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ORIGINAL ARTICLE

Association of a genetic variant in angiopoietin-like 3 with serum HDL-C and risk of cardiovascular disease: A study of the MASHAD cohort over 6 years

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Abstract

Background: Loss-of-function (LOF) variants of the angiopoietin-like 3 (ANGPTL3) gene are reported to be associated with serum triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) concentrations and thereby affect the risk of cardiovascular disease (CVD).

Objective: In the present study, we examined the association of rs10789117 in the ANGPTL 3 gene locus and the risk of CVD in the group of people who were part of the Mashhad-Stroke and Heart-Atherosclerotic-Disorders (MASHAD) cohort.

Methods: One thousand and two healthy individuals enrolled in this study of whom 849 subjects were healthy and 153 subjects developed CVD outcomes after 6 years of follow-up. After a 12-h overnight fasting, 20 mL of blood samples were collected for the measurement of fasting blood glucose and lipid profile. DNA was extracted, and the Tetra-ARMS PCR (amplification refractory mutation system)

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was used for genotyping of rs10789117 in the ANGPTL3 gene. The genotype frequencies of the variant of rs10789117 in the ANGPTL3 gene were estimated using χ^2 tests. Eventually, the statistical analysis was done by SPSS version 20.

Results: Individuals with AC/CC genotypes (rs10789117) were found to have to greater risk of CVD events compared to AA genotype (OR = 1.43, 95%CI = 1.01–2.02, $p = 0.041$). There was a 1.3-fold increase in cardiovascular events in individuals carrying the C allele of rs10789117 variant compared to non-carriers (OR = 1.32, 95%CI = 1.06–1.72, p value = 0.038). There were significant differences between different genotypes for serum triglyceride levels within the control group, but this difference was not significant in the group with CVD. Moreover, there was a significant association between CC genotype and CVD risk in the individuals with a normal serum HDL-C.

Conclusion: We have found that a rs10789117 C>A in ANGPTL3 gene polymorphism was associated with incident CVD events, and this may be of value as a risk stratification biomarker in CVD in the Iranian population.

KEYWORDS

ANGPTL3, cardiovascular disease, HDL-C, polymorphism

1 | INTRODUCTION

Genome-wide association studies (GWAS) have identified novel loci associated with CVD risk and serum HDL-C concentrations (Helgadottir et al., 2016). According to the literature review, a LOF polymorphism of the angiopoietin-like 3 (ANGPTL3) gene locus located on chromosome 1, is significantly associated with lipid profile (Lupo & Ferri, 2018; Oldoni et al., 2016). Several studies have reported that ANGPTL3 LOF mutation carriers have lower serum triglycerides (TGs) and cholesterol. This ultimately reduces the risk of atherosclerosis in these individuals (Gusarova et al., 2015; Stitziel et al., 2017). The ANGPTL3 gene is highly expressed in the liver and to a lesser degree in the kidney (Zandbergen et al., 2006). This gene is a member of the angiopoietin family, encoding a 70 kDa-secreted protein, which is composed of two domains: an N-terminal coiled-coil-domain and a C-terminal-fibrinogen-angiopoietin like domain (Camenisch et al., 2002). The efficacy of ANGPTL3 protein on lipid metabolism is probably mediated through its CCD (Coiled-coil domain) which is involved in inhibiting LPL (lipoprotein lipase) (Ono et al., 2003) and EL (endothelial lipase) activity, thereby reducing the release of phospholipids from HDL-C and free fatty acids from VLDL (Lupo & Ferri, 2018). As a result, TG plasma levels and TG-rich lipoproteins increase and lead to hypertriglyceridemia and increased risk of atherosclerotic plaque formation as previous experimental studies have reported (Dewey et al., 2016; Gusarova et al., 2015; Romeo et al., 2009).

