





RESEARCH ARTICLE

Association of ANGPTL3 polymorphisms with high-density lipoprotein cholesterol uptake capacity in patients with cardiovascular disease

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Abstract

Introduction: Previous studies have shown the importance of angiotensin-like 3 (ANGPTL3) as a modulator of lipid profiles. Cholesterol uptake capacity (CUC) is one means for assessing high-density lipoprotein (HDL) functionality. This study for the first time has investigated the relationship between genetic ANGPTL3 polymorphism and CUC in patients with cardiovascular disease.

Methods: Five hundred three subjects comprising 350 healthy subjects and 153 individuals who developed a cardiovascular disease (CVD) event during follow-up were recruited as part of the Mashhad Stroke and Heart Atherosclerotic Disorder (MASHAD) cohort study. A modified CUC method was used to determine the CUC of serum samples. Applied amplification refractory mutation system PCR was performed for

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The study protocol has been approved by Mashhad University of Medical Sciences (ID 971062).

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ANGPTL3 variants genotyping including: rs10789117, rs1748195, and rs11207997. Sanger sequencing was applied to confirm the genotypes.

Results: The results showed that there was a significant relationship between the rs1748195 genotypes and HDL concentration in the CVD group ($p = 0.02$). Moreover, individuals with a GG genotype of the rs1748195 were associated with a lower risk of CVD (OR = 0.49, 95% CI = 0.24–0.98, $p = 0.04$) compared with CC genotype in the CUC ≤ 1.7 a.u subgroup. Moreover, the CT genotype of rs11207997 was associated with a lower risk of CVD (OR = 0.74, 95% CI = 0.41–1.3, $p = 0.01$) compared with CC genotype in CUC > 1.7 a.u subgroup.

Conclusion: The results showed that the CT genotype of the rs11207997 variant was associated with a lower risk of incident CVD in patients with higher HDL functionality. As well, the rs1748195 gene variant may contribute to a reduced risk of CVD.

KEYWORDS

angiopoietin-like protein, cardiovascular disease, cholesterol uptake capacity, polymorphism

1 | INTRODUCTION

Cardiovascular disease (CVD) is a major cause of death, globally.¹ CVD was responsible for 30% of deaths with 80% of deaths result from CVD occurs in middle-income and low-income countries.² However, the application of appropriate interventions to reduce and modify the attributable risk factors to the CVD may prevent a high proportion of heart and peripheral vascular disease and stroke.³ Modifiable risk factors for CVD include the following: hypertension, tobacco consumption, glucose intolerance, obesity, dyslipidemia, physical inactivity, and unmodifiable risk factors such as age, gender, and ethnicity.¹ Dyslipidemia (high level of low-density lipoprotein (LDL-C) and triglyceride (TG), low level of HDL-C) is one of the risk factors for CVD.¹ There is an inverse correlation between serum HDL-C concentration and cardiovascular risk.^{4–6} More recent attention has focused on the different functions of HDL that include the following: anti-inflammatory, antioxidant, and cholesterol efflux.⁷ HDL removes cholesterol from macrophages within the arterial intima.⁸ Clinical and animal investigations have demonstrated that reverse cholesterol transport (RCT) through this process may be a stronger predictor of CVD events than serum HDL-C concentration.^{9,10} Since HDL is a pleiotropic particle and has different properties, evaluation of its functions has been difficult in clinical human studies. HDL's ability to remove cholesterol from the macrophages through the RCT pathway may be an important function of HDL that depends on HDL ability to accept cholesterol.⁹ Cholesterol uptake capacity (CUC) measurement is a new and developed cell-free, throughput, and sensitive assay that reflects HDL functionality without the utilization of cells and radio-isotope labeling.¹¹

Studies have demonstrated the key role of a combination of environmental and genetic factors such as genetic polymorphisms as risk factors for CVD, for example, coronary heart disease (CHD).¹²

