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Research paper

There is an association between a genetic polymorphism in the ZNF259 gene involved in lipid metabolism and coronary artery disease☆

Seyed Reza Mirhafez[a,1], Amir Avan[b,1], Sara Khatamianfar[b,1], Faezeh Ghasemi[c,1], Mohsen Moohebati[d], Mahmoud Ebrahimi[d], Hamideh Ghazizadeh[b], Majid Ghayour-Mobarhan[b,*,2], Alireza Pasdar[b,e,**,2]

[a] Noncommunicable Diseases Research Center, Neyshabur University of Medical Sciences, Neyshabur, Iran
[b] Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
[d] Cardiovascular Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[e] Division of Applied Medicine, Medical School, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK

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ABSTRACT

Background: Recent genome-wide association studies (GWAS) have identified several genetic variants that influence the risk of dyslipidemia and coronary artery disease (CAD). In this study, we have examined the potential association of five SNPs variants related to lipid pathway, previously identified in GWAS studies (ZNF259 C > G, CETP I405VA/G, LPA C > T, LPLS447X and PSRC1 A > G) with CAD.

Methods: Two hundred and ninety subjects including 194 patients with coronary artery disease and 96 controls were enrolled, followed by the analyses of anthropometric/biochemical parameters. Genotyping was carried out using Taq-Man real-time PCR based method. The association of the genetic polymorphisms with CAD was determined using univariate and multivariate analyses.

Results: CAD patients had a higher ($p < 0.05$) fasting blood glucose (FBG), total cholesterol (TC), high sensitivity C-reactive protein (hs-CRP), low-density lipoprotein cholesterol (LDL-C) and waist circumference. Results showed that subjects with CETP rs5882 genetic variant, AA&AG genotypes, had a higher risk of developing Coronary artery disease [OR: 2.1, 95% CI (1.2–4.1), p value = 0.015]. Also subjects who carried the G allele of the ZNF259 polymorphism were at an increased the risk of developing CAD [OR 1.86, 95% CI: 1.06–3.25, p value = 0.029] and had an increased TC, LDL and TG levels ($p < 0.05$). Furthermore, no statistically significant association was found between genetic polymorphisms of PSRC1 A > G, LPL S447X and LPA C > T and CAD.

Conclusion: We identified a relationship between a genetic variant in CETP and ZNF259 gene with CAD and CAD and lipid profile, respectively. Further investigation in a larger population may help to investigate the value of emerging marker as a risk stratification marker in CAD and its risk factors.

Abbreviations: GWAS, Genome-wide association studies; CAD, Coronary artery disease; HDL-C, High-density lipoprotein cholesterol; SNPs, Single nucleotide polymorphisms; lipoprotein, LP; VLDL, Very low density lipoprotein cholesterol; CETP, Cholesterol ester transfer protein; TG, Triglyceride; CE, Cholesterol ester; 3′UTR, 3′ untranslated region; PSRC1, Prolin/Serin rich coiled coil 1; CELSR2, EGF LAG seven-pass G type receptor 2; MYBPHL, Myosin binding protein H-like; SORT1, Sortilin 1; IDF, International diabetes federation; MUMS, Mashhad University of Medical Sciences; BMI, Body mass index; SBP or DBP, Systolic and diastolic blood pressures; LDL, Low density lipoprotein cholesterol; FBG, Fasting blood glucose; CRP, C-reactive protein; SD, Standard deviation; ZNF259, ZPR1 zinc finger 259; LPA, Lipoprotein a; LPL, Lipoprotein lipase; APOA5, Apolipoprotein A-V; CE, Cholesterol ester; PSRC1, Prolin/Serin rich coiled Coil-1; SORT1, Sortilin 1

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* Correspondence to: M. Ghayour-Mobarhan, Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
** Correspondence to: A. Pasdar, Division of Applied Medicine, Medical School, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK.
E-mail addresses: ghayourm@mums.ac.ir (M. Ghayour-Mobarhan), Pasdara@mums.ac.ir (A. Pasdar).
[1] Equally contributed as first author.
[2] Equally contributed as corresponding author.

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1. Introduction

Coronary artery disease (CAD) is the leading cause of death globally (Lopez and Murray, 1998). Dyslipidemia is one of the major risk factors for coronary artery disease (Genest Jr. et al., 1992). A low plasma high density lipoprotein cholesterol (HDL−C) level is a common dyslipidemia associated with CAD (Ballantyne et al., 1999; Ballantyne et al., 2001; Goldbourt et al., 1997; Zhu et al., 2014; Wood et al., 2011; Hatmi et al., 2007).