In recent years, more than 250 SNPs (single nucleotide polymorphisms) have been identified in the ANGPTL3 locus located in non-coding (NC) regions. It has been shown that allele-specific regulation is likely to be through effects on regulatory sites within the gene such as the promoter, enhancer, or silencer elements (Kundaje et al., 2015). Recent evidence has confirmed associations of NC angiopoietin-like 3 gene variants with levels of low-density lipoprotein cholesterol (LDL-C), HDL-C, TG, ANGPTL3 mRNA transcription, and coronary artery disease (Oldoni et al., 2016). Strategies are required to improve the prevention of heart disease and identify individuals at risk of CVD because it accounts for a high percentage of causes of mortality and disability in Iran (Sarrafzadegan & Mohammadifard, 2019; Townsend et al., 2016) and globally. There have been investigations of the association between haplotype of ANGPTL3 polymorphisms including rs10789117 with incident CVD and CVD risk factors including diabetes mellitus, hypertension, obesity, metabolic syndrome, and dyslipidemia (Aghasizadeh, Zare-Feyzabadi, et al., 2021; Oldoni et al., 2016). But to date, there is currently no data on the association between the rs10789117 variant of ANGPTL3 and lipid profile.

Given that there are studies limited data from cohort studies on the association between rs10789117 polymorphism and CVD risk, this paper will focus on the

association between this variant with serum HDL-C concentrations and the incidence of CVD within the MASHAD study cohort.

2 | MATERIALS AND METHODS

2.1 | Population

The Mashhad-Stroke and Heart-Atherosclerotic-Disorders (MASHAD) cohort study was started in 2010 and continued until 2020 with about 9700 participants without cardiovascular disease (CVD), stroke, and peripheral arterial disease at baseline. The follow-up examinations of this cohort study are taken every 3 years (Ghayour-Mobarhan et al., 2015). Of the original cohort, 215 individuals developed CVD outcomes over the 6 years of follow-up period. The diagnoses of these patients were confirmed by a cardiologist. In our study, 1002 healthy individuals, of whom 849 were healthy without clinical cardiovascular disease and 153 subjects who then developed CVD outcomes confirmed by a specialist cardiologist. The exclusion criteria at the time of recruitment into this study were cancer, cardiovascular disease (CVD), stroke, peripheral arterial disease, and chronic kidney disease. Two groups of subjects were identified: (1) those with low HDL-C (539 subjects had an HDL < 40 or 50 mg/dL in men and women, respectively) and those with normal HDL-C (463 normal HDL subjects with HDL ≥ 40 or 50 mg/dL in men and women, respectively); (2) those with CVD event (153 patients) and those without a CVD event (849 healthy) that occurred during the 6 years of follow-up period. Ethics committee approval was obtained from the Mashhad University of Medical Sciences, and written informed consent was obtained from all participants.

2.1.1 | Blood sampling

After a 12-h overnight fasting, 20 mL of blood was collected from individuals in the baseline study and then into plain blood collection tubes for examination of fasting blood glucose and lipid profile. In this study, parameters such as demographic, anthropometric, smoking status, blood pressure measurement, fasting blood glucose (FBG), history of hypertension and diabetes, lipid profile as well as HDL-C, LDL-C, TG, and TC were evaluated.

2.2 | Screening and genotyping

DNA was extracted from a blood sample using the salting-out extraction approach (Mardan-Nik et al., 2019). The extracted DNA was assessed by the use of a NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, DE, USA). Genotyping was undertaken for rs10789117 in the ANGPTL3 gene using the ARMS PCR (amplification refractory mutation system PCR). Tetra-ARMS PCRs were undertaken in a 20 μL volume containing 2 μL of DNA samples, 10 μL of PCR Master Mix, 4.5 μL ddH_2O, 0.5 μL for outer primers and 1.0 μL, 1.5 with the following primers (Forward outer primer 5′-AAAACTCTCATAGGACATGTTTCA T-3′; Forward inner primer 5′-CCCTTTTATCTCTCT ACTACTTAATAACAA-3′; Reverse outer primer 5′-GA GGGTC AGTGTAGAAAAGATGA-3′; Reverse inner primer 5′-CCCTTTTATCTCTCTAC TACTTAATAACA A-3′; Three band patterns were identified: 194 bp for AA, 291 bp for CC and 432 bp) (Table S1). Genotyping reagents were bought from Applied Biosystems (ABI-Veriti 96-well Thermal Cycler). The primers were designed using Primer-1 and Oligo 7 version 7.24 software. PCR cycling protocols were 94°C for 5 min, 31 cycles at 94°C for 1 min, 56°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 5 min. Gel electrophoresis was attempted using a 2% agarose gel. Finally, the genotypes were confirmed by Sanger sequencing as shown in Figure 1. All sequenced samples were analyzed using Finch TV version 1.4.0.