Angiopoietin-like proteins (ANGPTLs) have been proved as one of the important lipoprotein metabolism modulators. Thus, they have appeared as significant targets for lipid levels regulation and CVD risk.¹³ ANGPTL3 is an endogenous protein that originates in the liver exclusively. ANGPTL3 is one of the angiopoietin family members of vascular endothelial growth factors and is of 460 amino acids. ANGPTL3 has two regions, the N terminal coiled-coil domain that this region has a key role in the inhibition of lipoprotein lipase activity. The other domain is the C terminal fibrinogen region.¹⁴ The role of lipoprotein lipase (LPL) is TG-rich lipoprotein breaking down into FFAs for uptake and usage of energy in peripheral tissues.^{15,16} As well, it can inhibit endothelial lipase activity. This enzyme mediates HDL remodeling by breaking down phospholipids into lysophospholipids.^{17–19} The gene locus of ANGPTL3 is on chromosome 1 (1p31.1-p22.3).^{3,13} Studies have indicated that genetic variants of ANGPTL3 are correlated to lipid profiles such as TG levels, HDL cholesterol, and LDL cholesterol levels in studies of the general population.^{20–23} As well, recent studies of loss-of-function variants in the ANGPTL3 gene have emphasized its correlation with CVD.^{24,25} Studies have been shown ANGPTL3 inhibition does not promote LDL lowering through elevated clearance, while this effect occurred through decreased production of LDL consequence of VLDL-TG lowering.²⁶

Previous studies have been established an association between ANGPTL3 polymorphisms including rs1748195, rs10789117, and rs11207997 with incident cardiovascular disease and cardiometabolic disorders.^{27,28} The cohort study analyses have examined that carrier of a rare allele of the rs10789117 on ANGPTL3 gene had a lower risk of obesity, hypertension, HTN, metabolic syndrome, and diabetes mellitus. Also, they found that individuals with the rs1748195 variant of the ANGPTL3 gene had a lower risk of obesity.²⁹ Also, cholesterol uptake capacity is a good criterion

for evaluation of HDL functionality to CVD risk assessment.³⁰ Therefore, in this study for the first time, we aimed to investigate the relationship between an ANGPTL3 genetic polymorphism including rs1748195, rs10789117, and rs11207997 and CUC in patients with cardiovascular disease.

2 | METHOD AND MATERIAL

2.1 | Population

The Mashhad Stroke and Heart Atherosclerotic Disorder (MASHAD) cohort study was started in 2010 and subjects were followed up for 10 years until 2020 with 9704 (35–65 years old) individuals without CVD, peripheral arterial disease, and stroke. The examinations of follow-up for this cohort study were done every three years. During a minimum of 6-year follow-up, 215 participants developed a CVD outcome. In this study, 503 subjects randomly selected from the MASHAD study included 350 healthy randomized subjects without clinical CVD and 153 individuals without cardiovascular disease at the baseline who then developed CVD outcomes that were diagnosed by a specialist cardiologist. The initial exclusion criteria in this study were CVD, peripheral arterial disease, stroke, cancer, and chronic kidney disease (CKD).

The demographic and anthropometric data were collected from each participant. Also, fasting blood glucose (FBG), blood pressure (BP), CVD risk factors (history of hypertension, diabetes, syndrome metabolic, obesity, dyslipidemia, and smoking status), and lipid profile (TG, TC, LDL-C, and HDL-C) were assessed. All subjects provided written consent. The used protocols of the study were approved by the Ethics Committee of the Mashhad University of Medical Sciences (ID: 971062).

