Poursalehi *et al.*
Egyptian Journal of Medical Human Genetics (2022) 23:161
https://doi.org/10.1186/s43042-022-00366-x

Egyptian Journal of Medical Human Genetics

RESEARCH

Open Access

Association of the ANGPTL3 gene polymorphisms and haplotypes with cardiovascular diseases in Birjand longitudinal aging study (BLAS)

Forough Poursalehi[1,2†], Malihe Aghasizadeh[1,2,6†], Somaye Ghorbanzadeh[1,2], Farzaneh heydari[1,2], Tooba Kazemi[2,3], Farshad Sharifi[4], Mitra Moodi[5], Hossen Fakrzadeh[4] and Ebrahim Miri-Moghaddam[2*]

Abstract

Subject: Cardiovascular disease is now well established as an interaction between genetic and environmental components. Newly identified single nucleotide polymorphisms of angiopoietin-like 3 (ANGPTL3) influence lipid concentrations and risk of coronary artery disease. The current study aimed to determine the association between ANGPTL3 gene variants with incident CVDs in elderly population of the Birjand longitudinal aging study (BLAS).

Method: Totally, 360 individuals were recruited in baseline of BLAS including 128 patients with CVD and 153 control subjects. DNA extraction of samples and genotyping were performed by Tetra-ARMS PCR (amplification refractory mutation system polymerase chain reaction). The association between three polymorphisms of ANGPTL3 gene (rs1748195, rs11207997, and rs10789117 variants) with CVD and its risk factors were evaluated using multivariate analysis.

Results: Univariate and multiple analyses showed that individuals carrying the GG genotype of rs1748195 and those carrying the TT genotype of rs11207997 directly increased the risk of CVD. CC genotype of rs1748195 and rs11207997 polymorphisms had a significant negative relationship with the disease. In addition, the findings of this study indicate a significant difference in LDL, HDL, cholesterol levels between different genotypes of the rs1748195 and rs10789117 in the healthy group. Individuals with haplotypes CAC, CCC and CCT showed a significant positive relationship with CVD, CVA, AMI and CHD. As well as haplotype was associated with a 1.7-fold increase in risk of CVA, AMI, and CHD.

Conclusion: We found that polymorphism of ANGPTL3 gene might support to identify individuals with a cardiometabolic and genetic disorders susceptibility. Three haplotypes CAC, CCC and CCT associated with CVD, CVA, AMI and CHD were reported.

Keywords: CVD, ANGPTL3, Cohort, Polymorphism, Haplotype

Introduction

Cardiovascular diseases (CVDs) are a leading cause of death and a common cause of disability worldwide. Their prevalence is expected to increase, which applies a significant economic burden [1]. It has been predicted that by 2030, more than 23.3 million people will die each year due to CVD [2]. CVD burden will increase sharply in Iran

†Forough Poursalehi and Malihe Aghasizadeh contributed equally to this work

*Correspondence: moghaddam4@yahoo.com

[2] Department of Molecular Medicine, Cardiovascular Diseases Research Center, Faculty of Medicine, Razi Hospital, Birjand University of Medical Sciences (BUMS), Birjand, Iran
Full list of author information is available at the end of the article

over 2005–2025, primarily because of the elderly population [3].

Environmental contacts and genetic variation together control the spreading of common diseases within a population. Genome-wide association studies (GWAS) of cohort samples suggest a chance to analyze the etiology and epidemiology of such traits [4]. Candidate genes have diagnosed several genetic loci associated with cardiovascular traits features, and GWAS examined the number of common variants, principally in case–control studies in populations of different heritage and ethnicity [5]. According to the literature, newly identified loci that influence lipid concentrations and risk of coronary artery disease had been shown [6].

An explicit family of secretory proteins has been entitled 'angiopoietin-like proteins' (ANGPTLs), which share a structural resemblance to angiopoietins, the important factors that adjust angiogenesis. The ANGPTLs comprise eight members who play an important role in regulating plasma lipid metabolism in humans and animals [7]. ANGPTL3 is a 70 kDa-secreted protein (54 KDa before glycosylation) primarily expressed in the liver during embryonic growth and in the adult phase [8]. Several studies have already confirmed ANGPTL3 as an ideal target in the pharmacological therapy of CVD and dyslipidemia. Both individuals with loss of function (LOF) mutations in ANGPTL3 or mice with Angptl3 deficiency have lipid-lowering lipoprotein profiles and decreased plasma levels of TG and LDL-C. These results have shown a powerful image of the relationship between ANGPTL3 and CVD risk [7]. Many studies were concentrating on genetic variants of ANGPTL3 in humans in the past decade. The GWASs shown three SNPs at loci near ANGPTL3, containing rs1748195, rs12130333, and rs2131925, have shown three SNPs loci near ANGPTL3, holding rs1748195, rs12130333 and rs2131925, associated with plasma lipid concentrations [7].

The current study aims to determine the association between ANGPTL3 gene variants with incident CVD in elderly population of the Birjand study cohort. Therefore, we investigate the polymorphisms variants of the ANGPTL3 gene, including rs11207997, rs10789117, and rs1748195, in the individual with and without cardiovascular disease.