2.3 | Statistical analysis

The statistical analysis was done by SPSS version 20 (IBM Corp, 2011). Baseline characteristics of individuals in studied groups were compared by *t*-test and chi-square for normally distributed parameters and categorical ones, respectively. The genotype frequencies of the variant of rs10789117 in the ANGPTL3 gene were evaluated with χ^2 tests. We used a logistic regression test to determine the association between the rs10789117 variant and the risk of cardiovascular disease after adjustment for confounder's parameters including sex, age, BMI, dyslipidemia, hypertension, diabetes, and smoking status. The confounding factors were considered for the HDL group without dyslipidemia. p values were deemed statistically significant if <0.05. Furthermore, we determined the strength of our study by odds ratio (OR) with a 95% confidence interval (CI).

3 | RESULTS

3.1 | Allele and genotype frequency

The clinical characteristics of the population in the MASHAD study are summarized in Table 1. The study population sample comprised 153 individuals with a CVD

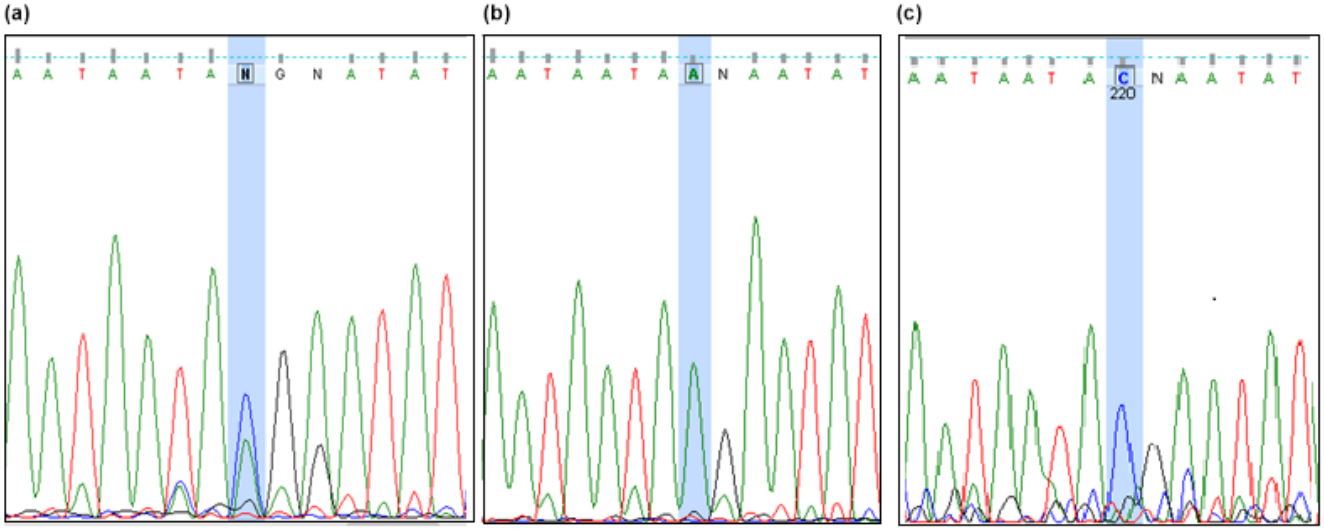

FIGURE 1 The results of DNA sequencing of rs1078917, ANGPTL3 gene; (a) AC genotype, (b) AA genotype, (c) CC genotype.