2.2 | Reagents and materials

Fetal bovine serum (FBS) and anti-apoA1 antibody (clone311, number of catalog MIA1402) were purchased from Thermo Scientific. BODIPY-cholesterol was obtained from Avanti Polar Lipids. Methyl- β -cyclodextrin was bought from Sigma-Aldrich. The preparation of BODIPY-cholesterol stock solutions was performed by dissolving every one reagent in dimethyl sulfoxide (DMSO) amount 0.5 mmol/L and solutions stored at -20°C . Phosphate-buffered saline (PBS) tables provided from Sigma-Aldrich. The preparation was performed as follows: dissolving 1 table of PBS buffer in one liter of deionized water that resulting in 10 mmol/L phosphate buffer, 140 mmol/L NaCl, and 3 mmol/L KCl in pH 7.4 at 25°C . PEG (Polyethylene glycol) 6000, Casein as blocking buffer, and Tris HCl were bought from Merck. The liposome stock solution (as a reaction buffer regular component) was produced from 25 mmol/L of hydrogenated soy phosphatidylcholine, 12.5 mmol/L of 1,2-dimyristoylsn-glycerol-3-phosphoglycerol, and 12.5 mmol/L cholesterol.

2.3 | High-density lipoprotein preparation from human serum

To remove lipoproteins containing apo-B, 100 μl of every serum sample was mixed with 20 μl of 45% PEG (polyethylene glycol) 6000 solution in Tris HCL 0.2 mol/L; after vortex mixing, samples were stored for 15 min at room temperature. Then, samples were centrifuged at 11,200 g at 4°C for 30 min in a refrigerated centrifuge, and finally, supernatant was collected as HDL fraction.

2.4 | Cholesterol uptake capacity assay

The cholesterol uptake capacity (CUC) assay was carried out according to our previously described method.¹¹ There are 3 main steps in this method: (A) plate preparation of containing antibody; (B) serum mixture preparation; and (C) CUC measurement.

First, 100 (μl) of 5 ($\mu\text{g}/\text{ml}$) of anti-apoA1 antibody, clone 311, in PBS with pH 7.4, was poured out to each well of the microplate (96 wells). Microplate incubated overnight at temperature of 4°C and antibody solutions were removed, and PBS solution containing 2% casein as the blocking buffer was added and incubated for 2 h at 37°C . The blocking buffer was detached, and the well of the microplate was washed with PBS 2 times. Subsequently, 10 μl of every one Apo-B-depleted sample of serum was incubated by 100 μl of 5 μm BODIPY-cholesterol in PBS with 2% BSA and solution of 0.8% liposome stock. Microtubes shook in an incubator (37°C , 280 rpm) for 20 h. Then, 100 μl of apo-B-depleted serum mixture was moved into wells that were covered by an antibody. Then, the plate was incubated at 37°C (350 rpm) for three hours. The wells were washed with 200 μl PBS 5 times. Then, 100 μl of 20 mmol/L cyclodextrins in PBS was added to increase the fluorescence signal extracted from BODIPY-cholesterol. The plate was incubated at 25°C (140 rpm) for 30 s. The intensity of the fluorescence signal was measured at 535 nm by excitation at 485 nm with a microplate reader.

We demonstrated cholesterol uptake capacity in the form of CUC % or BODIPY-cholesterol uptake percent by HDL (a.u or arbitrary unit defined for CUC). This parameter is computed by deducting the background signal from BODIPY-cholesterol uptake discovered for samples of the apo-B-depleted serum after washing and divided by signals of BODIPY-cholesterol for the same at zero time. Finally, the CUC value is normalized by HDL concentration.

2.5 | DNA extraction and genotype screening

DNA of all subjects extracted from a whole blood sample using a standard method of salting-out extraction.³¹ Control of extracted DNA quality determined using electrophoresis of agarose gel (Pars Tous Biotechnology). These quantitative determined applying NanoDrop 1000 Detector in Wavelength of 280 and 260-nm. For genotype detection of rs1748195 and rs10789117 used Tetra and genotype detection of rs11207997 applied amplification

