAD factors including physical activity, diet, and emotional stress should be cautiously considered and controlled during sample collection. Although controlling the aforementioned factors seems to be difficult, this is one of the most pivotal limitations of the present and most of the performed studies, leading to considerable bias in obtaining valuable results. Finally, the role of other genetic factors of CAD such as modifying factors should not be neglected but can be included in further studies considering larger sample sizes.

Ethical Approval

All enrolled patients filled the free and informed consent form according to the protocol of the Ethical Review Board of Science and Research Branch, Islamic Azad University of Tehran (Ethics code: IR.IAU.SRB.REC.1397.103) and the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the Institution's Human Research Committee. Eventually, the names of the cases and controls were replaced by numeric codes for data confidentiality.

Conflict of Interest Disclosure

None.

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