Methods and materials

Population

The target statistical population was the baseline of Birjand longitudinal aging study (BLAS) in Birjand, collected between 2018 and 2019 by multi-stage random cluster sampling [9]. A total of 1420 the population ≥ 60 years of BLAS. Informed consent was obtained from all subjects, and the study used protocols approved by the Ethics Committee of the Birjand University of Medical Science (IR.bums.rec.1400.062). Their personal information, including demographics factors, anthropometric parameters and lipid, and inflammatory factors, was recorded, and blood samples were taken. A total of 359 samples of this project used, divided into two groups of cardiovascular patients ($n=128$) and non-cardiovascular subjects ($n=231$).

Screening and genotyping

DNA of all subjects was extracted from buffy-coat blood samples using a standard salting-out extraction technique [10]. Quality control of the extracted DNA was determined by using agarose gel electrophoresis (Pars Tous biotechnology), and the quantitative of these was undertaken using Nano Drop 1000 Detector in 280, 260 nm Wavelength (Nano Drop-Technologies, Wilmington, DE, USA). Genotyping was done by applying Tetra ARMS-PCR (amplification refractory mutation system PCR) ofrs10789117& rs1748195, and rs11207997 the ANGPTL3 gene. Tetra ARMS-PCRs were undertaken in a 20 µl volume including ten µl of PCR Master Mix (Pars To us biotechnology), two µl of DNA samples, 4.5 µl ddH_2O, and 1.0 µl & 0.5 µl for inner and outer primers, respectively. The primers were designed using Primer-1 and oligo 7 version 7.24 software and are available in an Additional file 1: Table S1. The PCR program protocols were 94 °C for 5 min as the first phase, 32 cycles at 94 °C for 1 min, 56 °C (rs10789117), 62 °C (rs1748195), and 58 °C (rs11207997) for 1 min, 72 °C for 1 min and a final extension at 72 °C for 5 min. The post-PCR step was done by gel electrophoresis (2% agarose gel), and three bands were identified: 194&291 bp AA and CC in rs10789117, 223&332 bp CC and GG in rs1748195, and 179 and 154 bp CC and TT in rs11207997. Finally, the genotypes were approved by Sanger sequencing. All sequenced samples were analyzed by Finch TV version 1.4.0.

Statistical analysis

SPSS version 20 (IBM Corp, 2011) was used to perform the statistical analysis. T- and chi-square tests were used to investigate the relationship between baseline characteristics of subjects in the CVD and control groups for normally distributed parameters and categorical ones. A $\chi 2$ test was used to genotype frequencies of the ANGPTL3 gene along with the percentage. Multivariate logistic regression was used to predict the association between variants and cardio metabolic disorders after adjusting for confounding parameters, including sex and age. *P* values were measured statistically significant if less than 0.05 (< 0.05). Besides, we undertake the strength of the relationships using an odds ratio (OR) with a confidence interval (CIs) 95%.

Results

Demographic and biochemical characteristics of individuals with CVD and without CVD are presented in Table 1. In this study, 75 individuals with CVD events (58.6% female) and 103 individuals without CVD events (44.6% female) participated. According to the data obtained, patients were older than healthy individuals ($P=0.029$). The results had been demonstrated lipid profile levels (HDL, LDL, TG, TC, and Apo-B) were significantly higher in the healthy group ($P<0.05$ for all). In addition, the investigation indicated that the level of hypertension in the two groups was significantly different ($P=0.001$).

According to Table 2, there is a relationship between rs1748195 ($P=0.008$), rs11207997 ($P=0.021$) the ANGPTL3 gene locus and CVD. Based on the performed analysis, we concluded a significant difference between rs10789117 genotypes in the population-divided CVA group ($P=0.006$). In addition, the results showed that the genotype of the rs1748195 polymorphism is a significant difference between individuals with and without AMI ($P=0.030$).In contrast, no significant difference was reported between the ANGPTL3 genotypes CHD incident.

The frequency of ANGPTL3-related polymorphisms genotypes and alleles in CVD, CVA, AMI, CHD, and healthy groups is shown in Fig. 1. Our result showed significant differences in ANGPTL3 genotypes between CVD and healthy individuals and AMI and healthy individuals. Table 3 shows the association of ANGPTL3 genotype and CVD risk. We found that the GG genotype of rs1748195 was directly associated with CVD risk based on univariate and multiple regression analyses ($P<0.05$). However, the G allele frequency of this variant was significantly higher in healthy individuals than in patients (OR = 1.72, 95% CI = 1.25–2.37, $P=0.001$). Association between SNP and CVD was performed using the multiple logistic regression model after adjusting for age, sex, smoking, hypertension, HDL, LDL, TG, TC. Univariate regression analysis demonstrated that the TT genotype of rs11207997 variant was associated with CVD ($P=0.01$). Also, after adjusting the parameters of age, sex, smoking, hypertension, HDL, LDL, TG, and TC in the Multiple regression model, this data remained significant (OR = 4.80, 95% CI = 1.27–18.11, $P=0.021$).No significant relationship was found in the evaluations performed between the frequency of genotypes and alleles of rs10789117 polymorphism and CVD.