refractory mutation system PCR (ARMS PCR). Tetra -ARMS PCRs performed in 20 μ l volume comprising 10 μ l of PCR Master Mix (Biotechnology of Pars Tous), 4.5 μ l ddH₂O, 2 μ l of DNA samples, and 1.0 μ l for inner and 0.5 μ l for outer primers. To perform cycling protocols of PCR, as the first step at 94°C for 5 min, 32 cycles 94°C for 1 min, 62°C (rs1748195), 56°C (rs10789117), and 58°C (rs11207997) for 1 min, at 72°C for 1 min, and final extension 72°C for 5 min. Instruments of genotyping used from Biosystem (ABI-Veriti 96-well Thermal Cycler). The next stage of PCR was performed by 2% agarose gel electrophoresis and 3 bands identified: in rs10789117, 194 and 291bp AA and CC, in rs1748195, 223 and 332bp CC and GG, in rs11207997, 179 and 154bp CC and TT. Finally, by applying Sanger sequencing the genotypes were confirmed. All the sequenced samples were analyzed using Finch TV version 1.4.0.

2.6 | Statistical analysis

SPSS version 20 (IBM Corp, 2011) and MedCalc (version 16.8- Bvba) statistical software were used for statistical tests. The normality of parameters was assessed by Kolmogorov-Smirnov (K-S) test. Chi-square and t tests were utilized to analyze the association between baseline characteristics of subjects in two groups for categorical parameters and normally distributed ones, respectively. Changes in genotypes frequency in the ANGPTL3 gene with percentage were evaluated and compared by Chi-square tests. Logistic regression was used to examine the relationship between variants and CUC.

p values regarded statistically significant if less than 0.05 (<0.05). We considered the correlation by odds ratio (OR) with a confidence interval of 95%. MedCalc Statistical Software was utilized to determine the cutoff value of CUC in serum samples.

3 | RESULTS

3.1 | Patient characteristics

According to Table 1 the CVD group included 76 females (28.4%) and age median 53.79 \pm 6.9 years and the control group included 192 females (71.6%) and age median 48.9 \pm 7.8 years. Based on Table 1, no significant differences (*p* > 0.05) were observed between groups at the baseline characteristics of MASHAD cohort study participants except in age (*p* < 0.001) and TC (*p* = 0.007), TG (*p* = 0.001), and LDL (*p* = 0.03).

Table 2. indicated the relationship of ANGPTL3 gene variants with concentration of HDL and cholesterol uptake capacity as HDL functionality. According to our results, there was a significant association between rs1748195 and CUC value (*p* = 0.006) but rs10899117 and rs11207997 variants have no significant relationship with the concentration of HDL and CUC (*p* > 0.05) in the total population.

Consequently, we evaluated the association between ANGPTL3 gene variants and the concentration of HDL and CUC in

TABLE 1 Clinical characteristics of cardiovascular disease versus control subjects, Mashhad stroke, and heart atherosclerotic disorder (MASHAD) study

Variable	CVD (n = 153, 30.4%)	No CVD (n = 350, 69.5%)	<i>p</i>
Female (%)	76 (49.6%)	192 (54.8%)	0.2
Age (years)	53.79 \pm 6.9	48.9 \pm 7.8	<0.001
BMI (kg/m ²)	28.6 \pm 4.6	27.7 \pm 4.9	0.058
PAL	1.54 \pm 0.29	1.57 \pm 0.28	0.2
Non-smoker	97 (63.3%)	239 (20.3%)	
Ex-smoker	25 (16.3%)	39 (11.1%)	0.2
Current smoker	31 (20.26%)	69 (19.7%)	
TC (mg/dl)	199.6 \pm 43.9	188.4 \pm 42.04	0.007
TG (mg/dl)	174.08 \pm 109.2	144.17 \pm 101.3	0.001
LDL-C (mg/dl)	120.7 \pm 36.9	112.8 \pm 38.1	0.03

Values are expressed as mean \pm standard deviation or median (interquartile) for normal and non-normal distribution data respectively. Bold values indicates *p* value <0.05 is considered as significant.

CVD and healthy subjects. Our results indicated that there was a significant correlation between genotypes of rs1748195 and HDL concentration (*p* = 0.02) and CUC (*p* = 0.001) healthy individuals, as well as, there was a significant relationship between rs1748195 genotypes and HDL concentration (*p* = 0.02) in the CVD group. But in rs10899117 and rs11207997 genetic variants, we observed no significant association with HDL function (*p* > 0.05; Table 3).