event (76 (14.2%) females, aged 53.79 ± 6.9 years) and 838 subjects without a CVD event (461 (85.8%) females, aged 48.86 ± 7.7 years) after 6 years of follow-up. The frequency of AA, AC, and CC genotypes was 416 (41.5), 478 (47.7), and 108 (10.8), respectively, Allele A and C (common and uncommon allele) frequencies for the rs10789117 variant were predicted as 65.5% and 34.5%, respectively. The minor allele frequency (MAF) is equal to 0.34. It also noted that genotype frequencies of the rs10789117 locus were in Hardy–Weinberg equilibrium (HWE) (p value > 0.05).

3.2 | Association of genotypes of rs10789117 with cardiovascular events

The distribution of rs10789117 genotype and allele frequencies and its association with CVD events are shown in Table 2. ANGPTL3 gene rs10789117 genotypes frequencies were assessed for two study groups (CVD events and control) as 18% and 42% for AA, 13.8% and 46.2% for AC, and 11.1% and 88.9% for CC, respectively. Allele A frequency was 16.5% and 83.5%, and the frequency of allele C was 13% and 87% in the CVD and control groups, respectively. Moreover, this SNP was associated with CVD in the dominant genotype ($p = 0.041$, CC&AC vs. AA), but in the recessive and additive genetic models, there was not a significant association ($p = 0.18$, $p = 0.2$). We found that about the rs10789117, CC&AC vs. AA genotypes were associated with an increased risk of CVD (OR = 1.43, 95%CI = 1.01–2.02, $p = 0.041$). It is noteworthy that there was a 1.43-fold increase in cardiovascular endpoints among individuals who carriers the rs10789117 polymorphism compared to the non-carriers. Also, the C allele of the genetic variant showed a significant association with the CVD event (OR = 1.32, 95%CI = 1.06–1.72, p value = 0.038) during 6 years of follow-up. Multivariate analysis of our results adjusted with age, sex, diabetes, BMI, smoking, hypertension, and dyslipidemia (traditional risk factors for CVD). We found a positive relationship between individuals possessing a C allele of the rs10789117 variant and CVD risk.

3.3 | Association of rs10789117 variant with traditional risk factor of CVD

Associations of rs10789117 variant with specific CVD risk factor parameters were compared in the CVD group using Pearson's χ^2 tests (Table 3). There was not an association between lipid profile (including HDL, LDL, and TC) and ANGPTL3 variant rs10789117 in both CVD and control groups. The results of our analysis showed that there was a significant difference in TG level between variants genotypes ($p = 0.043$). Our analysis indicated that there was a significant difference between sex and the rs10789117 variant in the control group. (Female %; AA: 60.6%, AC: 48.8%, CC: 56.2%, p value = 0.006). However, no significant difference in age, BMI, PAL, or smoking habits was observed in the rs10789117 variant.

3.4 | Association of rs10789117 polymorphism genotypes with HDL concentration

Five hundred and thirty-nine participants were found to be in the low serum HDL-C category and the rest ($N = 463$) in the normal HDL-C category. The ANGPTL3 rs10789117 genotype (AA, AC, and CC) frequencies were assessed for the low HDL-C group as 39.7%, 49.2%,