Genome wide association (GWA) studies have shown that genetic variants of ZNF259, CETP, LPA, LPL, and PSRC1 are associated with dyslipidemia and CAD (Aung et al., 2014; Braun et al., 2012; Santos et al., 2014; Tang et al., 2010; Wang et al., 2011; Clarke et al., 2009). Clarke and colleagues recognized some single nucleotide polymorphisms (SNPs) of the LPA are strongly associated with serum Lp(a) lipoprotein and coronary risk (Clarke et al., 2009). Other studies have shown that one important loci associated with coronary artery disease is near the APOA5 gene locus (Waterworth et al., 2010a). The LPL gene encodes lipoprotein lipase which is involved in the lipolysis of triglyceride rich lipoproteins (Merkel et al., 2002). Lipoprotein lipase is a multifunctional protein that catalyzes the hydrolysis of triacylglycerol component of chylomicrons and VLDL (Mead et al., 2002). CETP (Cholesterol ester transfer protein) is an important hydrophobic glycoprotein involved in lipoprotein metabolism in man. Therefore, the CETP gene is probably also associated with coronary artery disease risk. CETP is expressed in adipocytes and pre-adipocytes where its expression regulates by cellular cholesterol. This protein mediates the exchange of triglyceride (TG) to cholesterol ester (CE) between apo A1 and apo B lipoproteins from HDL to very low density lipoprotein in hepatocyte (Bruce et al., 1998; Todur and Ashavaid, 2013; Oliveira and de Faria, 2011). To assess the effect of CTEP on atherosclerosis, genetic variants of CETP have been evaluated in several studies. Heterozygotes for CETP gene polymorphisms have been reported to have an increased risk of coronary artery disease (Zhong et al., 1996). One SNPs, rs599839, located in the 3′ untranslated region (3′UTR) of the PSRC1 gene and was found to be in linkage disequilibrium with several SNPs in the chromosomal region 1p13.3, that includes the Prolin/Serin rich coiled coil 1 (PSRC1), cadherin, EGF LAG seven-pass G type receptor 2 (CELSR2), myosin binding protein H-like (MYBPHL) and sortilin 1 (SORT1) (Kleber et al., 2010). Sortilin 1 is involved in the uptake of LDL by the liver and lower serum LDL-C levels. The significant positive correlation between PSRC1 and plasma LDL-C and a negative association between CELSR2, SORT1, and plasma LDL-C have been reported (Schadt et al., 2008). Several studies have shown that an intragenic variant near the BUD13-ZNF259 (rs964184) locus is associated with serum TG level (Braun et al., 2012). The ZNF259 gene is nearby APOA5 and has been connected with several lipid abnormalities (Waterworth et al., 2010b). ZNF259 is one of the nuclear regulatory proteins that plays a fundamental role in cell proliferation and signal transduction (Galcheva-Gargova et al., 1998). It has been demonstrated that the promoter for the ZNF259 gene has an impact on obesity and insulin resistance (Mangelsdorf et al., 1995). In this study, we have studied the potential association of five SNPs variants related to lipid pathway, previously discovered in GWAS studies (ZNF259 C > G, CETP I405VA/G, LPA C > T, LPLS447X and PSRC1 A > G) with CAD.

2. Material and method

2.1. Study population

At total of 290 Iranian subjects were recruited of whom one hundred and nighty four were patients referred to Qaem Hospital, Mashhad, Iran. These subjects were categorized into two groups dependent on the results of angiography: those with ≥50% stenosis, or < 50% stenosis. In addition, ninety-six individuals were selected by randomized clustering as the control group. The individuals who suffer from acute infection, heart and renal failure and women who were pregnant were excluded. All subjects were investigated for LPL, LPA, PSRC1, CETP and ZNF259 gene polymorphisms and their association with the presence of CAD determined. The informed written consent was obtained from all participants, and the project was approved by the Ethics Committee of Mashhad University of Medical Sciences (MUM).