The associated genotypes with CVD risk were evaluated according to the optimum cutoff value for CUC that were determined according to the previous study by decision tree.³⁰ Based on Table 4, CUC value as HDL functionality, in 409 subjects, was lower than the cutoff (CUC \leq 1.7) and in 96 subjects higher than the cutoff (CUC > 1.7). We found that individuals with GG genotype compared with CC of rs1748195 were correlated to lower risk of CVD (OR = 0.49, 95% CI = 0.24–0.98, *p* = 0.04) in CUC \leq 1.7 group. As well, individuals with CT genotype of rs11207997 were associated to lower risk of CVD (OR = 0.74, 95% CI = 0.41–1.3, *p* = 0.01) compared with CC genotype in CUC > 1.7 group.

Table 5 showed the associated genotypes with CVD risk according to HDL concentration: Low HDL (<40 mg/dl in male & <50 mg/dl in female) and Normal HDL (\geq 40 mg/dl in male & \geq 50 mg/dl in female). These results showed individuals with GG genotype were less likely to be at CVD risk (OR = 0.45, CI = 0.21–0.95, *p* = 0.03) than individuals with the CC genotype of the rs1748195 variant in the low HDL-C group. There were not a significant association of CVD risk and rs10899117 genotypes in the HDL-C group. These results for the rs11207997 variant showed individuals with CT genotype were less likely to be at CVD risk (OR = 0.4, CI = 0.21–0.77, *p* = 0.006) than individuals with the CC genotype in the low HDL-C group, but this relationship was not shown in normal HDL-C group.

TABLE 2 Relationship of the genetic variant with concentration of HDL and cholesterol uptake capacity in studied population

SNP/Variables	Genotypes			p
	AA (N = 224)	AC (N = 231)	CC (N = 44)	
Rs10789117				
HDL	43.5 ± 12.84	41.43 ± 12.7	40.11 ± 11.7	0.1
CUC	1.1 ± 0.55	1.24 ± 0.6	1.2 ± 0.57	0.05
Rs1748195	CC (N = 177)	CG (N = 225)	GG (N = 97)	p
HDL	41.64 ± 12.5	43.3 ± 12.9	40.8 ± 12.3	0.2
CUC	1.22 ± 0.56	1.09 ± 0.55	1.3 ± 0.64	0.006
Rs11207997	CC (N = 81)	CT (N = 389)	TT (N = 31)	p
HDL	42.45 ± 13.3	41.9 ± 12.8	45.5 ± 12.1	0.3
CUC	1.15 ± 0.53	1.2 ± 0.59	1 ± 0.49	0.1

Values are expressed as mean ± standard deviation or median (interquartile) for normal and non-normal distribution data respectively.

Bold values indicates p value <0.05 is considered as significant.

TABLE 3 Relationship of the genetic variant with concentration of HDL and cholesterol uptake capacity in CVD and healthy individuals

SNP/Variables	No CVD				CVD			
	AA (N = 149)	AC (N = 167)	CC (N = 32)	p	AA (N = 75)	AC (N = 66)	CC (N = 12)	p
Rs10789117								
HDL	44.5 ± 14.1	41.6 ± 13.5	41.45 ± 12.1	0.1	41.3 ± 9.6	41.1 ± 10.3	36.54 ± 10.4	0.3
CUC	1.15 ± 0.6	1.24 ± 0.6	1.23 ± 0.57	0.1	1.03 ± 0.43	1.13 ± 0.47	1.11 ± 0.57	0.4
Rs1748195	CC (N = 115)	CG (N = 156)	GG (N = 77)	p	CC (N = 63)	CG (N = 70)	GG (N = 20)	p
HDL	41.46 ± 13.7	45.06 ± 13.9	40.5 ± 12.61	0.02	41.9 ± 10.7	39.3 ± 9.4	42.2 ± 11.6	0.02
CUC	1.29 ± 0.6	1.09 ± 0.58	1.38 ± 0.65	0.001	1.09 ± 0.4	1.1 ± 0.48	0.097 ± 0.49	0.4
Rs11207997	CC (N = 47)	CT (N = 281)	TT (N = 20)	p	CC (N = 34)	CT (N = 108)	TT (N = 11)	p
HDL	45.09 ± 13.2	42.14 ± 13.8	47.7 ± 12.1	0.1	38.8 ± 10.2	41.3 ± 9.8	41.57 ± 11.5	0.4
CUC	1.14 ± 0.56	1.24 ± 0.63	1.09 ± 0.54	0.3	1.15 ± 0.48	1.08 ± 0.46	0.84 ± 0.34	0.1