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

Variable	CVD ($n=153$)	No CVD ($n=849$)	p
Anthropometrics			
Female (%)	76 (14.2%)	461 (85.8%)	0.29
Age (years)	53.79 ± 6.9	48.86 ± 7.75	<0.001*
BMI (kg/m^2)	26.6 ± 4.6	27.7 ± 4.8	0.032*
FBG (mg/dL)	118.3 ± 62.7	92.79 ± 37.5	<0.001*
PAL	1.54 ± 0.29	1.59 ± 0.28	0.046*
Hypertension, % (*N*)	87 (23.5%)	284 (76.5%)	<0.001*
Diabetes, % (*N*)	56 (30.4)	128 (69.6%)	<0.001*
Smoking status			
Non-smoker	97 (13.9%)	602 (86.1%)	
Ex-smoker	25 (23.1%)	83 (76.9%)	0.038*
Current smoker	31 (17%)	151 (83%)	
Blood pressure			
BSP (mmHg)	131.6 ± 20.8	122.1 ± 19.1	<0.001*
BDP (mmHg)	83.1 ± 10.13	79.5 ± 11.6	<0.001*
Lipid profile			
TC (mg/dL)	199.6 ± 43.9	190.4 ± 41.6	0.013*
TG (mg/dL)	585.86 (89,636)	479.59 (401,900)	<0.001*
LDL-C (mg/dL)	120.7 ± 36.9	113.7 ± 37.4	0.031*
HDL-C (mg/dL)	40.8 ± 10.01	43.35 ± 13.2	0.022*

Note: Values are expressed as mean ± standard deviation or median (interquartile) for normal and non-normal distribution data, respectively. p value <0.05 is considered as significant. We grouped persons based on HDL-C concentration into two types: Low HDL (<40 in male & <50 in female) and normal HDL (≥40 in male & ≥50 in female).

*$p \leq 0.05$.

and 11.1%, respectively, whereas for the normal HDL-C group 43.6%, 46%, and 10.4%, respectively. The relationship between CVD and different genotypes in the two HDL-C groups is shown in Table 4. As regards multivariate analyses adjusted for traditional CVD risk factors (including age, sex, BMI, smoking, diabetes, hypertension, and physical activity). The results showed individuals with the CC genotype were more likely to be at CVD risk (OR = 8.59, CI = 1.15–69.48, $p = 0.036$) compared to the individuals carrying the AA/AC genotype of the rs10789117 variant in the normal HDL-C group using a multivariate logistic regression test. The result did not show a significant association between CVD risk and individuals carrying the CC genotype in the low HDL-C group. The C allele of the rs10789117 showed a significant association with the CVD event (p value < 0.001 and 0.001 for the low and normal HDL-C group, respectively) during follow-up.

4 | DISCUSSION

To the best of our knowledge, this is the first study exploring the value of a genetic variant ANGPTL3 rs10789117 in a large and cohort-based program for evaluating its value as a risk stratification biomarker. Our data showed a significant relationship between ANGPTL3 rs10789117 and increased risk of CVD event, which is in line with recent GWASs (Jiang et al., 2018; Oldoni et al., 2016). The results of the correlational analysis suggest that individuals with a CC genotype had higher serum TG levels compared to AA/AC. Also, prior clinical and genetic studies have established that loss-of-function variants in ANGPTL3 are related to decreased plasma TG, LDL-C, and HDL-C, which leads to a significant decline in cardiovascular risk (Camenisch et al., 2002; Koishi et al., 2002; Minicocci et al., 2012; Ono et al., 2003). However, the mentioned variant has previously been examined in a few other studies but, the findings of this study were confirmed by GWASs with respect to the ANGPTL3 gene (Di Costanzo et al., 2017). Our study also showed that the frequency of C alleles in rs10789117 was 34.5% as the risk allele for CVD. These results are consistent with the minor allele frequency of other studies like the 1000 Genomes project ($C = 0.435/2182$) (Siva, 2008) and in the East Asian population ($C = 0.233/235$) (Stitziel et al., 2017). Our results indicate a significant association between genotypes of rs10789117 and sex so females are more likely to have genotype CC compared to AA/AC in the control group ($p = 0.006$). This finding is in agreement with Asselbergs et al.'s (2012) findings which showed a statistical difference between genders, with SNP rs10789117 in DOCK7 being significant only in females (Asselbergs et al., 2012).