According to the results, rs1748195 ($C>G$) ANGPTL3 was associated with CVD in the dominant model (OR = 1.82, 95% CI = 1.13–2.91, $P=0.014$), while the variants rs10789117 and rs11207997 showed no significant relationship with the disease (Table 4). In contrast, the TT genotype of SNP rs11207997 in the codominant model increased CVD risk by 4.80-fold. On the other hand, carriers of the mentioned variants, except rs10789117 according to the recessive model, are at higher risk of contracting the disease.

According to Table 5, analyses have revealed that lipid profile levels (including Ch, TG, HDL, LDL, Apo-A1, and Apo-B) are not related to CVD in any of the ANGPTL3 polymorphisms ($P>0.05$ for all). However, the difference in the mean LDL level of individuals carrying different genotypes of the rs1748195 variant in healthy individuals was reported to be significant ($P=0.042$). In addition, evaluation of lipid profiles of healthy individuals possessing rs10789117 indicated that Ch ($P=0.021$) and HDL ($P=0.040$) levels in different genotypes of this SNP were related to the health of the study population.

Table 1 Basic and biochemical characteristics of individuals with CVD and without CVD

Variable	Total	CVD ($N=128$)	No CVD ($N=231$)	*P*
Age	68.00 (11.00)	68.50 (12.50)	68.00 (11.00)	0.029
Female (*n*%)	178 (49.6)	75 (58.6)	103 (44.6)	0.011
HDL	45.00 (46.00)	44.00 (5.00)	46.00 (7.00)	0.008
LDL	113.00 (52.00)	96.00 (50.00)	121.00 (47.00)	< 0.001
TG	143.00 (64.00)	134.00 (81.00)	148.00 (65.00)	0.013
TC	190.00 (58.00)	176.81 ± 42.43	199.25 ± 40.29	< 0.001
SBP	132.59 ± 20.81	134.67 ± 20.71	131.44 ± 20.82	0.159
DBP	77.00 (16.00)	78.00 (16.00)	77.00 (14.00)	0.210
BMI	26.35 (6.68)	26.75 (6.59)	26.15 (6.95)	0.080
Apo-A	116.00 (22.00)	115.00 (21.00)	116.00 (22.00)	0.230
Apo-B	93.00 (32.00)	86.00 (26.50)	95.00 (34.00)	0.037
Hypertension (*n*%)	167 (46.5)	74 (57.8)	93 (40.3)	0.001
Diabetes (*n*%)	100 (27.9)	39 (30.5)	61 (26.4)	0.411

CVD Cardiovascular disease, *HDL* High-density lipoprotein, *LDL* Low-density lipoprotein, *TG* Triglyceride, *TC* Total cholesterol, *SBP* Systolic blood pressure, *DBP* Diastolic blood pressure, *BMI* Body mass index

Table 2 Genotype frequencies for the SNPs in the control and CVD groups (360 samples) in different genetic models

Genotype/Allele	CVD			CVA			AMI			CHD		
	Yes ($n=128$)	No ($n=231$)	*P*	Yes ($n=25$)	No ($n=322$)	*P*	Yes ($n=78$)	No ($n=277$)	*P*	Yes ($n=39$)	No ($n=320$)	*P*
rs1748195												
CC	50 (39.1)	122 (52.8)	0.008	11 (44.0)	160 (48.0)	0.815	28 (35.9)	142 (51.3)	0.030	18 (46.2)	154 (48.1)	0.370
CG	49 (38.3)	82 (35.5)		9 (36.0)	122 (36.6)		32 (41.0)	97 (35.0)		12 (30.8)	119 (37.2)	
GG	29 (22.7)	27 (11.7)		5 (20.0)	51 (15.3)		18 (23.1)	38 (13.7)		9 (23.1)	47 (14.7)	
C	149 (58.2)	326 (70.6)	0.001	31 (62.0)	442 (66.4)		88 (56.4)	381 (68.8)	0.004	48 (61.5)	427 (66.7)	0.361
G	107 (41.8)	136 (29.4)		19 (38.0)	224 (33.6)	0.529	68 (43.6)	173 (31.2)		30 (38.5)	213 (33.3)	
rs10789117												
AA	61 (47.7)	109 (47.2)	0.083	12 (48.0)	157 (47.1)		33 (42.3)	134 (48.4)	0.099	19 (48.7)	151 (47.2)	0.984
AC	44 (34.4)	98 (42.4)		5 (20.0)	137 (41.1)	0.006	29 (37.2)	112 (40.4)		15 (38.5)	127 (39.7)	
CC	23 (18.0)	24 (10.4)		8 (32.0)	39 (11.7)		16 (20.5)	31 (11.2)		5 (12.8)	42 (13.1)	
A	166 (64.8)	316 (68.4)	0.331	29 (58.0)	451 (67.7)		95 (60.9)	380 (68.6)	0.071	53 (67.9)	429 (67.0)	0.871
C	90(35.2)	146 (31.6)		21 (42.0)	215 (32.3)	0.159	61 (39.1)	174 (31.4)		25 (32.1)	211 (33.0)	
rs11207997												
CC	28 (21.9)	65 (28.1)	0.021	5(20.0)	88 (26.4)		20 (25.6)	71 (25.6)		9 (23.1)	84 (26.3)	
CT	91 (71.1)	162 (70.1)		19 (76.0)	233 (70.0)	0.858	52 (66.7)	199 (71.8)	1.000	28 (71.8)	225 (70.3)	0.801
TT	9 (7.0)	4 (1.7)		1 (4.0)	12 (3.6)		6 (7.7)	7 (2.5)		2 (5.1)	11 (3.4)	
C	147 (57.4)	292 (63.2)	0.128	29 (58.0)	409 (61.4)	0.633	92 (59.0)	341 (61.6)		46 (59.0)	393 (61.4)	
T	109 (42.6)	170 (36.8)		21 (42.0)	257 (38.6)		64 (41.0)	213 (38.4)	0.560	32 (41.0)	247 (38.6)	0.677