2.2. Anthropometric and biochemical measurements

Anthropometric parameters (height, body weight, waist and hip circumference) were measured as described previously (Mardan-Nik et al., 2014). Body mass index (BMI) was calculated as body weight (kg) divided by squared height in meters (m^2), and BMIs of 20–25, 25–30 or > 30 were considered as normal, over-weight or obese, respectively. Systolic and diastolic blood pressures (SBP or DBP) were measured in duplicate using standard sphygmomanometers. Total cholesterol (TC), HDL, LDL and TG, and fasting blood glucose (FBG) concentrations were evaluated by standard enzymatic techniques, while serum C-reactive protein (CRP) levels were determined by polyethylene glycol-enhanced immunoturbidimetry, as described previously (Mirhafez et al., 2016).

2.3. DNA extraction and genotyping

Genomic DNAs were isolated from whole blood samples using the QIAamp® DNA Mini-Kit according to the manufacturer's protocol (Qiagen, San Diego, CA). The quality and purity of DNAs were distinguished by the NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, USA). Genotype analysis of PSRC1 rs599839, LPL rs328, Lp (a) rs3798220, ZNF259 rs964184, and CETP rs5882 polymorphism was carried out using Taqman®-probes-based PCR reactions accomplished in 12.5 μl total volume, using 20 ng of DNA diluted in TaqMan® Universal Master Mix with specific primers and probes. The ABI PRISM-7500 instrument equipped with the SDS version-2.0 software was used to determine the allelic content of the samples.

2.4. Statistical analysis

All statistical analyses were performed using SPSS-20 software (SPSS Inc., IL, USA). The clinical data are presented as means ± SD where appropriate or median and interquartile range. The Kolmogorov-Smirnov test was used to determine the normality of distribution. The t-student test was utilized to survey the clinical and baseline characteristics between the groups for normally distributed variables. For continuous variables with non-normal distribution, the Mann-Whitney *U* test was used. Categorized variables were analyses by the Chi-square or Fisher exact tests. The relation of SNPs and CAD (in present of factors including age, sex, and smoking) was used by Logistic regression analysis. We then analyzed the variables with a $p < 0.05$ in the univariate analyses by Multivariate analyses. All the analyses were two-sided and statistical significance was set at $p < 0.05$.

3. Result

3.1. Characteristics of the population

The characteristics of the participants are shown in Table 1. The patients with coronary artery disease were categorized into two groups depending on the results of angiography: those with ≥50% stenosis as angiogram positive (Angio+) ($n = 98$), or < 50% stenosis as angiogram negative (Angio−) ($n = 96$). In addition, ninety-six individuals were selected by randomized clustering as control group. Of the patients who had positive angiography, 68.4% were males, and 31.6% were females. Also of the subjects who angiogram negative and control, 41.7% and 32.3% were male, and 58.3% and 67.7% were female, respectively. Individuals with angiogram negative had significantly higher age, serum total cholesterol (TC), low density lipoprotein

Table 1
Characteristics of subjects in the Angio+, Angio− and control groups.

Characteristics	Control (n = 96)	Angio− (n = 96)	Angio+ (n = 98)	P1	P2	P3
Age, year	50.1 ± 10.5	55.2 ± 10.9	58.4 ± 10.7	0.003	< 0.001	0.093
Gender, No (%) Male	31 (32.3)	40 (41.7)	67 (68.4)	0.178	< 0.001	< 0.001
Smoking, No (%) Yes	13 (13.5)	10 (10.4)	24 (24.5)	0.505	0.052	0.010
BMI (kg/m^2)	25.9 ± 4.1	26.8 ± 4.8	26.2 ± 4.1		0.527	
Weight (kg)	71.9 ± 11.1	69.1 ± 12.3	70.6 ± 13.1		0.278	
HC (cm)	102.1 ± 7.7	100.8 ± 10.2	98.4 ± 14.3		0.078	
WC (cm)	91.7 ± 11.2	95.2 ± 14.8	96.9 ± 13.7	0.182	0.027	0.667
Height (cm)	159.4 ± 8.9	160.7 ± 8.0	163.8 ± 9.3	0.562	0.002	0.051
TC (mg/dl)	149.2 ± 36.9	155.5 ± 39.8	161.6 ± 50.4	0.011	0.009	0.609
TG (mg/dl)	128.0 (99.5–171.0)	118.0 (86.2–153.5)	137.5 (105.7174.2)		0.160	
HDL-C (mg/dl)	43.5 ± 9.1	39.4 ± 11.5	35.7 ± 10.7	0.20	< 0.001	0.048
LDL-C (mg/dl)	122.9 ± 39.5	161.7 ± 44.8	157.3 ± 55.5	< 0.001	< 0.001	0.222
FBG (mg/dl)	88.7 ± 30.1	117.2 ± 63.1	141.9 ± 72.9	0.003	< 0.001	0.013
SBP (mm Hg)	124.2 ± 20.8	120.0 ± 18.1	118.4 ± 16.1		0.078	
DBP (mm Hg)	74.6 ± 10.8	72.8 ± 9.7	73.1 ± 8.4		0.229	
hsCRP (mg/dl)	1.8 (1.0–3.4)	3.3 (1.8–5.7)	4.1 (2.1–10.0)	< 0.001	< 0.001	0.105