Exome sequencing studies have identified polymorphisms of ANGPTL3 that are associated with serum HDL-C, LDL-C, and TG (Di Costanzo et al., 2017). A LOF ANGPTL3 mutation has been reported to result in FHBL2. Following this mutation, a decrease in all lipoproteins (except lipoprotein a) and ultimately a decrease in the risk of atherosclerotic CAD will be observed (Di Costanzo et al., 2017; Stitziel et al., 2017; Wang et al., 2015). In reviewing the literature, an association between ANGPTL3 and HDL-C was found in humans (Graham et al., 2017; Musunuru & Kathiresan, 2016). By binding to EL and LPL (through its coiled-coil domain), the target gene inhibits its ability to release phospholipids and FFAs from HDL-C and VLDL (respectively). This will lead to an increase in triglyceridemia and the creation of atherosclerotic plaques (Lupo & Ferri, 2018). To our knowledge, the association of ANGPTL3 variant and CVD events and HDL-C level has not been assessed in a cohort study with available clinical follow-up for CVD end-points. Another important finding was that there was a positive association

TABLE 2 Genotype frequencies and association of the rs10789117 SNP in control and CVD group (1002 sample) in different genetic models.

				Univariate		Multivariate[a]	
Genotype/allele	Total *N* (%)	CVD *N* (%)	No CVD *N* (%)	Odds ratio (95%CI)	*p*	Odds ratio (95%CI)	*p*
AA	416 (41.5)	75 (18)	341 (82)	Ref.		Ref.	
AC	478 (47.7)	66 (13.8)	412 (46.2)	1.37 (0.95–1.96)	0.086	1.27 (0.86–1.8)	0.22
CC	108 (10.8)	12 (11.1)	96 (88.8)	1.76 (0.91–3.37)	0.083	1.91 (0.94–3.8)	0.07
AA	416 (41.5)	75 (18)	341 (82)	Ref.		Ref.	
CC&AC	586 (58.5)	78 (13.3)	508 (86.7)	1.43 (1.01–2.02)	0.041	1.36 (0.94–1.96)	0.098
A	1310 (65.5)	216 (16.5)	1094 (83.5)	Ref.		Ref.	
C	694 (34.5)	90 (13)	604 (87)	1.32 (1.06–1.72)	0.038	–	–

Abbreviation: CI, confidence interval; Ref, reference.

[a]Association between SNP and CVD was performed using the logistic regression model after adjusting for age, sex, BMI, smoking, diabetes, hypertension, PAL, dyslipidemia.

TABLE 3 Association of the genetic variant of the rs10789117 SNP with clinical characteristics of population in studied groups.

	Genotype							
	CVD				No CVD			
Variable	AA	AC	CC	*p*	AA	AC	CC	*p*
Anthropometrics								
Age (year)	54.9 ± 7.07	52.7 ± 6.8	52.2 ± 6.4	0.13	48.7 ± 7.7	48.9 ± 7.7	48.9 ± 7.7	0.95
Female (%)	38 (50.7%)	32 (48.5%)	6 (50)	0.96	206 (60.6%)	201 (48.8%)	54 (56.2)	0.006
BMI (kg/m^2)	29.1 ± 4.7	28.1 ± 4.6	28.2 ± 4.03	0.41	27.8 ± 4.7	27.6 ± 4.9	27.44 ± 4.6	0.76
PAL	1.56 ± 0.3	1.53 ± 0.3	1.45 ± 0.12	0.49	1.59 ± 0.28	1.58 ± 0.29	1.62 ± 0.29	0.45
Smoking *n*								
Non-smoker	54 (72%)	38 (57.6%)	5 (41.7%)	0.13	252 (74.1%)	350 (69.2%)	73 (76%)	0.38
Ex-smoker	10 (13.3%)	13 (19.7%)	2 (16.7%)		35 (10.3%)	41 (10.2%)	7 (7.3%)	
Current smoker	11 (14.7%)	15 (22.7%)	5 (41.7%)		53 (15.6%)	82 (20.5%)	16 (16.7%)	
Lipid profile								
Cholesterol (mg/dL)	201 ± 46	200 ± 42	178 ± 29	0.22	190.7 ± 40.8	189.4 ± 43	192.9 ± 36.3	0.75
TG (mg/dL)	177.1 (100.3)	164.2 (118.7)	209 (109.2)	0.31	135.8 (90.09)	154.07 (113.4)	143.8 (70.58)	0.043
HDL-C (mg/dL)	41.34 ± 9.6	41 ± 1.27	36.54 ± 3.01	0.31	44.3 ± 13.01	42.47 ± 13.31	44.05 ± 13.4	0.14
LDL (mg/dL)	123.26 ± 35.7	122.43 ± 36.5	98 ± 41.7	0.08	115 ± 37.4	112.5 ± 37.8	113.9 ± 35.9	0.65