Values are expressed as mean ± standard deviation or median (interquartile) for normal and non-normal distribution data respectively.

Bold values indicates p value <0.05 is considered as significant.

4 | DISCUSSION

We aimed to the evaluation of the correlation between cholesterol uptake capacity and ANGPTL3 polymorphism in patients with CVD. Our results showed that the rs11207997 gene variant with CT genotype in CUC > 1.7 group was associated with a lower risk of CVD than the CC genotype. Unexpectedly, individuals with GG genotype in rs1748195 ANGPTL3 gene in CUC ≤ 1.7 group were correlated with a lower risk of CVD than CC genotype. We concluded that the effect of genetic polymorphisms along with HDL functionality in the prediction of CVD risk assessment is important.

In a study carried out by Park et al.³² in 7358 subjects, they found that individuals with T allele of rs11207997 had low serum levels of TG and TC compared with CC. The other investigation performed in 1144 adolescents and 1155 adults indicated that participants with rs11207997 variant (CT, TT vs. CC) were correlated to lower HDL-C concentration and lower levels of apo-A1 in both groups.³³

As well, a cohort study was performed in 7384 Korean adults without CVD, cancer, and DM at baseline. Findings revealed that the rs11207997 had a significant adverse relationship with plasma levels of TG and TC.³⁴

In addition, we found that the CT genotype of rs11207997 had a lower risk for CVD in CUC > 1.7 group, it means increasing of HDL functionality result in improvement in RCT reverse cholesterol transport pathway, the reduction of total cholesterol, and eventually toward lower risk of CVD.⁸ The importance of HDL functionality compared with its concentration has been confirmed in the prediction of CVD.^{4,5} Findings from other studies have been shown that HDL function independent of HDL-C concentration was correlated with a predictor of CAD (coronary artery disease).^{35,36} Few studies were carried out for assessment of HDL functionality with the CUC method to evaluate the risk of CVD. The first study was conducted by Harada et al. into the evaluation of CUC on 156 patients in 2017. They reported that cholesterol uptake capacity has

TABLE 4 Association of ANGPTL3 polymorphism with CVD risk in the two groups according to CUC cutoff

SNP	Genotype	CUC ≤ 1.7 (N = 409)		CUC > 1.7 (N = 96)	
		Odds ratio (95%CI)	p	Odds ratio (95%CI)	p
Rs10899117	AA	Ref.		Ref.	
	AC	0.91 (0.57–1.47)	0.7	0.91 (0.23–3.5)	0.8
	CC	0.62 (0.26–1.4)	0.2	4.3 (0.61–30.96)	0.1
Rs1748195	CC	Ref.		Ref.	
	CG	0.75 (0.64–1.23)	0.2	1.64 (0.41–6.4)	0.4
	GG	0.49 (0.24–0.98)	0.04	0.35 (0.05–2.3)	0.2
Rs11207997	CC	Ref.		Ref.	
	CT	0.74 (0.41–1.3)	0.3	0.15 (0.03–0.73)	0.01
	TT	1.4 (0.52–3.7)	0.5	0.23 (0.05–1.38)	0.4

Abbreviations: CI, Confidence interval; Ref, Reference; CUC, cholesterol uptake capacity. Bold values indicates *p* value <0.05 is considered as significant.