CVD Cardiovascular disease, *CVA* Cerebrovascular accident, *AMI* Acute myocardial infarction, *CHD* Coronary heart disease

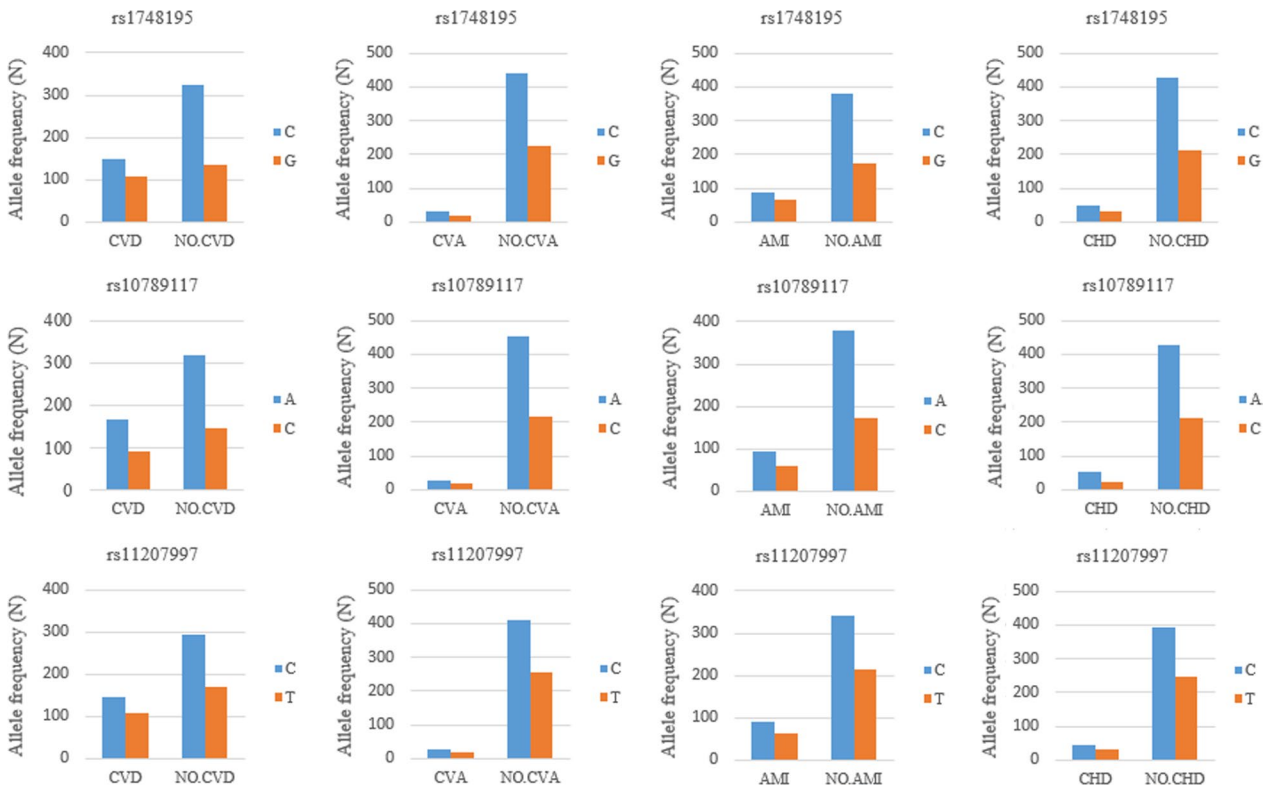

Fig. 1 Evaluation of allele frequency of ANGPTL3 gene polymorphisms with CVD, CVA, AMI, and CHD compared with healthy individuals