Abbreviations: BDP, blood diastolic pressure; BMI, body mass index; BSP, blood systolic pressure; HDL-C, high-density lipoprotein cholesterol; LDL, low density lipoprotein; PAL, physical activity level; TG, triglyceride; WC, waist circumference.

between rs10789117 CC genotype and, CVD risk in the group of individuals with normal HDL-C serum levels. Furthermore, the C allele of the rs10789117 variant confirmed a significant association with the incidence of a CVD event (in the low and normal HDL-C groups). It can be hypothesized that the association between CVD and the rs10789117 polymorphism of the ANGPTL3 gene in addition to being dependent on levels of HDL-C may additionally rely on its function as shown in previous studies (Samadi et al., 2019). Hence, it could conceivably be suggested that the functionality of HDL plays an important role as a predictor for clinical CVD risk (Brunham & Hayden, 2015). This finding supports previous clinical research that has demonstrated the efficacy of increasing HDL-C concentration to decrease cardiovascular risk has failed (Aim-High Investigators, 2011; Schwartz et al., 2012). In another study, the results showed a significant relationship between the rs1748195 genotype of the ANGPTL3 gene and HDL concentration in the CVD group ($p = 0.02$) (Aghasizadeh, Nosrati, et al., 2021).

In reviewing the literature, in 2021, Chen et al. stated that ANGPTL3 inhibition can effectively increase the

TABLE 4 Association of rs10789117 genotype with CVD in the low and normal HDL group.

				Univariate		Multivariate[a]	
HDL group	**Total *N* (%)**	**CVD *N* (%)**	**No CVD *N* (%)**	**Odds ratio (95%CI)**	***p***	**Odds ratio (95%CI)**	***p***
Low HDL (*N* = 539)							
AA	214 (39.7)	46 (21.5)	168 (75.5)	Ref.			
AC	265 (49.2)	40 (15.1)	225 (84.9)	1.54 (0.96–2.46)	0.07	1.64 (0.97–2.75)	0.06
CC	60 (11.1)	10 (16.6)	50 (83.4)	1.36 (0.64–2.9)	0.41	1.48 (0.64–3.3)	0.35
A	693 (64.3)	132 (19)	561 (81)	Ref.			
C	385 (35.7)	60 (15.5)	325 (84.5)	2.36 (1.7–3.28)	<0.001		
Normal HDL (*N* = 463)							
AA	202 (43.6)	29 (14.3)	173 (85.7)	Ref.			
AC	213 (46)	26 (12.2)	187 (87.8)	1.2 (0.68–2.12)	0.51	1.2 (0.64–2.22)	0.56
CC	48 (10.4)	2 (4)	46 (96)	3.85 (0.88–16.7)	0.072	8.95 (1.15–69.48)	0.036
A	617 (66.7)	84 (13.6)	533 (86.4)	Ref.			
C	309 (33.3)	30 (9.8)	279 (90.2)	1.7 (1.1–2.64)	0.01		

[a]Association between rs10789117 genotype and CVD in HDL group using the logistic regression model after adjusting for age, sex, BMI, smoking, diabetes, hypertension, and PAL; the HDL 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).