TABLE 5 Association of ANGPTL3 polymorphism with CVD risk in the two groups according to HDL concentration.

SNP	Genotype	Low HDL (N = 289)		Normal HDL (N = 215)	
		Odds ratio (95%CI)	p	Odds ratio (95%CI)	p
Rs10899117	AA	Ref.		Ref.	
	AC	0.66 (0.39–1.11)	0.1	0.96 (0.51–1.79)	0.9
	CC	0.81 (0.35–1.89)	0.6	0.42 (0.09–2.01)	0.2
Rs1748195	CC	Ref.		Ref.	
	CG	1.13 (0.66–1.95)	0.64	0.52 (0.26–1.01)	0.05
	GG	0.45 (0.21–0.95)	0.03	0.51 (0.2–1.29)	0.15
Rs11207997	CC	Ref.		Ref.	
	CT	0.4 (0.21–0.77)	0.006	0.76 (0.34–1.96)	0.5
	TT	1.21 (0.28–3.5)	0.9	0.77 (0.22–2.7)	0.6

Abbreviations: CI, confidence interval; Ref, reference. The HDL-C group was categorized based on HDL-C concentration into two types: Low HDL (<40 mg/dl in male & <50 mg/dl in female) and Normal HDL (≥40 mg/dl in male & ≥50 mg/dl in female). Bold values indicates *p* value <0.05 is considered as significant.

inversely correlated to the coronary lesions recurrence rate after revascularization in subjects with the optimal levels of LDL concentrations. They mentioned CUC potentially can be used for CVD risk assessment.¹¹

Whereas, increasing levels of TG, LDL-C, and reduced levels of HDL-C are important adjustable risk factors for CVD³⁷; hence, disturbance in components of any lipid profiles is important in the development of CVD. According to our results, effect of rs1748195 was higher than HDL functionality in CVD risk assessment (CUC ≤ 1.7, *p* = 0.01). One study by Nakayama et al.³⁸ reported a significant reverse correlation between rs1748195 and the concentration of TG in the Japanese population. Another study was conducted by Shen et al.³⁹ in 3503 Chinese children to identify the association between lipid profiles and SNPs. They indicated the a strong correlation (*p* = 0.016) between rs1748195 and TG levels. Also, several GWAS described a reverse relationship between rs1748195 and plasma

concentration of TG.^{40–42} Based on these results, we concluded that the rs1748195 gene variant maybe contributing to the reduction of CVD risk.

The novelty of our study was the first study for the evaluation of ANGPTL3 polymorphism and correlation of HDL functionality for CVD risk assessment. The limitation of our study was a sample size that it was smaller than the total MASHAD cohort study. Also, apolipoprotein-A1 measurement is better to use for normalized CUC than HDL concentration. The thesis does not engage with complete data about the use of lipid-lowering drugs since they might have an influence on HDL functionality. In future investigations, should be considered more studies on new ANGPTL3 loci to estimate the main biomarker for cardiometabolic disorders risk. Furthermore, additional experimental investigations are needed to evaluate HDL functionality along with HDL-C serum for early detection of CVD and tracking the effectiveness of the treatment.

5 | CONCLUSION

Our findings concluded that the CT genotype of rs11207997 variant was associated with a lower risk of CVD in patients with higher HDL functionality. As well, the effect of the rs1748195 gene variant may be higher than HDL functionality in CVD risk assessment.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

We declare that we contributed significantly toward the research study; MA, TK, EMM, MGM, GAF, and AA designed the experiments and revised the manuscript. MA, HS, and PA performed the experiments. MN, PA, and HS wrote the manuscript. MA, MSK, and EA carried out the data analysis. All authors reviewed, considered, and approved the manuscript.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Informed consent was obtained from all subjects using protocols approved by Ethics Committee of the Mashhad University of Medical Sciences (IR. MUMS. Medical. rec. 1386.250) and the Ethics Committee of the Birjand University of Medical Science (IR.bums. rec.1398.51).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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