Table 3 Association of ANGPTL3 genotype and CVD risk in the studied population

Genotype/allele	Total *N* (%)	CVD *N* (%)	No CVD *N* (%)	Univariate		Multivariate*	
				Odds ratio (95%CI)	*P*	Odds ratio (95%CI)	*P*
rs1748195							
CC	172 (47.9)	50 (39.1)	122 (52.8)	Ref.1	Ref.1	Ref.1	Ref.1
CG	131 (36.5)	49 (38.3)	82 (35.5)	1.46 (0.90–2.36)	0.126	1.58 (0.94–2.67)	0.084
GG	56 (15.6)	29 (22.7)	27 (11.7)	2.62 (1.41–4.87)	0.002	2.43 (1.25–4.71)	0.008
C	475 (66.2)	149 (58.2)	326 (70.6)	Ref.1	Ref.1	Ref.1	Ref.1
G	243 (33.8)	107 (41.8)	136 (29.4)	1.72 (1.25–2.37)	0.001	NA	NA
rs10789117							
AA	170 (47.4)	61 (47.7)	109 (47.2)	Ref.1	Ref.1	Ref.1	Ref.1
AC	142 (39.6)	44 (34.4)	98 (42.4)	0.80 (0.50–1.29)	0.362	0.90 (0.55–1.49)	0.695
CC	47 (13.1)	23 (18.0)	24 (10.4)	1.71 (0.89–3.29)	0.106	1.66 (0.81–3.40)	0.163
A	482 (67.1)	166 (64.8)	316 (68.4)	Ref.1	Ref.1	Ref.1	Ref.1
C	236 (32.9)	90 (35.2)	146 (31.6)	1.17 (0.85–1.62)	0.330	NA	NA
rs11207997							
CC	93 (25.9)	28 (21.9)	65 (28.1)	Ref.1	Ref.1	Ref.1	Ref.1
CT	253 (70.5)	91 (71.1)	162 (70.1)	1.30 (0.78–2.17)	0.310	1.45 (0.84–2.51)	0.188
TT	13 (3.6)	9 (7.0)	4 (1.7)	5.22 (1.48–18.38)	0.010	4.80 (1.27–18.11)	0.021
C	439 (61.1)	147 (57.4)	292 (63.2)	Ref.1	Ref.1	Ref.1	Ref.1
T	279 (38.9)	109 (42.6)	170 (36.8)	1.27 (0.93–1.74)	0.128	NA	NA

* Association between SNP and CVD was performed using the logistic regression model after adjusting for Age, Sex, Smoking, Hypertension, HDL, LDL, TG, TC. *Ref* Reference, *CI* Confidence interval

Table 4 Genetic models association of ANGPTL3 variants with CVD risk in the studied population ($n=360$)

Variant	Allele	Genetic models							
		Dominant OR (95%CI)	*P*	Codominant OR (95%CI)	*P*	Recessive OR (95%CI)	*P*	Additive OR (95%CI)	*P*
rs1748195	C > G	1.82 (1.13–2.91)	0.014	CG: 1.58 (0.94–2.67)	0.084	1.98 (1.07–3.67)	0.029	2.55 (1.31–4.98)	0.006
				GG: 2.43 (1.25–4.71)	0.008				
rs10789117	A > C	1.05 (0.66–1.68)	0.82	AC:0.90 (0.55–1.49)	0.695	1.74 (0.88–3.43)	0.110	1.61 (0.80–3.24)	0.183
				CC1.66 (0.81–3.40)	0.163				
rs11207997	C > T	1.54 (0.89–2.66)	0.123	CT: 1.45 (0.83–2.51)	0.188	3.63 (1.03–12.78)	0.044	6.58 (1.53–28.42)	0.012
				TT: 4.80 (1.27–18.11)	0.021				

Logistic regression model after adjusting for Age, Sex, Smoking, Hypertension, and HDL, LDL, TG, TC

Table 5 Relationship of the genetic variant with clinical characteristics of the population in studied groups