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

activity of EL and LPL, modulate lipid metabolism, and ultimately lead to a reduction in the risk of cardiovascular events (Chen et al., 2021). These results agree with the findings of other studies (Wang et al., 2019), which was shown 2 nonsense mutations in ANGPTL3 in humans will lead to familial combined hypolipidemia. On the other hand, mentioned that subjects completely deficient in ANGPTL3 showed no evidence of atherosclerotic plaque (Stitziel et al., 2017). Other studies have considered the relationship between ANGPTL4 and cardiovascular risk. According to the ANGPTL3-4-8 model, reviews confirmed that LOF variants in ANGPTL4 reduced cardiovascular risk. According to the ANGPTL3-4-8 model, reviews confirmed that LOF variants in ANGPTL4 reduced cardiovascular risk and Other researchers corroborated these findings (Dewey et al., 2016; Folsom et al., 2008; Peloso et al., 2014; Quagliarini et al., 2012; Romeo et al., 2007, 2009; Smart-Halajko et al., 2011). Smith's study (a cross-sectional study) which was conducted on 72,868 CAD patients and 120,770 controls reported that the E40K variant and other inactivating mutations of ANGPTL4 were associated with a 53% lower risk of CAD compared with non-carriers (Dewey et al., 2016; Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators et al., 2016). The results of the study of Dewey et al. (on 10,552 patients with CAD and 29,223 as a control group) were consistent with the observations of Stitziel. It was found that there is an inverse relationship between CAD and E40K and other ANGPTL4 inactivating mutations (an inactivating mutation in ANGPTL4 reduces the risk of CAD by 44%) (Dewey et al., 2016).

Furthermore, structure evaluation of the ANGPTL3 gene confirmed that this protein has coiled-coil, signal peptide, and FBG domains. The cleavage site of the signal peptide sequence is between positions 16 and 17 amino acid which helps to secrete ANGPTL3 into extracellular space (Camenisch et al., 2002). Consequently, ANGPTL3 inhibits LPL potency to release phospholipids from HDL-C and free fatty acid (FFA) from VLDL via binding LPL with its coiled-coil domain (Wang et al., 2015). This process can enhance TG levels and cause atherosclerotic plaque (Romeo et al., 2009). Also, analysis tools indicated ANGPTL3 has physical interactions with some proteins such as integrin subunit beta 3 (ITGB3) and integrin subunit alpha V(ITGAV) (Lupo & Ferri, 2018; Viney et al., 2016). ITGB3 and ITGAV have regulations on lipid storage and transport. According to this, defecting in ANGPTL3 can cause problems in storing and transporting lipids through ITGB3 and ITGAV (Lupo & Ferri, 2018).

5 | CONCLUSION

We have demonstrated that carriers of the rs10789117 polymorphism of the ANGPTL3 gene had a higher risk for incident CVD. The minor allele frequency of this SNP was 0.34 in the MASHAD cohort study. According to our analysis based on serum HDL concentration, there was

an association between the rs10789117 variant and CVD events in normal HDL-C groups. Therefore, further studies tend to examine HDL functionality along with HDL-C serum in order to early detection of cardiovascular disease and also track the effectiveness of the treatment. Furthermore, ANGPTL3 inhibitors can be used for the treatment of CVDs through some signal pathways.

6 | LIMITATIONS AND STRENGTHS

The project was limited in several ways. First, is that the numbers of patients and controls were relatively small. Also, since this study was a small part of an academic study, other aspects including gene expression were not investigated at this stage. The key strength of this study is that a genetic point associated with CVD was introduced, which could be used in personalized medicine in the future.

AUTHOR CONTRIBUTIONS

We declare that we contributed significantly towards the research study; M.A., T.K., E.M.-M., M.G.-M., and A.A. designed the experiments and revised the manuscript. M.A., R.S., and P.A.-S. performed the experiments. M.A., P.A.-S., and G.A.F. wrote the manuscript. M.A., H.E., S.S., S.K., and H.G. carried out the data analysis. All authors reviewed, considered, and approved the manuscript.

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The authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

ETHICS STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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