Values are expressed as mean ± SD, median and interquartile range for normally and non-normally distributed variables, respectively. Comparisons were performed by one-way ANOVA and Kruskal–Wallis test. Also the post hoc test was used for comparisons between groups and X^2 test for categorical data.
BMI: body mass index; WC: waist circumference, TC: total Cholesterol, TG: triglycerides, HDL−C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, FBG: fasting blood glucose, HC: hip circumference, SBP: systolic blood pressure; DBP: diastolic blood pressure, hsCRP: high sensitivity C-reactive protein, Angio−: Angiogram negative, Angio+: Angiogram positive.
P1: comparison between groups of control and Angio−, P2: comparison between groups of control and Angio+, P3: comparison between groups of Angio− and Angio+.

cholesterol (LDL-C), fasting blood glucose (FBG), and high sensitivity C-reactive protein (hsCRP) ($p < 0.05$), whereas no significant differences were discovered for gender, smoking, BMI, weight, height, hip circumference (HC), waist circumference (WC), triglyceride(TG), high density lipoprotein cholesterol (HDL−C), and systolic and diastolic blood pressure (SBP and DBP). In contract, subjects with angiogram positive compared with control group had remarkably higher age, WC, height, TC, LDL-C, FBG and hsCRP and lower HDL-C ($p < 0.05$), however, no discrepancy was found for smoking, BMI, weight, HC, TG, SBP and DBP between this two groups. There was a significant difference between Angio+ and control groups as gender ($p < 0.001$). Also the comparisons between Angio+ and Angio− groups showed that just in gender, smoking, HDL-C and FBG were significant differences ($p < 0.05$) (Table 1).

3.2. Association of genetic polymorphisms and CAD

The frequency of genotype AA of CETP rs5882 was 11.6%, whereas the AG and GG genotype frequencies were 42.1% and 46.3% in the control group, respectively. On the other hand, these frequencies in angiogram positive group were 11.2% (AA) and 38.8% (AG) and 50% (GG). Also frequencies AA and AG and GG in angiogram negative group were 15.2%, 55.4% and 29.3%, respectively. We showed that subjects with AA&AG genotypes were at an increased risk of being coronary artery disease in angiogram negative before and after correction for age, sex and smoking, respectively [OR: 2.1, CI (1.1–3.7), p value = 0.018] and [OR: 2.1, CI (1.2–4.1), p value = 0.015] (Table 2). The frequency of ZNF259-rs964184 genotype CC (wild-type) was 78.9% whereas the CG and GG genotype were 17.9% and 3.2% in control group, respectively. On the other hand, these frequencies in angiogram positive group were 66.3% (CC) and 26.5% (CG) and 7.1% (GG). Furthermore, carriers of the G allele of the ZNF259–rs964184 SNP were at a remarkably increased the risk of having coronary artery disease (p value = 0.029) (Table 2). Moreover, no statistically significant association was found for PSRC1 A > G, LPL S447X and LPA C > T between groups.

3.3. Association of rs964184 (ZNF259) genotype distribution with features of dyslipidemia and metabolic syndrome

We analyzed the association between rs964184 (ZNF259) genotype distribution with features of dyslipidemia and metabolic syndrome (Table 3). In particular subjects carrying GG and GC genotypes had an increased TC and LDL levels in comparison to subjects carrying CC genotype ($p < 0.05$). The TG level was significantly higher in subjects carrying GG genotype, compared to subjects carrying CC genotype ($p < 0.05$). This analysis did not reveal a significant association between BMI, HDL, FBG and hs-CRP and rs964184 (ZNF259) (Table 3).