Polymorphisms	CVD ($N=129$)				No CVD ($N=231$)			
Lipid profile (mg/dl)	**CC**	**CG**	**GG**	***P***	**CC**	**CG**	**GG**	***P***
rs1748195								
Cho	174.50 (52.00)	175.00 (40.00)	158.00 (83.00)	0.653	201.00 ± 40.32	202.02 ± 36.62	182.89 ± 47.95	0.079
TG	140.50 (79.00)	130.00 (87.00)	127.00 (60.00)	0.266	148.00 (66.00)	148.00 (66.00)	138.00 (111.00)	0.627
HDL	43.40 ± 4.41	44.94 ± 3.98	44.24 ± 4.59	0.215	46.00 (6.00)	45.50 (9.00)	46.00 (7.00)	0.493
LDL	94.50 (49.00)	101.00 (39.00)	94.00 (68.00)	0.758	123.61 ± 34.32[a]	124.10 ± 33.47	105.81 ± 39.61[b]	0.042
Apo-A1	109.82 ± 19.18	116.68 ± 11.90	104.91 ± 18.23	0.138	117.00 (17.5)	117.00 (25.00)	115.00 (21.00)	0.950
Apo-B	86.00 (35.30)	88.00 (34.30)	80.00 (50.00)	0.409	97.65 ± 27.74	91.76 ± 25.72	90.56 ± 24.19	0.226
Polymorphisms	**CVD ($N=129$)**				**No CVD ($N=231$)**			
Lipid profile (mg/dl)	**AA**	**AC**	**CC**	***P***	**AA**	**AC**	**CC**	***P***
rs10789117								
Cho	181.00 (54.00)	162.00 (50.00)	180.00 (76.00)	0.165	193.96 ± 40.36[a]	207.64 ± 37.95[b]	188.96 ± 44.32	0.021
TG	134.00 (75.00)	137.00 (84.00)	128.50 (95.00)	0.947	146.00 (55.00)	148.00 (86.00)	148.00 (56.00)	0.281
HDL	45.00 (6.00)	43.50 (5.00)	45.00 (6.00)	0.340	45.00 (7.00)[a]	47.00 (7.00)	47.00 (7.00)[b]	0.040
LDL	103.00 (47.00)	93.00 (46.00)	106.50 (53.00)	0.102	117.64 ± 35.40	128.00 ± 33.05	114.46 ± 38.17	0.058
Apo-A1	116.00 (20.00)	107.00 (27.00)	105.00 (16.50)	0.564	115.00 (18.50)	118.00 (20.00)	116.50 (25.30)	0.130
Apo-B	86.00 (40.00)	81.00 (22.00)	92.00 (31.00)	0.325	93.27 ± 26.92	98.10 ± 24.82	86.32 ± 31.27	0.122
Polymorphisms	**CVD ($N=129$)**				**No CVD ($N=231$)**			
Lipid profile (mg/dl)	**CC**	**CT**	**TT**	***P***	**CC**	**CT**	**TT**	***P***
rs11207997								
Cho	177.26 ± 39.94	178.32 ± 43.16	160.33 ± 43.51	0.482	197.42 ± 41.66	199.86 ± 40.18	204.25 ± 25.93	0.891
TG	128.00 (89.00)	138.00 (84.00)	138.00 (75.00)	0.724	149.00 (76.00)	148.00 (60.00)	136.50 (232.00)	0.754
HDL	44.74 ± 4.32	44.04 ± 4.52	43.67 ± 2.87	0.718	45.00 (8.00)	46.00 (6.00)	40.00 (13.00)	0.164
LDL	102.00 (50.00)	96.00 (47.00)	74.00 (60.00)	0.379	122.00 (50.00)	120.50 (47.00)	131.00 (14.00)	0.836
Apo-A1	116.00 (26.30)	115.00 (18.50)	104.50 (25.80)	0.676	116.00 (23.30)	117.00 (21.00)	123.50 (12.00)	0.200
Apo-B	88.00 (43.50)	86.00 (26.50)	89.50 (49.80)	0.863	94.50 (37.50)	95.50 (33.80)	75.50(25.50)	0.250

Cho Cholesterol; *TG* Triglyceride; *HDL-C* High-density lipoprotein cholesterol; *LDL* Low-density lipoprotein

Note: regarding rs1748195 letter "a" means significantly difference between CC and CG and letter "b" means significantly difference between CG and GG. Moreover, regarding rs10789117 letter "a" means significantly difference between AA and AC and letter "b" means significantly difference between AC and CC

Table 6 Association between the non-coding ANGPTL3 haplotype and the risk of CVD, CVA, AMI, CHD

Haplotype	OR (95%CI)							
	CVD	*P*	CVA	*P*	AMI	*P*	CHD	*P*
H1 (CAC)	0.6 (0.44–0.83)	0.002	0.6 (0.4–0.83)	0.002	0.6 (0. 4–0.8)	0.002	0.6 (0.4–0.8)	0.002
H2 (CAT)	1.47 (0.92–2.03)	0.1	1.4 (0.9–2.3)	0.1	1.4 (0.9–2.3)	0.1	1.47 (0.92–2.34)	0.1
H3 (GCC)	1.35 (0.83–2.21)	0.2	1.3 (0.8–2.2)	0.2	1.3 (0.8–2.2)	0.2	1.35 (0.83–2.2)	0.2
H4 (GCT)	1.15 (0.68–1.81)	0.66	1.1 (0.6–1.84)	0.6	1.15(0.68–1.8)	0.6	1.1 5 ((0.68–1.8)	0.6
H5 (GAC)	1.08 (0.51–2.3)	0.83	1.08 (0.5–2.3)	0.8	1.08 (0.5–2.3)	0.8	1.08 (0.5–2.3)	0.8
H6 (CCC)	2.55 (1.17–5.54)	0.014	2.5 (1.17–5.5)	0.01	2.5 (1.17–5.5)	0.01	2.5 (1.17–5.54)	0.01
H7 (GAT)	1.75 (1.03–2.98)	0.1	1.71 (1.03–2.09)	0.03	1.75 (1.03–2.98)	0.03	1.75 (1.03–2.9)	0.03
H8 (CCT)	0.45 (0.23–0.89)	0.02	0.4 (0.2–0.88)	0.02	0.4 (0.2–0.8)	0.02	0.45 (0.23–0.89)	0.02

Haplotype and cardiovascular disorders

Association between the non-coding ANGPTL3 haplotype and the risk of CVD, CVA, AMI, CHD is indicated in Table 6. According to the performed analyzes, three haplotypes CAC, CCC and CCT related to CVD, CVA, AMI and CHD were reported. Although haplotype GAT was not significantly associated with CVD, this haplotype was associated with a 1.7-fold increase in risk of CVA, AMI, and CHD. The other haplotypes studied did not show any significant association with cardiovascular disorders.

Discussion

The present study examined the association between haplotypes of ANGPTL3 gene variants and cardiovascular. According to our results, the CC genotype of rs1748195 and rs11207997 polymorphisms had a significant negative relationship with the disease. Furthermore, univariate and multiple analyses showed that individuals carrying the GG genotype of rs1748195 and those carrying the TT genotype of rs11207997 directly increased CVD risk. In addition, the findings of this study indicate a significant difference in LDL levels between different genotypes of the rs1748195 genotypes in the healthy group and Ch and HDL levels in different genotypes of different genotypes rs10789117. Haplotypes of CAC, CCC and CCT showed a significant positive relationship with CVD, CVA, AMI and CHD. Studies have reported that ANGPTL3 is strongly associated with the risk of developing the disease [11–13]. The ANGPTL3 genetic variants were associated with serum HDL, LDL, TG, TC, Apo-A1, and Apo-B levels in our analysis so reviewing the literature demonstrated that ANGPTL3 deficiency reduces the mentioned cases, and finally, the risk of CVD is reduce [14, 15]. A study conducted in 2018 by Park et al. on 7358 participants (including 3931 females and 3427 males) showed that the genetic variants of the rs11207997 polymorphism at the ANGPTL3 gene locus correlated with TC and TG levels, so that individuals minor allele carriers ($C > T$) had lower TC and TG levels ($P = 0.029$ for TC and $P < 0.001$ for TG) [16]. Also, another study conducted on participants from several European countries (consisting of 1155 adults and 1144 adolescents) reported that this variant was associated with lower levels of Apo-A1 and HDL ($P < 0.05$) in both groups' adults and adolescents. Contrary to these cases, no significant relationship was found between this variant and TG level [17]. A study by Aghasizadeh et al. stated that rs1748195, rs10789117, and rs11207997 genotypes are related to CVD events. Also, the results showed that rs10789117 polymorphism was associated with HTN in the studied patients ($P = 0.01$). On the other hand, the CC genotype of this polymorphism showed a negative relationship with MetS risk in healthy individuals ($P = 0.03$). No association was found between rs1748195 with MetS, HTN and dyslipidemia [18]. Based on the results of a meta-analysis study (includes < 100,000 people), it was revealed that ANGPTL3 has an obvious effect on LDL and TG levels [19].A study conducted by Li et al. (2018) on 1107 patients (539 IS patients and 568 CAD patients) showed that rs12563308 and rs1748195 of the ANGPTL3 gene would be reduce and increase the risk of CAD, respectively. In addition, rs1748195 was found to increase the risk of atherosclerosis, while no association was found between rs12563308 and atherosclerosis [20]. Some other genetic studies have reported that ANGPTL3 with the LOF mutation reduces CVD risk by 34% [21]. According to the results of several studies, loss of function in the ANGPTL3 gene will lead to a reduction in plasma LDL, HDL and TG [8], as well as a 41% reduction in CAD risk [15].

Although many studies have been performed on ANGPTL3, the mechanism of action of this gene is not yet fully understood. Therefore, it is suggested that more detailed studies be performed on the polymorphisms of this gene in the future so that its therapeutic

potential in the prevention and treatment of cardiovascular disorders can be fully utilized. The limitation of this study is that the numbers of patients and controls were relatively small. This article is derived from a medical student's thesis, and access to limited resources was one of the problems that a small number of samples were used in this experiment. Therefore, more samples need to be undertaken to explore better result in this regard. Also, the findings in this report were unable to analyze clinical variables. Although the study has successfully demonstrated that haplotypes of ANGPTL3 gene have a significant relationship with cardiometabolic disorders, it has certain limitations in terms of gene expression. Further studies on more loci of the ANGPTL3 gene are needed for the evaluation of haplotypes as the main biomarker for cardiometabolic disorders risk estimation.

Conclusion

According to our results, univariate and multiple analyses showed that individuals carrying the GG genotype of rs1748195 and those carrying the TT genotype of rs11207997 directly increased the risk of CVD CC genotype of rs1748195 and rs11207997 polymorphisms had a significant negative relationship with the disease. In addition, the findings of this study indicate a significant difference in LDL, HDL, cholesterol levels between different genotypes of the rs1748195 and rs10789117 in the healthy group. The data obtained showed that haplotypes of CAC, CCC, and CCT have a significant relationship with CVD, CVA, AMI, and CHD.

Abbreviations

CVDs: Cardiovascular diseases; GWAS: Genome-wide association studies; ANGPTLs: Angiopoietin-like proteins'; ARMS-PCR: Amplification refractory mutation system PCR.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43042-022-00366-x.

Additional file 1: Table S1. Primers sequences for Tetra ARMS-PCRs for detecting ANGPTL3 gene polymorphisms.

Acknowledgements

We gratefully acknowledge the contributions of the data collection team and the individuals who participated in this study.

Author contributions

We declare that we contributed significantly towards the research study; MA, TK, and EMM designed the experiments. MA, SGH, FH and FP performed the experiments. FP and MA wrote the manuscript, and EMM revised the manuscript. MA, MM carried out the data analysis. All authors reviewed, considered, and approved the manuscript.

Funding

There was no funding.