4. Discussion

Our results shows an association between the rs964184 (ZNF259) genotype distribution and features of dyslipidemia and metabolic syndrome such as TC, TG and LDL. Additionally, we showed that subjects with AA&AG genotypes of CETP rs5882 were at an increased risk of being coronary artery disease in our population. Recently, many genes previously involved in lipid regulation were recognized by genome-wide association scans (GWAS) and meta-analyses. Lipoprotein metabolism has a prominent role in atherosclerotic cardiovascular disease by affecting arterial lipid accumulation and atherosclerotic plaque formation (Rader and Daugherty, 2008). The elevation of serum cholesterol and LDL-C level and decreased HDL-C level have been recognized as important risk factors for Coronary artery diseases (Clarke et al., 2009). In particular, plasma level of HDL-C is noticeably inversely associated with the risk of cardiovascular disease (Franceschini, 2001). In this regard in our previous studies we also detected a relationship between metabolic syndrome (MetS) and its components such as HDL with vascular endothelial growth factor genetic variants (Ghazizadeh et al., 2018; Ghazizadeh et al., 2017). The present research was performed to assess the role of five of the most strongly associated with the risk of having CAD (PSRC1 rs599839, LPL rs328, LPA rs3798220, ZNF259 rs964184 and CETP rs5882) in lipoprotein pathway in an Iranian cohort. Our study demonstrated that only G allele of ZNF259 rs964184 was correlated with CAD risk. Van de Woestijne et al. illustrated that the log plasma triglycerides was powerfully related to the

Table 2
Association of lipid pathway SNPs and coronary artery disease.

	Control	Angio−	Angio+	Odds ratio (CI)[a]	p value[a]	Odds ratio (CI) [a,c]	p value[a,c]	Odds ratio (CI)[b]	p value[b]	Odds ratio (CI)[b,c]	p value[b,c]
CETP rs5882	96	92	98								
GG	44 (46.3)	27 (29.3)	49 (50)	Ref Cat				Ref Cat			
GA	40 (42.1)	51 (55.4)	38 (38.8)	2.1 (1.1–3.9)	0.024	2.1 (1.1–4.1)	0.023	0.85 (0.46–1.55)	0.605	0.99 (0.49–2.01)	0.989
AA	11 (11.6)	14 (15.2)	11 (11.2)	2.1 (0.8–5.2)	0.122	2.3 (0.9–6.1)	0.081	0.89 (0.35–2.27)	0.820	0.88 (0.29–2.64)	0.823
GA + AA	51 (53.7)	65 (70.7)	49 (50)	2.1 (1.1–3.7)	0.018	2.1 (1.2–4.1)	0.015	0.8 (0.4–1.5)	0.609	0.9 (0.5–1.8)	0.928
HWE	0.918	0.452	0.688								
G allele	128 (67)	105 (57)	136 (69)	Ref Cat				Ref Cat			
A allele	62 (33)	79 (43)	60 (31)	1.5(0.8–2.7)	0.146			0.91 (0.59–1.39)	0.670		
LPL rs328	96	85	98								
CC	77 (80.2)	73 (85.9)	79(80.6)	Ref Cat				Ref Cat			
CG	17 (17.7)	12 (14.1)	18 (18.4)	0.7 (0.3–1.6)	0.473	0.8 (0.3–1.8)	0.614	1.03 (0.49–2.14)	0.933	1.17 (0.48–2.86)	0.718
GG	2 (2.1)	0	1(1)	0.6 (0.2–2.0)	0.612	0.6 (0.2–1.9)	0.712	0.48 (0.4–5.48)	0.561	1.28 (0.06–24.0)	0.867
HWE	0.671	0.782	0.999								
C allele	171 (89)	158 (93)	176 (90)	Ref Cat				Ref Cat			
G allele	21 (11)	12 (7)	20 (10)	0.6(0.2–1.6)	0.327			0.92 (0.48–1.76)	0.814		
PSRC1 rs599839	96	94	97								
AA	78 (81.2)	76 (80.9)	77 (79.4)	Ref Cat				Ref Cat			
AG	15 (15.6)	16 (17)	18 (18.6)	1.1 (0.5–2.3)	0.818	1.1 (0.4–2.4)	0.813	1.21 (0.57–2.58)	0.612	1.08 (0.44–2.68)	0.855
GG	3 (3.1)	2 (2.1)	2 (2.1)	0.6 (0.1–4.2)	0.682	0.6 (0.1–4.3)	0.695	0.67 (0.11–4.15)	0.672	0.69 (0.07–6.24)	0.747
HWE	0.152	0.597	0.749								
A	(89) 171	168 (89)	172 (89)	Ref Cat				Ref Cat			
G	21 (11)	20 (11)	22 (11)	1 (0.4–2.4)	1.000			1.04 (0.55–1.96)	0.900		
ZNF259 rs964184	95	95	98								
CC	75 (78.9)	73 (76.8)	65 (66.3)	Ref Cat				Ref Cat			
CG	17 (17.9)	20 (21.1)	26 (26.5)	1.2 (0.5–2.4)	0.607	1.1 (0.5–2.4)	0.691	1.76 (0.88–3.53)	0.110	1.54 (0.71–3.36)	0.270
GG	3 (3.2)	2 (2.1)	7 (7.1)	0.6 (0.1–4.2)	0.683	0.7(0.1–4.6)	0.738	2.69 (0.66–10.83)	0.163	2.51 (0.55–11.43)	0.233
HWE	0.300	0.903	0.192								
C	167 (88)	166 (87)	156 (80)	Ref Cat				Ref Cat			
G	23 (12)	24 (13)	40 (20)	1.1 (0.4–2.5)	0.831			1.86 (1.06–3.25)	0.029		
LPA rs3798220	88	94	95								
TT	75 (85.2)	83 (88.3)	79 (83.2)	Ref Cat				Ref Cat			
TC	13 (14.8)	11 (11.7)	16 (16.8)	0.7 (0.3–1.8)	0.541	0.7 (0.3–1.9)	0.577	1.16 (0.52–2.59)	0.702	1.50 (0.58–3.90)	0.360
HWE	0.755	0.833	0.669								
T	163 (93)	88 (94)	174 (92)	Ref Cat				Ref Cat			
C	13 (7)	6 (6)	16 (8)	0.8 (0.2–2.6)	0.848			1.15 (0.53–2.47)	0.714		

Ref Cat: Reference category, CI: Confidence interval, HWE: Hardy-Weinberg Equilibrium, Angio−: Angiogram negative, Angio+: Angiogram positive.
[a] Comparison between groups of control and Angio−.
[b] Comparison between groups of control and Angio+.
[c] After correction for age, sex and smoking.

Table 3
Comparison between rs964184 (ZNF259) genotype distribution and features of dyslipidemia and metabolic syndrome among the total population.

Characteristics	CC (n = 213)	CG (n = 63)	GG (n = 12)	P1	P2	P3
BMI (kg/m^2)	27.2 ± 4.6	27.3 ± 4.1	25.6 ± 4.5		0.563	
TC (mg/dl)	163.7 ± 42.2	185.0 ± 46.6	256.4 ± 56.6	0.003	< 0.001	< 0.001
TG (mg/dl)	121.0 (91.0–164.0)	136.0 (98.2–167.7)	177 (90.5–241.5)	0.343	0.034	0.205
HDL-C (mg/dl)	39.2 ± 10.1	40.5 ± 13.5	41.1 ± 10.3		0.658	
LDL-C (mg/dl)	95.1 ± 34.9	111.4 ± 40.4	197.2 ± 98.4	0.020	< 0.001	< 0.001
FBG (mg/dl)	112.2 ± 58.9	126.0 ± 73.2	128.7 ± 42.0		0.255	
hsCRP (mg/dl)	4.7 ± 4.9	4.2 ± 4.0	5.5 ± 4.8		0.638	

Values are expressed as mean ± SD, median and interquartile range for normally and non-normally distributed variables, respectively. Comparisons were performed by one-way ANOVA and Kruskal–Wallis test. Also the post hoc test was used for comparisons between groups and X^2 test for categorical data.
BMI: body mass index; TC: total Cholesterol, TG: triglycerides, HDL−C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, FBG: fasting blood glucose, hsCRP: high sensitivity C-reactive protein.
P1: Comparison between groups of CC and CG, P2: Comparison between groups of CC and GG, P3: Comparison between groups of CG and GG.

minor allele of ZNF259 rs964184 (β 0.12; 95% CI 0.10–0.15, $p = 1.1 \times 10^{-19}$) in patients with clinically manifesting vascular disease (van de Woestijne et al., 2014) and in other study an association found between plasma triglycerides with vascular risk in normal group (Deloukas et al., 2013). In particular, Braun and et al. showed the strongest signal for TG remained with ZNF259 (rs964184: $p = 1.06 \times 10^{-39}$) (Braun et al., 2012). It is indicated that this locus is related to with plasma TG and V-LDL levels in several studies including Caucasian GWAS and a meta-analysis (Willer et al., 2008; Kathiresan et al., 2008) as well as in Middle Eastern population (Ken-Dror et al., 2010).