Availability of data and materials

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

Declarations

Ethics approval and consent to participate

Informed consent was obtained from all subjects using protocols approved by the Ethics Committee of the Birjand University of Medical Science (IR.bums.rec.1400.062).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Student Research Committee, Department of Molecular Medicine, Faculty of Medicine, BUMS, Birjand, Iran. [2]Department of Molecular Medicine, Cardiovascular Diseases Research Center, Faculty of Medicine, Razi Hospital, Birjand University of Medical Sciences (BUMS), Birjand, Iran. [3]Razi Clinical Research Development Unit (RCRDU), Faculty of Medicine, BUMS, Birjand, Iran. [4]Elderly Health Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran. [5]Department of Education and Health Promotion, Social Determinants of Health Research Center, School of Health, BUMS, Birjand, Iran. [6]International UNESCO Center for Health-Related Basic Sciences and Human Nutrition, Mashhad University of Medical Sciences, Mashhad, Iran.

Received: 21 April 2022 Accepted: 6 November 2022
Published online: 14 December 2022

References

1. Li W-J, Yin R-X, Cao X-L, Chen W-X, Huang F, Wu J-Z (2018) DOCK7-ANGPTL3 SNPs and their haplotypes with serum lipid levels and the risk of coronary artery disease and ischemic stroke. Lipids Health Dis 17(1):30
2. Capewell S, Ford ES, Croft JB, Critchley JA, Greenlund KJ, Labarthe DR (2010) Cardiovascular risk factor trends and potential for reducing coronary heart disease mortality in the United States of America. Bull World Health Organ 88:120–130
3. Sadeghi M, Haghdoost AA, Bahrampour A, Dehghani M (2017) Modeling the burden of cardiovascular diseases in Iran from 2005 to 2025: the impact of demographic changes. Iran J Public Health 46(4):506
4. Sabatti C, Service SK, Hartikainen A-L, Pouta A, Ripatti S, Brodsky J et al (2009) Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nat Genet 41(1):35
5. Fiatal S, Adany R (2018) Application of single-nucleotide polymorphism-related risk estimates in identification of increased genetic susceptibility to cardiovascular diseases: a literature review. Front Public Health 5:358
6. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R et al (2008) Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet 40(2):161–169
7. Su X, Peng DQ (2018) New insights into ANGPLT3 in controlling lipoprotein metabolism and risk of cardiovascular diseases. Lipids Health Disease 17(1):1–9
8. Lupo MG, Ferri N (2018) Angiopoietin-like 3 (ANGPTL3) and atherosclerosis: lipid and non-lipid related effects. J Cardiovasc Dev Disease 5(3):39
9. Moodi M, Firoozabadi MD, Kazemi T, Payab M, Ghaemi K, Miri MR et al (2020) Birjand longitudinal aging study (BLAS): the objectives, study protocol and design (wave I: baseline data gathering). J Diabetes Metab Disord 19(1):551–559

10. Mardan-Nik M, Saffar Soflaei S, Biabangard-Zak A, Asghari M, Saljoughian S, Tajbakhsh A et al (2019) A method for improving the efficiency of DNA extraction from clotted blood samples. J Clin Lab Anal 33(6):e22892
11. Francula-Zaninovic S, Nola IA (2018) Management of measurable variable cardiovascular disease risk factors. Curr Cardiol Rev 14(3):153–163
12. Kathiresan S, Melander O, Guiducci C, Surti A, Burtt NP, Rieder MJ et al (2008) Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet 40(2):189–197
13. Atsma F, Bartelink M-LE, Grobbee DE, van der Schouw YT (2006) Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. Menopause 13(2):265–279
14. Tikka A, Metso J, Jauhiainen M (2017) ANGPTL3 serum concentration and rare genetic variants in Finnish population. Scand J Clin Lab Invest 77(8):601–609
15. Dewey FE, Gusarova V, Dunbar RL, O'Dushlaine C, Schurmann C, Gottesman O et al (2017) Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. N Engl J Med 377(3):211–221
16. Park CY, Moon J, Jo G, Lee J, Kim OY, Oh H et al (2019) The association between genetic variants of angiopoietin-like 3 and risk of diabetes mellitus is modified by dietary factors in Koreans. Sci Rep 9(1):1–9
17. Legry V, Bokor S, Cottel D, Beghin L, Catasta G, Nagy E et al (2009) Associations between common genetic polymorphisms in angiopoietin-like proteins 3 and 4 and lipid metabolism and adiposity in European adolescents and adults. J Clin Endocrinol Metab 94(12):5070–5077
18. Aghasizadeh M, Zare-Feyzabadi R, Kazemi T, Avan A, Ferns GA, Esmaily H et al (2021) A haplotype of the ANGPTL3 gene is associated with CVD risk, diabetes mellitus, hypertension, obesity, metabolic syndrome, and dyslipidemia. Gene 782:145525
19. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M et al (2010) Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466(7307):707–713
20. Li W-J, Yin R-X, Cao X-L, Chen W-X, Huang F, Wu J-Z (2018) DOCK7-ANGPTL3 SNPs and their haplotypes with serum lipid levels and the risk of coronary artery disease and ischemic stroke. Lipids Health Dis 17(1):1–12
21. Stitziel NO, Khera AV, Wang X, Bierhals AJ, Vourakis AC, Sperry AE et al (2017) ANGPTL3 deficiency and protection against coronary artery disease. J Am Coll Cardiol 69(16):2054–2063

Publisher's Note