We showed that subjects with AA&AG genotypes of CETP rs5882 were at an increased risk of being coronary artery disease. Some studies in China and United States presented that CETP rs5882 is the susceptibility allele for the risk of having CAD (p value = 0.0002) while it was in accordance with our results and for reducing of HDL-C level (p value < 0.001) (Sun et al., 2014; Peloso et al., 2010). The minor allele of this variant has been associated with decrease CETP activity, increased lipoprotein particle size, and exceptional longevity in Ashkenazi Jewish probands (Barzilai et al., 2003).

Furthermore, in the present study no statistically significant association was found between genetic polymorphism of PSRC1 A > G with

CAD and its risk factors. In this regards Arvind and colleagues reported that rs599839 variation from PSRC1 gene has been involved in the pathogenesis of coronary artery disease (OR = 0.422, 95% CI 0.181–0.981, $p = 0.045$) and they showed that homozygous and heterozygous subjects had 30% and 15% higher PSRC1 expression, respectively (Arvind et al., 2014). Other study was showed that the G allele of rs599839 variant associated with reduced plasma LDL-C levels and lower cardiovascular disease risk. They reported that subjects who were homozygous for G allele, had a higher expression of the SORT1, CELSR2 and PSRC1 genes in peripheral white blood cells. The strongest correlation was discovered for SORT1 mRNA level. These results were emphasized in human embryonic kidney cells (HEK293) overexpressing SORT1 that revealed the lower plasma LDL-C level (Linsel-Nitschke et al., 2010). The minor allele G of the rs599839 SNP is associated with low LDL-C serum levels, therefore it was suggested to be associated with the early onset of CAD in the Chinese population (Huang et al., 2008). However, Karvanen et al. were not able to find an association between CAD and rs599839 in a study in Finland, Sweden, France and Northern Ireland population (p value = 0.242 Finland ATBC, p value = 0.239 Finland Finrisk, p value = 0.245 Northern Sweden, p value = 0.0219 France, p value = 0.271 Northern Ireland) (Karvanen et al., 2009) as we have demonstrated it. They detected that rs599839 SNP is related to higher values of non-HDL cholesterol (Karvanen et al., 2009). Also, Clarke and coworkers showed the effects of the LPA variant on the risk of coronary disease correlated with the effects on the LPA lipoprotein level (Clarke et al., 2009). Several studies in accordance with our results found no association between LPA rs3798220 with CAD in Chinese (Li et al., 2013) and Brazilian population (Li et al., 2013). In 2010, Agirbasli et al. examined the impact of polymorphisms in the LPL gene and CAD severity documented by coronary angiography. They found LPL S447X variants play a protective role against the severity of CAD detected by coronary angiography (25 ± 30, p value = 0.048) (Agirbasli et al., 2011). Previous studies demonstrated the X447 allele is associated with higher levels of HDL-C, lower plasma TG levels and reduced risk of CAD (Corella et al., 2002; Razzaghi et al., 2000). Moreover, no association was detected for other markers, LPA, LPL, PSRC1, and CETP among Iranian cohorts, which can be explained at least in part by ethnicity (Chretien et al., 2006; Deo et al., 2011; Cobbaert and Kesteloot, 1992; Utermann, 1999; Lanktree et al., 2010). Another possible hypothesis for the lack of this association could be the lower value of linkage disequilibrium between SNPs identified in the Iranian patients compared with some studies on patients predominantly from Europe (Luke et al., 2007; Li et al., 2011). Another reason may be due to the allele frequencies differences or apo (a) size heterogeneity, resulting in functional copy number variation within these SNPs (Kraft et al., 1992). Hence we have shown an association between a CETP rs5882 genetic variant, AA&AG genotypes, with a high risk of coronary artery disease, as demonstrated by angiogram, and in particular association between ZNF259 genetic variant with an increased TC, LDL and TG levels ($p < 0.05$). However, further studies in a larger population are required to explore these findings.

Ethical approval and consent to participate

Informed consent was obtained from all subjects using protocols approved by the Ethics Committee of the Mashhad University of Medical Sciences.

Conflict of interests

The authors declare that they have no competing interests.

Financial disclosure

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